EXHIBIT 33

IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF VIRGINIA
ALEXANDRIA DIVISION

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NICHOLAS HARRISON, et al., :
Plaintiffs, :
vs. : Case No.
PATRICK M. SHANAHAN, et al., : 1:18-cv-641-LMB-IDD
Defendants. :
- - - - - - - - - - - - - - - - - - -x
RICHARD ROE, et al., :
Plaintiffs, :
vs. : Case No.
PATRICK M. SHANAHAN, et al., : 1:18-cv-1565-LMB-IDD
Defendants. :
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Washington, D.C.
Tuesday, May 7, 2019

Deposition of WILLIAM DAVID HARDY, M.D., a witness herein, called for examination by counsel for the Defendants in the above-entitled matter, pursuant to notice, the witness being duly sworn by KAREN YOUNG, a Notary Public in and for the District of Columbia, taken at the offices of Winston & Strawn

LLP, 1700 K Street, Northwest, Washington, D.C., at 9:19 a.m. on Tuesday, May 7, 2019, and the proceedings being taken down by stenotype and transcribed by KAREN YOUNG.

APPEARANCES:

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C O N T E N T S

E X H I B I T S

- - -

at any time for any reason, just tell me or your attorney or Scott. We will finish your answer. If I've already asked a question and once your answer is complete, we'll talk about when to have a break. Does that work for you?

A. Yes.

Q. Sometimes it happens you give an answer and you think you've answered it completely but later on you remember there's some additional information or you perhaps -- you think some clarification is in order. If that happens, please tell us at any time and if you'd like to add something to an earlier answer, and we'll try to take care of it right away while it's still on your mind. Does that make sense?

A. Yes.

Q. In addition, sometimes it occurs to you that your previous answer might not have been entirely accurate. Kind of the same thing, if that happens, just tell us and we will take the time to make the necessary corrections to your answer, okay?

A. Okay.

Q. Now, I've got a few other preliminary matters to take care of. This is kind of pro forma in a deposition, but I want to make sure that you're fully capable of testifying today. Are you taking

any medications or drugs of any kind that might make it difficult for you to either understand my questions or answer truthfully today?

A. No.

Q. Have you had anything alcoholic to drink in the last eight hours?

A. Not in the last 16 years.

Q. Okay. Pretty competent in that one. Is there any other reason that you can think of why you would not be able to answer my questions fully and accurately?

A. No.

Q. And as I mentioned before, there's some water and coffee and soda over there. If you want, let me know if you need to take a break to get anything, and I'm happy to oblige, okay?

A. Yes, okay.

Q. Okay, so let's talk about your involvement in this case. When were you first retained as an expert in the Harrison or in the Roe cases?

A. I was retained officially -- it's in one of the reports because it was an addendum to one of the reports that we created when I became a -- a expert witness. I don't remember the exact date, but it was sometime in April this year.

Q. And were you involved in these cases before you were officially retained?
A. Yes.
Q. When did your involvement begin?
A. Again, probably in late -- sometime in March of this year.
Q. March of 2019?
A. March of 2019.
Q. You weren't involved at all before March of 2019?
A. No.
Q. How were you contacted to become involved in this case?
A. Mr. Scott Schoettes contacted me by e-mail.
Q. And what's -- what are the terms of your engagement as an expert?
A. Again, this was a subject of one of the addendum that we sent in I know. I think I am being remunerated at a rate of $150 an hour for depositions and other kinds of work, or I think there's a daily rate of $1,500 for courtroom days.
Q. Is there a duration of your retainer? Does it end after a certain day or is it continuous through the case?
A. As far as I know, it's continuous through

replication cycle.

Q. Do you know how many FDA-approved pill regimens there are?

A. Oh, my God. I've kind of lost count. I've lost count probably -- I'm going to say there are well over 35 now.

Q. Are there pill regimens that require more than one pill a day?

A. Yes.

Q. Why would someone be on those regimens instead of a one pill a day regimen?

A. That's a good question. Many people ask themselves that every day in my profession. As far as what people start medication on now, the -- the preponderance of patients start on one pill or at most two pills once a day. The persons who are -- and that is becoming less and less all the time. So I would say probably 90 percent of people who start treatment these days start on one pill.

Those who didn't start on one pill are being -- there's a great interest in offering those persons one pill with newer coformulated medications.

So part of it is that in the world of HIV, one is -- is loathe to change a regimen that is working well and being well tolerated, but one of the

very allowable and favorable things to do is to look at a patient's regimen and say we could simplify this to one pill a day and ask the patient if that would be something they would like to happen, and if they can, then we can offer them a prescription for a different pill that oftentimes may contain all the medications someone's taking by taking multiple pills maybe even multiple times a day, but we can actually give them, offer them a much simpler once-a-day regimen.

If that can happen, I for one do that frequently to simplify a patient's life. It's usually done -- I should say it's always done in the -- in my practice in concert with the patient's agreement to do this because even though I think it would be a great idea, if the person doesn't think it's a good idea, it's not going to work.

So -- and I've learned over time of course that the people get attached to their pills in different ways. They have different relationships with their pills that have to be learned -- learned and respected. So that's why someone may not be on one, is because they could be on a older regimen that was started when the single-tablet regimens were not available, because we get one of these new ones about

two or three times a year, and so that's why people may not be on single-tablet regimens.

Q. So you talked a little bit about reasons the patient might not want to switch for various personal reasons. Are there medical reasons why you would not want to switch from -- switch them to a one pill a day regimen?

A. There can be. There can be, but those reasons are -- are interestingly becoming less and less all the time. The two single-tablet regimens that I use most frequently now and try to switch patients as a -- as a starting point for therapy or switch patients to as a change have very, very few side effects, have very, very few drug interactions, and so they're really what I would call -- at this point in time have really created a pinnacle of where we have been able to offer people very simple, very well tolerated, very -- regimens that have very little drug-drug interaction. So the reasons for not being able to put someone on a single-tablet regimen are getting less and less and less all the time.

Q. What -- what are those reasons though?

A. Rarely -- God, it's hard to think of now. For example, one of our regimens that is commonly used today which is a combination of three

A. Patient population that I have seen here in D.C. and continue to see is a rather complex patient population, meaning that not every single patient in the -- in the -- in every -- in every -- not every -- answering your question, probably about 50 percent of my patients are on -- are on a single-tablet regimen, and increasingly more so all the time.

Q. And you were about to explain that your population is more complex. Why is the other 50 percent not on a single-tablet regimen?

A. Many of the patients that I deal with are individuals who have had discontinuous medical care for a variety of reasons, either because they didn't have insurance or they lost insurance, and so the ability for -- their access to continuous medical care and continuous medications has been such that they would take the medication, stop the medication.

While they were off the medication, resistance may have developed, especially if they were on older medication, therefore, the virus that we're dealing with with them has more resistance because of discontinuous therapy, either because they lost that access, they went to jail and lost access, or they just stopped taking the medication for reasons that can happen for lots of different things.

So discontinuous therapy is associated in some cases, not every case, with resistance, and when resistance occurs in a virus, we have to take that into account for the next regimen that we use.

The places that I have worked have been taking care of individuals who classically have been termed individuals who have -- who have encountered challenges to health care, and therefore, their regimens tend to be -- their viruses tend to be more complex, more complicated, and therefore, their regimens have to be a bit more like that as well because of the fact the clinics I've worked in usually care for people who do not have a great history of continuous medical care.

Q. Okay. I think in your next paragraph, paragraph 16, if you want to turn the page, you say after beginning an ART regimen, within four to six weeks, most people's HIV will become virally suppressed, as defined as 200 -- less than 200 copies of the virus per millimeter -- milliliter, excuse me, of blood; is that correct?

A. Correct.

Q. What does this mean in layman's terms?

A. What does it mean? What I was referring to here specifically was using the guideline HHS and IAS

USA guideline recommended medications. One of the reasons that the integrase class has been so highly recommended is because it drops viral load the fastest of any of our classes of antiretroviral medication such that we have seen in -- in multiple studies that by four weeks, upwards of 65 to 70 percent of patients' virus has reached undetectability.

As they continue to take the medication, usually by 12 weeks, 95 percent -- 90 to 95 percent of individuals taking the medication will have suppressed virus. That used to take as long as 24 weeks for that to happen. With integrase inhibitors, that suppression across the board in more people happens very quickly, so at four weeks, it's about 80 percent. By six weeks, it's close to -- by four weeks, it's about 70 percent. By six weeks, it's about 80 percent, and by 12 weeks, it's usually about 90 percent.

Q. So this opinion is based on integrase inhibitor --

A. Yes.

Q. -- class of ART?

A. This is integrase inhibitor ART.

Q. Okay, and then -- but even though there --

these percentages become virally suppressed in four to six weeks, what is recommended regarding testing their viral load again?

A. Usually we test a viral load several -- between four to six weeks after starting therapy. That's our first check, to make sure the medication, number one, is being taken. It's rare for -- rare to nonexistent that the medication will not work even though it's being taken. So it's a double-check that we have a patient come back to, number one, make sure they're taking the medication, or if they encountered any side effect or problem or access issue or whatever, if their dog ate their food -- medication, who knows.

So we check to make sure the medication actually was picked up, taken, is in the person's body, and our best way of doing that is checking their viral load in four to six weeks. If that is -- if that is at undetectable or is near undetectable, what I generally do and what is usually recommended is the next visit is somewhere about three months later, and that one is -- has a very high probability on integrase inhibitor of being undetectable.

The first one should be probably less than a thousand at four to six weeks, and the second one

at, say, probably -- no, let's say four plus another three, which would be seven weeks -- four plus three, yeah, seven weeks, would be undetectable. Not seven weeks. Four weeks plus three months, sorry, three months. Twelve weeks -- 16 weeks would be undetectable.

Q. And what's the standard of care to test after that?

A. Usually about every four months for the first two years, and because if undetected -- if -- if there is a consistent -- say 16 weeks is undetectable, 20 weeks is undetectable, 24 weeks is undetectable, many practitioners will start to stretch out that monitoring out to six months at first, and if there's a couple undetectable at six months and there is a good feeling that the continuation of the patient's therapy, primarily the continuation of the access to therapy is going to be very high and very stable, then some practitioners will be able to stretch that out to once a year. After about two years of every six-month indetectables, they can stretch it out to even longer if they feel like that there has been a very stable and continuous treatment experience.

Q. Let's talk about that first two-year

period. Why does the standard of care require more frequent testing?

A. The main reason is to make sure it's working, because if, for example -- if, for example, medication was started and the patient didn't come back for six months, what could potentially happen, although it doesn't happen very often, is if the patients, for example, became non-adherent during that six months, and usually that first six months is a time the patient is learning to take a pill every day because in large part -- not in all part, but in large part, we are most commonly diagnosing people who are starting therapy under the age of 30, and many people under the age of 30 have never taken medications in their entire life on a regular basis, and so HIV's the first medication that they start taking and are told they have to take every day without exception for a long time.

So there's a real learning curve that people have to go through in terms of sort of getting their head around how do I remember to take this pill every single day? And so not only is there a reason to have them come back at four to six weeks just to make sure that their viral load's going down, but also to talk to them about what technique, what

lifestyle changes they have incorporated into their life to remember to take that pill every day, because that's the learning curve that's so important.

And to tell you the truth, the physician does very little of that these days. The persons who actually do that are medical cases managers, nurses, adherence counselors, people who actually work in the field who might be positive themselves who offer their own experience to this new patient and says here, here's what I do.

So doctors prescribe, doctors check and watch viral loads, but a lot of the more socio -- what do I call it? Behavioral work is actually now done in large part by people who work in concert with the prescriber to help the person learn to take the medications, because we learned early on that just having the patient come in and hear from the doctor and walk out the door is never enough, is never enough, because this not a ten-day or 14-day course of antibiotics, which would probably work if -- even if they got half of it in their body. This is a situation where we expect the adherence, number one, to be good. At least 85 percent is what we're looking for, and long term. So we're really trying to do -- instill a long-term behavioral change in

these people who become HIV positive.

Q. Also in this paragraph, you talk about consistent adherence leads to a CD4 T cell count rebound. Does the -- the CD4 cell count of every person taking this therapy rebound?

A. Good question. I would say in my experience, yes. I've not had a patient -- other than the ones that die from opportunistic infections because they got treated too late, that one percent, I've had -- I've been amazingly surprised that patients who even come in to start seeing me who have initially five T cells, as long as they -- in concert with them taking the medication and bringing their viral load down consistently undetectable, that their T cells even starting that low will build. It takes -- it may take a longer period of time, but they will build even starting as low as five T cells, and clinical trials have shown the same thing. It takes longer if you're starting lower, but it will actually occur. What is key here is getting the virus and keeping the virus undetectable.

Q. And by a longer period of time, what do you mean by that?

A. The period of time that T cells -- it depends on where a T cell count is starting. Our

goal of the T cell counts are to get them over 500. 500 is considered to be the normal range. 500 up are in the normal range. So if someone is starting with 400, they'll be in the normal range probably within six months. If someone's starting at 200, it may take a year. If someone's starting at a hundred, it may take a year and a half. If someone is starting at six T cells, it may take two years.

Q. I think we've talked about this already, but you state -- let's see. It goes from virally suppressed to shortly after, undetectable. What is that time range that you're referring to in here?

A. Let me just -- say that again.

Q. Yeah, between virally suppressed and undetectable.

A. Okay, what paragraph are you --

Q. Still in 16.

A. Okay.

Q. The second sentence, you state that you go from virally suppressed, and shortly after that --

A. Oh.

Q. It becomes undetectable.

A. I guess what I was kind of referring to there is that -- well, virally suppressed defined at less than 200 copies. The definition of what is

considered to be undetectable has changed over time as our assays to be able to measure HIV in the blood have gotten more and more sensitive. There was a time when less than 200 was in fact undetectable, but since that time, better tests have come along that have been able to measure virus down as low as 50, 40 and 20 copies of virus per drop of blood.

So we consider less than 200 to be virally suppressed, and that's primarily coming from research studies that have documented that there's really no difference between less than 200 and less than 50, but in clinical practice, what patients and treating practitioners shoot for is not less than 200, because that test is no longer available and they can't even order it. What they shoot for is less than 50, less than 40 or less than 20, depending upon what test they are using, which is all of course dictated by the financing of medicine and which group they're in, but less than 50, less than 40 or less than 20 is really probably not different at all, but those have really become what is considered to be undetectable. We know that less than 200 is in fact virally suppressed, but what we really call undetectable technically now is less than 50, less than 40 or less than 200 -- less than 20.

tell, yeah. Currently what we're seeing is that long-term detectability is really depending upon several issues, but it's very simply the fact the patient who has committed to take the pill every day. Access to the pill is another important part of this because even though the patient wants to take the pill every day and can take the pill every day, if the patient doesn't have access to the pill, that's a problem. So it's really, you know, the issue of a patient being and remaining adherent to taking the pill every day, number one. Number two, having access to that pill.

Q. Can you define what you mean by consistent adherence?

A. Daily. Daily, but I'll say at least 85 percent. What studies have shown is that even with -- with our integrase inhibitors, we know that if even 85 percent adherence is reached, taking 85 percent of the pills over, say, a three-month period, that -- that undetectability will be preserved, and that's kind of what I would call consistent, at least 85 percent.

Q. Okay. I think in the -- yeah, in paragraph 17, if you want to turn to paragraph 17 --

A. Uh-huh.

Q. You state there factors such as lack of consistent access to health care, which we've talked about, unstable housing, and food insecurity as factors that make adherence more difficult; is that correct?

A. True.

Q. What's the basis for your opinion on this?

A. Well, the basis for this have been studies which have -- which have sought out reasons that patients were asked why they became undetectable -- I mean to why they became detectable again, because one of the -- ever since adherence was identified as being a critical issue in keeping virus suppressed, the -- a tremendous amount of research has been done to investigate the reasons why patients who were undetectable became un -- were undetectable became detectable.

So these are the reasons that have usually popped up when patients were interviewed in clinical research studies to say your -- you were doing great, but what happened? It was because I got kicked out of my house, I didn't have any place to live, I didn't have -- I was looking -- looking for a place to live, I hadn't eaten in three days and any money I got I was spending on food, not buying my

medications, things like that.

Q. Can you explain just in a little bit more detail beyond that other circumstances that would create these types of issues?

MR. SCHOETTES: Objection, vague. You can answer.

A. The ones I would say are losing a job, losing a home, losing a place to live, I'd say, not being able to have access to food. Losing insurance has been one that's been a problem for many people. Losing the medication somehow, having medication and then having it whisked away for one reason or another. Sometimes pharmacies will give them new medication, which isn't always -- which is not always -- which is -- remedies that problem very quickly, but that may depend upon their insurance company. Being put in jail. That's one that has occurred with many of my patients. Once they get thrown in jail, they lose -- they -- medications are usually taken away from them and not represcribed until they leave -- leave the jail, or -- yeah, those are the main ones that I've encountered.

Q. Would you consider living in a tent unstable housing?

A. No.

do.

If, for example, they find themselves in a situation where they are about to run out of medication and they have, say, for example, five pills left, and they think well, I'm going to make these last for ten days. I'll take one today, none tomorrow, one the next day. We say don't do that. Take it every day as -- as directed, and then when you run out, you run out.

A -- a clean stop is the best way to stop HIV medications, because in that way, what happens is is the levels of the medication drop quickly, and we know that that is not associated with viral resistance for some reason. It's been done many times and studied many times. So we tell patients if you run out or you find yourself near the end of your bottle, just run out and then come in and tell -- start again because that's the way we train people to avoid resistance.

Q. You mentioned when you first started talking that the clinical studies involved a couple of classes of drugs. Trying to remember which ones.

A. Integrase.

Q. Right.

A. And boosted protease.

Q. Boosted protease. Is this opinion about stopping or discontinuing the medication limited to those, or is it a opinion for all treatment regimens?

A. Just those two classes.

Q. Your next sentence -- actually, let's -- you relied on this article. Let me pull it out. We'll call it Exhibit 6.

(Hardy Exhibit No. 6 was marked for identification.)

BY MS. CUTRI-KOHART:

Q. Before we get into this article, it talks about U equals U. What does that mean?

A. U equal U is just a acronym for the short little phrase of undetectable equals untransmittable, which means that a viral load that is undetectable -- a person who has a viral load that is undetectable is unable to transmit virus to another person by a sexual route.

Q. Okay. You look to the -- kind of the top left-hand corner box on the back side, yeah, sorry, on the reverse side, the fourth bullet down says, "Stopping therapy negates the validity of assuming that U equals U." Can you explain what that means?

A. Yes, stopping therapy means that the viral

load suppression will be lost, and that the viremia will occur.

Q. So even in situations where resistance doesn't develop on these classes of drugs we were just talking about, it does negate the validity of assumptions regarding transmissibility?

MR. SCHOETTES: Objection, vague. You can answer if you understand.

A. That is part of the assumption, that U equals U is based upon a -- the concept that there will be continuous suppression of virus by continuous taking of antiretroviral therapy by HIV positive individual. If that taking of therapy is -- is interrupted, then there -- there will eventually not be undetectability and there will be measurable virus in the blood again, yes.

Q. How fast can resistance possibly develop?

A. How fast. It depends upon the class of the medication again. We know, for example, that when integrase inhibitors and protease inhibitors have been stopped or even taken intermittently, that resistance does not seem to occur at all, which is why those two classes of medications have been recommended for treatment, because of their resistance to resistance.

However, other classes of medications, such as the non-nucleoside reverse transcriptase inhibitor class, intermittent medication is associated with resistance very easily, as well as the nucleoside class. So it is very much a class-dependent sort of situation.

Q. You can put that aside. We're going to refer back to that article again a couple more times. Leave that out for now. You state in paragraph 18 -- put it aside myself there. In paragraph 18, the second to last sentence, that you would expect that someone who stopped taking their medication would continue to have a suppressed viral load for four to 12 weeks, can you explain the basis of that opinion?

A. Two of them came from -- you know, where that came from was the fact that -- part of it's been my clinical experience, and part of it has been looking at the results of different studies in which what's called analytic treatment interruptions or structured treatment interruptions have been done, and it's been variable from study to study, but it's usually been usually within as quick as two to four weeks and sometimes as long as eight to no -- no resumption. So it's a very broad area overall, but the number I chose was sort of in the middle of that,

somewhere between about four to eight weeks that most patients who go off medication will have detectable virus in their blood within that period of time for the first time again.

Q. And then you rely on this -- now this study from Nature, if you want to mark this as Exhibit 7?

MR. SCHOETTES: Can we clarify something that I think is just going to create a messier record --

MS. CUTRI-KOHART: Sure.

MR. SCHOETTES: -- for you? At the end of the doctor's statement, he said he picked the number -- or he chose four to eight weeks, and that is not what is in the report as what he chose.

BY MS. CUTRI-KOHART:

Q. That's correct. Do you want to correct what's in the report to four to eight weeks or do you want to stand by your opinion?

A. Four to eight's fine. Four to eight will be fine to correct that, sure.

Q. Okay.

A. Just to make sure that it actually jives with the reference.

MR. SCHOETTES: Wait. So are you saying that -- well, I can come back and do this later if

first thing. There are several studies that check viral load every two weeks. I'm sorry, some studies that check viral load as often as two to three times a week and with more frequent checking, and also the other thing -- the other variable that's important is what is considered to be the threshold for calling it detectable again, is it above 50, is it above 200, is it above a thousand.

Different studies have used different thresholds for what they consider to be a rebound of viral load. Different studies have used different time points when they're checking for virus after someone goes off medication. That's what has really created I think a lot of variability as to when the people will call a rebound. There's not been a standardization of what that is like.

So if, for example, someone who's checking viral load once every two weeks after medication is stopped, and the first time they find it is after four weeks, but they didn't find it at two weeks, they don't know exactly when exactly it occurred between two and four. If they're checking it every month, for example, it could be even worse discrimination.

So if they're checking it every -- on the

other end, if they're checking it, say, twice a week, they can get blips up to 200, then go back down, then they get a blip and it goes back down. That could be -- that's been a pattern that's been seen as well. So the difficult thing about this and the way that it's been hard to generalize is that we know that there's about 20 percent of patients who when they go off their medication, especially if they were -- started treatment within a fairly early time after they were diagnosed, 20 percent of patients will never rebound, interestingly, and a couple -- couple studies have actually borne that out pretty well.

Other patients who have a very large viral reservoir, meaning that they usually were started treatment after they'd been on -- had been infected for a long period of time, a large reservoir of -- of -- of latent virus usually predicts a fast -- a faster rebound.

So there's lots of variables that are found in here, all of which we don't know, but we do know that probably 80 percent of people will have a rebound of virus off the medication as early as two to four weeks but maybe as far out as 24 weeks too.

Q. What's the fastest a viral load rebound can occur in?

A. It depends upon how often you check it.

Q. Could you see a viral load rebound in a few days, or would that only be a blip?

A. When I use the word "blip," we're talking about something pretty specific. A blip has to be under 200 and it has to be undetectable to begin with and undetectable again. So a blip is just an up and down but never greater than 200. If it goes -- if it goes on what we call undetectable, a thousand, and undetectable, that's not really a blip. We still consider that to be a breakthrough, but there's been resumption of some control to account for the second undetectable.

So blips just mean that something's coming up, going down, coming up, going down. The word "blip" is -- has been coined to say that this is probably not active viral replication that we are picking up on by our very sensitive viral load test. It is probably what's called heterogeneous -- excuse me, homogeneous proliferation of cells, which means that latently infected cells will increase in number, and one of them will reactivate and react to -- in response to an antigen and a little virus is released. It doesn't mean the virus is replicating. It just means that it's been released into the blood,

none. So they take two of the medications but not the third because the pharmacist didn't give it to them yet because they hadn't come in. We call that selectively -- selective nonadherence, meaning that they took two of the pills but not all three.

Single-tablet regimens get around that because they put all three pills into one pill, all three medications in the same -- in one pill, so there can't be this situation of I'll take two of the three, or I don't like the way this one pill makes me feel when I take it so I'm just not going to take it. I'll take the other two. Single-tablet regimens get around it. So it is all or nothing, which is how we feel like medications work best with integrases and with protease inhibitors.

Q. What would you consider minimal side effects?

A. You know, in the world of medicine, there's not a medication alive today that doesn't have some sort of side effect that some person will say I feel different when I take it. So transient -- why -- why I say minimal and transient is because almost every medication that we have used ever, someone who has taken it says I feel different, I feel nauseous, I feel bloated, I feel like I'm going to have diarrhea,

I have a headache, my skin feels itchy.

That usually lasts for no more than a week to two weeks, but that's a lot better than where we were ten, 15 years ago with medications in which side effects were very pronounced, were very predictable across the majority of patients, such as profuse diarrhea, such as severe anemia, such as very bad stomach upsets. HIV medications have progressed fantastically from being very difficult medications to take to being very easy medications to take.

Q. And so are you primarily basing your opinion as stated here about the side -- the single-tablet side effects being minimal and transient and well tolerated on your experience prescribing them?

A. Both that and clinical trials.

Q. Okay.

A. Clinical trials have documented that very nicely.

Q. And different people taking the same medication can potentially have different side effects; is that correct?

A. Could.

Q. And different HIV medicines, we've talked about a number of different regimens that have been approved by the FDA, those can have differing side

effects.

A. They can, but what has been interesting is that in the past five years, since -- especially since our last two integrase inhibitors have come out, dolutegravir or Tivicay, and bictegravir in Biktarvy, the percentage of patients having side effects has gotten to be so low, especially side effects that have caused patients to stop taking the medications, that over 95 percent of people don't have side effects really at all. It's been remarkable how we have landed upon medications which have the least amount of side effects we've ever seen before in HIV, and that's pretty remarkable if it's across the board.

Q. And your opinion here about the minimal transient and well tolerated -- minimal and transient side effects and well tolerated medicines, does that apply to all the single-tablet regimens available?

A. No.

Q. What are the exceptions?

A. The exceptions are the very first single-tablet regimen that was licensed in 2006 called Atripla has an annoying what's called -- has a very annoying neuropsychiatric medication in it called Sustiva or efavirenz that causes people to get groggy

when they first start taking it. It makes them feel uncomfortable when they wake up. It makes them feel cloudy headed when they wake up. It causes them to have sometimes very vivid and sometimes violent dreams, and because of those side effects, that medication has dropped out of the preferred category to three categories down because of side effects. So -- but of course, that was the first one that we had.

Other ones along the way have had much less side effects, which is why they were developed. I would say the majority of our medications, of our single-tablet regimens have minimal side effects.

Q. Are there other single-tablet regimens besides that one that you would not say have minimal or transient side effects?

A. Only the ones that I would say that are ones that contain what's called a booster. One of the strategies that has been used in HIV therapy to make a medication last 24 hours so that it only has to be dosed once a day has been to couple it with a what's called pharmacoenhancer, a medication that causes the medication to last longer in the patient's blood, and that booster works to prolong the exposure of the patients to the medication for a 24-hour period so it can be dosed once a day.

A booster, however, oftentimes brings on side effects, such as in ten to 15 percent of patients, stomach upset, bloating and diarrhea. So there are one, two, three -- three single-tablet regimens that do have boosters in them, and because of the boosters, they have less tolerability.

Q. Is it true that you can -- some of the antiretroviral therapy medications may have side effects that could appear months or even years after the treatment starts?

A. Yes. I'll qualify that by saying though that the medications we use today have been honed and chosen because of both their short-term and long-term minimal side effects. So although we can never say never, this is not going to occur, experience with medications like Tivicay, which came out in 2012, seven years ago, has not evidenced any new longer term side effects since that time. There's a new medication called TAF that came out in 2015 that is an improvement over an older medication that has reduced kidney and bone mineralization side effects, and that's been out now for four years, and that seems to be well tolerated.

But so far what -- what -- what is becoming -- and I should say there is a new regimen that was

just -- just licensed about three weeks ago called Dovato, which is for the first time not a three-drug regimen, but a two-drug regimen, and that is becoming a regimen -- a -- a strategy of using just two drugs rather than three, and the reason the FDA decided to approve that regimen was because it had equal efficacy but actually less toxicity than a three-drug regimen. So the movement in HIV treatment is to maintain good viral suppression but offer patients regimens that even -- have even less side effects, both short term and long term.

Q. But it is true that some of these medications do have long-term side effects, even if they're rare?

MR. SCHOETTES: Objection, mischaracterizes the prior testimony. To the extent you can answer.

A. Older ones do, yes, older ones have had. The newer ones have less and less.

Q. So there's no kidney problem side effects from the current medications?

A. The TAF medications that I mentioned that are becoming more and more common today have minimal kidney side effects by design. The medication -- that particular -- those medications that contain TAF, those single-tablet regimens that contain TAF

because they can stay on the medications continuously and they don't have side effects anymore.

Q. You qualify this statement with referring only to a person that's diagnosed with HIV in a timely manner. How do you define timely manner?

A. That they're diagnosed as soon as possible, because we do know that the earlier someone's diagnosed in the course of the disease and started on therapy, the better outcome -- the better life span they seem to -- it seems they will have.

We know very clearly that by treating people as early as possible after diagnosis, that the least amount of damage is done to the immune system under a short -- under a shorter period of time, the lesser amount of what's called latent viral reservoir is formed, and the -- the return to health is much faster than someone who's been infected for, say, ten years before they're actually treated. So that's where rapid identification of HIV infection and treatment is really important.

Q. So by rapid, do you mean within a few months of acquiring the infection or a few years?

A. Really as soon as possible. I mean, that's why -- you know, that's why the recommendation is that persons who know that they're at risk for HIV

infection or a health care provider recognizes their risk for HIV infection tests that person at least annually if not more often, so that if a positive test is recognized, that action is taken immediately, not somewhere down the road. But it's really all about screening, which is not unlike what we do for annual cholesterol checking, blood pressure checking, checking someone's weight, looking at some sort of measurement of health, and HIV testing really needs to be part of that.

Q. In the same paragraph in the very last sentence, you talk about the higher prevalence of things like cardiac disease, kidney disease and bone demineralization.

A. Yeah.

Q. We just talked a little bit about the side effects of antiretroviral therapy.

A. Yes.

Q. Is this sentence saying that these things aren't related to those side effects?

A. We don't know. We don't know. This is where things have been really kind of murky, because while we know that, for example, tenofavir has been associated with kidney disease, and that lots of patients around the world have been on tenofavir or

were on tenofovir when it became available in 2003 to where people were switched over to TAF in 2015, and there's a long history there of people being on the medication. We also know that there are other factors inside of a HIV positive person's body, such as the fact that HIV itself can cause kidney disease, and then when we treat HIV infection, kidney disease actually improves. So we have kind of conflicting evidence here sometimes.

We also know that one of the factors that may be associated with more co-morbid conditions in general, heart disease, bone disease, kidney disease, liver disease, might be the fact that there's a condition called immune activation going on in people who's HIV positive, meaning this is the result of HIV itself, and it causes inflammation in lots of organs throughout the body that cause them to change and become less functional.

Bringing that level -- that inflammation down with therapy is very helpful, and it's the most helpful thing to deal with it as far as we know. So the medications are a benefit in that way of taking the pressure that HIV causes organs all over the body, and we've always outweighed the benefit over any toxicity of the medications, but at the same

time, we've worked to make the medications more and more tolerable, like no longer using tenofavir, but using TAF instead, and maybe with this in two-drug regimen, we won't even use TAF anymore. So there's movements in terms of trying to make -- really looking at long-term treatment of HIV.

But the thing we may not be able to fix with -- even with our treatment is the chronic inflammation that still persists at a very low level and treat HIV positive people. That's something that may cause some of -- may account for some of the heart, liver, kidney, bone disease even in treated people. It's a complex situation, still doing a lot of research.

MS. CUTRI-KOHART: This would be a good quick break.

(Recessed at 2:17 p.m.)

(Reconvened at 2:38 p.m.)

BY MS. CUTRI-KOHART:

Q. Okay, I'd like to turn to the next segment of your opinions on the transmission of HIV, which I believe starts on page 7 of your disclosure --

A. Right.

Q. -- part C. Can you tell me what the primary routes of transmission of HIV are?

A. Yes, primary route is sexual, followed by what we call parenteral, meaning blood-to-blood contact, and the third one is by mother-to-child contact. Those are the three primary routes.

Q. What are other routes of transmission?

A. That's really the only three that have been proven to really have chances of transmitting HIV.

Q. You talk in your opinion that you could also transmit via rectal fluids and --

A. Oh, I consider that to be sexual.

Q. Okay.

A. Sexual transmission, yeah, blood, semen, presemen. So the big pictures are sexual transmission with sexual secretions, including semen, preseminal fluid, rectal fluids, vaginal fluids, blood-to-blood contact, and then mother to child either through pregnancy, birth or breast milk.

Q. Okay, great. Can we turn to paragraph 22 of your opinion, which starts on page 8? You mention that the risk of transmission through blood transfusion -- you talk about the risk of transmission through blood transfusion, and you say that such a transmission is rare; is that correct?

A. Correct.

Q. What is the basis for that belief?

A. Basis for that belief are publications from the FDA that has demonstrated that in the United States since the ability to screen blood for HIV in 1985, the transmission of HIV via blood products has dropped dramatically to be less than one in a million.

Q. So the basis is assuming that there is screening in place?

A. Correct, that's what -- that's what I was referring to here.

Q. Okay, and you -- so you would agree that recipients of unscreened blood or blood products from HIV infected donors would be at a higher risk for HIV infection.

A. If blood is unscreened and is coming from an HIV positive person, yes.

Q. And that would be true even if the donor is on antiretroviral therapy?

A. That's unknown, unknown. That's never been -- as far as I know, that's never been tested. Never been tested.

Q. However, if a person receives an HIV -- a single donor blood product from somebody who is HIV infected, the likelihood of them also becoming HIV infected would -- would be high?

A. I wish --

MR. SCHOETTES: Objection. Sorry, lack of foundation, vague. You can answer.

A. That is unknown. That is unknown, and the reason that's unknown right now is that in our current blood system in the United States, if an HIV positive person who has an undetectable viral load donates blood, their blood product would be not allowed for human -- not -- not be allowed for further transfusion because their antibody in the blood would still be positive. It would still be identifiable as a positive blood unit. So I do not know of any data that has ever tested that concept, that with an undetectable plasma viral load, would there be transmission of HIV infection, that's never been tested as far as I know.

Q. When you're at a detectable viral load amount though or above virally suppressed, say, above 200 milliliters per -- or 200 cells per milliliter --

A. Yeah, yeah, 200 viral particles --

Q. Thank you.

A. -- per milliliter.

Q. Milliliter, would -- would transmission occur then in a blood transfusion?

MR. SCHOETTES: Objection, calls for

A. I would include -- I would differentiate needle sticks from drug injection equipment. Needle sticks -- there I was using needle sticks in the -- in the -- in the context of being in a health care setting, yes.

Q. Are there other routes of transmission in health care settings that are not on this list that you considered?

A. Usually what goes along with needle sticks are splash, body fluid splash situations that have a lower quantitative risk factor, risk percentage, I should say, but that's why I didn't include it, because it was even less than needle sticks, but splash injuries could be the same.

Q. Let's turn to paragraph 24 of your opinion. Would it be correct to say that paragraph 24 of your opinion is a summary of the undetectable means untransmittable work that the CDC has been put out recently?

A. Yes.

Q. The CDC study -- is that focused primarily on sexual conduct -- or sexual transmission?

A. Yes.

Q. And so are you taking the results of the CDC studies on sexual transmission and applying that

to your opinions on other forms of transmission?
A. No.
Q. So you're not assuming that the CDC results undetectable means untransmissable applies to other routes of transmission besides sexual conduct?
A. The only thing we can really apply it --
Q. Contact
A. -- to is sexual contact. The only thing that U equals U can be applied to is sexual transmission.
Q. Okay. Can a mother transmit HIV via breast feeding even if she is at undetectable levels of HIV?
A. Not to my knowledge, although I wouldn't be surprised if there were cases of that, but as far as I know, transmission via breast milk is low in a -- in a breast feeding mother who is -- who has suppressed viral load, but it probably can -- probably has occurred.
Q. And then likewise, undetectable means untransmittable is not meant to apply in the transfusion of HIV infected blood contacts, correct?
A. It's not been tested, no.
Q. And again, undetectable means untransmittable is not meant to be used in the context of exposure to blood in other ways, such as

by needle sticks or splash.

A. Has not been tested, no.

Q. Okay. Just want to make sure I've covered all of this. In the context of sexual transmission, the U equals U or undetectable equals untransmittable concept depends on the undetectable patient achieving and maintaining an undetectable viral load. That's correct, right?

A. Correct.

Q. And that includes taking the antiretroviral medication as prescribed, correct?

A. Correct.

Q. And once that person has achieved the undetectable viral load, the person needs to continue to take that medication as prescribed to maintain their undetectable viral load, correct?

A. Correct.

Q. Does the risk of sexually transmitting HIV continue during the first six months of antiretroviral therapy?

A. There seems to be at least a few variables, and that's how long it takes for the viral load to fall from being detectable to being undetectable. There has been at least one or two cases in which someone was on antiretroviral medication but the

viral load was still detectable that transmission did occur. So that's where the U equals U is important, because it doesn't mean on ART equals U. It means U equals U, assuming that the person has been on ART long enough and with a great enough adherence to be able to have produced multiple undetectable laboratory tests. So whether it's 24 months or whether it's something shorter than that can only be proven by when the first undetectable virus -- undetectable blood test is -- is established.

Q. Does the U equals U context define any specific viral load testing intervals, the six-month interval, the 12-month interval?

A. No.

MR. SCHOETTES: I'm sorry -- let me -- objection, vague. You can answer.

A. No.

Q. Okay, let's turn to the next segment of your opinions on neurocognitive impairment --

A. Uh-huh.

Q. -- which starts on page 10, in paragraph 27. Can you first, can you define neurocognitive impairment?

A. Sure. Neurocognitive -- neurocognitive impairment is something that was recognized early in

important. So what U equals U is really trying to express is that it is the viral load at the time of sexual contact that's important to determine whether or not sexual transmission can or cannot happen.

Q. So let me ask another question. Does it matter when the person was last tested if they have an undetectable viral load -- let me try that again. Does the recency of the last test matter in terms of transmission risk if the person has an undetectable viral load at the time of the sexual exposure?

A. I'll try to answer this the best I can. If it's known that the patient has an undetectable viral load at the time of sexual encounter, then that's what really matters. What the viral load was two months ago, three months ago or would be in the future is not as important as what it was at the time of the sexual encounter. That's what counts.

Q. If you look at the statement that's made here that stopping therapy negates the validity of assuming that U equals U, does that say that as soon as the person stops therapy, they are no longer noninfectious?

A. It's not an immediate situation because we know it does take time for viral load to become detectable in blood after medication is stopped.

There is a period of time anywhere from as -- perhaps as short as two weeks to as long as six months or longer that it will take for viral load to become detectable once again after therapy is stopped.

Q. So if you look at the statement itself, it says that stopping therapy negates the validity of assuming that U equals U. Is it ever true that undetectable does not equal untransmittable?

A. Not based upon the concept that we have in clinical trial research.

Q. Is this statement actually saying that you can't assume that you are noninfectious or that you -- I'm sorry, does this statement actually mean that you can't assume that you have an undetectable viral load if you have stopped therapy?

A. Well, I think what U equals U is saying is once there's a correlation between taking therapy on a daily basis or at least -- as long as there's an undetectable viral load while someone's taking medication on a regular basis with regular access to it, that as long as someone is continuing that medication at the same rate and at the same kind of basis, then the viral load will stay undetectable. If the medication is stopped, it is also presumed that at some point, the large majority of people who

stop medication will once again have a detectable viral load at some point down -- into the future.

Q. And just to get a little more clarity here, if you look at the statement with me, it says that stopping therapy negates the validity of assuming that U equals U.

A. Right.

Q. Does U, undetectable, ever not equal untransmittable?

MS. CUTRI-KOHART: Objection, asked and answered.

A. Only if the person has stopped taking the medication, but when it's going to occur is unknown.

Q. I'm going to try one more time.

A. Okay.

Q. Just take the statement out of context, and maybe this is what I did before, but does an undetectable viral load --

A. Uh-huh.

Q. Regardless of whether you took your medication -- the last time you took your medication --

A. Yeah.

Q. -- always mean an untransmittable virus?

A. Yes, yes.

correct?

A. Correct.

Q. Two weeks is less than 12 days, correct? I'm sorry. Two weeks is more than 12 days, right?

A. Fourteen days, right.

Q. So if someone went off of their medication for 12 days, would you expect to see viral rebound?

A. Probably not, no.

MR. SCHOETTES: That's all.

MS. CUTRI-KOHART: Okay.

(Whereupon, at 5:52 p.m., the taking of the instant deposition ceased.)

UNITED STATES OF AMERICA)

ss:

DISTRICT OF COLUMBIA)

I, KAREN YOUNG, a Notary Public within and for the District of Columbia, do hereby certify that the witness whose deposition is hereinbefore set forth was duly sworn and that the within transcript is a true record of the testimony given by such witness.

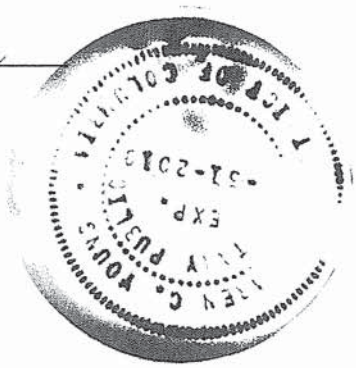
I further certify that I am not related to any of the parties to this action by blood or marriage and that I am in no way interested in the outcome of this matter.

IN WITNESS WHEREOF, I have hereunto set my hand this _____day of_____, 20___.

Karen Young

My Commission Expires:

July 31, 2019



Notice Date: 05/21/2019

Deposition Date: 05/07/2019

Deponent: William David Hardy, M.D.

Case Name: Harrison, et al. v. Shanahan

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CERTIFICATE OF DEPONENT

I hereby certify that I have read and examined the foregoing transcript, and the same is a true and accurate record of the testimony given by me. Any additions or corrections that I feel are necessary, I will attach on a separate sheet of paper to the original transcript.

_____

Signature of Deponent

I hereby certify that the individual representing himself/herself to be the above-named individual, appeared before me this _____ day of _____, 20__, and executed the above certificate in my presence.

_____

NOTARY PUBLIC IN AND FOR

_____

County Name

MY COMMISSION EXPIRES:

EXHIBIT 34

IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF VIRGINIA
ALEXANDRIA DIVISION

RICHARD ROE, ET AL.,	)	
	)	
Plaintiffs,	)	
	)	
VS.	)	CIVIL ACTION NUMBER:
	)	1:18-CV-01565
PATRICK M. SHANAHAN, ET AL.,	)	
	)	
Defendants.	)	

****

ORAL/VIDEO DEPOSITION OF

LT. COL. JASON OKULICZ, M.D.

MARCH 20, 2019

****

ORAL DEPOSITION OF LT. COL. JASON OKULICZ, M.D., produced as a witness at the instance of the Plaintiffs, was duly sworn, was taken in the above-styled and numbered cause on the MARCH 20, 2019, from 1:36 p.m. to 5:30 p.m., before Chris Carpenter, CSR, in and for the State of Texas, reported by machine shorthand, at the offices of Hoffman Reporting, 206 E. Locust Street, San Antonio, TX 78212, pursuant to the Federal Rules of Civil Procedure and the provisions stated on the record or attached hereto.

A P P E A R A N C E S

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ALSO PRESENT:

Major Gregory Morgan
Steve Lopez, videographer
Chris Carpenter, CSR, RPR

INDEX

EXHIBITS

NO.	DESCRIPTION	PAGE MARKED
Exhibit 1	Deposition Notice	8
Exhibit 2	Article: Physical Fitness Characteristics of Active Duty Air Force Members with HIV Infection	76
Exhibit 3	Study: Evaluation of HIV Post Exposure Prophylaxis for Occupational and Non Occumational Exposure at a Deployed U.S. Military Trauma Hospital	83
Exhibit 4	Article: Occupational Exposures and Prevalence of Blood-Borne Pathogens in a Deployed Setting: Data from a U.S. Military Trauma Center in Afghanistan	101
Exhibit 5	Special Report: Operation United Assistance: Infectious Disease Threats to Deployed Military Personnel	105
Exhibit 6	E-Mail Chain Ending June 2, 2017	108
Exhibit 7	Memorandum for Honorable Members of the Board (CONFIDENTIAL)	121
Exhibit 8	Asymptomatic HIV Case Summaries (CONFIDENTIAL)	126

and HIV would be the third.

Q. I can't remember now whether I saw in this article or another, but there is a reference to the rarity or the lack of provision of PEP in the context of blast injuries. Can you explain why PEP would not be offered after potential exposure through blast injuries?

MS. BERMAN: Objection, vague and calls for speculation.

You can answer if you know.

A. The CDC has specific guidelines for blast injury, mass casualty scenarios which could be -- an example of that could be an in-theater explosion environment or it could be a civilian event, an unfortunate -- let's say a terrorist event where there's an explosion. And for lack of better description, material from human beings can be embedded into other human beings, and essentially be an exposure, an occupational or nonoccupational exposure, essentially.

The reason why those guidelines exist was to try to -- in my opinion, is to try to frame this difficult to assess circumstance. But to my knowledge, there is not really good evidence to -- to guide us in these types of scenarios.

Q. (By Mr. Schoettes) And without that guidance, in the absence of that guidance, the CDC does not

recommend provision of PEP to individuals who have been a part of a blast zone?

A. My recollection of that guidance is that PEP is not -- is generally not recommended unless there is a suspicion of HIV. I am not certain how I would know, have a suspicious of HIV, but the guidelines say -- they use the word I believe generally again saying which would -- which would assume that general practice would be just to not use PEP in -- in most of these scenarios.

Q. Okay, we'll do one more and then we'll take a break.

MR. SCHOETTES: If you would, mark this as the Exhibit 5.

(Exhibit 5 marked for identification.)

Q. (By Mr. Schoettes) Do you recognize Exhibit 5?

A. Yes, I recognize Exhibit 5.

Q. And what is Exhibit 5?

A. This is an opinion piece that a number of infectious disease physicians, including myself, in the services wrote and published in 2015 regarding infectious disease threat to deployed military personnel. Specifically, for Operation United Assistance, was when -- for the Ebola outbreak.

Q. And that's Operation United Assistance?

A. Correct.

2019, in response to the promulgation of the DODI on the subject?

A. I believe I have seen that document, yes.

Q. You used the term deployable with limitations to describe airmen living with HIV; is that correct?

A. That is correct.

Q. Do you know if the Air Force has adopted use of that terminology?

A. I'm not aware of the Air Force adopting that terminology. That has been my assessment on someone with HIV who can go overseas with a waiver, someone with HIV who can have a deployment that's not CENTCOM. If that person is deployable, then -- then my -- I tag -- my assessment is that they're deployable with limitations, the limitations essentially being the CENTCOM AOR at this point. So that's not an official Air Force position that I've heard.

Q. And if they were deployable with limitations, is it your understanding that under the new DODI on this topic, they would not be subject to the retention review called for by the new policy?

MS. BERMAN: Objection, calls for speculation.

You can answer.

A. My understanding is that if the deployable with

limitations description exists on a DOD, on a DODI level, my personal opinion would be is that would also apply on the service level, as well, to HIV infection, as I previously mentioned.

Q. (By Mr. Schoettes) I want to switch back up for a moment to talk about treatment interruptions in a deployed environment. What happens if an HIV-positive service member with viral suppression and preserved immune function experiences a treatment interruption in their HIV medications?

MS. BERMAN: Objection, calls for speculation.

You can answer.

A. The vast majority of individuals will become viremic again. Essentially meaning that their -- their suppressed virus will no longer be suppressed, it will detectable and at a higher -- high level.

Q. (By Mr. Schoettes) Assuming the person has an undetectable viral load to start, how long of a treatment lapse is generally required before a person's viral load is no longer virally suppressed?

MS. BERMAN: Objection, calls for speculation.

You can answer.

A. That would vary upon the individual. It could

be in the order of weeks. There are some individuals who have not had a viral rebound after stopping their medication. Although, those individuals are very rare.

Q. (By Mr. Schoettes) Are there individuals for whom it would be shorter than weeks?

MS. BERMAN: Same objection.

A. My opinion, my medical opinion would be that it would take weeks for us to visualize a viral rebound after cessation of medications.

Q. (By Mr. Schoettes) Approximately, when you say weeks, can you put me in a range of how many?

A. I would think it may be possible to see as early as two weeks, but maybe -- maybe four to six weeks. I believe that there may be a more concrete answer to the usual appearance of viremia following cessation of antiretroviral, so I'd have to refer to the literature to get a specific -- a specific number.

Q. Again, assuming a person had an undetectable viral load to start and preserved immune functions, how long of a treatment lapse is generally required before a person's viral load is significant enough to conduct a genotype of the virus?

MS. BERMAN: Same objection.

You can answer.

A. A viral load has to reach a level of

essentially, manifest or visible possibly even decades later. That being said, it could -- it's highly variable when -- when these effects may occur. It's unlikely to be in the short-term for the majority of individuals.

MR. SCHOETTES: I'm done.

MS. BERMAN: Okay. Do you want to talk -- take two like minutes to see if I have any follow-up questions?

MR. SCHOETTES: Sure.

THE VIDEOGRAPHER: Off the record at 5:27.

(Recess.)

THE VIDEOGRAPHER: Back on the record at 5:29.

MS. BERMAN: Okay. I also don't have any questions, but I wanted to let the court reporter know that we want the witness to read and sign the deposition.

THE VIDEOGRAPHER: Thank you. This concludes the testimony given by Jason Okulicz, M.D. Time off the record is 5:30.

(Deposition concluded at 5:30 p.m.)

CHANGES AND SIGNATURE

RE: RICHARD ROE, ET AL., VS. SHANAHAN ET AL.

PAGE	LINE	CHANGE	REASON

I, LT. COL. JASON OKULICZ, M.D., have read the foregoing deposition and hereby affix my signature that same is true and correct, except as noted above.

_____

LT. COL. JASON OKULICZ, M.D.

THE STATE OF _____)

COUNTY OF_____)

Before me,_____, on this day personally appeared LT. COL. JASON OKULICZ, M.D., known to me (or proved to me under oath or through_____ (description of identity card or other document) to be the person whose name is subscribed to the foregoing instrument and acknowledged to me that they executed the same for the purposes and consideration therein expressed.

Given under my hand and seal of office this_____day of _____, 2019.

_____
NOTARY PUBLIC IN AND FOR
THE STATE OF _____

IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF VIRGINIA
ALEXANDRIA DIVISION

RICHARD ROE, ET AL.,	)	
	)	
Plaintiffs,	)	
	)	
VS.	)	CIVIL ACTION NUMBER:
	)	1:18-CV-01565
PATRICK M. SHANAHAN, ET AL.,	)	
	)	
Defendants.	)	

REPORTER'S CERTIFICATION
ORAL/VIDEO DEPOSITION OF LT. COL. JASON OKULICZ, M.D.
MARCH 20, 2019

I, Chris Carpenter, Certified Shorthand Reporter in and for the State of Texas, hereby certify to the following:

That the witness, LT. COL. JASON OKULICZ, M.D., was duly sworn by the officer and that the transcript of the oral deposition is a true record of the testimony given by the witness;

That the deposition transcript was submitted on the _____day of _____, 2019, to the witness or to the attorney for the witness for examination, signature and return to_____, by _____, 2019; and if returned, the original transcript will forwarded to Scott Schoettes, the custodial attorney;

That the amount of time used by each party at the deposition is as follows:

Mr. Schoettes: 3 hours, 56 minutes

I further certify that I am neither counsel for, related to, nor employed by any of the parties or attorneys in the action in which this proceeding was taken, and further that I am not financially or otherwise interested in the outcome of the action.

Certified to by me this 1st day of April, 2019.

Chris Carpenter, Texas CSR 1151
Expiration Date: 6/30/2021
206 East Locust Street
San Antonio, TX 78212
(210) 736-3555

Firm Registration No. 93

EXHIBIT 35

IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF VIRGINIA
ALEXANDRIA DIVISION

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NICHOLAS HARRISON and :
OUTSERVE-SLDN, INC., :
Plaintiffs, :
vs. : No. 1:18-cv-00641
JAMES N. MATTIS, In His : LMB-IDD
Official Capacity As Secretary:
of Defense; MARK ESPER, In His:
Official Capacity As the :
Secretary of the Army; and the:
UNITED STATES DEPARTMENT OF :
DEFENSE, :
Defendants. :
- - - - - - - - - - - - - - - - - - x

VIDEOTAPED 30(b)(6) DEPOSITION OF
UNITED STATES ARMY
GIVEN BY JASON BLAYLOCK

DATE: Wednesday, February 27, 2019
TIME: 9:04 a.m.
LOCATION: Winston & Strawn
1700 K Street, N.W.
Washington, D.C.
REPORTED BY: Denise M. Brunet, RPR
Reporter/Notary

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(Appearances continued on the next page.)

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C O N T E N T S

(*Exhibits attached to the transcript.)

years.

Q Not a bad posting.

A Not bad at all.

Q And then prior to that?

A Prior to that, I was in medical school for four years at Georgetown University school of medicine.

Q Georgetown is also my alma mater, law school. And what year did you obtain your M.D.?

A I obtained my M.D. -- it would have been 2005.

Q And did you have a specialty at that point?

A No. So upon obtaining my medical degree, I completed my internal medicine residency at Tripler, so completed that in 2008. And then went to my infectious disease fellowship at the former Walter Reed from 2008 to 2011. Then spent one year in the viral diseases branch right after that, prior to coming to Walter Reed National Military Medical Center.

Q Obviously, as a part of your training, you spent some amount of time learning about HIV transmission and diagnosis, correct?

A Correct.

Q Do you have any training that is specific to service members living with HIV? I'm talking about training that related to that specific context.

A I'm not aware that there is any training specific to service members living with HIV.

Q So some of these next questions -- we're going to talk a little bit about HIV -- may seem basic to you and -- but it's still important to sort of get down on the record. So what bodily fluids are capable of transmitting HIV?

A Some bodily fluids that are capable of transmission include blood, also via sexual exposure, so semen, vaginal fluids. I can tell you that, you know, there's a lot of body fluids that are not found to really be highly transmissible for HIV: Saliva, sweat, tears.

Q Any other bodily fluids that do present a significant risk of transmission besides blood, semen, vaginal fluids?

A When I say blood, I mean, you know, blood components, you know, as well. So -- but other...

Q What about breast milk?

A Okay. Breast milk, yes.

Q Should be on that list as well?

A Yes, it should.

Q Okay. What factors influence the risk of HIV transmission?

A So the major factors that influence risk include, probably most importantly, the patient's viral load, so number of copies per milliliter of blood. Also, how -- with that, how adherent the patient is to taking their antiretroviral therapy also affects that.

Q And so those two things are related --

A Yes.

Q -- viral load is related to medication adherence?

A Correct.

Q While we're there, is it true that a patient who adheres to their treatment regimen will obtain an undetectable viral load?

A Not in all cases, but to some extent, in the majority of cases, if somebody adheres to what today is considered first-line regimens for antiretroviral therapy, they should be able to attain an undetectable viral load.

Q And if someone is unable to reach an undetectable or suppressed viral load on the first-line regimens, are there second-line

Q What approximately is the per-act risk of transmission from receptive anal intercourse?

A I would have to have the CDC's -- the CDC has a nice table that depicts these different modes of transmission. It's fairly low, but it's not -- I want to say, off the top of my head, like ten out of a hundred thousand, or something close to that. Or maybe it's out of 10,000. I'd have to see the document right in front of me, but...

Q What is the risk of transmission via oral sex?

A Oral sex is very, very low risk of transmission. I'm not sure if the CDC document says negligible for oral sex, but it's -- as far as modes of sexual transmission, that's the lowest.

Q Is it possible to transmit HIV by biting someone?

A It is possible. A couple of factors would have to play. You would have to -- it would have to be a decent bite that breaks whatever mucosal surface you're biting. And that person who's biting would typically have to have also a break in their mucosal surface somewhere in their mouth, whether a lesion in their gumline or in

their oral pharynx somewhere that was bleeding.

Q Has there ever been a documented case of HIV transmission via biting?

A I have not reviewed the literature recently on that, so I wouldn't be able to tell you definitively.

Q What is the risk of transmission of HIV via blood splash?

A Again, assuming an intact mucosal surface, it's negligible.

Q And actually, will you define blood splash for us?

A When I -- my definition of blood splash is if blood or -- splashed onto a person who -- let's say it was a health care provider caring for a patient, and the blood landed on intact skin, then that's a negligible risk of transmission.

Q Has there ever been a documented case of transmission via blood splash?

A Not to my knowledge, but I have not reviewed the literature on that recently.

Q How is HIV treated?

MS. BERMAN: Objection. Vague.

BY MR. SCHOETTES:

Q What is the common treatment for HIV?

A So combination antiretroviral therapy is the preferred and first-line treatment regimen for HIV infection. And there's a handful of them. A couple of them are slated as first-line regimens.

Q And the first-line treatments today, are those all single-tablet regimens?

A The first-line regimens today, there are two single-tablet, one pill one a day, regimens, and then there's a -- it ends up being three, three pills a day regimen that is also still on the first line.

Q And are there some individuals who are on a regimen that's two pills once a day?

A Two separate pills once a day? Yes, one of those pills being a pill that you do take twice a day, though, as well. I don't know if you're thinking of raltegravir as a BID dosing.

Q Okay.

(Discussion held off the record.)

THE WITNESS: Twice daily dosing, BID.

BY MR. SCHOETTES:

Q Can you tell us what -- you referred earlier to pre-exposure prophylaxis. Can you tell us how pre-exposure prophylaxis works?

A So pre-exposure prophylaxis for HIV

involves taking one medication. It's a combination pill of emtricitabine and tenofovir disoproxil fumarate.

THE REPORTER: You need to say those slower, please.

THE WITNESS: And this pre-exposure prophylaxis for HIV involves taking one pill that is a combination of two drugs, emtricitabine, E-M-T-R-I-C-I-B-I-N-E [sic], and tenofovir, T-E-N-O-F-O-V-I-R, disoproxil, D-I-S-I-P-R-O-X-I-L [sic], fumarate, F-U-M-A-R-A-T-E.

BY MR. SCHOETTES:

Q I'll try to avoid asking questions that involve medication names in the answers.

A Once daily to prevent -- if someone were to get exposed to HIV infection, to prevent that virus from being transmitted to that person.

Q And without using medication names, can you explain how post-exposure prophylaxis, or PEP, works?

A Yes. So post-exposure prophylaxis for HIV infection is designed in the sense that if a person were to present for medical care after being potentially exposed to somebody with HIV infection or a high suspicion of HIV infection,

they would be started on at least a three-drug regimen for HIV empiric treatment within 72 hours, is what the guidance is for that exposure. And you continue on that regimen for 28 days, for four weeks. And during that time, you undergo subsequent follow-up, HIV testing and toxicity testing based on the regimen that you started on to ensure that you're tolerating it well and that you do not acquire HIV infection after that exposure.

Q Are the side effects of PEP that is administered currently considered significant?

A No.

Q Have you heard the treatment as prevention?

A I have.

Q Can you tell us what treatment as prevention is?

A So the idea of treatment as prevention is identifying patients or personnel that are at high risk of HIV infection, identifying them and identifying if they are actively infected with HIV and getting them on treatment as quickly as possible and, by doing that, preventing transmission to other -- other individuals.

circumstance, it's -- it depends on a lot of variables that we've already discussed, if they're taking their HIV medications regularly, what kind of environment they're in, what access they have to medications and lab testing. It depends on a lot of different variables, where they are in the world.

Q So the risk of transmission in that context would have to take into account all of those different variables?

A Correct.

Q And the likelihood that each of those circumstances would be occurring at that time?

A Correct.

Q Is the probability of HIV transmission affected by the role in which the HIV-positive soldier is serving?

A To -- to some extent, yes.

Q And can you describe how the role affects the risk of transmission?

A So based on where a service member may be in the world -- let's say, for example, if a service member is deployed to a combat environment with little to no capabilities for access to pharmacy, access to laboratory testing and medical

services in general, and based on the stresses in that combat environment, that would potentially affect a service member's HIV infection -- ability to maintain control of their HIV infection.

Q And are both of those factors that you just cited, access to pharmacy/medical services and stressors, are you saying that those things would affect the soldier's ability to adhere to their medications?

A Potentially, yes.

Q And that that's the mechanism by which there could then be a risk to other personnel?

MS. BERMAN: Objection. Mischaracterizes the testimony.

You can answer.

THE WITNESS: Yes, to other personnel or themselves, yes.

BY MR. SCHOETTES:

Q So let's just focus on other personnel for a moment.

A Okay.

Q Can you describe the mechanism by which, then, the increased viral load creates a risk to other personnel?

MS. BERMAN: Objection. Lack of

foundation.

You can answer.

THE WITNESS: So an increase in viral load -- you wanted to refer to other personnel, how -- its effects on other personnel first, you said?

BY MR. SCHOETTES:

Q Yes.

A So somebody with an increased viral load in a deployed setting, the biggest concern is the potential need for activation of emergent blood transfusions in the deployed setting. So should that person donate blood in a deployed setting where we don't have the -- oftentimes the appropriate resources to exclude HIV infection prior to blood donation, then that would pose a significant risk to somebody receiving that unit of blood or units of blood.

Q Setting aside blood donation, which we will return to, what are the other mechanisms by which a person with a non-suppressed viral load would present a risk to the health of other personnel?

A Via sexual transmission is the other most likely.

Q Anything else?

A No. We don't allow pregnant, breast-feeding women in theater, so it would not include that.

Q So if a soldier has an undetectable viral load, what is the probability that that soldier will endanger the health of other personnel?

A Again, it depends on the scenario. If we take that previous scenario of an HIV-infected service member in a deployed setting and in the potential setting of an emergent blood transfusion need, there is still a significant risk of transmission of HIV infection.

Q Then again, setting aside -- or not even setting aside -- any other potential risk other than the service member donating blood?

A So the other risk we didn't mention, other than blood donation, would still be blood exposure, but a needle stick exposure would also be a significant -- could be a significant risk. Sexual exposure with an undetectable viral load would be a very, very low risk, but again, not zero.

Q But approximately zero, correct?

A Approximately zero, correct.

their home duty station.

Q So you referred to appointments every six months --

A Correct.

Q -- at which an evaluation is done and blood is drawn?

A Uh-huh.

Q Would the time that is required for that care in theater be considered excessive time lost?

A When you say "in theater," mean in a deployed setting?

Q Yes. Within a theater of operations.

A So currently, in a theater of operations, we don't have access to care at a military treatment facility that would include an infectious disease specialist and all of the laboratory testing required that would need to be obtained for somebody's biannual visit with HIV.

Q And where -- sorry.

Is an infectious disease specialist required to provide the follow-up evaluation for an individual living with HIV in a deployed setting?

A So again, in a deployed setting, we -- there's no precedent for that. We don't have

capabilities to -- you know, as far as lab capabilities and stuff needed. So there has never been an evaluation of an HIV-infected person in a deployed setting. So...

Q So because you're not deploying people living with HIV, there are not the mechanisms set up --

A Right.

Q -- to do that --

A Correct.

Q -- right now?

MS. BERMAN: Objection. Mischaracterizes the testimony. Sorry.

THE WITNESS: Correct. Yes. That's what I'm...

BY MR. SCHOETTES:

Q So let me back up and make my question more general. Is it required that a physician be an infectious disease specialist in order to provide an individual living with HIV with this type of follow-up care?

A So by our Army regulation, it is specified that they are followed by an infectious disease provider. On a national level, there are providers that are not infectious disease

specialists who still are slated as HIV specialists who do follow patients. And there are a handful of primary care providers in the -- on a national level that do follow HIV patients.

Q Can the -- is it possible to do a blood draw within theater?

A Yes, it's possible to do a blood draw in theater. However, it depends on what you want to do with that blood draw in theater.

Q So --

A It depends on what you're testing for.

Q Okay. So tell me -- explain that to me. Explain why that makes a difference.

A So different tests, depending on what you would like to order in theater, require different processing of the specimen and different ways to preserve that specimen in order to do the appropriate testing based on where you need that specimen to go to get that testing accomplished, if that makes sense.

Q I think it does. Let me unpack it a little bit more. So there could be particular ways that you have to conduct the blood draw or preserve the specimen --

A Yes.

Q -- in order to make it viable for the kind of test that's going to be performed?

A Correct.

Q And are you saying that, for HIV, there are currently not the capabilities to do those types of preservation of the specimen within theater?

A At most levels of care within theater. So if I -- can I explain to you --

Q Sure.

A -- the different roles of care for patients in theater?

Q Yes.

A So we have role one capabilities, which is our most minimal capability for care, which is usually at the front line where the unit is in -- very near to combat operations. So that essentially is what we typically call it as a battalion aid station. It's a tented facility that really is designed for acute trauma care, life -- lifesaving care to get them to the next level of care. So it's run by, most of the time, a PA, sometimes a medical physician, and a handful of medics, to sometimes include, like, a senior medic. So there's that level of care which really

typically has little to no lab testing capability at all.

So the next level of care --

Q I'm sorry. Follow-up question there.

A Yes.

Q You said lab testing capability.

A Correct.

Q Is that differentiated from the actual draw of the blood?

A They kind of go hand in hand because you wouldn't draw somebody's blood if you weren't going to be able to test it from there.

Q So that's part of my question, is can --

A Oh, you're saying --

Q Can blood be shipped -- can a specimen be shipped to a different location?

A Yes, but you would need to know -- you would need to be able to process that blood at that role one facility. And those capabilities are typically not in place.

Q The processing capabilities --

A Correct.

Q -- for the draw?

A Yes.

Q Okay. Go ahead. You were going to talk

about, I think, role two.

A Okay. But if I could also clarify. So processing -- typically what it involves is spinning down the blood to separate it out between -- there's different, you know, compartments of the blood. There's plasma, serum, and there's actually whole, you know, red blood cells. So they separate that out. Then they typically need to freeze it, particularly for HIV testing. They freeze it to minus 20 degrees Celsius. And that capability is not present at role one facilities.

BY MR. SCHOETTES:

Q Thank you. Let's talk about the next level of care.

A Sure. So the next level of care is role two facilities. That is typically run by a medical company, which could be anywhere from, you know, 30 to as many as, like, a hundred personnel in that medical company. It includes essentially the capability maybe to hold somebody for a 24 to maybe 48-hour period, depending on the type of environment you're working in. We also include our forward surgical teams as kind of a role two facility. And that's -- they provide acute

surgical care fairly close to the front line. And so they're considered, just because of their surgical capabilities, a role two level of care.

Again, at role two facilities, very limited laboratory services as well. Maybe chemistries, like checking somebody's electrolytes or -- I -- I can't speak to the exact lab assets at the role two, but they're fairly limited and -- your more, like, benign, day-to-day lab testing capabilities.

Q And then -- so they also would not have the capability of processing the blood, spinning it down, as you say?

A For the most part. Again, it probably depends on what assets they decide to deploy with in a given circumstance, but typically not.

Q Can you talk about the next level of care?

A So role three is the next level of care, and that's your combat support hospitals, which are -- in the deployed setting, they're usually at major hubs of military bases in the deployed setting. So, for example, in our current theater in Afghanistan, we've got role three facilities in Bagram and I think also in Kandahar. And that's

current theater of operations, their ability to fly aircraft in and out to transport specimen.

Historically, and what we've seen, what I've seen over the past, you know, ten years now, is that it's typically at -- the least amount of time we've seen is about 28 days to get a result for an HIV test, an HIV -- and that's an HIV antibody test from theater.

Q And you don't have the -- any type of metric by which to measure the other types of testing related to an HIV follow-up because you're not generally currently doing those kinds of tests; is that correct?

A Correct, yeah.

Q How would a 28-day delay in receiving the results of the test affect the ability to monitor a person's HIV while in a deployed setting?

MS. BERMAN: Objection. Calls for speculation.

You can answer.

THE WITNESS: I just want to make sure I understand. So we're assuming somebody who already has HIV infection in the deployed setting, if they needed to get blood work to --

BY MR. SCHOETTES:

Q Yeah. So --
A I just want to make sure we're talking about the same --
Q Yeah.
A -- scenario.
Q Yeah. Let's assume a person who has been diagnosed with HIV and they're on a regimen and they've been virally suppressed for some period of time and relatively stable, and they're going in to get their six-month evaluation. Doctor does follow-up. You referred to the fact that it takes a while to get the results back. Does that in any way impact the validity of those results or the ability to monitor that relatively stable person in the deployed setting?
MS. BERMAN: Objection. Calls for speculation and compound.
Go ahead.
THE WITNESS: So we're assuming that there's a lot of variables that fall into place here too. So we're assuming that that individual can get to a role three facility fairly easily to -- which also depends on the combat environment in a deployed setting. We're assuming that, once that blood test, the lab work is drawn, we would

be sending, in this case, for a viral load, which also requires, you know, spinning down the blood, freezing it to minus 20 -- so this is assuming that the cold chain for this specimen is preserved as the specimen gets all the way back to the United States, which -- we know that there's a handful of times that it's -- lab specimens frequently can get lost in theater or are not usable by the time they get to the lab for processing. So we're assuming that all of these variables occur successfully.

Then, in that setting, we're looking about -- anywhere from a month to longer, depending on any hiccups in the travel of the specimen -- because it typically goes through Landstuhl in Germany and then into the United States. It may be processed in Germany, but right now, our -- the algorithm is that it goes to our HIV diagnostics and research laboratory, or HDRL, in Silver Spring and Rockville, Maryland.

BY MR. SCHOETTES:

Q Can you explain why that is? Why does it come all the way back to the United States if it could be processed in Germany, for instance?

A So far as the -- I can't speak to the lab

capabilities in Germany. I think they actually may send it out to the civilian sector in Germany for some HIV testing because of their lab capabilities at Landstuhl, but our -- for the Army, and we do -- for the Army, our HIV diagnostics and research lab that has -- there's a few different antibody tests for HIV as well as viral load testing, and those are all processed at our HDRL in Maryland, in Silver Spring.

Q I guess I'm still wondering -- I understand that that is what happens. Is there a reason that it needs to be that way as opposed to having this testing done in Germany?

A I'm not a lab personnel so I don't want to speak on something that I'm not aware of the nuanced details of, but that is our current Army processing --

Q Presumably --

A -- algorithm.

Q Go ahead. Presumably, other places know how to do this testing that is required for people living with HIV and it could be done in Germany.

MS. BERMAN: Objection. Argumentative. Calls for speculation.

Go ahead.

THE WITNESS: I mean, I'm sure, you know, different European countries are probably very savvy at obtaining a viral load and the appropriate HIV diagnostics, but the Army does not have an agreement with any of these countries, so...

BY MR. SCHOETTES:

Q And is that what would be required, some type of agreement?

A I don't know. That's not my thing.

Q Okay. Going back to my original question, which is, let's say the physician who is conducting this follow-up evaluation receives these results 28 days after the blood is drawn, or even 45 days or 60 days --

A Sure.

Q -- what effect would that lapse of time have on their ability to effectively monitor the individuals with HIV?

MS. BERMAN: Objection. Calls for speculation. It's outside the scope of what this witness is being offered to testify about.

Go ahead.

THE WITNESS: So, I mean, it's -- the effects are -- you know, there are several

different, you know, effects that can occur in that span of -- it depends on a lot of variables, too. It depends on was that service member taking his medication regularly. If he had been somewhere where he lost access to his antiretroviral regimen and, say, it took him a month to two months to get back to this role three facility to actually get his labs drawn, I mean, in that span of time, he probably had a fairly high viral load and, in that setting, depending on what's going on in that combat environment, that might affect his ability to perform his job. It certainly would become an issue in an emergent blood transfusion setting.

I mean, there's -- it depends on the scenario and it depends on a lot of different variables again.

BY MR. SCHOETTES:

Q And I want to talk about all those things, but I'm still not hearing -- the doctor who is evaluating this individual, I want to know what effect the delay in receiving those test results has on that doctor's ability to monitor this individual.

MS. BERMAN: Same objections.

medication, that it would be important to get that individual back on their medication.

A Yes.

Q And that would not be reliant upon the -- getting the test results from that patient, correct?

A Correct.

Q So I want to know what the effect would be on the doctor's ability to provide the type of monitoring and care that the doctor is being asked to provide if they got the labs back 30 days later, 45 days later?

MS. BERMAN: Same objections and asked and answered.

You can answer.

THE WITNESS: I don't see an effect to the doctor managing the patient in that setting.

BY MR. SCHOETTES:

Q You talked about -- would it likely result in excessive time lost from duty for an individual with HIV to get to a role three medical facility twice a year?

A So again, I think you're mixing up -- so a role three medical facility in our current Army policy does not have an infectious disease

specialist there. So they would never go to a -- a role three facility, that's only what we talk about in the deployed setting.

In the United States, we have role four facilities, which include all of our major medical treatment facilities where, at the -- most of them infectious disease specialists reside.

Q Can you tell -- can you describe a role four medical facility?

A Yeah. So I can use -- Walter Reed, where I work, for example, is a role four military treatment facility. Has, you know, robust services that you would expect in any other civilian hospital in the United States, great laboratory capabilities, radiology capabilities, primary care and subspecialty care capabilities.

Q So does the Army believe that an individual living with HIV must return to the United States from a deployed setting in order to get their six-month follow-up evaluation?

A Currently, yes.

Q And I understand that that's the current policy, but what I'm asking is, is there any reason why an individual living with HIV would not be able to get the kind of follow-up care they

need at a role three facility?

A Yes. So again, it depends on what lab capabilities they have at the role three facility. And not every primary care physician is knowledgeable and knows how to treat a patient with HIV infection. So it really is dependent on exactly who is at that role three facility and their comfort and their experience with managing an HIV-infected patient.

Q And that includes the type of follow-up care we're talking about for an individual who has well-controlled HIV?

A Yes.

Q A primary care physician couldn't be expected to handle that type of care?

A No, it's not they couldn't be expected. It's just some -- some are more savvy and knowledgeable about our newest HIV regimens and some are not at all, have no experience. So it really depends on the individual experience level of that provider.

Q And you're saying that kind of training potentially could be provided to those providers?

MS. BERMAN: Objection. Mischaracterizes the testimony.

that could be deemed an excessive loss of time for that unit.

BY MR. SCHOETTES:

Q Are soldiers with HIV medically capable of satisfactorily completing required training?

MS. BERMAN: Objection. Calls for speculation.

You can answer.

THE WITNESS: Yes. Assuming they are well controlled and otherwise asymptomatic HIV-infected service members, yes.

BY MR. SCHOETTES:

Q Are HIV-positive soldiers -- I'm sorry. Yes. Is an HIV-positive soldier adaptable to the military environment without the necessity of geographic area limitations?

MS. BERMAN: Objection. Calls for speculation.

THE WITNESS: Currently, no.

BY MR. SCHOETTES:

Q And why is that?

A Currently, because we do not deploy service members into combat operations or contingency operations. They are -- for the various variables that we've already discussed, it

depends on the austerity of the environment, the access to laboratory capabilities and medical service capabilities, the access to pharmacy capabilities should that service member require refills of his medications or lose his medications.

And I would also add the confidentiality issue that we had already discussed as well.

Q So we already talked about the access to care and we talked about the confidentiality provision. Let's talk about the austerity of the environment. What factors influence an individual living with HIV in terms of the austerity of the environment?

MS. BERMAN: Objection. Vague.

THE WITNESS: So when we talk about austere environments, so -- it depends on, you know, again, the austerity in the environment kind of goes hand in hand with what capabilities are available in that environment.

If all -- I guess I'll -- I can use an example from personal experience. Being deployed in the middle of the desert in Afghanistan only next to a role one facility that has essentially no diagnostic laboratory capabilities and no

access to pharmaceuticals for treatment of HIV infection at that role one, outside of a short supply of antiretrovirals for use as PEP if needed -- so that's a pretty austere environment where there's a lot of variables that could come into play where an HIV-infected service member might need care at that role one and not be able to receive it.

BY MR. SCHOETTES:

Q So that's what I -- I -- what I want to know about is what those factors are. So I understand -- and I want to set aside access to care and access to medication. We're going to talk about that in a moment. And I just want to know what the austere environment factors, environmental factors, are that would create a need for more immediate care.

MS. BERMAN: Objection. Vague.

You can answer.

THE WITNESS: I guess -- I mean, that's -- I kind of lump all of this access to austerity, if you get what I'm saying. Are you talking about, like, extremes in temperature, extremes in the -- you know, just maybe staying up for 48 hours straight without sleep?

You can answer.

THE WITNESS: To clarify, this is assuming on a deployment?

BY MR. SCHOETTES:

Q Yes, I'm sorry.

A If -- on a deployment, the service member would be given whatever number of medications they needed to span that deployment, in most cases.

Q How long are the longest deployments?

A The longest deployments currently are slated as nine-month deployments, but it varies based on combat operations.

Q So for a nine-month deployment, the soldier would be given 270 days' worth of medication, approximately?

A Yes.

Q If a deployed soldier's medications were lost, stolen or destroyed, how would they be provided with a replacement supply?

A That depends on the scenario and where they're located in -- in theater.

Q Can we do it for each -- for someone with a role one, someone with a -- a soldier with a role two and then a soldier near a role three?

A Sure.

Q Okay.

A So at a role one facility, there would be no pharmaceutical capability to immediately replenish that medication supply. The role one provider would probably reach out to a role three facility, the closest role three, and ask for their capability to supply an immediate course of that medication until that service member can actually order it through TRICARE Express Scripts and have that new shipment sent out to him.

Q How long would it likely take for the supply at the role three to reach a soldier at a role one?

A Again, that completely depends on combat operations at the time, whether there's no-fly restrictions, what that unit is doing. If they're out in the field somewhere and not even going back to that role one battalion aid station for a week or so, then it might span anywhere from 48 hours to get that supply to them out to a couple of weeks to a month, depending on what they're doing.

Q Are there currently any HIV medications that are stocked or on the formulary within theaters of operations?

MS. BERMAN: Objection. Vague.

You can answer if you know.

THE WITNESS: Yes. There are usually stockpiles for PEP of HIV medications, so for post-exposure prophylaxis, but it usually is as minimal as, like, one course of PEP. So like a 28-day supply of an antiretroviral regimen.

BY MR. SCHOETTES:

Q So a change in the policy that would allow soldiers living with HIV to deploy would require a change to the formularies to include HIV treatment regimens, correct?

MS. BERMAN: Objection. Calls for speculation. It's outside the scope of what this witness is being offered to testify about.

But you can answer.

THE WITNESS: Yes.

BY MR. SCHOETTES:

Q I didn't let you finish before. We talked about a role one -- a soldier at a role one unit, but what about a soldier in a role two unit or at a role three unit?

MS. BERMAN: Can you clarify which question?

BY MR. SCHOETTES:

Q Yeah. So we were talking about how a

soldier who -- living with HIV who had lost -- actually, scratch the HIV part. Try again.

If a deployed soldier's medications are lost, stolen or destroyed, how are they provided with a replacement supply? You indicated that it would depend in part on the type of medical facility to which they were adjacent. So could you please answer the question with respect to role two and role three facilities?

A Yes. So similar to a role one, a role two facility would have to likely reach out to a role three facility and see if that medication was readily available at that pharmacy there. And if -- if so, they would give them a temporary supply, typically like a 30-day supply or so, until Express Scripts can actually deliver a -- that medication to that service member's location.

Q And then if they were adjacent to a role three facility, I'm assuming that they would be able to get it readily from that role three facility if they had the medication in stock?

A That's correct. So if they had that particular medication at that role three, it would be given to them.

Q Until they were able to get their full prescription from Express Scripts?

A Correct.

Q How long approximately does it take for an Express Scripts prescription medication to reach a soldier at a role one facility, or at a post with a role one medical facility?

A So that completely depends on the location of that role one facility. If -- it depends on how remote it is and what the -- what the plane flight route is of Express Scripts. And that could be very variable.

Q Can you give us a range? Can you give me a range?

A A range would be anywhere from two weeks out to over a month.

Q Can you put a top end on -- you say over a month. How much over a month?

MS. BERMAN: Objection. Calls for speculation.

THE WITNESS: I mean, again, I can't -- I can't give you an upper range because it depends on -- if that unit is hunkered down somewhere and engaged in combat with the enemy, they're not going to fly an Express Scripts shipment in to a

service member. So -- I mean, it could be outwards of a month to -- I can't put a top end range to it.

BY MR. SCHOETTES:

Q It could be more than two months?

A Sure.

Q It could be more than three months?

A Potentially. Unlikely, but potentially.

Q So would three months be a top-end range?

MS. BERMAN: Objection. Mischaracterizes the testimony.

THE WITNESS: I can't definitively give you a top-end range because I don't know -- I can't account for every variable that could occur in that setting.

BY MR. SCHOETTES:

Q But more than three months is unlikely?

A Correct.

Q Is more than two months unlikely?

A Less likely than more than three months. Less unlikely than more than three months.

Q Do antiretroviral medications have any storage or handling restrictions?

A So all medications that are FDA-approved come with a package insert that lists storage

requirements. And antiretrovirals are no exclusion to that.

Q Are they -- do HIV antiretroviral medications have any special storage or handling restrictions that are not -- that are out of the ordinary in some way?

MS. BERMAN: Objection. Vague.

You can answer.

THE WITNESS: Not to my knowledge.

BY MR. SCHOETTES:

Q Do they tolerate -- do any of them require refrigeration?

A Not any of the commonly used regimens that we use today.

Q Do they tolerate heat relatively well?

MS. BERMAN: Objection. Vague.

You can answer.

THE WITNESS: As well as antimalarials or other medications we take in a deployed setting.

BY MR. SCHOETTES:

Q Do they tolerate cold very well?

MS. BERMAN: Same objection.

THE WITNESS: Same response.

BY MR. SCHOETTES:

Q I'm going to move on to topics 24 and 25.

You described earlier concerns over transmission via the blood supply. Are soldiers who have been diagnosed with HIV told that they are not to donate blood?

A Yes.

Q Given that soldiers are told not to donate blood, what are the concerns with transmission via a donation from a soldier living with HIV?

A So the biggest concern that we've seen actually has been concerns for the soldier breaching confidentiality of his HIV status to fellow service members. And we have seen cases -- at least one case that I am aware of -- of a service member who attempted to donate blood within the United States who knew he was HIV-infected and was flagged by the blood bank, because we have very good blood testing capabilities for HIV infection in the United States. And that was brought to the attention of the Army public health -- or the -- I believe this was a marine soldier, so it was a -- whatever public -- the public health service for -- that governs them. And it was also brought to the soldier's attention too. They notified the

individual donating blood, obviously.

Q And you said that was done in the United States --

A Uh-huh.

Q -- where the requisite testing is done on the blood to identify a bloodborne pathogen like HIV, correct?

A Correct.

Q And that's how the blood was flagged and discarded, correct?

A Correct.

Q In the setting of deployment, what kind of testing is done on the blood that is drawn for transfusion?

A So in a deployed setting, in preparation for the potential of a need for emergent blood transfusions, there's kind of two groups of personnel. So there's a blood donor pool, so people who sign up at the start of the deployment or prior to deployment and say, yeah, I'm going to be ready and willing to donate blood if needed. And then there's also an emergent blood donor pool, so folks who are asked on the spot, hey, we need blood, you weren't in the original donor pool, can you emergently give us blood?

So there's kind of two separate pools. That first pool is screened, obviously, for HIV, hepatitis B, hepatitis C, all the potential bloodborne transmitted infections at -- when they sign up for that, to be in that donor pool. And then as -- there's -- the guidance says there's attempts to get them rescreened throughout the deployment, but that is typically not possible, so -- but they are, you know, it's a pool that we know at the start of this deployment they are HIV uninfected, or hepatitis B and C uninfected.

Q And presumably someone who was diagnosed with HIV would not volunteer to become a part of the blood donor pool, correct?

A Correct.

MS. BERMAN: Objection. Calls for speculation.

THE WITNESS: Correct.

BY MR. SCHOETTES:

Q The second pool that you talked about, is that sometimes referred to as the walking blood bank?

A Correct.

Q What kind of testing is done on the blood drawn for the -- as a part of the walking blood

bank?

A So there are attempts to test for hepatitis B, hepatitis C and HIV in those settings. Those tests are not always run reliably before blood is transfused in the activation of a walking blood bank.

Q And can -- why is that?

A It's based on the emergence of the need for blood. So each of those tests take approximately 15 minutes to run, to get results, and sometimes you don't have 15 minutes before you need to give somebody blood.

Q Now, anyone who is participating in the walking blood bank is presumably asked to consent to giving blood, correct?

MS. BERMAN: Objection. Calls for speculation.

You can answer.

THE WITNESS: To my knowledge, there's no written consent when a walking blood bank is activated.

BY MR. SCHOETTES:

Q No soldier is asked to donate blood against his will?

A No.

Q So a soldier has the ability to decline to donate blood?

MS. BERMAN: Objection. Calls for speculation.

You can answer.

THE WITNESS: Yes. But you can imagine how that's perceived by the unit in a setting of a MASCAL if a soldier declines to give blood for a fellow service member.

BY MR. SCHOETTES:

Q But it is -- my question remains, it is possible for a soldier to decline to give blood.

A Yes.

Q And then there's a concern that you've expressed with something that happened in the United States, that a solder, in an effort to maintain confidentiality around his HIV status, might choose to give blood even though he knows he has HIV?

A Correct.

Q Would there be a method for a soldier to indicate or identify that his blood was not to be used for transfusion even though it had been donated as there is in the United States for people in similar situations?

Let me give you an example. They're doing a blood drive at work at a place of employment. And there's a person living with HIV who doesn't want to be seen as not -- who wants his employees [sic] to believe that he is a person who would give blood, but he doesn't want to reveal his HIV status. And lots of blood collection centers have a box you can check that says, don't use this blood.

Is there any mechanism or would it be possible for a soldier to indicate that so that their blood would not be used?

MS. BERMAN: Objection. Form. And compound.

But you can answer.

THE WITNESS: To my knowledge, there is no mechanism in place, but that would certainly not be feasible to fill out a checklist to donate blood if it was needed urgently in a MASCAL scenario.

BY MR. SCHOETTES:

Q Is there -- are there other individuals -- are there other reasons why a person could not donate blood?

A Yes.

A Correct.

Q This sentence says, "Some HIV-infected, virally suppressed patients on ART will develop illnesses associated with premature aging."

Do you know that to be true?

A Yes. There's quite a bit of literature to demonstrate that HIV-infected service members are more likely to develop cardiovascular disease, osteoporosis, yes.

Q Do you know at what -- how long it takes for those illnesses to develop?

A It's very variable. It depends on a lot of different factors.

Q When it talks about premature aging, is there an age at which we would start to see that kind of premature aging or is it contingent upon -- well, I'll just stop there. Is there a particular age at which we would be likely to see these illnesses associated with premature aging for virally suppressed, HIV-positive people?

A No, it's very variable.

Q Is there a time from infection, amount of time from infection, that would be an indication of when we might expect to see illnesses associated with premature aging?

A Again, it's variable on several factors.
Q Can you tell me what those factors are?
A So if somebody is taking their ART regularly, is probably the biggest factor, how well controlled their HIV is. However, on the flip side, there's also bodies of literature that express concern about certain ART regimens and their effects on the cardiovascular system as well.
Q So I just want to pause for a moment and say --
A And osteoporosis. I'm sorry.
Q -- that in the sentence, though, it talks about someone virally suppressed.
A Okay.
Q So wouldn't that indicate that that person is regularly taking their HIV medications?
A Yes.
Q So that wouldn't be a factor for that individual.
A Correct.
Q But now I heard you say the regimen they're on could affect when we might see the possibility of illnesses associated with premature aging, correct?

that I have seen this individual study.

Q Okay. The next sentence admits that, "The impact of these potential neurocognitive impairments on a service member's readiness, resilience and/or retention is currently unknown."

Would you agree with that statement?

A Yes.

Q The next sentence talks about the HIV-positive population on -- "As the HIV-positive population on ART ages" -- ART is capitalized, A-R-T -- "there is greater recognition that cerebrovascular disease risk factors such as hypertension, diabetes and hypercholesterolemia may become risk factors for cognitive impairment."

Are those also risk factors for cognitive impairment for people who are not HIV-positive?

A Yes. They certainly are.

Q So right now, we're not sure the extent to which HIV-positive service members will be disproportionately impacted with neurocognitive impairments; is that correct?

MS. BERMAN: Objection. Again, he's -- the scope of his testimony will only be for the Army. And also this calls for speculation.

But you can answer.

A Yes, that's correct. But even --

Q What other --

A Even in virally suppressed patients there is evidence to show that HIV-infected persons, even with suppressed viral loads, still have -- whether it's related to an increased inflammatory state in their body or their immune system, for lack of a better term, is ramped up to control the HIV virus, that that has effects on the cardiovascular system and other organ systems as well.

Q Which is indeed what this sentence says, "Some HIV-infected virally suppressed patients on ART will develop illnesses associated with premature aging."

A Yes.

Q So what I'm trying to understand is, are there factors -- what the factors are that you described that would influence if that happened? One thing you said would be the treatment regimen that they were on. Are there other factors that would influence when we might see that?

MS. BERMAN: Objection. Form.

You can answer.

THE WITNESS: So I had mentioned the

body's own kind of inflammatory state. So there's various inflammatory chemicals in the body, cytokines in particular and interleukins and these different factors that -- a lot of research has looked at their role in inducing conditions like cardiovascular disease, renal disease, osteoporosis in the setting of HIV infection.

So even with a suppressed viral load, perhaps, the thought is -- and some of the research is showing that these -- this ramp-up of these inflammatory factors are affecting an individual's cardiovascular system or bone system or renal system.

BY MR. SCHOETTES:

Q Am I -- is it correct that those response -- the level of those responses may vary from individual to individual?

A Yes, correct.

Q So that would be another factor is how -- a particular person's response, immune system response in terms of these inflammatory conditions?

A Yes.

Q Is there other factors that would influence how rapidly one might see the onset of

these illnesses associated with premature aging?

A So the other, you know, very common comorbid factors to a lot of Americans: Smoking, alcohol use, lifestyle habits. Those, combined with HIV infection, might portend a higher likelihood of development of cardiovascular disease and osteoporosis.

Q Anything else?

A Not that I can think of right now.

Q Further down, it talks about, "Some patients may experience a fluctuating course of neurocognitive impairments over time, including symptom normalization; however, it is unknown whether these changes reflect biological alterations induced by responses to (or failures) of ART, or occur independently of viral load and changes to ART regimens."

So we're still figuring this all out. Is that an accurate statement?

A Yes. That's very accurate.

Q And some of this is speculative as to how these things are associated?

MS. BERMAN: Objection. Vague.

THE WITNESS: Some of it is -- could be speculative, but a lot of it is based on studies

that have been done as well. So it's -- I think there's data on both sides of the spectrum so far, and we're waiting for more data to make firmer -- firmer conclusions from this.

BY MR. SCHOETTES:

Q Actually, I'm going to go back to the factors. You did not identify length of time that the person has been infected with HIV. Is that not a factor in whether we -- you would see illnesses associated with premature aging in that individual?

A There are studies that have shown length of time on ART is -- more likelihood of development of cardiovascular disease. And the issue is, is it due to the HIV itself or is it due to a bunch of regimens that that person had seen over the course of their HIV illness, and is it an ART-related issue or is it both? I mean, there's...

Q Is it also possible that the onset of the illnesses associated with premature aging could be affected by the regimen used on a particular individual?

MS. BERMAN: Objection. Asked and answered.

You can answer.

THE WITNESS: Yes.

BY MR. SCHOETTES:

Q Are some of the people that are now aging with HIV, did they take regimens that are no longer used?

MS. BERMAN: Objection. Calls for speculation.

You can answer.

THE WITNESS: Yes.

BY MR. SCHOETTES:

Q And we have newer medications to treat HIV on a fairly regular basis, correct?

MS. BERMAN: Objection. Calls for speculation.

You can answer.

THE WITNESS: Correct.

BY MR. SCHOETTES:

Q And because we're not sure exactly what the cause of this is, no one can say whether the newer regimens will have the same types of effect as some of the older regimens, correct?

A Correct. The newer regimens have not been around long enough either for us to have that body of data to show. So I think time will tell

any potential long-term side effects of our newer regimens.

Q How many regimens, if you know, have been phased out of use over the course of the 35 years of the HIV epidemic?

A So there have been a handful of antiretroviral medications that are no longer being produced and have certainly been taken off of our formulary within the military health care system. A couple of them -- like didanosine and stavudine are two that come to the top of my mind.

Q And, before, you talked about first-line regimens and second-line regimens. Are there third-line regimens?

A So I believe I would have to refresh my memory of the DHHS guidelines for ART treatment, but I believe they list them as first-line and then alternative regimens, there's a category for. And that includes, I think, all of the -- and then there are -- there is a not recommended category as well for ART.

Q And the two medications that you just identified would fall into the not recommended category at this point?

A They would, yes.

Q And how many medications are in the not recommended category at this point?

A I would be speculating off the top of my head, but I'm sure it's a handful.

Q Okay. There's a sentence here that says, "A longitudinal cohort observation study found that numerous patients with asymptomatic neurocognitive impairment (ANI), even with a suppressed plasma viral load, eventually developed symptomatic neurocognitive impairment."

Are you familiar with the longitudinal cohort observation study referenced there?

A Perhaps not this exact study, but I am aware of different studies that have looked at this and have shown that this is to be the case, that even HIV-infected service -- or personnel with suppressed viral loads can still demonstrate asymptomatic neurocognitive impairment.

Q Would you look to page 30 and it's -- the longitudinal study is identified there.

A Reference 8.

Q Yes. Is this one with which you are familiar?

A Again, I'd have to see the exact -- I'd have to see the article to -- to really confirm

THE WITNESS: Yes, I would say that's correct.

BY MR. SCHOETTES:

Q Is there any reason the Army would not be able to identify and address neurocognitive impairments in HIV-positive people just as it would in the population not living with HIV?

A So the diagnosis of HAND, HIV-associated neurocognitive disorder, is fairly complicated. It's actually -- by definition, it's a clinical diagnosis. We don't have MRI imaging or electroencephalogram -- EEG -- readouts that say, yes, based on this study, this person definitively has HIV-associated neurocognitive disorder.

It's based on more of what's called neuro -- neuropsychiatric testing or neuropsychological testing profiles that are pretty complex and have a lot of different variables to them. So it's more of a clinical diagnosis based on that testing. And we use brain imaging, like MRIs and EEGs and lumbar punctures, to rule out other infections, to make sure we're not missing another diagnosis.

So it's very -- bottom line, it's very -- it's a very unique testing panel that goes into

formal diagnoses of HAND.

Q So that's specific with respect to HAND, which is HIV-associated neurocognitive disorder, which, I'm assuming, a person who is not living with HIV would not have -- could not have. So what I actually am asking is just neurocognitive impairments as they occur in the general population, is there any reason why the neurocognitive impairments that might be the result of HAND could not be addressed in the same way as neurocognitive impairments that occur in the general population?

MS. BERMAN: Objection. Assumes facts not in evidence.

You can answer the question.

THE WITNESS: I don't think so. I'm a little confused, though, at what you're trying to ask.

BY MR. SCHOETTES:

Q Well, I'm assuming that the Army has some way of identifying and addressing neurocognitive impairments that might occur to service members in general. Correct?

A I mean, we do -- neuropsychologic testing is our battery that is performed for most cases of

neurocognitive impairment, whether it's HIV-related or not. So -- so in answer to your question, yes, there is a way to identify that.

Q And what I'm asking is, is there any reason that HIV-associated neurocognitive impairment would not be diagnosed using those tests in the same way?

A The only issue is that there's a lot of unknowns, as this is stating, regarding, you know, the extent of neurocognitive impairment and, you know, further details of exactly the pathophysiology and the mechanism of what's causing this neurocognitive impairment.

So I think -- I think that's what you're -- I mean, so that's the issue that I have, I guess, is that --

Q Yeah.

A -- based on what they are saying in here as well, that there's -- there's a lot of unknowns regarding this. So giving everybody a formal diagnosis of, yes, you have neurocognitive impairment or, no, you do not, I don't think it's as -- it's not black and white because of all these unknowns.

Q And I understand, in terms of a diagnosis

and being able to say it is HIV-associated or not and -- I guess what I'm asking is, to the extent that it's having an impact on a person's memory, concentration, attention, motor skills, it seems to me that that could be diagnosed in a person with HIV or without HIV in the same manner. Am I correct?

MS. BERMAN: Objection. Form.

You can answer.

THE WITNESS: I don't know how to best answer that, to be honest with you. I mean, I -- a service member without HIV infection who had these similar symptoms would undergo similar testing, if that answers your question.

BY MR. SCHOETTES:

Q Yeah. So I guess what I'm asking is, you offered neurocognitive impairments as a reason why HIV-positive individuals should not be deployed or are not fit to serve. And I'm asking why, given its relatively low occurrence in all populations, why wouldn't it just be diagnosed as if the person didn't have HIV, and addressed as a neurocognitive impairment separately from the fact that the person has HIV?

MS. BERMAN: Objection. Mischaracterizes

the testimony. And form.

THE WITNESS: I see what you're -- I think I see what you're saying. The issue with -- regardless of HIV infection, if we take that even out of the picture here, whether somebody has HIV infection or not, it's still a clinical diagnosis. So it's fairly subjective based on the results of testing.

So there's certainly potential for some people to get diagnosed, some people not to get diagnosed formally. Right? So I guess in that setting, it's not -- again, it's not a black or white, like, you obviously have neurocognitive impairment, we're not going to deploy you, versus you're cleared -- you're cleared to deploy, if that's what you're...

BY MR. SCHOETTES:

Q Do you know what the current prevalence of HIV-associated neurocognitive disorder is among the population in the military living with HIV?

A In the military living with HIV overall, no, I don't know that. So when you talk about HIV-associated neurocognitive disorder, it's a spectrum, so everything from asymptomatic neurocognitive disorder to HIV-associated

dementia. So every -- and there's a couple, you know, gray areas between there.

So I mean, it's kind of -- obviously, you can see that if somebody has asymptomatic neurocognitive disorder, it's very hard to calculate that because they're not demonstrating any obvious symptoms or signs to suggest that. So it's --

Q And is that the reason why you don't have an estimate of prevalence within the military or is that why you don't have an estimate across the general population?

MS. BERMAN: Objection. Compound. Lack of foundation.

You can answer.

THE WITNESS: Exactly the latter, that we don't have a good estimate of that nationwide, let alone within the military.

BY MR. SCHOETTES:

Q And do you have an estimate of the percentage of the population with HIV in the military who are exhibiting symptoms in a way that has impacted their ability to perform their duties?

MS. BERMAN: Objection. Vague.

"Pharmaceutical supplies intended for emergency nPEP will not be compromised for PrEP."

So nPEP is, as you said, nonoccupational exposures. So nPEP is kept on hand for potential sexual exposures?

A So I think this could classify as nPEP or PEP, you know, in general, needle stick exposures. Like I had mentioned before, we do keep -- currently the pharmacies that are in OCONUS settings keep a short supply of antiretrovirals -- and it's typically Truvada plus another antiretroviral -- for use as PEP and -- or nPEP if needed --

Q Okay. And nPEP --

A -- for these situations.

Q So -- I guess I'm wondering how nPEP is used in the military. I'm imagining potentially after a sexual assault?

A Correct.

Q Is it ever used for someone who comes in and says, I think I may have been exposed sexually to HIV?

A Yes.

Q Okay. But you're saying that really this sentence, the "n" could be taken off of PEP there?

A Yes. That's what I'm saying.

Q Okay. I think I'm good there. You can put that document aside. Give me a second. I need to find this document to do this part of my outline.

(Pause.)

BY MR. SCHOETTES:

Q We're going to continue on and I will come back to this after we've taken a break.

I'm going to talk about topic 23 which is accessions and deployment policies for other conditions requiring daily medication. Are you familiar with the medical condition of dyslipidemia?

A Yes, I am.

Q What kind of treatment does dyslipidemia generally require?

A Generally, it requires a cholesterol-lowering medication, typically from a class we call statins.

Q Taken daily?

A Yes.

Q Generally one pill?

A Yes.

Q Once a day?

A Yes.

Q What is the accession policy for individuals with dyslipidemia?

A I don't believe it specifically mentions dyslipidemia as a limiting restriction to joining the military.

Q And if it's not listed specifically, then it would not be a bar to enlisting or commissioning?

A I believe there's a line in our accessions policy that says every medication can be looked at on an individual basis for consideration for whether it merits accessioning or not.

MS. BERMAN: Counsel, I just want to reiterate that this witness is here to provide medical testimony as it applies to these questions. We did offer someone to talk about accessions in a more policy-specific way. And deployment as well.

MR. SCHOETTES: Okay.

MS. BERMAN: But you --

MR. SCHOETTES: I just want to try to understand what portion of the topic -- I mean, I don't want to waste our time if he's not

testifying on the topic. So...

MS. BERMAN: As we discussed earlier, it's just -- he's going to talk about these conditions and how they might be different than HIV, might be treated same or different than HIV. But I think it's fine. You can --

MR. SCHOETTES: Okay.

MS. BERMAN: -- continue your questioning.

BY MR. SCHOETTES:

Q All right. So if a soldier is diagnosed with dyslipidemia after enlisting or commissioning, are they discharged?

A No.

Q If a soldier is required to start taking daily medication for dyslipidemia, are they discharged?

A No.

Q What is the deployment policy for individuals with dyslipidemia?

A So I don't know exactly what the deployment policy is with [sic] somebody with dyslipidemia, but if it is very well controlled on a once-daily statin regimen, they would be allowed to deploy.

BY MR. SCHOETTES:

Q And they're certainly not referred into the DES automatically upon diagnosis?

A No.

Q Are you familiar with the medical condition of hypothyroidism?

A Yes.

Q What kind of treatment does hypothyroidism generally require?

A It generally requires a pill called levothyroxine. It's a -- it's a synthetic thyroid replacement medication taken once daily.

Q I'm going to go back for a moment. What are the consequences of not taking one's dyslipidemia medication?

A So the consequences of not taking that daily, it depends on the duration of time that you go without it, but your cholesterol levels increase and, over time, you may be at higher risk of heart disease, stroke, all the other complications of dyslipidemia that goes untreated.

Q Including some potentially fatal conditions?

A Yes.

Q So what are the consequences of untreated

Q And are individuals with dyslipidemia supplied with their medication during deployment -- well, let me go back.

How are individuals living with dyslipidemia supplied with their medication during deployment?

A So if they don't already bring a 270-day or whatever duration their deployment is, supply with them, they can sign up via TRICARE Express Scripts to have it mailed to them throughout their deployment.

Q If they go with 180-day supply on a 270-day deployment, how long into their deployment before they can request a refill of their prescription?

A I don't know the exact time frame that TRICARE would -- mandates for you before you can get a refill of a medication.

Q Would it take into account the fact that it might take some time for that refill to get to the individual who is deployed?

A I would hope so, but I can't tell you definitively.

Q And if the medication of a person with dyslipidemia was lost, stolen or destroyed while

deployed, would they be resupplied in the manner that we discussed earlier for all of their medications?

A Yes.

Q Besides for [sic] receiving treatment, are soldiers handled -- are soldiers with dyslipidemia handled differently in any respect?

MS. BERMAN: Objection. Vague.

BY MR. SCHOETTES:

Q I can ask a more specific question. Are individuals living with dyslipidemia referred into the DES under 1332.18?

A I don't know -- I don't know how to exactly address that, because I would imagine it would -- if they have very uncontrolled dyslipidemia that -- despite being on appropriate statins or other agents to reduce cholesterol, then they very well may be referred to the DES.

Q And to clarify, the DES is the Disability Evaluation System?

A Correct.

MS. BERMAN: And I want to reiterate that this witness is not being offered to talk about retentions, so...

MR. SCHOETTES: Right.

hypothyroidism?

A So if it goes untreated, then you develop -- in many cases, you can develop symptomatic hypothyroidism which is manifested by a number of symptoms to include extreme fatigue, weight gain, cold intolerance, constipation.

Q All things that could affect one's ability to perform one's duties as a soldier, correct?

A Correct.

Q What is the accession policy for individuals with hypothyroidism?

A Individuals with hypothyroidism are, if it's well controlled, are allowed to accession.

Q And if a service member is diagnosed with hypothyroidism after enlisting or commissioning, are they discharged?

A No.

Q If they're required to start taking daily medication for their hypothyroidism, are they discharged?

A No.

Q Do you know what the deployment policy is for individuals with hypothyroidism?

A As long as it's well controlled, then

noticeable symptoms as a result of that loss of medication?

MS. BERMAN: Objection. Calls for speculation.

You can answer.

THE WITNESS: So that time frame is very variable. It depends on a lot of factors that we've already discussed, in particular, the environment that that person is working in with regards to their potential exposure to environment stimulants that affect the immune system.

Also, what that person's baseline CD4 count was at the time they stopped taking the medication. And that person's individual immune system, how it reacts to not being on antiretroviral therapy for a period of time.

BY MR. SCHOETTES:

Q Is there a -- are you able to provide what the average time would be before you would see a significant increase in the person's viral load assuming they were -- had a suppressed viral load before the loss of medication?

A So there are studies that have looked at this before, kind of what's called viral load rebound, or rebound viremia, after stopping

antiretroviral therapy. The majority of the studies seem to show that within even as early as nine days, you can see evidence of viral load, significant viral load rebound after stopping antiretroviral therapy.

There is some data to show that the earlier somebody is started on antiretroviral therapy shortly after their diagnosis, if they do stop it later on, that rebound may not occur for outwards of 50 days or so. But the -- most of the studies have shown what I've seen to be an average of about nine days.

Q And when you say significant viral rebound, do you have a demarcation point at which you are setting significant viral --

A No, I don't. I'd have to look closer at those studies to see what the actual viral loads are that they're depicting.

Q Are you familiar with genotype testing to identify resistance in the HIV virus?

A Yes.

Q Do you have a sense -- or can you tell us how long it would take before there was enough virus in the blood to do genotype testing after stopping treatment?

their HIV as a result of stopping medication?

MS. BERMAN: Objection. Vague.

You can answer.

THE WITNESS: I mean, again, that -- it depends on what you're categorizing as symptoms and then also it depends on that person's baseline immune status. And there's a very broad range of time period between symptom onset after stopping -- or when having uncontrolled HIV infection.

BY MR. SCHOETTES:

Q If HIV is left untreated, for how long after infection does it remain asymptomatic? Again, it would have to be an average, because I understand everyone is different --

A Yes.

Q -- but what's the average amount of time?

MS. BERMAN: Objection. Vague.

Go ahead.

THE WITNESS: So average amount of time in our early studies of HIV infection prior to treatment were anywhere from two years to ten years. So on average, maybe around five years, you could say. But typically, again, those studies were conducted prior to combination

antiretroviral therapy and those -- we're talking pretty severe symptoms, like opportunistic infections, malignancy, to include lymphoma, Kaposi's sarcoma, so pretty significant infections and disease processes that occur with untreated for that long.

Q The things that could actually lead to a person being disabled by their HIV?

A Yes.

Q What is the course of treatment after treatment interruption? What would the normal plan be for such an individual?

A To restart your antiretroviral therapy as soon as possible.

Q To restart the regimen you were on?

A Correct. And then hope to obtain a genotype on -- after being on that therapy for typically three weeks to a month, because that would be our best assessment of if you've developed any resistance to that regimen after stopping it for periods of time.

Q You would do a genotype at that point or would a viral load test indicate whether the same regimen was being -- was still effective?

A So you can't do a genotype without

getting a viral load. So they kind of go hand in hand, because you get the genotype based on that viral load, if that makes sense.

Q So the individual who goes back on treatment would be on treatment for some period of time, the same treatment?

A Yes.

Q And you would see if the viral load went back to suppressed or undetectable?

A Correct. So you would assess that at the three to four-week period after they restart that regimen. You would want to get a viral load with a genotype at three to four weeks after being back on that regimen.

Q What if they didn't have enough viral -- virus at that point to do a genotype?

A Then that would be great. Then we wouldn't need a genotype.

Q Right.

A The goal is to see that they've either suppressed their viral load again or, if not, we're going to try to get a genotype to see if they've developed resistance.

Q Got it. Is it possible that they would have -- that it would have been reduced, but they

maybe had not gotten all the way back down to suppressed at that point, in which case what do you do?

A We would still --

MS. BERMAN: Objection. Form.

THE WITNESS: We -- can I still answer?

MS. BERMAN: Yes.

THE WITNESS: We would still want to check a genotype because it might give us a glimpse into a new resistance panel and it may prompt us to switch that regimen.

BY MR. SCHOETTES:

Q But that would require at least a thousand copies if you used the one test?

A Right, 1,000 to 2,000 copies, yes.

Q Okay. What concerns, if any, is there around -- well, I guess we were just talking about this. Do you know what the likelihood is of resistance after treatment interruption?

MS. BERMAN: Objection. Vague and calls for speculation.

You can answer.

THE WITNESS: Yeah. I mean, it depends on a lot of different variables. So it's -- you are more likely to acquire resistance if you're

intermittently taking your medications. So one thing that we always try to couch [sic] our patients about is when you're -- sometimes what our patients do -- because, for various reasons, they may not come back in to get refills of their medications or run out of their meds -- sometimes what they do is they start spacing out the dosing of their medication. So they'll say, oh, I'll take it every other day or every third day to make it last longer. That's exactly how you develop resistance. So it's actually better to stop it cold turkey and then restart it up later. That's your best option for not developing resistance.

So it depends on if somebody is taking their meds intermittently to space them to -- or stops it altogether.

BY MR. SCHOETTES:

Q So in the context of lost medication, and it's a sustained stop, that would be less likely to develop resistance than someone intermittently taking their medication?

A Correct. The other thing that you have to take into account is the half life of the particular drug in the body. So there's different antiretroviral regimens that have longer half

lives or shorter half lives. So missing one or two medications of one drug might be not as -- might be less forgiving than missing one or two days of another drug.

Q And that's more of a concern in that intermittent drug-taking scenario --

A Yes.

Q -- than it would be in a full stop?

A Yes.

Q Because the half life on all of them is going to sort of run out relatively quickly and then there won't be anything that the virus could mutate around, because there's only one of the medications left, right?

A Correct.

MR. SCHOETTES: I think I'm done.

MS. BERMAN: Okay. I think we want to talk for a minute about whether I have any follow-up questions. And -- so if we want to go off the record for a minute, I may have a few more.

MR. SCHOETTES: Sounds good.

THE VIDEOGRAPHER: The time is 3:28 p.m. We are going off the record.

(Whereupon, a short recess was taken.)

THE VIDEOGRAPHER: The time is 3:35 p.m. We are back on the record. Please proceed, Counsel.

EXAMINATION BY COUNSEL FOR THE U.S. DEPARTMENT OF JUSTICE

BY MS. BERMAN:

Q Colonel Blaylock, I just want to clarify a few things. You and counsel were talking about the CDC guidelines earlier about -- that state there's essentially zero risk, or approximately zero risk, of transmission of HIV from a person with a suppressed viral load. Does that guideline specifically concern sexual transmission?

A Yes. That guideline does concern sexual transmission only.

Q Is there any similar statement about transmission by blood transfusion from the CDC?

A No, there is not.

Q Okay. And I think this is in the record, but before, when you were talking about the risk of transmission by blood transfusion, I believe you and counsel were talking about the scenario of someone with an increased viral load, someone who was not fully suppressed. Is there also a significant risk of transmission from a person

with a suppressed viral load by blood transfusion?

A Yes. There would still be a significant risk, even if the viral load was suppressed under 20 copies because, again, that's not zero copies; that's -- could be ten copies per milliliter of blood.

So when you're looking at a total of 250 to 300 milliliters of blood in a whole unit of blood, that risk becomes more substantial.

Q Okay. Do you have any estimate of the percentage risk of transmission in both of those scenarios, both the suppressed viral load scenario and the unsuppressed viral load scenario?

A So, unfortunately, the only solid data that we have is pretty old CDC data from -- I believe it was 1990 when it was published that talked about the risk via blood transfusion. And they did not separate it out into folks who had very low viral loads, or even undetectable viral load, and those that had high viral load.

We just know across the board, across everybody with HIV, there was upwards of 90 percent transmission rate from somebody who was HIV-infected donating blood.

Q Okay. And we talked about this somewhat,

between a person with a high viral load and one with a suppressed viral load?

A I think it's -- I don't think there are studies that have looked at somebody with an undetectable viral load, their ability to transmit virus to a seronegative person. That would be a very unethical study.

Q Right.

MR. SCHOETTES: Okay. That's all I have.

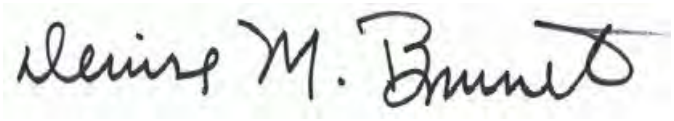
MS. BERMAN: Okay. We're done, then.

THE VIDEOGRAPHER: The time is 3:54 p.m. This concludes today's testimony given by the United States Army and Dr. Jason Blaylock. We are now off the record.

(Whereupon, at 3:54 p.m., the deposition of JASON BLAYLOCK was concluded.)

CERTIFICATE OF NOTARY PUBLIC

I, Denise M. Brunet, the officer before whom the foregoing deposition was taken, do hereby certify that the witness whose testimony appears in the foregoing deposition was sworn by me; that the testimony of said witness was taken by me stenographically and thereafter reduced to print by means of computer-assisted transcription by me to the best of my ability; that I am neither counsel for, related to, nor employed by any of the parties to this litigation and have no interest, financial or otherwise, in the outcome of this matter.



Denise M. Brunet
Notary Public in and for
The District of Columbia

My commission expires:
December 14, 2022

Veritext Legal Solutions
1100 Superior Ave
Suite 1820
Cleveland, Ohio 44114
Phone: 216-523-1313

March 12, 2019

To: Keri L. Berman, Esq.

Case Name: Harrison, Nicholas, et al. v. Mattis, James N., et al.

Veritext Reference Number: 3235700

Witness: Jason Blaylock Deposition Date: 2/27/2019

Dear Sir/Madam:

Enclosed please find a deposition transcript. Please have the witness review the transcript and note any changes or corrections on the included errata sheet, indicating the page, line number, change, and the reason for the change. Have the witness' signature notarized and forward the completed page(s) back to us at the Production address shown above, or email to production-midwest@veritext.com.

If the errata is not returned within thirty days of your receipt of this letter, the reading and signing will be deemed waived.

Sincerely,
Production Department

NO NOTARY REQUIRED IN CA

DEPOSITION REVIEW
CERTIFICATION OF WITNESS

ASSIGNMENT REFERENCE NO: 3235700
CASE NAME: Harrison, Nicholas, et al. v. Mattis, James N.
DATE OF DEPOSITION: 2/27/2019
WITNESS' NAME: Jason Blaylock

In accordance with the Rules of Civil Procedure, I have read the entire transcript of my testimony or it has been read to me.

I have made no changes to the testimony as transcribed by the court reporter.

_____ _____
Date Jason Blaylock

Sworn to and subscribed before me, a Notary Public in and for the State and County, the referenced witness did personally appear and acknowledge that:

They have read the transcript;
They signed the foregoing Sworn Statement; and
Their execution of this Statement is of their free act and deed.

I have affixed my name and official seal

this _____ day of_____, 20____.

_____
Notary Public

_____
Commission Expiration Date

DEPOSITION REVIEW
CERTIFICATION OF WITNESS

ASSIGNMENT REFERENCE NO: 3235700
CASE NAME: Harrison, Nicholas, et al. v. Mattis, James N.
DATE OF DEPOSITION: 2/27/2019
WITNESS' NAME: Jason Blaylock

In accordance with the Rules of Civil Procedure, I have read the entire transcript of my testimony or it has been read to me.

I have listed my changes on the attached Errata Sheet, listing page and line numbers as well as the reason(s) for the change(s).

I request that these changes be entered as part of the record of my testimony.

I have executed the Errata Sheet, as well as this Certificate, and request and authorize that both be appended to the transcript of my testimony and be incorporated therein.

_____ _____
Date Jason Blaylock

Sworn to and subscribed before me, a Notary Public in and for the State and County, the referenced witness did personally appear and acknowledge that:

They have read the transcript;
They have listed all of their corrections in the appended Errata Sheet;
They signed the foregoing Sworn Statement; and
Their execution of this Statement is of their free act and deed.

I have affixed my name and official seal this _____ day of_____, 20____.

_____
Notary Public

_____
Commission Expiration Date

ERRATA SHEET

VERITEXT LEGAL SOLUTIONS MIDWEST

ASSIGNMENT NO: 2/27/2019

PAGE/LINE(S) / CHANGE /REASON

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Date Jason Blaylock

SUBSCRIBED AND SWORN TO BEFORE ME THIS _____

DAY OF _____, 20_____ .

_____

Notary Public

_____

Commission Expiration Date

EXHIBIT 36

IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF VIRGINIA
Alexandria Division

NICHOLAS HARRISON, *et al.*, Plaintiffs, v. MARK ESPER, Secretary of Defense, *et al.*, Defendants.	No. 1:18-cv-641 (LMB/IDD)

RICHARD ROE, *et al.*, Plaintiffs, v. MARK ESPER, Secretary of Defense, *et al.*, Defendants.	No. 1:18-cv-1565 (LMB/IDD)

DECLARATION OF LIEUTENANT COLONEL JASON M. BLAYLOCK

I, Jason Michael Blaylock, hereby state and declare as follows,

1. I am an active duty member of the United States Army, currently serving as the Chief of Medicine at Walter Reed National Military Medical Center and the Army clinical lead of the Defense Health Agency (DHA) HIV Tri-Service Working Group. I have served in the latter position since July 2017. As the former Chief of Infectious Diseases at Walter Reed from May 2018 to January 2020, I oversaw all aspects of inpatient and outpatient infectious disease care at the medical center. As the Army's Clinical Lead for

HIV Infection and co-chair of the DHA's HIV Tri-Service Working Group, I continue to serve as a subject matter expert for the development of policy regarding clinical management of HIV within the DHA. Since July 2018, I have also served as the Infectious Diseases consultant to the White House Medical Unit and the Office of the President, providing clinical support and information briefs regarding HIV infection and other infectious diseases as needed.

2. Between 2013 and 2017, I served as the Associate Program Director for Walter Reed's Infectious Disease Fellowship Program while serving as the officer in charge of the Infectious Disease Outpatient Clinic. In the latter role, I stood up Walter Reed's HIV Pre-Exposure Prophylaxis (PreP) Clinic. On June 30, 2011, I completed a three year fellowship in Walter Reed's Infectious Disease Fellowship Program.

3. Based on my education as an Infectious Disease doctor and my official duties and responsibilities as an Army Infectious Disease specialist with experience treating HIV, I have an understanding of HIV infection, transmission, progression, treatment, and management within the United States military.

4. I make this declaration based upon my own personal knowledge and upon information that has been provided to me in the course of my official duties. I submit this declaration in support of the Defendants' Motion for Summary Judgment in the above-captioned case.

HIV Disease Progression

5. The clinical progression of HIV infection varies greatly based on if and when effective treatment is initiated and on subsequent compliance with antiretroviral therapy (ART). Intervention with ART generally has significant implication for viral load,[1] CD4 lymphocyte count,[2] and rate of disease progression. However, ART is not a cure for

[1] Viral load is the number of copies of a virus per milliliter of blood.
[2] CD4 lymphocyte count is the number of white blood cells (T cells) per milliliter of blood. The number of cells provides an indication of immune function.

HIV infection.

6. Most patients who become infected with HIV and are not immediately treated will present with evidence of an acute infection including early rapid viral replication and infection of CD4 lymphocytes. Although approximately 60% of such patients present clinically as asymptomatic, most will typically display very high viral loads, often more than one million copies per milliliter of blood, accompanied by a transient drop in CD4 count.

7. Individuals infected with HIV develop detectable antibodies against the infection within the first several weeks of infection, a process known as seroconversion. Within approximately six months of infection, an untreated individual's viral set point will generally be established. A higher viral load set point and lower early CD4 count in the absence of ART are commonly associated with an increased rate of disease progression. Although viral load typically stabilizes over time for untreated patients, at an average of 30,000 to 50,000 copies per milliliter of blood, an untreated HIV-infected individual's CD4 lymphocyte count continues to progressively decline, rapidly within the first year, and then more slowly thereafter.

8. For patients who are not treated with ART, the average time from infection to development of AIDS is eight to ten years. The rate of disease progression is influenced by a number of factors, including subtype of infection, coinfection with other diseases such as tuberculosis and syphilis, and increased age of the patient. A diagnosis of AIDS is premised on a patient's CD4 count falling below 200 lymphocytes per milliliter of blood and/or the presence of an AIDS-defining illness. In this setting, continuous high-level replication of HIV virus causes viral and immune-mediated destruction of CD4 lymphocytes,[3] and ultimately results in such

[3] In immune-mediated destruction of T cells, the human body's natural immune response to HIV infection causes the body's immune cells to attempt to kill the HIV virus. Because the virus is essentially hiding in CD4 cells, however, in doing so the immune system can also destroy those cells.

opportunistic infections as esophageal candidiasis and Kaposi's sarcoma.

9. HIV infection is also associated with the development of comorbid conditions at younger ages than are generally observed in uninfected populations, including cardiovascular disease, osteoporosis, cognitive dysfunction, and certain cancers. There is data to suggest that untreated HIV infection results in immune activation that causes inflammatory cascades and accelerated aging of T cells (immunosenescence). The implication of this is that persons living with HIV (PLWH) are predisposed to comorbid conditions associated with aging, such as atherosclerosis, cardiovascular disease, neurodegeneration, and certain malignancies. It is not clear that these inflammatory effects on T cells are reversed by initiation of ART. *See* T. Sokoya, et al. *HIV as a Cause of Immune Activation and Immunosenescence*. Mediators of Inflam., Oct. 25, 2017;[4] Steven G. Deeks, *HIV infection, inflammation, immunosenescence, and aging*, 62 Ann. Rev. of Med. 141 (2011).[5]

10. With the advent of more effective and well-tolerated ART regimens, PLWH have longer life expectancies similar to the uninfected population. However, several studies continue to demonstrate that PLWH remain at increased risk for other comorbidities, to include hypertension, myocardial infarctions, peripheral vascular disease, liver disease, renal disease, and non-AIDS cancers (e.g. non-Hodgkins lymphoma, hepatocellular carcinoma, and anal and cervical cancer) that could reduce life expectancy. *See* J. Schouten, et al., *Cross-sectional comparison of the prevalence of age-associated comorbidities and their risk factors between HIV-infected and uninfected individuals: the AGEhIV cohort study*. 59 Clin. Infect. Dis. 1787 (2014);[6] Robert S Rosenson, et al., *Excess Risk for Atherosclerotic Cardiovascular Outcomes Among US Adults With HIV in the Current Era*, 9(1)

[4] A true and correct copy of this document is attached as Exhibit A to this Declaration.
[5] A true and correct copy of this document is attached as Exhibit B to this Declaration.
[6] A true and correct copy of this document is attached as Exhibit C to this Declaration.

J. Am. Heart Assoc., Jan. 2020.[7] While these comorbidities occur earlier in life and are more commonly diagnosed in untreated or poorly-controlled HIV infection, they also remain prevalent in well-controlled HIV infection. *Id.*

11. Neurocognitive issues are also of concern in PLWH. It is still unknown whether mild cognitive impairment in virally suppressed HIV-infected persons is a residual effect of the virus itself, the particular ART regimen, and/or other comorbidities such as substance abuse and depression. *See* Kevin Robertson, et al., *Antiretroviral Neurotoxicity*, 18(5) J. Neurovirology 388 (2012);[8] RK Heaton, et al., *Neurocognitive change in the era of HIV combination antiretroviral therapy: the longitudinal CHARTER study*, 60(3) Clin. Infect. Dis. 473 (2015).[9] Diagnosis of HIV-associated neurocognitive disorders is typically conducted via formal neurocognitive testing. However, it is difficult to completely exclude other concomitant diagnoses such as psychiatric disorders. In addition, the testing requires administration by specialized professionals who are generally not readily accessible.

12. As newer ART regimens are being utilized, more data is accruing to suggest that more commonly used regimens are associated with bone mineral density loss, renal dysfunction, weight gain, and increased lipid profiles. *See* Jason J Schafer, et al., *Changes in Body Mass Index and Atherosclerotic Disease Risk Score After Switching From Tenofovir Disoproxil Fumarate to Tenofovir Alafenamide*, 6(10) Open Forum Infect. Dis., Oct. 4 2019;[10] GD Huhn, et al., *Atherosclerotic Cardiovascular Disease Risk Profile of Tenofovir Alafenamide Versus Tenofovir Disoproxil Fumarate*, 7(1) Open Forum Infect. Dis. Nov. 11 2019;[11] SK Gupta, et al., *Renal safety of tenofovir alafenamide vs. tenofovir disoproxil fumarate: a*

[7] A true and correct copy of this document is attached as Exhibit D to this Declaration.
[8] A true and correct copy of this document is attached as Exhibit E to this Declaration.
[9] A true and correct copy of this document is attached as Exhibit F to this Declaration.
[10] A true and correct copy of this document is attached as Exhibit G to this Declaration.
[11] A true and correct copy of this document is attached as Exhibit H to this Declaration.

pooled analysis of 26 clinical trials, 33(9) AIDS 1455 (2019);[12] CA Moran, et al., *Bone Loss in HIV Infection*, 9(1) Curr. Treat. Options Infect. Dis. 52 (2017);[13] A Carr, et al., *The rate of bone loss slows after 1–2 years of initial antiretroviral therapy: final results of the Strategic Timing of Antiretroviral Therapy (START) bone mineral density substudy*, 21 HIV Med. 64 (2020).[14] The clinical implications of these findings are as yet unknown, however, there is concern within the military infections disease (ID) community about the potential effects, particularly of weight gain, on the ability of an HIV-infected service member to maintain standards for military medical readiness.

13. With ART, HIV infection becomes a manageable condition for most patients. Although factors such as older age, lower pre-ART CD4 count, delay in initiation of ART, and the presence of coinfections can complicate the ability to attain viral load suppression, in general effective initiation of ART results in sustained viral suppression. In most cases, effective ART can reduce the HIV viral load to levels below our current limits of detection and can result in, on average, an increase of 50-150 CD4 lymphocytes per milliliter of blood each year for the first four years of treatment. However, strains of HIV do exist in circulation that have developed extensive resistance to current ART. While relatively rare, this can result in inability to effectively maintain viral suppression in a small number of PLWH.

Recommended Treatment Guidelines

14. Use of ART for treatment of HIV infection is strongly recommended by various expert medical organizations including: the United States Department of Health and Human Services, National Institutes of Health, the United States Panel of the International Antiviral Society, and the World Health Organization. These organizations recommend that all patients with acute or early HIV infection initiate

[12] A true and correct copy of this document is attached as Exhibit I to this Declaration.
[13] A true and correct copy of this document is attached as Exhibit J to this Declaration.
[14] A true and correct copy of this document is attached as Exhibit K to this Declaration.

ART as soon as possible and regardless of CD4 cell count because the potential benefits to individual and public health outweigh any potential drawbacks of ART initiation.[15]

15. Early initiation of ART improves public and individual health outcomes in both HIV morbidity and HIV mortality. ART allows for rapid suppression of viral load in most patients; approximately 97% of patients treated with ART achieve an undetectable viral load within 11 weeks of beginning treatment. ART also allows for maintenance of viral suppression; approximately 92% of patients who have initial success with ART remain virally suppressed 18 months after beginning treatment.

16. Early initiation of ART can also reduce the ability of an HIV-infected individual to transmit infection, as approximately 50% of new HIV infections are transmitted from individuals in the acute stage of infection. In addition, initiation of ART immediately after infection can result in decreased viral reservoir and immune activation in the patient.

17. While use of HIV pre-exposure prophylaxis by an HIV-uninfected sexual contact can further reduce risk of transmission, other factors in an HIV-infected individual, such as infections, including concomitant sexually transmitted diseases, can increase viral load, viral shedding, and risk of transmission.

18. In most cases, ART can be initiated safely and effectively on the same day that HIV infection is diagnosed. At the time of diagnosis, baseline resistance to common ART regimens should be tested to allow for modification of treatment if necessary when the results become available.

19. Once a patient initiates ART, the United States Department of Health and Human Services recommends ongoing monitoring of the patient's viral load at two weeks and

[15] To avoid duplication, the various CDC/HHS guidelines related to HIV discussed in this declaration are attached as exhibits to the Defendants' Brief in Support of their Motion for Summary Judgment.

at four to eight week intervals until the patient's viral load becomes undetectable. After viral load suppression is achieved, viral load should be monitored at least every three to six months until the patient's CD4 count has been measured as greater than 300 cells per milliliter of blood for two years, after which viral load can be measured every six months. If a patient changes his or her ART regimen or experiences a deterioration of clinical status, viral load should be measured at two to eight weeks and more frequently thereafter, as needed, to ensure suppression.

20. ART treatment also requires monitoring of a patient's CD4 lymphocyte count. CD4 monitoring is recommended every three to six months following ART initiation, until the patient's CD4 count has been measured as greater than 300 cells per milliliter of blood for two years, after which viral load can be measured every twelve months.

21. The CDC has also established guidelines for Post-exposure Prophylaxis (PEP) for HIV seronegative individuals who are exposed to the blood or other bodily fluids of individuals with known or suspected HIV infection. To maximize effectiveness of HIV prevention, it is recommended that PEP be initiated as soon as possible, and within 72 hours of known or suspected exposure. The CDC guidelines also instruct that individuals being treated with PEP be monitored during the course of the treatment to determine whether the individual has contracted HIV and if he or she is tolerating the regimen.

Management of HIV in the Military

22. The Army, Navy, Marines, and Air Force follow the Department of Health and Human Services' guidelines for HIV treatment and monitoring.

23. A recent study tracked use of ART and viral suppression among Army service members with HIV between January 2012 and June 2018. During that period 1050 Army service members were newly diagnosed with HIV; of this group 89.4% received

ART within 6 months of diagnosis; 95.4% within 12 months; and 98.7% by end of surveillance period. During the period of the study, 793 service members remained on ART for over one year and 99% of those achieved viral load suppression within one year. *See* S Stahlman, et al., *Antiretroviral Therapy and Viral Suppression Among Active Duty Service Members with Incident HIV Infection - United States, January 2012-June 2018*. 69(13) MMWR Morb. Mortal Wkly. Rep. 366 (2020).[16] It is important to note that none of these service members were taking ART in the deployed setting.

24. For service members with HIV, clinical evaluations are performed by an infectious disease specialists at least every six to twelve months after diagnosis in addition to the lab monitoring recommended by the treatment guidelines established by the Department of Health and Human Services.

Management of HIV in a Deployed Setting

25. The availability of medical assets in deployed settings varies widely and is dependent on the resources available in the particular deployed setting and the current pace of military operations.[17] Military medical facilities are designated by their capabilities as Role 1, Role 2, Role 3, or Role 4 with Role 4 having the most extensive capabilities. In general, less mature theaters of operation have less extensive medical capabilities. The availability of medical and supporting logistical resources is also typically more limited the closer a military position is to active combat.

26. Laboratory capabilities such as those needed to conduct HIV viral load testing and CD4 count monitoring are currently not available at Role 1 facilities and most Role 2 facilities. Under current conditions, sending blood and other products to laboratories at higher level facilities with more expansive resources typically creates a delay of two

[16] A true and correct copy of this document is attached as Exhibit L to this Declaration.
[17] The pace of operations or "ops tempo" is a highly variable measure reflecting current conditions, such as engagement in active combat, need to change locations or urgently evacuate, no-fly zones, 24-hour operations, and numerous other potentialities.

to four weeks for results depending on the location of the forward military position from which they are being sent. Future near-peer conflicts or operations in less mature theaters could face additional complications. It is not possible to send out products or people for laboratory testing from certain locations or under certain circumstances due to potential mission operation tempo, safety or logistical concerns.

27. Role 1 and Role 2 facilities have a limited number of medical personnel and are unlikely to have access to an infectious disease specialist. Although Role 3 facilities have more resources, most do not have an infectious disease specialist available. Non-specialist medical personnel often do not have substantial familiarity with HIV monitoring and treatment including CD4 count and viral load testing, appropriate implementation of PEP, and knowledge of potential drug interactions and side effects of various ART regimens. The current Army standard of care for PLWH includes every 6 month evaluations by an Infectious Diseases specialist, which is not feasible in a deployed setting due to the limited numbers of such personnel.

28. Forward military positions also have less access to pharmacy capabilities, limiting the variety of medications available and the possibility of resupply or replacement of medications as needed. In far forward positions close to front lines, and in austere settings, there is minimal or no access to mail-order pharmacy or other resupply. Even in more established areas of operation, pharmacies must often be limited to common medications for operational reasons including mobility needs and the cost of maintaining pharmacy supplies. If a service member's ART were lost or destroyed in most deployed settings, it is very unlikely that that individual's specific medication would be readily available from existing medical supplies.

29. There are scenarios in which combat operations or circumstances at forward military positions interfere with movement in and out of those locations, such as no fly restrictions. These restrictions can result in interruptions to medication adherence,

either because an individual cannot timely obtain an anticipated resupply, or because an individual has lost his or her supply of medication and resupply is unavailable. Supplies may also be destroyed by attacks targeting logistical operations. Additionally, moving service members to higher levels of care can result in both interruptions in the mission as well as degradation of mission readiness.

30. Interruptions in medication adherence for HIV-infected individuals on ART result in a rising viral load that increases with the duration of the interruption. For patients who are virally suppressed on ART, an interruption to medication will result in a detectable viral load, typically within two to eight weeks, but rebound has been observed in as little as nine days. *See* KJ Bar, et al., *Effect of HIV Antibody VRC01 on Viral Rebound after Treatment Interruption*, 375(21) N. Engl. J. Med. 2037 (2016).[18]

31. An increased viral load results in an increased ability to transmit HIV infection via blood exposure, blood transfusion, and sexual contact. Interruptions to ART adherence can also lead to the development of antiretroviral resistance to an individual's ART regime. In this case, it would be important to obtain an HIV viral load and, if elevated to > 1000 copies/mL, to obtain a resistance panel to assess for development of resistance to the current ART regimen and need for switching to another regimen. Typically, a PLWH is more likely to develop resistance to a particular ART regimen if taking intermittently and inconsistently, as opposed to stopping the regimen altogether. The duration of intermittent ART use and association with development of resistance varies based on several factors, including the exact frequency of ART use and the half-life of the specific antiretroviral.

32. Generally, high stress settings have a known negative effect on viral and bacterial infections, for example reactivation of shingles, mononucleosis, and tuberculosis disease processes. The effects of a high-stress setting such as on a military deployment

[18] A true and correct copy of this document is attached as Exhibit M to this Declaration.

on an HIV infection are unknown. Given the potential for sleep disruptions, dehydration, and increased stress of mission requirements and pace of operations, it is possible that these concomitant stressors could contribute to immune dysregulation and affect the body's ability to adequately suppress HIV infection.

33. Medical needs in a deployed setting also risk the confidentiality of an HIV-infected service member. Service members may be required to submit to a medical evaluation (e.g. as result of laceration or other significant trauma) during which the member would need to divulge their HIV status to the physician and any other exposed members. Confidentiality could also be breached by medication resupply or the unavailability of resupply and its consequences in the deployed setting. Additionally, if it is necessary for the military to activate a walking blood bank and call for immediate blood donations from service members who have not previously or recently been screened, an HIV-infected service member may feel significant pressure to disclose his or her status to avoid donating blood.

Transmission of HIV

34. Most new HIV infections are acquired through sexual intercourse or exposure to infected blood. Risk of infection varies based on the type of exposure, including type of sexual contact or blood exposure, and by the viral load of the HIV-infected source. A higher viral load increasing the risk of transmission in the setting of either a sexual or blood exposure. Other cofactors, such as the presence of other sexually transmitted infections, may also affect the risk of transmission.

35. Exposure to HIV through blood transfusion has, by far, the highest risk of HIV transmission, with early studies indicating risk of infection in 9 out of 10 exposures. Other potential blood exposures at risk of transmission of HIV infection include percutaneous needle stick (one infection per 435 exposures) and mucous membrane exposure to blood (one infection per 1,000 exposures). Observational studies have

determined that receptive anal intercourse is the sexual exposure with the highest risk of transmission (one transmission per 72 sex acts).

36. Although studies conducted since widespread use of ART indicate that viral suppression vastly reduces the risk of sexual transmission of HIV, no similar studies have been conducted concerning blood exposure transmissions. It is unknown to what extent the risk of transmission via blood exposure might be reduced by use of ART. In addition, currently available commercial diagnostics are only able to quantitatively assess HIV viral load down to a threshold of 20 copies per milliliter of blood. Therefore, the risk of HIV transmission via a blood transfusion remains substantial even in the setting of reported viral suppression as diagnosed by current testing capabilities. CDC guidance requires that individuals with undetectable viral loads refrain from donating blood.

37. In the deployed setting, risks of blood borne transmission of HIV, even from service members who are virally suppressed, are real yet unquantifiable. There is a negligible risk of transmission via mucous membrane or by exposure of intact skin to HIV-infected blood. However in battlefield trauma circumstances, such as a mass casualty event, there remains an unknown non-negligible risk of transmission from blood exposure to another service member's non-intact skin.

38. There is also a significant risk of transmission through blood transfusion, even from an HIV-infected service member with an undetectable viral load,[19] in an emergent situation where donated blood cannot be reliably pre-screened, such as the activation of a walking blood bank. Under such circumstances, when asked to donate urgently needed blood, an HIV-infected service member would be put in the untenable position of either disclosing his or her HIV status or proceeding to donate blood to avoid

[19] Due to current limits viral load detection, only down to 20 copies/mL of blood, it is entirely feasible that a whole blood transfusion of 250mL (1 unit), might transmit HIV from a well-controlled HIV-infected Soldier to an uninfected Soldier.

disclosure. Rapid HIV tests are not readily available at all deployed locations, and there currently are no rapid point-of-care HIV tests that are FDA-approved for use in screening whole blood for planned transfusion. I am aware of at least one case where an HIV-infected service member knowingly donated blood in the United States which was fortunately identified by American Red Cross screening procedures that are not readily available in combat situations.

* * *

In accordance with 28 U.S.C. § 1746, I declare under penalty of perjury that the foregoing is true and correct. Executed this 2nd day of June 2020.

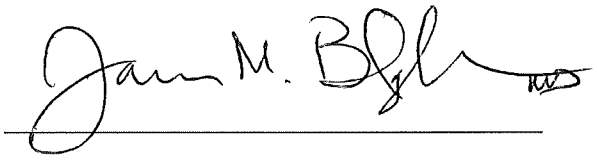

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EXHIBIT A

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Review Article

HIV as a Cause of Immune Activation and Immunosenescence

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Systemic immune activation has emerged as an essential component of the immunopathogenesis of HIV. It not only leads to faster disease progression, but also to accelerated decline of overall immune competence. HIV-associated immune activation is characterized by an increase in proinflammatory mediators, dysfunctional T regulatory cells, and a pattern of T-cell-senescent phenotypes similar to those seen in the elderly. These changes predispose HIV-infected persons to comorbid conditions that have been linked to immunosenescence and inflamm-ageing, such as atherosclerosis and cardiovascular disease, neurodegeneration, and cancer. In the antiretroviral treatment era, development of such non-AIDS-defining, age-related comorbidities is a major cause of morbidity and mortality. Treatment strategies aimed at curtailing persistent immune activation and inflammation may help prevent the development of these conditions. At present, the most effective strategy appears to be early antiretroviral treatment initiation. No other treatment interventions have been found effective in large-scale clinical trials, and no adjunctive treatment is currently recommended in international HIV treatment guidelines. This article reviews the role of systemic immune activation in the immunopathogenesis of HIV infection, its causes and the clinical implications linked to immunosenescence in adults, and the therapeutic interventions that have been investigated.

1. Introduction

More than 3 decades following the discovery that the human immunodeficiency virus (HIV) causes the acquired immune deficiency syndrome (AIDS), there is an increasing evidence that systemic immune activation plays a significant role in the disease pathogenesis [1]. High levels of systemic immune activation and inflammation not only promote viral replication and $CD4^+$ T-cell apoptosis, but also may lead to more rapid decline of immune function and competence. This resembles the phenomenon of immunosenescence that has been associated with ageing [2]. While combination antiretroviral therapy (cART) has improved the quality of life and reduced mortality and morbidity in HIV-infected persons, long-term virally suppressive treatment has not been successful in normalizing elevated markers of systemic immune activation [3]. HIV-infected individuals remain at a high risk of developing degenerative, dysfunctional, or malignant non-AIDS-defining diseases; many of which have been linked to immunosenescence and inflamm-ageing [4].

An ageing immune profile is characterized by decreased production of naïve T-cells and an increase in the proportion of memory T-cells with oligoclonal expansion [5]. The senescent T-cell phenotype is marked by a lack of CD28 expression, decreased homing receptors (e.g., CD62L and CCR7), and increased expression of the senescence marker, CD57 [6]. In addition, senescent cells are characterized by decreased proliferative capacity as indicated by shortened telomere length (TL), cell cycle arrest, increased β-galactosidase activity, limited proliferation in response to antigen stimulation, and activation of proinflammatory secretory pathways [6]. Several immunological changes seen in HIV-1-infected people are comparable to those observed in the aged. Proinflammatory cytokines, which are increased in HIV infection, including tumour necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6, are known to play a role in

ageing [7, 8]. Increased secretion of interferon (IFN)-α and reduced production of IL-2 are observed in both HIV infection and ageing [9]. Similarities in T-cell differentiation also exist, such as a reduction in the longevity of $CD4^+$ and $CD8^+$ T-cells, reduced production of naïve $CD4^+$ T-cells, increased numbers of late-differentiated $CD4^+$ and $CD8^+$ T-cells, and shortened TL [9].

In HIV-infected persons, systemic immune activation and $CD4^+$ T-cell function are inextricably linked to immunosenescence, in what appears to be a self-perpetuating cycle. The changes in immune and cytokine release resulting from HIV-induced immune activation increase susceptibility to activation-induced cell death [10–13]; consequent immune exhaustion results in senescence and programmed $CD4^+$ T-cell death, which further drive immune activation [14–17]. In both the aged and in HIV, immunosenescence has been associated with negative immune outcomes, such as thymic involution, reduction in the overall T-cell repertoire, autoimmunity, and poor antigen responsiveness [6]. Immunosenescence seems to be of particular importance in the pathogenesis of conditions where inflammation represents a significant risk factor, such as atherosclerosis and cardiovascular disease (CVD), neurodegeneration, and cancer [6]. Indeed, in the ART era, development of non-AIDS-defining, age-related comorbidities, such as osteoporosis, atherosclerosis, and neurocognitive decline, is a major cause of morbidity and mortality in HIV-infected persons [18]. The Strategies for Management of Antiretroviral Therapy (SMART) study demonstrated that deaths were mostly due to non-AIDS-defining malignancies (19%) and CVD (13%), while opportunistic diseases only accounted for 8% [19].

This study reviews the role of systemic immune activation in the immunopathogenesis of HIV infection and the causes of systemic immune activation and inflammation. We also review the clinical implications of accelerated ageing and age-related morbidity in adults and therapeutic interventions investigated to date. Data for this review were identified through searches of publicly available databases, for example, Medline and Pubmed, and in the references of studies found through these searches. Particular attention was paid to biologically *mechanistic* studies and review articles focused on systemic immune activation in HIV-infected persons. Preference was given to recent studies, that is, published in the last decade, but earlier studies that were relevant were also included.

2. Systemic Immune Activation in the Immunopathogenesis of HIV Infection

Introduction of HIV into host cells activates a complex network of protective responses originating from both the innate and adaptive immune systems [20]. These responses are either insufficient or too late to eliminate the virus. This enables life-long viral latency and chronic infection, which drives ongoing immune activation and progressive immunodeficiency, characterized by high cell turnover, apoptosis, and activation-induced death of immune cells [21].

Studies of pathogenic and nonpathogenic models of simian immunodeficiency virus (SIV) infection have provided insights into the role of systemic immune activation in the progression to AIDS [22]. The natural hosts of SIV, such as the African green monkey and sooty mangabey, are able to live normally with the virus and do not progress to immunodeficiency, regardless of high levels of viral replication. On the other hand, inoculating other nonhuman primates, such as rhesus macaques and Asian pigtailed macaques, with SIV results in immunodeficiency and progression to AIDS similar to that in HIV-infected humans [23–26]. During both pathogenic SIV (pSIV) and nonpathogenic SIV (npSIV) infection, robust viral replication and early antiviral responses occur during the acute phase of infection. However, it appears that the natural hosts have devised an evolutionary strategy to maintain an effective response, which enables symbiotic coexistence [27, 28]. This adaptive response appears to be associated with early resolution of acute T-cell activation, rather than an improved viral control.

It is thought that differences in immune response determine whether pSIV or npSIV infection develops. pSIV studies have demonstrated substantial loss of mucosal T-helper (Th) 17 cells, with subsequent microbial translocation as evidenced by high levels of plasma lipopolysaccharide (LPS) and soluble CD14 (sCD14) [28]. pSIV is associated with dysregulation of cell cycle and T regulatory cell (Treg) loss. This indicates a failure to the control of T-cell activation/proliferation and contributes to poor outcome [28]. Other characteristics distinguishing natural from unnatural hosts include superior cell homeostasis, higher numbers of $CD4^+$ T-cells, the presence of anti-inflammatory mechanisms such as attenuated IFN signalling, maintenance of progenitor cell regeneration, and more limited immune activation, and T-cell apoptosis [27, 28].

In humans, elite controllers are a unique yet heterogeneous group of people that maintain adequate control of viral replication even in the absence of cART [22, 29]. Unlike in npSIV, elite controllers are able to downregulate viral replication in lymphoid tissue. They also have powerful and durable anti-HIV immune responses, with significantly higher activation of T-cells compared to uninfected individuals. However, this is relatively less than that seen in HIV-infected persons who are not elite controllers [29, 30]. Many elite controllers do eventually experience immune-mediated depletion of $CD4^+$ T-cells and develop AIDS-defining diseases. It has been shown that basal levels of immune activation determine this progression [31].

3. Causes of Systemic Immune Activation in HIV

3.1. Direct Effects of Virions and/or Viral Proteins. HIV gene products, such as gp120 and Nef, directly stimulate the activation of lymphocytes and macrophages, resulting in the secretion of proinflammatory cytokines and chemokines [32]. Certain HIV proteins imitate and/or enhance TNF-receptor signalling, causing persistent HIV replication in infected cells through activation of nuclear factor (NF)-κB, a prototypical proinflammatory signalling pathway [33], and apoptosis of uninfected bystander T-cells [34].

3.2. Viral Coinfections. Coinfection with other viruses, such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), and hepatitis B virus (HBV) and hepatitis C virus (HCV), is common in HIV-infected individuals. Pathogenic gene products enhance the replication of HIV by transactivation of HIV long terminal repeats (LTRs) [35]. HIV-induced immunodeficiency and replicative senescence, which result in the loss of $CD8^+$ T-cell populations that control viral replication, may, in turn, reactivate other pre-existing viruses or exacerbate infection by increasing viral load (VL) and consequent viral persistence [2]. This accelerates disease progression and contributes to systemic immune activation [36, 37]. CMV accounts for approximately 10% of the circulating memory T-cell repertoire in healthy, asymptomatic, HIV-uninfected CMV-seropositive individuals. The vast majority of HIV-infected individuals, between 75% and 90%, elicit significant CMV-specific T-cell responses [37, 38]. Chronic coinfection with CMV has been associated with immunological senescence, that is, gradual age-related deterioration of the immune system, homeostatic changes, and low $CD4^+$ T-cell counts. It is noteworthy that the latter is particular for naïve T-cell counts, possibly due to decreased T-cell renewal capacity and thymic involution, which lead to inadequate T-cell reconstitution [39].

HIV-1-infected individuals normally have a higher content of EBV in their lymphoid tissues, or a larger number of EBV-infected cells in their peripheral blood mononuclear cells (PBMCs), than HIV-uninfected individuals. It is thought that the expansion of EBV-positive B-cells may be caused by chronic B-cell stimulation driven by HIV proteins and impaired immune surveillance against EBV secondary to immunodeficiency [40]. A strong association has been found between HIV viremia, markers of immune activation, and EBV DNA load in PBMCs [41].

Hepatocytes and Kupffer cells, the latter of which are liver macrophages, are derived from blood monocytes, phagocytose, and clear particles draining through the portal system. Decreased Kupffer and $CD4^+$ T-cell counts have been found in individuals coinfected with HIV and HCV [42–44]. This cell loss may be due to the direct cytotoxic effects of HIV, specifically induced programmed cell death due to soluble viral or host factors, and altered Kupffer cell trafficking to target sites [44]. In coinfected people, elevated levels of sCD14 and LPS are found in the blood, due to a decrease in the clearance of particles and microbial products following diminished Kupffer cell numbers [42–44]. The reduction in $CD4^+$ T-cells occurring during HIV-1 infection may also lead to inadequate viral control, thereby permitting reactivation of HCV, which perpetuates the cycle of viral replication and immune activation [32].

3.3. Persistent Elevation of Type I and II Interferons (IFNs). IFNs I and II are produced by the innate immune system during HIV infection. IFN I plays an important role in mediating continuous inflammation. It is produced by plasmacytoid dendritic cells (pDCs) following direct activation of toll-like receptor (TLR)-7 and toll-like receptor (TLR)-8 by HIV RNA [45–47]. IFN I levels increase with plasma HIV-1 RNA levels and decrease with $CD4^+$ T-cell counts [48]. IFN I leads to the synthesis and recruitment of more target cells for HIV by upregulating the HIV coreceptor, C-C chemokine receptor type 5 (CCR5), and inducing pDC production of CCR5 ligands. IFN I also suppresses thymic output, limits $CD4^+$ T-cell recovery, induces $CD4^+$ T-cell apoptosis, and limits antigen-specific $CD4^+$ and $CD8^+$ T-cell responses [49]. IFN I further stimulates expression of the immunosuppressive enzyme, indoleamine 2,3-dioxygenase (IDO), leading to dysfunctional and immunosuppressive Tregs [48]. The elevated production of IFN-α leads to upregulation of proapoptotic molecules [50]. The administration of IFN II to HIV-infected individuals reduces the number of $CD4^+$ T-cells [49]. There is a close association between the elevation of types I and II IFN, IL-12, monocyte- and DC-derived inflammatory cytokines, and T-cell activation in HIV-infected individuals on ART [51]. The inadequate regulation of IFN responses drives chronic immune activation [52, 53].

3.4. Microbial Translocation. In the early stages of infection, HIV preferentially infects and depletes CCR5-expressing $CD4^+$ T-cells in the gastrointestinal tract (GIT) [54–58]. The accumulation of inflammatory cells, such as pDCs, neutrophils, and monocytes, and a concomitant decrease in cells that regulate epithelial homeostasis, such as IL-17 and IL-22-producing $CD4^+$ T-cells, progressively compromise mucosal integrity [59–64]. In turn, this inflammatory environment may lead to alterations in tight junction protein expression, decreased expression of claudins, upregulation of channel-forming claudins (e.g., claudin 2), and possibly increased epithelial and enterocyte apoptosis [65–69]. Dysfunction of the epithelial barrier in the GIT then allows translocation of microbial products from the intestinal lumen into the systemic circulation [70].

Pattern recognition receptors, such as nucleotide-binding oligomerization domains (NODs) and TLRs, detect microbial-associated molecular patterns (MAMPs), such as peptidoglycan, LPS, flagellin, and CpG DNA. The binding of microbial products to these receptors on cells of the innate immune system, most notably monocytes, macrophages, and DCs, activates a signalling cascade resulting in the production of proinflammatory cytokines, such as IL-1β, IL-6, TNF-α, and type-1 IFNs, such as IFN-α and IFN-β [43, 71]. For example, when TLR-4 recognises LPS, peripheral macrophages and DCs are directly stimulated to secrete proinflammatory cytokines [32]. This results in local and systemic immune activation and inflammation [65, 72–74].

Elevated levels of intestinal fatty acid-binding protein (I-FABP), originating from enterocytes, are found in the bloodstream of HIV-infected individuals [75]. I-FABP is a marker of enterocyte damage, which is associated with impaired intestinal function and microbial translocation. Enterocyte loss may be due to their reduced glucose uptake and increased expression of proinflammatory markers, such as TNF-α [43]. In response to the interaction between cell surface TLR-4 and monocyte activation, sCD14 is secreted into the blood [76–78]. sCD14 is a marker of LPS bioactivity and monocyte activation and is an independent predictor of mortality in HIV infection [75]. It may consequently be a

clinically useful surrogate marker of immune activation [51]. The interaction between LPS and LPS binding protein (LBP) leads to activation of NF-κB and increased cytokine expression. LPS-induced monocyte activation may also trigger the coagulation cascade through increased production of procoagulant tissue factor (TF), which correlates with increased levels of sCD14, D-dimer, and LBP [79]. Microbial translocation correlates with poor $CD4^+$ T-cell recovery, HIV disease progression, and susceptibility to non-AIDS conditions such as CVD and neurocognitive impairment [80].

4. The Detrimental Consequences of Systemic Immune Activation

The detrimental consequences of systemic immune activation are multifaceted. While some are particular to HIV, for instance immune system dysregulation, many are similar to the human ageing process and affect multiple organ systems.

4.1. Immune System Dysregulation. Immune dysregulation is characterized by a shift in leukocyte activity and an imbalance in cytokine levels. Derangement of both the innate and adaptive immune systems is associated with increased apoptosis of $CD4^+$ T- and B-cells, immunoparalysis of monocytes, and endotoxemia following microbial translocation [81]. In addition, continuous viral replication leads to a loss of T-cell homeostasis, characterized by increased T-cell turnover, an increase in the differentiation of naïve to antigen-exposed cells, T-cell replicative exhaustion, and apoptosis.

Immune activation also leads to depletion of T-cells and memory T-cell pools by other mechanisms, such as a decrease in the overall half-lives of $CD4^+$ and $CD8^+$ T-cells, irregular T-cell trafficking within T-cell subsets, and selective T-cell clonal exhaustion [21, 57]. A reduction in $CD4^+$ T-cells compromises the host's ability to combat pathogens and results in frequent and recurrent opportunistic and nonopportunistic infections. Inhibition of the normal functions of B-cells, NK, and other antigen-presenting cells also results in suboptimal viral control, further contributing to continuous activation of the immune system [82]. T-cells reach a state of persistent replicative senescence and immune exhaustion with the loss of antigen specificity in the immune system [83].

Cytokines play a vital role in coordinating host inflammatory response and are consequently useful markers of inflammation and systemic immune activation. Excessive production of either proinflammatory, for example, IL-1β, IL-2, IL-6, IL-8, and TNF-α, or anti-inflammatory cytokines, for example, IL-4, IL-10, and IL-13, imbalances immune responses [84]. Activation of T-, B-, and NK cells by HIV antigens and their components may increase the secretion of proinflammatory cytokines, chemotactic cytokines, for example, macrophage inflammatory protein (MIP)-1α, and adhesion molecules associated with inflammation, such as intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) [85–87]. Activation of monocytes, pDCs, and myeloid DCs may increase secretion of CXCL9, (monokine induced by gamma interferon (MIG)), CXCL10 (IFN gamma-induced protein 10 (IP-10)), CCL2 (monocyte chemoattractant protein-1 (MCP-1)), and TNF-α [51]. This culminates in T-cell activation and cytokine-driven T-cell apoptosis [88]. Increased proinflammatory cytokine levels increase susceptibility to inflammation-related conditions, such as osteoporosis, arteriosclerosis, cardiovascular conditions, and cancers [32].

Infection of pDCs by HIV may also increase immunosuppressive IDO and transforming growth factor (TGF)-β1, which impact immune dysregulation and T-cell homeostasis. The predominant origin of TGF-β1 is likely to be Tregs, but platelets, macrophages of the M2 phenotype, and immunoregulatory $CD8^+$ T-cells may also produce it [88]. Activation of TGF-β1 signalling in fibroblasts triggers increased procollagen and chitinase 3-like-1 production. This leads to collagen deposition, tissue fibrosis, and fibroblastic reticular cell network loss within the parafollicular T-cell zone of lymph nodes [89–91]. The interaction between mucosal intestinal myofibroblasts (IMFs) and LPS also leads to an increase in the soluble mediators of fibrogenesis (IL-6 and TGF-β1), which directly increase collagen deposition by IMFs [92]. This may contribute to the disproportionate depletion of $CD4^+$ T-cells in the GIT [90]. The ratio of Th17 to Tregs remains diminished during ART [93]. Such an imbalance may drive elevated IDO production by DCs, with subsequent impaired production of IL-17 and IL-22, leading to compromised antimicrobial immunity and tissue repair at barrier surfaces, with sustained immune activation [94, 95].

4.2. Thymic Function Alteration. During successful HIV suppression, $CD4^+$ and $CD8^+$ T-cell numbers are replenished, either through de novo thymic production, or through the proliferation of existing cells. As thymic output diminishes with age, naïve cells are mainly created through the latter process [96]. HIV infection can induce thymic damage through direct infection and killing of thymocytes, apoptosis, or disruption of the thymic stromal architecture, resulting in defective thymopoiesis and apoptosis of $CD4^+$ T-cells [97]. These changes mimic those induced by ageing, characterized by a decrease in the size and compartments of the thymus, and reduced thymopoiesis [5]. Thymic involution is associated with immunosenescence, with dysfunction of the immune system secondary to alterations in T-cell composition, most notably a shift from naïve to terminally differentiated cells [5, 98]. Thymic recovery may occur in some patients on ART; however, extensive thymic damage generally hampers immune reconstitution.

Systemic immune activation, independent of $CD4^+$ T-cell count and HIV VL, also results in inflammatory damage to the thymus [99]. In this case, thymic dysfunction through suboptimal production of naïve T-cells and greater differentiation of naïve into effector/memory cells occurs [100]. Immune reconstitution in HIV-infected individuals has been directly associated with thymic cellularity and volume, with the efficacy of reconstitution inversely correlated with age [101–103].

4.3. Systemic Inflammation. The proinflammatory state is associated with the development of major degenerative

diseases in the elderly [104]. In HIV-associated immune activation, there is an increase in proinflammatory mediators, TNF-α, IFN-α, IL-2, and IL-8, and dysfunctional Tregs, which lead to such an inflammatory state. HIV-infected individuals are predisposed to chronic inflammatory conditions leading to a host of progressive age-related diseases, so-called "Inflamm-ageing" [18]. This includes inflammatory bowel disease, osteoarthritis, heart disease, kidney and liver diseases, metabolic syndrome, dementia, cancer, and frailty [105, 106]. Inflammatory biomarkers, such as C-reactive protein (CRP), IL-6, and D-dimer, are elevated in HIV-infected persons compared to HIV-uninfected persons. Randomized clinical trials have demonstrated correlations between these biomarkers, disease progression, and mortality [18, 107].

4.4. Development of Non-AIDS-Associated Disease. The most significant consequence of systemic immune activation, especially in terms of long-term morbidity and mortality, is the development of non-AIDS-associated diseases. In fact, increased inflammatory biomarkers are predictive of the development of non-AIDS conditions, independent of $CD4^+$ T-cell count and HIV VL [32]. Many of these are also associated with ageing and have been linked to immunosenescence. The most common non-AIDS conditions related to immune activation include the following.

4.4.1. Cardiovascular Disease. Individuals in the chronic phase of HIV disease have a greater risk of endothelial dysfunction and subclinical atherosclerosis than HIV-uninfected persons [108]. Endothelial dysfunction is characterized by elevated levels of endothelial lesion biomarkers and endothelial cell adhesion molecules, such as ICAM-1, VCAM-1, E-selectin, P-selectin, thrombomodulin, class 1 tissue plasminogen activator, and class 1 tissue plasminogen activator inhibitor (PAI-1) [109]. When HIV infects endothelial cells, endothelial dysfunction may result from the release of cytokines by activated monocytes or directly by gp120 and transactivator of transcription (Tat) HIV proteins altering cell signalling pathways [110, 111].

Both HIV and its treatment have been associated with vasculopathy and hypercoaguability with subsequent thrombosis [112]. *In vitro* studies have demonstrated that HIV may affect the storage and secretion of proteins that affect homeostasis, such as von Willebrand factor. HIV may also affect the fibrinolytic system through the release of TNF-α, which in turn increases the expression of PAI-1 in endothelial cells, a known risk factor for thrombosis. HIV proteins, specifically gp120, activate arterial smooth muscle cells to release TF, triggering coagulation through the extrinsic pathway. Conversely, HIV infection is also associated with reduced levels of anticoagulant proteins C and S and antithrombin III [113]. Thrombosis, often in the context of the metabolic syndrome, has also been linked to the protease inhibitor (PI) class of HIV treatment [114]. High levels of TNF-α and PAI-1, and increased expression of the scavenger receptor, CD36, with subsequent increased absorption of cholesterol, have been found in PI-treated individuals [115, 116].

A key component of atherogenesis in both HIV and ageing is the formation of lipid-laden macrophages (i.e., foam cells), secondary to unregulated uptake of modified lipoproteins, especially oxidized low-density lipoprotein (oxLDL), under the influence of CD36 [117]. HIV-infected persons have been shown to have increased levels of oxLDL and higher expression of CD36 and TLR-4 in monocytes [118]. OxLDL levels correlate with markers of monocyte activation, for example, sCD14, and TF expression on inflammatory monocytes [118]. Oxidized lipids are thought to play a role in atherosclerosis through alteration of nitric oxide (NO) signalling, initiation of endothelial activation, and expression of adhesion molecules that promote leukocyte homing [119]. The ensuing inflammatory process releases downstream biomarkers, such as IL-6, VCAM-1, selectins, fibrinogen, D-dimer, CRP, and TF, that predispose the patient to accelerated coronary atherosclerosis and arteriosclerosis and subsequent CVD including myocardial infarction, heart failure, stroke, and sudden cardiac death [120–123]. A recent mouse model has shown that the pathological process is driven by macrophages in the subendothelial space expressing senescence markers, namely elevated senescence-associated β-galactosidase activity, $p16^{Ink4a}$, p53, and p21. This increases expression of key atherogenic and inflammatory cytokines and chemokines and promotes plaque instability by elevating metalloprotease production [124].

4.4.2. Renal Disease. Individuals living with HIV are at an increased risk of renal diseases such as acute tubular necrosis, HIV-associated nephropathy (HIVAN) [125], HIV immune complex kidney disease, hypertensive and atherosclerotic renal diseases, and toxicity secondary to potentially nephrotoxic medication, such as tenofovir disoproxil fumarate (TDF) [126]. HIVAN is one of the major risk factors of end-stage renal disease and is histologically defined as a collapsing form of focal segmental glomerulosclerosis (FSGS), microcystic tubular dilation, tubointerstitial inflammation, and fibrosis [127]. FSGS is similar to atherosclerosis and involves the buildup of cholesterol, activation of monocytes, release of lipid-laden macrophages, and fibrosis, suggesting that similar inflammatory processes may be involved [128]. The pathogenesis of HIVAN is not entirely understood; however, it has been suggested that it is triggered by direct viral infection of renal epithelial cells, Nef-induced podocyte dysfunction, renal tubular epithelial cell apoptosis induced by Vpr, and upregulation of proinflammatory mediators, especially those induced by NF-κB [127].

In ageing, senescent cells are important sources of inflammation and increased levels of biomarkers of inflammation, coagulation, and endothelial dysfunction, such as TNF-α, IL-6, MCP-1, CRP, and PAI-1, are commonly seen in this population [128]. Recruitment of T-cells into the renal tubulointerstitial compartment has been implicated in many renal inflammatory diseases and is an important mediator of tubular injury leading to progressive renal failure in HIVAN [129, 130]. Interactions between primary renal tubule epithelial cells (RTECs) and HIV-infected T-cells induce potent inflammatory gene responses. The consequent release of cytokines/chemokines from RTECs may then attract additional T-cells. Resident proximal tubular epithelial cells also

participate in the inflammatory process by enhancing cytokine/chemokine communication with interstitial immune cells [131]. Activation of RTECs by infiltrating T-cells perpetuate local inflammatory responses through upregulation of proinflammatory chemokine/cytokine production mediated by soluble factors or by direct cell-to-cell contact [132]. The HIV-upregulated cytokines/chemokines in the RTECs include inflammatory cytokines CCL20, IL-6-, and the IL-8 related chemokines: CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, and CXCL8 (IL-8). The receptors to these chemokines are expressed on certain populations of T-cells (reviewed in [133]) and, thus, may also be involved in promoting the mononuclear infiltration observed in HIVAN. The infiltration of HIV-infected cells into the kidney and activation of chemokines have been implicated in reduced survival of kidney allografts after transplantation, despite undetectable viremia [134] and the high prevalence of interstitial nephritis found in kidney biopsies in HIV-infected patients [135].

4.4.3. Cognitive Impairment. HIV-infected individuals manifest a spectrum of cognitive, motor, and psychological dysfunctions similar to that found in ageing, ranging from asymptomatic neurocognitive impairment to HIV dementia. Following infection, HIV is believed to enter the central nervous system (CNS) in infected mature $CD14^+CD16^+$ monocytes that traffic to the CNS as part of the turnover of perivascular macrophages [136]. Once inside the CNS, the virus infects microglia and may remain dormant for an extended period of time. HIV does not directly destroy cells of the CNS in large quantities; instead, it triggers a cascade of deleterious inflammatory changes affecting cellular signalling and resulting in oxidative stress [137]. Proinflammatory cytokines may damage neurons, while high levels of reactive oxygen species (ROS) may damage DNA and RNA [138]. The HIV VL in the brain does not determine the extent of the inflammatory response. In individuals on ART, minuscule amounts of residual virus may be sufficient to maintain a self-perpetuating inflammatory response [137]. High levels of macrophage activation markers, such as sCD163, sCD14, and CCL2 in cerebrospinal fluid and blood, together with inflammatory biomarkers, such as CRP, IL-6, TNF-α, IP-10, and neopterin, have been implicated in the development of HIV-associated neurocognitive disorders (HAND) [139, 140]. This is similar to what has been observed in the elderly, where inflammatory markers, particularly IL-6 and CRP, have been linked to cognitive decline and an increased risk of dementia [141].

The CNS and microglial cells may potentially serve as anatomical and cellular reservoirs, respectively, where HIV-1 may persist during chronic infection despite successful cART. The persistence of HIV in the CNS and microglia may result in immune activation with consequent microglia senescence [142]. Brain imaging of HIV-1-infected patients on cART using positron emission tomography imaging and ^{11}C-PK11195 as an *in vivo* marker of microglia activation reveals activation of microglia even in the absence of neurological symptoms [143]. The CSF from HIV-1 patients also contains increased levels of inflammatory cytokines including TNF-α, β2-microglobulin and neopterin, IL-1α, and S100β [144]. The latter, an intraneuronal calcium-inducing cytokine, could further contribute to neuronal degeneration [145]. Microglia have been demonstrated to undergo telomere shortening, which is a characteristic of senescence, in an animal model [146]. Emerging evidence from *in vitro* models also suggests that microglia could potentially develop a senescence-like phenotype characterized by reduced phagocytic and migratory capacities of microglia [147]. A dystrophic microglial phenotype has been observed to increase with ageing and has been detected in neuropathological conditions, such as Alzheimer's disease [148]. Although the progression and exact nature of microglial "ageing" remains unclear, activation and senescence appear to be integral parts of the process. Moreover, HIV-1 infection or bystander effects of HIV-1 infection seem to disrupt the delicate balance of cell survival, cell cycle progression, and apoptosis, which could contribute to the development of HAND [142].

4.4.4. Osteoporosis. HIV-infected persons have an increased prevalence of osteoporotic fractures compared to age-matched, HIV-uninfected individuals [149]. In addition to traditional risk factors, such as smoking, alcohol, low body weight, and vitamin D deficiency, HIV-infected patients have additional risk factors brought about by the virus' direct and inflammatory effects on bone resorption [150], as well as the effects of ART, especially TDF [151]. The major inflammatory pathways that have been identified involved cytokines that have also been shown to be elevated during senescence [152]. For example, TNF-α increases the expression of the receptor activator of NF-κB (RANKL), which accelerates osteoclastic bone resorption [150]. In addition, TNF-α and IL-1 inhibit osteoblast function and stimulate osteoblast apoptosis through activation of the inflammatory mediator, NO [152].

4.4.5. Cancer. Due to immune deficiency, HIV-infected persons are at an increased risk of developing non-AIDS-defining malignancies, such as Hodgkin's lymphoma, head and neck, lung, liver, kidney, skin, and anal cancers [153, 154]. Factors contributing to the development of non-AIDs defining cancers include the virus itself, tobacco exposure, and possibly ART [154]. HIV may activate proto-oncogenes, alter the regulation of the cell cycle, inhibit tumour suppressor genes, or cause endothelial abnormalities, such as proangiogenesis signalling that may facilitate tumour growth and metastasis [154]. Other persistent viral coinfections commonly found in HIV-infected persons, such as HBV, HCV, human papillomavirus, and EBV, also play a role. Elevated levels of EBV-positive B-cells, which express latent membrane protein 1, a key viral protein in EBV-mediated transformation of B-cells, correlate with an increased long-term risk for such individuals to develop Hodgkin's lymphoma [40].

The risk of cancer increases with lower $CD4^+$ T-cell counts; however, there appears to be an added risk even among infected people with well-preserved immune systems. $CD8^+$ T-cells and NK cells maintain surveillance of the body

and kill cells showing signs of anomalous growth or malignant modification. However, in HIV infection, the signalling cascades that control cell development and tissue restoration may be disrupted, leading to uncontrolled cell proliferation [155].

In HIV-infected and uninfected persons, inflammation contributes to cancer development, primarily by causing oxidative stress and DNA damage. ROS and proinflammatory cytokines, such as TNF-α, activate NF-κB, which induces the expression of genes involved in cell proliferation, apoptosis, and carcinogenesis. This leads to further production of proinflammatory cytokines [156]. Macrophages, platelets, fibroblasts, and tumour cells are all sources of inflammatory angiogenic mediators, for example, basic fibroblast growth factor, vascular endothelial growth factor, and prostaglandin-E_1 and E_2 that increase the production of ROS. Additionally, many oncogenes inhibit apoptosis and, in doing so, facilitate survival of preneoplastic and malignant cells [156]. This combination of DNA damage and unchecked proliferation contribute to an increased risk of cancer.

IL-7 is important in T-cell homeostasis as it maintains the survival of the naïve T-cell pool during HIV infection [157]. Increased IL-7 leads to abnormal B-cell differentiation [158] and the upregulation of both programmed cell death protein (PD-1) and its ligands [159]. Under physiological conditions, PD-1, a negative costimulatory molecule, prevents excessive T-cell activation and assists in peripheral tolerance through promotion of Tregs [160]. The expression of PD-1, together with its cognate ligand PD-L1, is upregulated during chronic HIV infection. This is caused by the HIV Nef protein via a p38 MAPK-dependent mechanism, the cytokine-rich microenvironment, T-cell receptor-independent stimulation, and persistent activation of the innate immune system [161]. Persistently elevated levels of PD-1 expression have been observed on exhausted $CD8^+$ T-cells. The PD-1/PD-L1 signalling pathway is critical in tumour immune surveillance. Tumours may escape host immune surveillance by expressing PD-L1 [162]. PD-1 signal inhibitors have emerged as a useful therapeutic strategy in the treatment of many cancers. They are also being investigated as approaches to reverse HIV latency and facilitate eradication [160, 162].

5. Immune Activation and Early Initiation of ART

Owing to improved ART access, the prognosis of HIV-infected patients has improved, although increased morbidity and mortality persist. This is caused by clinical events such as CVD, malignancy, and inflammatory conditions exacerbated by incomplete immune recovery and residual immune activation [29, 163]. The timing of ART initiation is thought to play an important role in immune activation [53]. Data indicate that an immunologic activation set point develops in the acute phase of HIV infection, which determines the rate at which $CD4^+$ T-cells are lost over time [164]. Early ART initiation may protect and preserve lymphoid gut homeostasis and reduce microbial translocation through maintenance of epithelial integrity, maturation of mucosal DCs, and conservation of intestinal lymphoid structures [165]. Other long-term benefits include conservation of HIV-specific $CD4^+$ T-cells, reduction of the turnover rate and activation of $CD4^+$ and $CD8^+$ T-cells, and in prevention of viral evolution [166–171].

6. Therapeutic Interventions

A number of therapeutic measures have been explored with the aim of reducing systemic immune activation in HIV-infected persons. To date, most studies have been observational in nature, making it impossible to rule out confounding factors, and to our knowledge, no human trials have used markers of immunosenescence as the primary outcome. Prospective interventional studies have rather focused on the causes of immunosenescence, such as immune activation and inflammation, linked with specific outcomes [6]. Unfortunately, there is no consensus regarding the optimal combination of biomarkers for measuring either immune activation or treatment success. No single strategy has been found effective in large-scale clinical trials, and no adjunctive treatment is currently recommended in international HIV treatment guidelines.

6.1. ART Intensification and Strengthening. Intensification with the integrase strand transfer inhibitor, raltegravir, in virally suppressed persons on ART has been found to lead to a rapid increase in 2-LTR circles with a significant decrease in levels of D-dimer [172]. Most studies have not shown any significant change in $CD8^+$ T-cell activation with this strategy [173–176]. Intensification with maraviroc, a selective, reversible CCR5-receptor antagonist that inhibits the binding and signalling of CCR5 ligands, produced no effect on $CD4^+$ or $CD8^+$ T-cell counts and actually increased LPS and sCD14 levels [177, 178].

6.2. Gastrointestinal Repair Strategy. The use of prebiotics and probiotics to modify the imbalance in the bacterial profile in the GIT of HIV-infected persons has been explored. Prebiotic use showed a significant reduction in levels of sCD14 and improved the functional capability of $CD4^+$ T-cells [179–181]. Supplementation with probiotics in infected macaques demonstrated reduced IDO-1 activity, indicating improved ability to maintain mucosal homeostasis [182, 183]. Other studies have shown increased $CD4^+$ T-cell counts and lower levels of IL-6 and LBP with probiotic use [180, 181]. Administering bovine colostrum containing LPS-specific antibodies/immunoglobulin did not yield any significant change in LPS, sCD14 levels, or $CD4^+$ T-cell counts [173, 184].

Recently, it has been reported that elite controllers, who spontaneously maintain sustained control of HIV, possess a microbiota that is richer and differs in predicted functionality from treatment naïve HIV progressors, resembling the micobiota of HIV-uninfected persons [185]. Therapeutic interventions that modulate gut microbiota richness, not only composition, are important in reducing HIV-related inflammation [185]. In addition to bacterial composition, other factors such as stability, resistance, resilience, and

redundancy contribute to the functional properties of the microbiota [186]. Confirmation of microbiota-related control of HIV infection in elite controllers by metabolomic studies may result in new intervention strategies, such as faecal transplants, to control HIV [185, 187].

6.3. Treatment of Coinfections. Treatment of CMV seropositive patients with valganciclovir has demonstrated significant decreases in CMV DNA expression and activation of $CD8^+$ T-cell, but had no effect on CRP, IL-6, and sCD14 [188]. The treatment of HCV with IFN-α and ribavirin did, however, correlate with a significant decrease in TNF receptor-1 and endothelial dysfunction markers, for example, soluble E-selectin and sVCAM-1 [189].

6.4. Interleukins. The coadministration of IL-21 and probiotics to SIV-infected animals was found to increase the production of polyfunctional Th17 and reduce pathobiont translocation [190]. Administering IL-7 to patients on ART restored functionality of $CD4^+$ and $CD8^+$ T-cells, enhanced $CD4^+$ T-cell production, and restored intestinal Th17 and Th22 populations [191]. In addition, IL-7 significantly decreased the viral reservoir by activating latent virus replication [192]. Reconstitution of the immune system with excitatory cytokines such as IL-2 or IL-15 has improved $CD4^+$ T-cell counts and HIV-specific T-cell responses [9, 193].

6.5. Immune Suppressive Agents. Administering cyclosporine A as a conjunctive therapy increases average $CD4^+$ T-cell counts, possibly through the inhibition of T-cell activation and proliferation [194].

6.6. Reducing Activation of Plasmacytoid Dendritic Cells. Chloroquine and hydroxychloroquine prevent the endosomal acidification and fusion in pDCs and also inhibit IDO, a regulator of T-cell responses [195]. There is some controversy regarding the effect of chloroquine and hydroxychloroquine in HIV-infected people. Studies on chloroquine report a substantial reduction in VL in newly ART-treated patients [196, 197], a reduction in memory $CD8^+$ T-cell activation and $CD4^+$ and $CD8^+$ T-cell proliferation [195, 198]. Additional beneficial effects, such as reduced levels of LPS, IFN-α, IL-6, and TNF-α and an increase in $CD4^+$ T-cell counts, have also been demonstrated [195, 198, 199]. On the other hand, there have also been reports of no significant changes in $CD4^+$ and $CD8^+$ T-cell activation and proliferation [200]. An increase in VL and a reduction, or no change, in $CD4^+$ T-cell counts have also been found [196, 197, 201].

6.7. Immune Modulators. Administering 3-hydroxy-3-methyl-glutharyl-coenzyme A (HMG-CoA) reductase inhibitors was found to reduce D-dimer and CRP [202–207]. A study of atorvastatin demonstrated a significant reduction in $CD8^+$ T-cells compared to the control group [202]. Another study observed that the addition of statins to ART correlates with a decline in the occurrence of non-AIDS-associated cancer, non-Hodgkin's lymphoma, and a decreased mortality rate [206]. Selective cyclooxygenase type 2 (COX-2) inhibitors have been found to reduce $CD8^+$ T-cell activation and immune activation levels [208]. The active metabolite of leflunomide, a disease-modifying antirheumatic drug, reduced activated T-cell proliferation in an *in vitro* study while no significant change was observed in HIV VL or $CD4^+$ and $CD8^+$ T-cell counts in patients treated with leflunomide in a randomised clinical trial [209–212]. Studies administering rapamycin and mycophenolate as a supplementary therapy with ART have shown to lower activation and proliferation of T-cells [213, 214].

6.8. Senolytics. Senescent cells are known to accumulate in various tissues during the aging process [215], and even a small number of these cells can cause adverse age- and disease-related phenotypes due to their "proinflammatory senescence-associated secretory phenotype" [216]. Senolytics are drugs that selectively promote apoptosis of senescent cells by temporarily disabling prosurvival signalling pathways, for example, those involving "PI3K/AKT, p53/p21/serpines, dependence receptor/tyrosine kinases, and BCL-2/BCL-X_L." This has delayed or alleviated the appearance of age- and disease-related phenotypes in several animal models [216]. These drugs consequently hold promise in attenuating the appearance of age-related cell phenotypes and chronic diseases, such as diabetes, pulmonary fibrosis, osteoporosis, cardiovascular disease, and cancers [216, 217].

Various drug candidates have been identified, for example, the tyrosine kinase inhibitor, dasatinib; the naturally occurring flavonoids and related compounds, such as quercetin, fisetin, and piperlongumine; drugs that target components of the BCL-2 pathway, for example, navitoclax; and the specific BCL-X_L inhibitors, A1331852 and A1155463 [215–219]. However, none of these drugs have demonstrated efficacy on all senescent cell types, significant side effects have been observed, none have yet successfully completed preclinical studies, and concerns exist regarding toxicity following long-term use. Fisetin, A1331852, and A1155463 appear to have more favorable side effect profiles and are potentially better candidates for use in humans [215, 216].

7. Conclusion

Systemic immune activation has become a focus of research into the immunopathogenesis of HIV. This immune activation is characterized by an increase in proinflammatory mediators, dysfunctional Tregs, and a pattern of T-cell-senescent phenotypes similar to those observed in the elderly. These changes predispose HIV-infected persons to comorbid conditions that have been linked to immunosenescence and inflamm-ageing. Treatment strategies aimed at curtailing persistent immune activation may help prevent the development of these conditions. At present, early ART initiation appears to be the most effective strategy although there is difficulty in achieving this in many settings. More studies of supplementary strategies are required. Consensus should also be reached regarding the optimal combination of biomarkers for measuring systemic immune activation and its successful treatment.

Disclosure

Opinions expressed and conclusions arrived at are those of the authors and are not necessarily attributed to NRF or SACEMA.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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EXHIBIT B

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HIV Infection, Inflammation, Immunosenescence, and Aging

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Abstract

Although antiretroviral therapy for HIV infection prevents AIDS related complications and prolongs life, it does not fully restore health. Long-term treated patients remain at higher than expect risk for a number of complications typically associated with aging, including cardiovascular disease, cancer, osteoporosis and other end-organ diseases. The potential effect of HIV on health is perhaps most clearly exhibited by a number of immunologic abnormalities that persist despite effect suppression of HIV replication. These changes are consistent with some of the changes to the adaptive immune system that are seen in the very old ("immunosenescence") and that are likely related in part to persistent inflammation. HIV-associated inflammation and immunosenescence have been implicated as causally related to the premature onset of other end organ diseases. Novel therapeutic strategies aimed at preventing or reversing these immunologic defects may be necessary if HIV infected patients are achieve normal life span.

INTRODUCTION

The development of antiretroviral therapy for the treatment of HIV infection is one of the greatest achievements of modern medicine. In a matter of a few years, the overall prognosis for HIV-infected patients shifted from years to decades. Although the initial regimens were complex and associated with significant short-term and long-term toxicity, current regimens are generally easy to administer, safe, and well tolerated. It is generally accepted that we are entering a phase of the epidemic in which we can achieve, and indefinitely maintain, control of HIV replication in the vast majority of patients.

Despite unquestioned success, combination antiretroviral therapy does not fully restore health. For reasons that remain poorly defined, long-term treated HIV-infected persons have an expected life span that is substantially shorter than that of their HIV-uninfected peers (1–4). This shortened life span is largely due to an increased risk of a number of "non-AIDS" complications, including heart disease, cancer, liver disease, kidney disease, bone disease, and neurocognitive decline. Many of these complications are similar to that observed among the elderly. Given the degenerative nature of most of these diseases, their impact on quality of life and function can be dramatic. These observations have led to growing concern that HIV-infected persons suffer from accelerated or premature "aging." This vaguely defined clinical scenario likely reflects a complex condition characterized by increased burden of comorbid diseases, higher prevalence of traditional behavioral risk factors (e.g., substance abuse), antiretroviral treatment toxicity, and chronic inflammation. These collectively result in functional decline and a higher than expected vulnerability to stressors or injury (5).

This review summarizes the complex epidemiologic, clinical, and pathogenesis data supporting the concept that (*a*) antiretroviral-treated HIV-infected persons are at higher than normal risk for certain age-associated diseases, and (*b*) this risk is due in part to irreversible HIV-associated immunologic dysfunction. This review does not seek to summarize the related but distinct effect of aging on HIV infection and its management, a topic that has been well-reviewed elsewhere (5, 6).

RISK OF AGE-ASSOCIATED DISEASE IS HIGHER IN ANTIRETROVIRAL-TREATED HIV PATIENTS THAN IN HIV-UNINFECTED PERSONS

Several recent studies have attempted to determine the ability of modern antiretroviral treatment regimens to fully restore health. Each of these studies has significant limitations, including short-term follow-up, lack of proper adjustment for unmeasured confounders and the failure to consistently exclude patients who failed to achieve durable viral suppression. Despite these limitations, the data collectively strongly suggest that the risk of non-AIDS morbidity is higher among antiretroviral-treated HIV-infected individuals than their age matched uninfected peers for reasons directly related to the disease or its treatment (1–4).

Cardiovascular Disease

Most (but not all) studies have found higher rates of cardiovascular disease in HIV-infected populations than in age-matched HIV-uninfected populations (7). For example, in one large U.S. healthcare system, the risk of a myocardial infarction was much higher in HIV-infected versus uninfected persons (8). Similar results have been reported by other groups using either clinical events or well-validated surrogate markers as the outcome measure (e.g., carotid intima thickening, brachial artery flow-mediated dilation) (7–9).

The mechanism that accounts for the higher than expected rates of cardiovascular disease is the focus of intense investigation. People with HIV often have more traditional risk factors for heart disease (e.g., hypertension, diabetes, dyslipidemia), but these factors do not account for all of the increased risk (8, 10, 11). Abacavir and the various protease inhibitors have been associated with cardiovascular toxicity (12, 13). Because HIV-associated biomarkers such as $CD4^+$ T cell nadir, proximal $CD4^+$ T cell count, and markers of inflammation consistently predict an elevated risk of cardiovascular disease independent of other factors (14, 15), it is almost certain that HIV infection contributes to the elevated risk of cardiovascular disease. Our observation that the HIV-infected persons who are able to durably control HIV infection in the absence of therapy (“elite controllers”) have more carotid disease than age-matched uninfected persons also argues for an effect of HIV-associated factors that is independent of direct toxicity, high viral replication, and advanced immunodeficiency (16).

Cancer

Another emerging dataset suggests that HIV infection is associated with a higher than expected rate of many non-AIDS cancers (17, 18). This risk is particularly evident for those non-AIDS-defining cancers that are known or believed to be caused by chronic infections (e.g., anal cancer, Hodgkin’s disease, liver cancer), while the risk of other cancers (e.g., lung, colorectal, melanoma) is only slightly higher. This higher cancer rate is apparent even among long-term antiretroviral-treated patients, and it is strongly related to the degree of immunodeficiency—as defined by the on-therapy $CD4^+$ cell count (19). Because the spectrum of cancers among HIV-infected persons is similar to that in the post-transplantation population (20), it has been argued that HIV-associated immune dysfunction may be the primary factor driving any excess risk.

Frailty

Aging is often defined on the basis of functional capacity rather than the collection of age-associated diseases (21). Aging is hence quantified by a series of metrics that cover specific domains. One such domain is frailty, which includes measures of sarcopenia (loss of muscle mass), osteoporosis, and muscle weakness. Although frailty has not been formally measured in HIV-infected populations, an estimate of frailty (the “frailty-related phenotype”) was

determined using interview data collected in the Multicenter AIDS Cohort Study (MACS). During the course of the study, ~14% of the HIV-infected population met the study definition of frailty at least once, whereas only 2% of the HIV-uninfected persons met this definition. The risk was most apparent after prolonged HIV infection and was strongly predicted by the peripheral $CD4^+$ T cell count (in both treated and untreated individuals) (22). Since clinically apparent frailty as seen in the very old is likely uncommon in younger HIV-infected individuals, it has been proposed that other, more subtle measures of functional capacity be used in future studies (5).

Liver, Kidney, and Bone Disease

HIV-infected persons have a higher risk of both liver and kidney disease than age-matched uninfected persons (23, 24). Among HIV-infected persons, untreated disease (or persistent viral replication) is associated with a higher risk than treated disease, suggesting that HIV replication directly or indirectly harms these organs (25). The extent of viral replication appears to be a strong determinant of kidney disease (26, 27), while the peripheral $CD4^+$ T cell count may be a more important determinant of either kidney or liver disease (14, 28). The extent to which effective antiretroviral therapy normalizes liver and kidney function is unknown and may be difficult to discern given that many antiretroviral drugs have are directly toxic to these tissues.

In some studies, the prevalence of osteopenia and osteoporosis is at least three times higher in HIV-infected persons than HIV-uninfected controls (29). Fractures are also more common in HIV-infected persons (30). Persistent inflammation during therapy may be causally related to disease, as many of the inflammatory markers known to be higher in HIV disease have been associated with bone disease in HIV-uninfected persons (31). Other factors—including antiretroviral drug toxicity and vitamin D deficiency—also contribute to bone disease.

Neurologic Complications

The harmful effect of untreated HIV on peripheral and central nervous system (CNS) function was apparent very early in the epidemic. HIV-associated inflammation is believed to be a central factor in CNS disease. Effective therapy clearly prevents and often reverses this process, but residual disease often persists (32, 33). One of the most contentious issues in clinical HIV medicine is whether harm continues to accumulate during therapy and, if so, whether this ongoing harm is due to inadequate penetration of certain drugs into the CNS (thus allowing ongoing viral replication) or to residual inflammation (34, 35). Although persistent defects noted in treated patients are often subtle and of unclear clinical relevance, even subtle increases in the rate of progression could over time result in the early onset of clinically relevant conditions such as dementia.

AGING OF THE IMMUNE SYSTEM (IMMUNOSENESCENCE)

As with any organ system, the immune system exhibits characteristic changes as people get older. These changes are most apparent (or at least most studied) in T cells. Compared to younger adults, the immune system in older adults is marked by a number of characteristics, including (*a*) reduced number and function of hematopoietic stem cells, (*b*) thymic involution, (*c*) reduced circulating naive T cells, (*d*) increased frequencies of well-differentiated memory $CD28^-$ T cells with limited proliferative potential, (*e*) increased levels of many proinflammatory cytokines, including interleukin (IL)-6 and TNFα, and (*f*) decreased CD4/CD8 ratios (36, 37). Although most of these changes pertain to the adaptive immune system, other aspects of the immune system—including NK cells—exhibit reduced

function in the very old, but data are limited. The few mucosa-based studies suggest that age-associated changes in peripheral blood are comparable to those seen in tissues.

These age-associated changes in immune function are often referred to as immunosenescence, a vaguely defined condition that refers to the age-associated changes in the immune system that are associated with morbidity and mortality. Among Swedish octogenarians and nonagenarians enrolled in a small, pathogenesis-oriented, population-based longitudinal cohort (the OCTO Immune Longitudinal Study), an inverted CD4/CD8 ratio was associated with short-term mortality (38). Comparable findings have been observed in other small cohorts (39). Other parameters from the OCTO cohort (and the subsequent NONA cohort) that predicted morbidity and mortality included reduced T cell proliferation, increased frequency of $CD28^-$ T cells and increased IL-6 (38, 40). The rare individuals who are able to survive to 100 or more years of age often lack these immunologic abnormalities (41).

The optimal T cell response is characterized by dramatic clonal expansion and generation of effector responses. The initiation of the T cell response requires interaction of antigen with the T cell receptor and at least one potent costimulatory receptor. Perhaps the most important costimulatory molecule is CD28, which is gradually downregulated as central memory cells differentiate into effector cells. The resulting $CD28^-$ cell population has shorter telomeres and is less able to proliferate. Although many of these cells rapidly die, some may become apoptosis resistant and long-lived (althoug *in vivo* data regarding the turnover of these cells is limited).. These so-called “senescent” cells are proinflammatory and hence may have a viable effector function, but their expansion can contribute to heightened systemic inflammation and collateral harm (42). Because chronic antigen exposure and inflammation result in the gradual expansion of these $CD28^-$ cells, it is not surprising that chronic viral infections are associated with a progressive expansion of these cells.

Cytomegalovirus (CMV) infection may prove to be very instructive with regard to the mechanism whereby HIV causes the premature onset of age-associated diseases. Among the very old, CMV seropositivity is associated with dramatic expansion of $CD8^+CD28^-$ T cells, with many of these cells directed at CMV (43). This remodeling of the immune system is associated with vaccine unresponsiveness, cardiovascular disease, and mortality (44–46).

HIV INFECTION AND IMMUNOSENESCENCE

Many of the T cell abnormalities associated with aging are similar to those observed in untreated HIV infection (42, 47, 48). These similarities are based on a diverse set of isolated nondefinitive observations, and hence the superficial similarities summarized here should be used to justify more intense investigation. Untreated HIV-infected adults and the elderly often exhibit low CD4/CD8 ratios, low naive/memory ratios, reduced T cell repertoire, reduced responsiveness to vaccines, and an expansion of $CD28^-$ effector T cells (see Table 1) (49, 50).The degree to which long-term antiretroviral therapy reverses these HIV-associated changes in the T cell compartment is the focus of ongoing investigation. In one recent study of treated HIV-infected adults and both young and old uninfected controls, the T cell phenotype ($CD57^+CD28^-CD8^+$ T cells, naive/memory T cell ratios, activated T cells) of the HIV-infected cohort was more similar to the much older uninfected cohort (48).

Many of the T cell characteristics associated with immunosenescence—including thymic dysfunction, T cell activation, and a reduced T cell regenerative potential—are more common among individuals who fail to exhibit robust $CD4^+$ T cell gains during therapy than among those who achieve a normal $CD4^+$ T cell count (51–53). Because a low $CD4^+$ T cell count on therapy is a consistent proximal predictor of non-AIDS morbidity (54), these

observations collectively suggest that HIV-associated immunosenescence contributes to persistent immunodeficiency and the early onset of age-associated diseases (see Figure 1). Focused investigation regarding this hypothetical pathway could lead to novel therapeutic interventions for both HIV-infected persons and the elderly.

The clinical significance of immunosenescence is often explored using vaccine responsiveness as the outcome. Effective antiretroviral therapy improves vaccine responsiveness, but residual defects remain, particularly if therapy is initiated late in the disease course. For example, among a cohort of 29 long-term treated patients (all of whom had high CD4 T cell counts), lymphoproliferatve responses to KLH, tetanus, and diphtheria toxoid were lower than that observed in HIV-seronegative controls, and predicted by both CD4 nadir and percentage of $CD28^{-}CD4^{+}$ T cells (but not current CD4) (55).

HIV INFECTION AND IMMUNOSENESCENCE

Untreated HIV infection is associated with persistently high levels of inflammation, as defined by levels of inflammatory cytokines such as IL-1β, IL-6, and TNFα. The coagulation system is also activated (23). Most if not all of these markers of inflammation decline with combination antiretroviral therapy, indicating that active HIV replication is either directly or indirectly responsible for this inflammatory response. Notably, the level of inflammation—as defined by IL-6, C-reactive protein (CRP), cystatin C- and D-dimers—remains elevated despite durable and possibly complete suppression of HIV replication with antiretroviral therapy. This persistent inflammation during therapy is probably due to a number of factors, including ongoing HIV production (if not HIV replication), increased copathogen load (particularly CMV, but likely other herpesviruses as well), translocation of lipopolysaccharide (LPS) across a damaged gut mucosa, loss of T regulatory cells and other immunoregulatory cells, and the irreversible fibrosis of the thymus and lymphoid infrastructure (see Figure) (56–59).

The association between HIV infection and inflammation shares many similarities with the association between advanced age and inflammation. Indeed, many of the markers now being studied in HIV disease were first validated in cohorts of older individuals (60, 61), and many of the mechanisms thought to be causally associated with inflammation in HIV disease are also thought to be causally associated with the inflammation of aging (56). The strength of the association between certain inflammatory markers (e.g., IL-6, D-dimers) and risk of age-associated diseases and mortality is very strong in both patient groups (62), and generally higher than that seen in younger HIV-uninfected populations.

BIOLOGY OF AGING

From a biologic perspective, aging is typically defined as the progressive deterioration in physiologic function that occurs as a consequence of cumulative molecular, cellular, and organ damage. These changes invariably result in an increased susceptibility to disease, decreased responses to stress, and death. The clinical manifestations of this process include loss in end organ function (e.g., liver, kidney, heart), bone loss (osteoporosis), muscle wasting (sarcopenia), neurocognitive decline, and loss in immunologic function (immunosenescence). There is a subtle distinction, however, between aging and age-associated diseases. The former is not generally considered a disease but rather a normal and well-conserved evolutionary process that is highly regulated and affects all organ systems. The latter is a series of clinical syndromes that increase in frequency as we age and are ultimately what cause death (to a purist, one cannot die of “old age”). This distinction can be confusing as many of the mechanisms known to regulate the aging process have been implicated in the pathogenesis of specific age-associated diseases. Also, the accumulation of

comorbid diseases may be causally associated with accelerated aging, defined biologically or clinically.

Genetics of Aging

The rate at which a person ages is defined in part by his or her genetic background. Studies performed on elderly Danish twins in the modern era suggest that ~25% of the variability in life expectancy can be attributed to genetic factors (63). The genetic basis for these observations has been the focus of extensive research. To date, only the defective lipid carrier apolipoprotein E4 (apoE4) gene and the FOXO transcription factors (see below) have been consistently associated with longevity (64). Mutations that influence the expression of insulin-like growth factor (IGF) receptor are enriched in Ashkenazi Jewish centenarians (65). Because many of the genes known to increase longevity in animal models may result in a higher risk of cancer (by preventing cell death) and/or reduced fertility (by shifting resources from reproduction and growth to maintenance), it is likely that strong evolutionary pressures are acting to prevent exceptional longevity (66).

Notably, the harmful apoE4 gene has been associated with mortality in HIV disease (67), whereas the protective transcription factor FOXO3a has been associated with central memory T cell persistence in HIV infection. FOXO3a may represent one mechanism whereby certain individuals are able to remain healthy without antiretroviral therapy for years (68).

Molecular Biology of Aging

The best-characterized external (and hence potentially modifiable) factor associated with healthy aging is moderate caloric restriction. In nearly all species studied to date, experimental restriction of caloric intake to levels below that when fed ad libitum but above that which causes starvation is associated with increased longevity (69, 70). In a recently published study that took decades to complete, rhesus monkeys randomized to a 30% reduction in caloric intake exhibited a reduced risk of dying from age-associated diseases (although this mortality benefit was not significant when a large number of non-age-associated deaths were included in the analysis) (71). Caloric restriction in these monkeys reduced the risk of cancer, diabetes, and heart disease. Caloric restriction may also enhance T cell function and prevent immunosenescence in aging nonhuman primates (72). Whether this approach will work in humans is not known because such diets are nearly impossible to maintain; however, in a recent short-term prospective clinical trial, calorie restriction resulted in reduced energy expenditure, increased mitochondrial content, and increased expression of many genes associated with mitochondrial function and longevity (73).

Many of the experimental mutations that prolong life expectancy affect the caloric and nutrient signaling pathways, providing definitive proof for the role of diet in aging. Genetic mutations that decrease the activity of the insulin/IGF pathway equivalents in *C. elegans* double the life spans of these worms. The FOXO transcription factors regulate a wide range of genes known to be involved in the stress response and are critically important in mediating the antiaging effects of caloric restriction and reduced insulin/IGF in worms (74). Similar albeit less dramatic observations have been made in flies and mice. The apparent enrichment of certain genetic mutations within the insulin and IGF pathways among human centenarians strongly suggests that the ability of these crucial pathways to regulate longevity is conserved from worms to humans (74).

The target of rapamycin (TOR) is also involved in regulating the cellular response to nutrients and is a key component of the insulin/IGF pathways. Activation of TOR in many experimental models results in a shift in metabolism toward growth and reproduction,

whereas inhibition of this enzyme results in a number of outcomes associated with cell maintenance, including higher levels of autophagy (i.e., the recycling of digested cellular components) (75, 76). TOR is of high interest clinically because the administration of low doses of rapamycin—an immunosuppressant approved by the U.S. Food and Drug Administration to prevent transplant rejections—prolongs the life of various species, including mice (77).

The silent information regulator protein deacetylases (sirtuins) are yet another family of proteins that influence the aging process. The sirtuins regulate many aspects of cellular metabolism, and therapeutic activation of at least one sirtuin (SIRT1) in experimental models is associated with reduced activity of cellular activation (via NFkB and other regulatory enzymes) and prolonged life span. The impact of caloric restriction on health may be mediated via the sirtuin family (73).

Cellular Biology of Aging

Normal cells cannot proliferate indefinitely. After multiple rounds of cell division, an irreversible state of replicative senescence occurs (the “Hayflick Limit”). This phenomenon is regulated in part by the progressive shortening of telomeres, the repeated DNA sequences (TTAGGG) that cap the ends of the chromosomes (78). In addition to stimulating specific pathways that influence the cell cycle (79), telomere length also influences the activity of p53 tumor suppressor pathways, resulting in either apoptosis or cell senescence and the prevention of malignant transformation. Genomic damage and mitochondrial damage—which are caused by many environmental exposures that are common in HIV infection—also activate many of these pathways, leading to either apoptosis or cell senescence and the prevention of cancer (80, 81).

The fate of senescent cells may be a key determinant of health outcomes. Campisi and colleagues have argued that senescent cells often secrete [**AU: word missing?**] inflammatory and other regulatory factors, resulting in chronic, low-level, “sterile” inflammation. These cells are known to accumulate in degenerating cells and may be causally associated with development of certain age-associated diseases (81). Although these observations have largely focused on stromal and epithelial cells, they may also apply to T cells and other immune cells. Chronic viral infections (e.g., HIV, CMV) cause excessive T cell turnover and the apparent accumulation of phenotypically senescent proinflammatory $CD8^+$ T cells, as described above.

BIOLOGY OF AGING AND ITS IMPLICATIONS FOR THE PATHOGENESIS OF NON-AIDS MORBIDITY DURING ANTIRETROVIRAL TREATMENT

Many of the biologic factors that are thought to accelerate aging have also been implicated in the pathogenesis of HIV disease. It is the central hope of this review that these two distinct fields of study could merge, as knowledge gained in one could accelerate progress in the other.

Visceral Fat, Insulin Resistance, and the Metabolic Syndrome

HIV infection and/or its treatment may cause peripheral fat wasting (lipoatrophy) and central fat gain. Visceral obesity is a well-established risk factor for many age-associated complications, including vascular disease and dementia. Visceral obesity is also a well-known source for many of the chronic inflammatory proteins known to influence both aging and HIV disease outcomes (82). Finally, visceral obesity is a strong predictor of insulin resistance, which is common in HIV-infected patients and is a strong determinant of aging (83, 84). Development of therapeutic agents aimed at reversing the complex effect of HIV

infection on visceral fat, insulin resistance, and the lipodystrophy syndrome is the focus of intense investigation.

Genotoxicity and Mitochondrial Dysfunction

DNA damage and telomere shortening are strong determinants of cellular aging; each can activate the p53 and other tumor suppressor pathways, leading to apoptosis or cellular senescence and the inability to maintain tissue homeostasis (81, 85). Mitochondria dysfunction may also contribute to cellular aging, either by the release of potentially harmful reactive oxygen species (86) or by directly activating p53 and similar pathways. Release of mitochondrial products into the circulation may result in harmful levels of inflammation (87).

Zidovudine, stavudine, and perhaps other nucleoside analogs inhibit mitochondria synthesis, cause the release of mitochondrial DNA, and increase the risk of oxidative damage. Mitochondrial toxicity is thought to be a major contributor to fat redistribution and other metabolic abnormalities that are commonly seen with certain antiretroviral drug regimens (88). It has also been suggested that certain nucleoside analogs might inhibit telomerase (which is a reverse transcriptase); this could theoretically contribute to cellular and tissue aging (89).

T Cell Regenerative Failure

A reduced ability to regenerate T cells is another feature common to both HIV disease and advanced age. The progressive loss of hematopoietic progenitor cells is likely a major factor in the normal aging process (90). Age-associated factors that may accelerate the loss of these cells include excessive turnover, damage to the microenvironment, exposure to oxidative stress, and the accumulation of genetic and epigenetic alteration. Cellular senescence or apoptosis is often the final outcome (91). HIV may be able to directly infect hematopoietic stems or may negatively affect their function via exhaustion and/or local damage to the stem cell microenvironment (92). The double insult of aging and HIV to hematopoietic stem cells can contribute to many of the factors associated with immunosenescence, including reduced number of naive T cells, reduced T cell proliferation, and reduced ability of the immune system to mount an effective response to vaccines and infection. A loss of these stem cells may also contribute to a reduction in the number of endothelial progenitor cells, which can contribute to vascular dysfunction and cardiovascular disease.

This same story could be told with regard to thymic function. Advanced age and HIV infection are both associated with a progressive and possibly irreversible loss of thymic function; this process in theory could contribute to progressive immunologic dysfunction (including chronic inflammation) and the development of many age-associated diseases (37, 92).

Inflammation

Chronic inflammation is strongly associated with the development of morbidity and mortality in the elderly and in those with HIV disease. Chronic viral infections such as the herpes and hepatitis viruses are an important cause of this persistent inflammation in both settings. CMV causes lifelong antigenic stimulation and the eventual development of an expanded population of well-differentiated, apoptosis-resistant, senescent T cells with limited proliferative potential (93, 94). The end result is an immune system with limited capacity to recognize novel antigens and hence prevent disease. Because copathogens are more common in people with HIV, and because they appear to have a deleterious immunologic and clinical impact in HIV disease (58, 95), it seems reasonable to postulate

coinfections may contribute to the "accelerated aging" syndrome now being observed in HIV-infected individuals (96).

CONCLUSION

The consistent observation that HIV-infected persons have a higher than expected risk for a number of conditions commonly associated with aging has led to the widespread assumption that HIV accelerates the aging process. It should be emphasized, however, that it is impossible to truly define the independent effect of HIV infection on this risk, as infected and uninfected persons differ with regard to many important and difficult to measure factors that influence the risk of developing an age-associated complication. Certain biomarkers that predict morbidity in the very old are higher than expected in younger HIV-infected persons, and this consistent observation provides indirect evidence that HIV infection might accelerate the aging process.

A careful integration of the basic biology of aging with the biology of HIV infection may lead to novel insights into the larger questions of why people age and why antiretroviral therapy fails to restore health. An integration of these two disciplines could provide the rationale for the codevelopment of novel therapeutics. For example, the National Institute of Aging has sponsored a collaborative program aimed at identifying possible therapeutic agents that delay physiologic aging (97). Any drug that advances into clinical trials among the elderly might also be considered in younger antiretroviral-treated HIV-infected patients. Interventions that have shown promise as antiaging therapeutics in experimental systems include resveratrol, rapamycin, acetyl-L-carnitine and alpha-lipoic acid, telomerase activators, caloric restriction, and stem cell therapy (97). Approved drugs that have an anti-inflammatory effect and are often used in older adults—including aspirin, omega-3 fatty acids, vitamin D, and the statins—are now being studied as adjuncts to antiretroviral therapy in younger HIV-infected individuals. It is expected, but not yet the focus of prospective investigation, that lifestyle modification—including moderate exercise and dietary changes—might also prove very beneficial as an adjunct to standard antiretroviral regimens.

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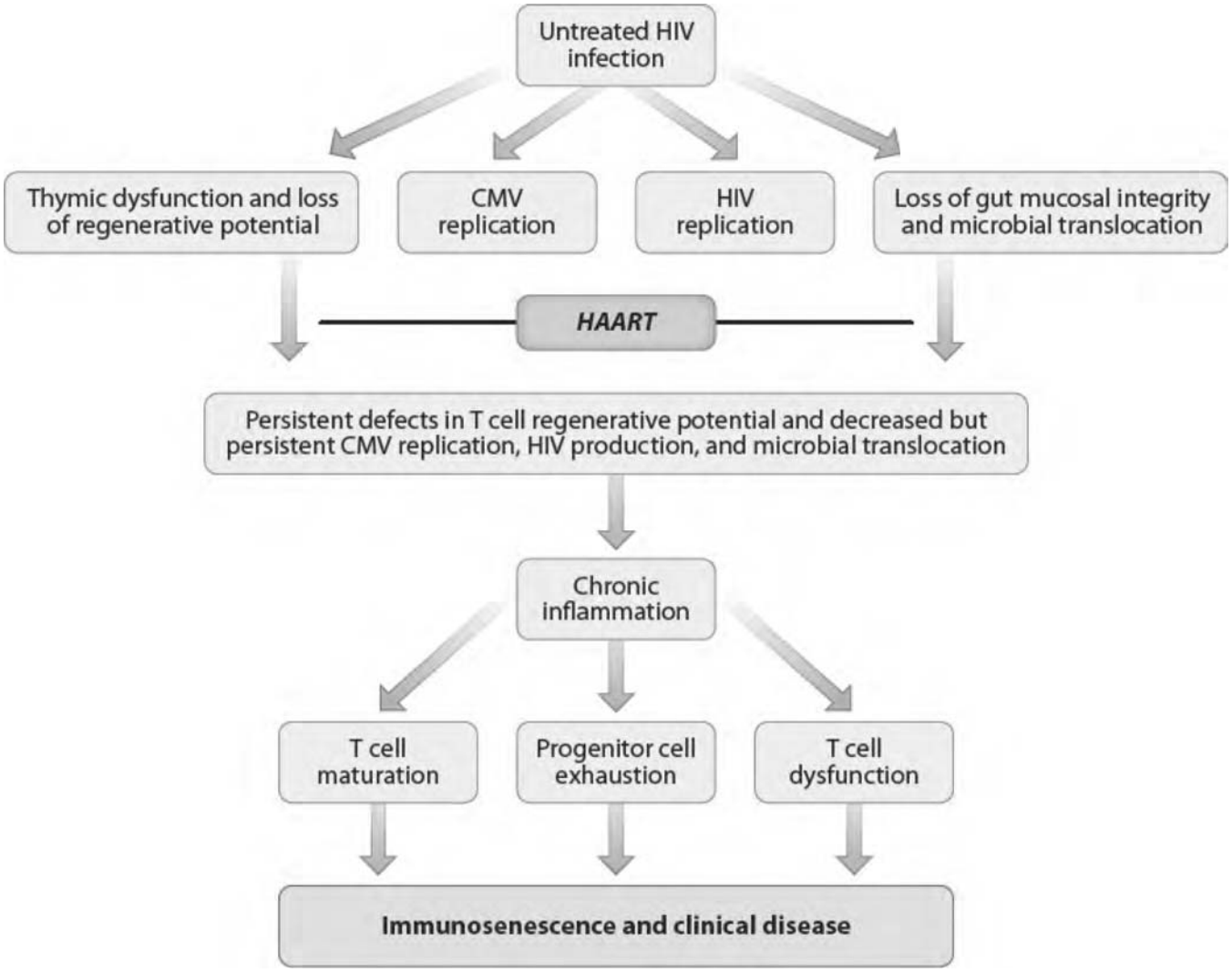

Figure 1.

Table 1

Possible similarities between HIV-associated and age-associated immunologic changes

Outcome measure	Age >70 years, HIV-uninfected [**AU: OK?**]	Untreated HIV infection	Long-term (5–10 years) treated HIV infection[a]
Low CD4/CD8 ratio	Yes	Yes	Unknown
Low naive/memory ratio	Yes	Yes	Possible
Low T cell proliferative potential	Yes	Yes	Possible (low CD4 nadir)
Expanded CMV-specific CD8 cells	Yes	Yes	Yes
Expanded $CD28^{-}CD8^{+}$ T cells	Yes	Yes	Unknown
Expanded $CD57^{+}$ T cells	Yes	Yes	Unknown
Reduced T cell repertoire	Yes	Yes	Possible
Increased IL-6	Yes	Yes	Possible
Increased T cell activation	Unclear	Yes	Possible
Reduced thymus function	Yes	Yes	Unknown
Low IL-2, high IFN-γ ($CD8^{+}$ T cells)	Yes	Yes	Unknown
Reduced response to vaccines	Yes	Yes	Possible (CD4 nadir)
Reduced T cell telomere lengths	Yes	Yes (CD8)	Controversial

[a] A number of studies have suggested persistent immunologic impairment during HAART, but the subjects of these studies have generally received therapy for only a short period of time (<3 years). Also, most subjects at the time of the study had lower than normal peripheral $CD4^{+}$ T cell counts.

EXHIBIT C

Cross-sectional Comparison of the Prevalence of Age-Associated Comorbidities and Their Risk Factors Between HIV-Infected and Uninfected Individuals: The AGE$_h$IV Cohort Study

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Background. Human immunodeficiency virus (HIV)-infected individuals may be at increased risk of age-associated noncommunicable comorbidities (AANCCs).

Methods. Cross-sectional analyses of AANCC prevalence (including cardiovascular, metabolic, pulmonary, renal, bone, and malignant disease) and risk factors in a prospective cohort study of HIV type 1–infected individuals and HIV-uninfected controls, who were aged ≥45 years and comparable regarding most lifestyle and demographic factors.

Results. HIV-infected participants (n = 540) had a significantly higher mean number of AANCCs than controls (n = 524) (1.3 [SD, 1.14] vs 1.0 [SD, 0.95]; $P < .001$), with significantly more HIV-infected participants having ≥1 AANCC (69.4% vs 61.8%; $P = .009$). Hypertension, myocardial infarction, peripheral arterial disease, and impaired renal function were significantly more prevalent among HIV-infected participants. Risk of AANCC by ordinal logistic regression was independently associated with age, smoking, positive family history for cardiovascular/metabolic disease, and higher waist-to-hip ratio, but also with HIV infection (odds ratio, 1.58 [95% confidence interval, 1.23–2.03]; $P < .001$). In those with HIV, longer exposure to CD4 counts <200 cells/μL, and, to a lesser extent, higher levels of high-sensitivity C-reactive protein and soluble CD14, and longer prior use of high-dose ritonavir (≥400 mg/24 hours) were each also associated with a higher risk of AANCCs.

Conclusions. All AANCCs were numerically more prevalent, with peripheral arterial, cardiovascular disease, and impaired renal function significantly so, among HIV-infected participants compared with HIV-uninfected controls. Besides recognized cardiovascular risk factors, HIV infection and longer time spent with severe immunodeficiency increased the risk of a higher composite AANCC burden. There was a less pronounced contribution from residual inflammation, immune activation, and prior high-dose ritonavir use.

Keywords. HIV infection; aging; comorbidity.

AIDS-associated morbidity and mortality have dramatically declined with the advent of combination antiretroviral therapy (cART) [1–3]. However, the life expectancy of individuals infected with human immunodeficiency virus (HIV) on average remains shorter than for the general population [3–5], and non-AIDS comorbidities have gained increasing importance as causes of death in cART-treated patients [2, 3, 6, 7]. As HIV-infected individuals on cART age, they increasingly experience non-AIDS comorbidities [7, 8], which in HIV may be both accentuated and/or accelerated, thereby possibly occurring at younger ages [8–10]. Potential contributors may include a higher prevalence of recognized risk factors, as well as ART exposure and

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toxicity, HIV infection, immune dysfunction/dysregulation, and chronic immune activation/inflammation associated with the infection [11–16].

By 2015, half of the HIV-infected population in the United States will be aged >50 years, with similar trends observed in Europe and resource-limited settings [7, 9, 17]. More insight into prevalence, incidence, and risk factors of non-AIDS comorbidity among HIV-infected individuals is therefore essential to optimize policy for prevention and management [18]. Most published studies thus far did not include a comparable uninfected control group. Whether different comorbidities occur more often and possibly at a younger age among HIV-infected individuals therefore remains unclear.

To clarify these issues, the AGE_hIV Cohort Study was implemented in 2010 in Amsterdam, the Netherlands, to compare the prevalence, incidence and risk factors of aging-associated noncommunicable comorbidities (AANCCs) and organ dysfunction among HIV type 1 (HIV-1)–infected individuals and HIV-uninfected controls. We report a cross-sectional comparison at the time of enrollment of AANCC prevalence between the HIV-infected and HIV-uninfected groups, and analyzed both recognized and potential HIV-associated risk factors.

METHODS

Study Design and Data Collection

HIV-1–infected participants were recruited from the HIV outpatient clinic of the Academic Medical Center in Amsterdam, and HIV-uninfected participants (controls) were recruited from the sexual health clinic of the Amsterdam Public Health Service or among uninfected participants in the existing Amsterdam Cohort Studies on HIV/AIDS [19]. To ensure comparability of the HIV-infected and HIV-uninfected study groups, we regularly monitored age, sex, and ethnicity in both study groups, and adjusted enrollment of underrepresented categories among HIV-uninfected participants accordingly.

All participants were aged ≥45 years with laboratory-confirmed presence or absence of HIV-1 infection. All subjects who provided written informed consent within the 2-year enrollment period were included. Of 1100 eligible patients from the HIV outpatient clinic, between 600 and 800 were expected to be enrolled, and we therefore aimed to include a similar number of uninfected controls. This sample size would provide sufficient statistical power to investigate associations between a broad range of AANCCs and potential risk factors.

At baseline, 2 years later, and depending on sufficient resources every 2 years thereafter, participants undergo standardized screening for AANCCs and organ dysfunction.

Participants are requested to complete a standardized questionnaire concerning demographics, (family) medical history, use of medications (both prescribed and over-the-counter), participation in population screening programs, substance use, quality of life, depression, sexual orientation/behavior/dysfunction, cognitive complaints, calcium/vitamin D intake, physical exercise, social behavior, and work participation/income. All participants undergo measurements of blood pressure, height, weight, and hip/waist circumference, as well as electrocardiography, measurement of vascular elasticity, spirometry, screening cognitive tests, frailty, bone densitometry, and quantification of advanced glycation end products in the skin. Blood and urine samples are obtained for extensive laboratory testing, and cryopreserved for future analyses.

Detailed information concerning HIV and ART history is obtained from the Dutch HIV Monitoring Foundation, formally responsible for capturing detailed HIV/ART-related data from all individuals in care for HIV at an HIV treatment facility in the Netherlands [20]. The study protocol was approved by the local ethics review committee and registered at www.clinicaltrials.gov (identifier NCT01466582). All participants provided written informed consent.

Study Participants

All study participants who underwent baseline assessments (between 1 October 2010 and 30 September 2012), and completed a study questionnaire were included in the analyses.

Definitions

Data were available on hypertension, angina pectoris, myocardial infarction, peripheral arterial disease, ischemic cerebrovascular disease, diabetes mellitus type 2, obstructive pulmonary disease, impaired renal function, non-AIDS cancer, and atraumatic fractures/osteoporosis.

Hypertension was considered to be present if diastolic blood pressure ≥90 mm Hg and/or systolic blood pressure ≥140 mm Hg in all 3 measurements (Omron 705IT) with a 1-minute interval, and if on antihypertensive medication [21]; diabetes mellitus type 2 if hemoglobin A1c (HbA1c) (International Federation of Clinical Chemistry and Laboratory Medicine [IFCC]) was ≥48 mmol/mol and/or elevated blood glucose (nonfasting ≥11.1 mmol/L or fasting ≥7.0 mmol/L), and if on antidiabetic medication [22]; obstructive pulmonary disease if 1-second forced expiratory volume (FEV_1) to forced vital capacity (FVC) ratio was <0.7 in all 3 forced expiratory spirometric measurements (MicroDirect SpiroUSB) without bronchodilation, in those on bronchodilators, or in those self-reporting obstructive pulmonary disease by questionnaire [23]; impaired renal function if estimated glomerular filtration rate was <60 mL/minute using the Chronic Kidney Disease Epidemiology Collaboration formula; atraumatic fractures/osteoporosis in case of a dual-energy X-ray absorptiometry (DXA) (Hologic QDR 4500 W and Hologic Discovery A densitometers, software versions

12.4–13.3) T score ≤ -2.5 standard deviations (postmenopausal women and men aged ≥ 50) or z score ≤ -2 standard deviations (premenopausal women and men aged <50), or in those reporting atraumatic fracture by questionnaire [24, 25].

Angina pectoris, myocardial infarction, peripheral arterial disease, ischemic cerebrovascular disease, and non-AIDS cancer (including nonmelanoma skin cancers) were diagnosed in participants self-reporting these diseases by questionnaire. All self-reported diagnoses were validated using hospital records for HIV-infected participants, and, general practitioners' records for controls, provided the latter had consented to contact with their general practitioner. If not, unvalidated diagnoses were used. This may result in a conservative estimate of the difference in AANCC prevalence between the HIV-infected and -uninfected cohorts by likely overestimating the true number of AANCCs among controls.

Physical activity was defined according to Dutch healthy physical activity guidelines ("Combinorm"): moderate physical activity ≥ 5 days per week for ≥ 30 minutes, or heavy physical activity at least twice a week for ≥ 20 minutes [26].

Statistical Analysis

Study groups were compared using the χ^2, Wilcoxon rank-sum, nonparametric test for trend, or Student t test as appropriate. All reported P values are 2-sided.

Multivariable ordinal logistic regression analysis (proportional odds model) was performed to assess the contribution of HIV and recognized risk factors toward AANCCs. The outcome measure was the total number of AANCCs per participant. All models were adjusted for age, sex, Dutch origin, sexual orientation, positive family history (for myocardial infarction, hypertension, diabetes mellitus type 2, or hypercholesterolemia), smoking, use of cocaine/ecstasy/cannabis, severe alcohol use, and hepatitis B/C coinfection. Biologically plausible determinants of AANCC (including HIV/ART-related factors, and markers of systemic inflammation/monocyte activation/coagulation) were explored using a stepwise model selection. Continuous variables were transformed or categorized when necessary. Exposure to HIV-related factors was set to zero for HIV-uninfected participants, as were pack-years for nonsmokers. All models used data from both study groups (including those exploring HIV-related risk factors), except where explicitly stated otherwise.

Analyses were performed using SAS version 9.2.

RESULTS

A total of 598 HIV-infected participants and 550 HIV-uninfected controls completed a baseline visit between 1 October 2010 and 30 September 2012. Data from 540 HIV-infected and 524 HIV-uninfected participants were available for analysis, after excluding 58 HIV-infected and 26 HIV-uninfected participants with a missing questionnaire. Age, DXA results, glucose/HbA1c, blood pressure, FEV_1/FVC ratio, and renal function were not significantly different between HIV-infected and -uninfected participants with or without a completed questionnaire.

Baseline Characteristics of Participants

Participants in both study groups were very comparable in terms of baseline characteristics; the median age was around 52 years, and the majority were male, men who have sex with men (MSM), and of Dutch origin. Significantly fewer HIV-infected participants were of Dutch, and more of African, origin (72.2% vs 81.3%; $P < .001$ and 7.4% vs 1.3%; $P < .001$, respectively). Significantly fewer controls were hepatitis B/C coinfected (0.6% vs 3.9%; $P < .001$ and 0.8% vs 2.8%; $P = .029$, respectively) (Table 1). No statistically significant difference in age distribution was found between the two study groups.

On average, HIV-infected participants were known to be infected for a prolonged period of time, and 30% had prior AIDS. Virtually all were on cART for many years, and currently had undetectable HIV-1 plasma viral loads. The majority had experienced immune recovery on treatment, with a median nadir CD4 count of 180 cells/μL and current median CD4 count of 565 cells/μL.

Significantly more HIV-infected participants were current smokers (32.0% vs 24.6%; $P = .007$), whereas ecstasy use was significantly more prevalent among controls (4.3% vs 8.6%; $P = .004$) (Table 2).

Body mass index (BMI) was lower (24.2 [interquartile range {IQR}, 22.3–26.6] vs 24.5 [IQR, 22.8–27.0] kg/m^2; $P = .019$) and above-normal waist-to-hip ratio was significantly more prevalent among HIV-infected participants (84.0% vs 62.4%; $P < .001$). Systolic (135 [IQR, 126–147] vs 133 [IQR, 125–143] mm Hg; $P = .006$) and diastolic blood pressure (81 [IQR, 75–89] vs 79 [IQR 72–85] mm Hg; $P < .001$) were significantly higher among HIV-infected participants. Significantly fewer HIV-infected participants were physically active (44.3% vs 53.0%; $P = .005$) and they had significantly lower levels of 25-hydroxy vitamin D2 + D3 (47 [IQR, 29–71] vs 54 [IQR, 39–72] nmol/L; $P < .001$).

AANCC Prevalence

All self-reported diagnoses of angina pectoris, myocardial infarction, peripheral arterial disease, ischemic cerebrovascular disease, and non-AIDS cancer could be validated among HIV-infected participants: of the total 155 self-reported diagnoses, 100 were confirmed and 55 rejected. Fourteen controls did not consent to contact their general practitioner for validation of 16 self-reported diagnoses, which accounted for 21.6% of 74 self-reported diagnoses among controls. Of the remaining 58 self-reported diagnoses that could be validated, 39 were confirmed and 19 rejected.

Table 1. Baseline Demographic and HIV-Related Characteristics

Characteristic	HIV-Uninfected Participants (n = 524)	HIV-Infected Participants (n = 540)	*P* Value
Age, y	52.1 (47.9–58.3)	52.9 (48.3–59.6)	.200*
Male sex	85.1%	88.1%	.146**
Dutch origin	81.3%	72.2%	<.001**
African origin	1.3%	7.4%	<.001**
MSM[a]	69.7%	73.9%	.125**
Hepatitis C RNA positive	0.8%	2.8%	.029**
Hepatitis B antigen and/or hepatitis B DNA positive	0.6%	3.9%	<.001**
Time since HIV-1 diagnosis, y	. . .	12.1 (6.2–17.1)	. . .
Diagnosed with HIV-1 prior to 1996	. . .	32.8%	. . .
CD4 count at enrollment, cells/μL	. . .	565 (435–745)	. . .
Nadir CD4 count, cells/μL	. . .	180 (78–260)	. . .
Known cumulative duration of CD4 count <200 cells/μL, mo	. . .	0.8 (0.0–9.6)	. . .
Plasma viral load >200 copies/mL among cART-treated participants within 4 mo before or at enrollment[b]	. . .	1.5%	. . .
Last plasma viral load within 4 mo before or at enrollment, $\log_{10}$ copies/mL[b]	. . .	1.6 (1.6–1.6)	. . .
Duration of plasma viral load ≤200 copies/mL, y[c]	. . .	5.8 (2.4–10.2)	. . .
Prior clinical AIDS[d]	. . .	31.3%	. . .
On cART[e]	. . .	95.7%	. . .
Time since ART was first initiated, y	. . .	10.4 (4.4–14.5)	. . .
Naive at start of cART	. . .	79.1%	. . .
High-dose ritonavir (≥400 mg daily) use			
Prior exposure among all non–ART-naive participants	. . .	31.5%	. . .
Cumulative exposure among all non–ART-naive participants, mo	. . .	0.0 (0.0–6.3)	. . .
Cumulative exposure among participants that used high-dose ritonavir, mo	. . .	17.6 (7.6–40.4)	. . .

Data are presented as median (interquartile range) or percentage.

Abbreviations: ART, antiretroviral therapy; cART, combination antiretroviral therapy; HIV-1, human immunodeficiency virus type 1; MSM, men who have sex with men.

[a] Male participants who stated in the questionnaire to feel mostly or exclusively sexually attracted to men.

[b] Only a plasma HIV-1 load that was measured ≤4 months prior to enrollment was used. If such a recent test result was not available, plasma HIV-1 load was measured at enrollment.

[c] Duration since last plasma viral load >200 copies/mL.

[d] Previous AIDS-defining condition following the US Centers for Disease Control and Prevention classification.

[e] Combination of ≥3 antiretroviral drugs, other than ritonavir used as a booster.

* Wilcoxon rank-sum test.

** χ^2 test.

HIV-infected individuals had a significantly higher mean number of AANCCs than controls (1.3 [SD, 1.14] vs 1.0 [SD, 0.95]; $P < .001$). The proportion of participants with ≥1 AANCCs was also significantly higher among those with HIV (69.4% vs 61.8%; $P = .009$).

The mean number of AANCCs within the 50–54, 60–64, and ≥65 age categories was significantly higher among HIV-infected than HIV-uninfected participants (Figure 1). Furthermore, the distribution of the number of AANCCs for HIV-infected participants in each 5-year age stratum resembled the distribution for controls who were 5 years older.

Each individual AANCC was numerically more prevalent among HIV-infected participants, with hypertension (45.4% vs 30.5%; $P < .001$), myocardial infarction (3.9% vs 1.5%; $P = .018$), peripheral arterial disease (2.6% vs 0.6%; $P = .008$), and impaired renal function (4.3% vs 2.1%; $P = .044$) being significantly more prevalent among HIV-infected participants (Figure 2).

Factors Contributing to the Risk of AANCC

HIV-Related Risk Factors

After adjustment for age, sex, Dutch origin, sexual orientation, positive family history (for myocardial infarction, hypertension,

Table 2. Prevalence of Comorbidity Risk Factors

Characteristic	HIV-Uninfected Participants (n = 524)	HIV-Infected Participants (n = 540)	*P* Value
Smoking status			
Never smoked	36.5%	33.0%	.028*
Ever smoked	38.9%	35.0%	
Currently smoking[a]	24.6%	32.0%	
Pack-years of smoking among ever-smokers	15.0 (4.5–28.8)	22.2 (7.8–36.8)	.001**
Severe alcohol use[b]	7.3%	4.8%	.098***
Daily to monthly use of cannabis	11.6%	13.5%	.356***
Daily to monthly use of cocaine	2.9%	3.7%	.442***
Daily to monthly use of ecstasy	8.6%	4.3%	.004***
BMI, kg/m^2	24.5 (22.8–27.0)	24.2 (22.3–26.6)	.019**
BMI categories, kg/m^2			
<20	3.3%	8.2%	.121*
20 to <25	54.1%	50.7%	
25 to <30	32.7%	33.2%	
≥30	9.9%	8.0%	
Waist-to-hip ratio higher than normal[c]	62.4%	84.0%	<.001***
Blood pressure, systolic, mm Hg	133 (125–143)	135 (126–147)	.006****
Blood pressure, diastolic, mm Hg	79 (72–85)	81 (75–89)	<.001****
Positive family history for myocardial infarction, hypertension, diabetes mellitus type 2, or hypercholesterolemia[d]	66.5%	70.8	.139***
Physical activity[e]	53.0%	44.3%	.005***
25-hydroxy vitamin D2 + D3, nmol/L	54 (39–72)	47 (29–71)	<.001**

Data are presented as median (interquartile range) or percentage.

Abbreviations: BMI, body mass index; HIV, human immunodeficiency virus.

[a] Smoked during the last month before completing the questionnaire.

[b] Alcohol intake >4 units (for men) or >2 units (for women) daily or almost daily.

[c] If ≥0.9 in males and ≥0.85 in females.

[d] Participants were considered to have a positive family history for myocardial infarction/hypertension/diabetes mellitus type 2/hypercholesterolemia when they stated in the questionnaire to have a first-degree family member who experienced a myocardial infarction before the age of 60, or to have a first-degree family member suffering from hypertension, diabetes mellitus type 2, or hypercholesterolemia.

[e] Physical activity was defined following the Dutch guidelines for healthy physical activity ("Combinorm"): at least 5 days per week at least 30 minutes of moderate physical activity or at least twice per week at least 20 minutes of heavy physical activity [26].

* Nonparametric test for trend.

** Wilcoxon rank-sum test.

*** χ^2test.

**** Student *t* test.

diabetes mellitus type 2, or hypercholesterolemia), smoking, use of cocaine/ecstasy/cannabis, severe alcohol use, and hepatitis B/C coinfection, HIV infection remained independently associated with a higher number of AANCCs (odds ratio [OR], 1.58; 95% confidence interval [CI], 1.23–2.03; $P < .001$). Age, positive family history, and smoking were also strongly independently associated with AANCCs. Analyzing the HIV-infected and HIV-uninfected study groups separately, the OR for age was higher in the HIV-infected study group (OR, 1.60; 95% CI, 1.41–1.81; $P < .001$) compared with the uninfected controls (OR, 1.41; 95% CI, 1.25–1.60; $P < .001$), the difference being borderline significant (P value for interaction = .06).

In univariable analyses (though adjusting conform previous models), several HIV-related variables were significantly associated with AANCCs: time since HIV diagnosis (OR, 1.03 per additional year; 95% CI, 1.02–1.05; $P < .001$), duration of ART use (OR, 1.04 per additional year; 95% CI, 1.02–1.06; $P < .001$), and duration of CD4 count <200 cells/μL (OR, 1.30 per additional year; 95% CI, 1.17–1.45; $P < .001$). In multivariable analysis, only duration of having CD4 counts <200 cells/μL remained an independent risk factor for AANCCs.

In multivariable analyses nadir CD4-count, prior AIDS, (cumulative) duration of undetectable plasma HIV-1 viral load, being diagnosed before 1996, and being pretreated with

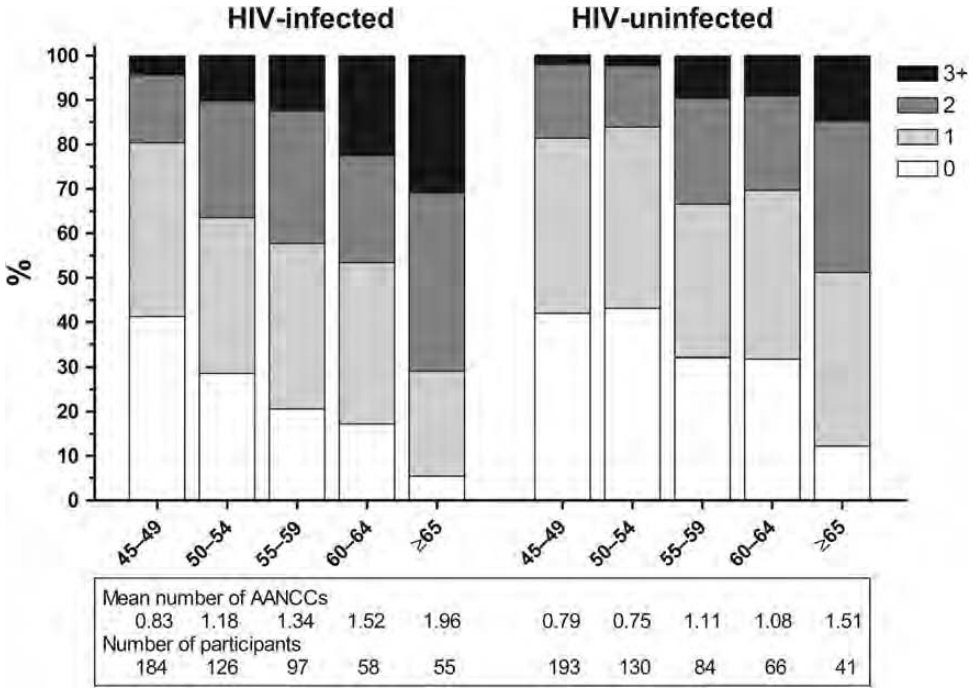

Figure 1. Distribution of the number of age-associated noncommunicable comorbidities stratified by age across both study groups. Abbreviations: AANCC, age-associated noncommunicable comorbidities; HIV, human immunodeficiency virus.

mono-/dual therapy before starting cART were not significantly associated with risk of AANCC.

Inflammation, Coagulation, and Innate Immune Activation

We subsequently analyzed the potential contribution of markers of systemic inflammation (high-sensitivity C-reactive protein [hs-CRP]), coagulation (D-dimer), and monocyte activation (soluble CD14 [sCD14] and soluble CD163 [sCD163]).

The median levels of each of these biomarkers, except D-dimer, were significantly higher among HIV-infected vs HIV-uninfected participants (Table 3). Adding hs-CRP and sCD14 to the above-mentioned regression model (analyzing both study groups jointly) showed both markers to be (borderline) significantly associated with AANCCs (hs-CRP: OR, 1.03/mg/L higher; 95% CI, 1.00–1.07; $P = .037$; sCD14: OR, 1.02 per 100 ng/mL higher; 95% CI, 1.00–1.03; $P = .057$), whereas this was not the case for hs-CRP >10 mg/L, D-dimer, D-dimer >0.5 mg/L, and sCD163. Analyzing the effect of hs-CRP and sCD14 in the 2 study groups separately, both were independent risk factors for AANCC in the HIV-infected cohort, but not in controls. None of these differences, however, reached statistical significance.

Other (Lifestyle-Related) Risk Factors

An above-normal waist-to-hip ratio was an independent risk factor for AANCCs, both in the cohorts combined (OR, 1.49 per 0.1 higher ratio; 95% CI, 1.23–1.80; $P < .001$) and in the HIV-infected (OR, 1.35 per 0.1 higher ratio; 95% CI, 1.04–1.76; $P = .024$) and HIV-uninfected groups separately (OR, 1.78 per 0.1 higher ratio; 95% CI, 1.34–2.37; $P < .001$). No significant interaction between waist-to-hip ratio and HIV infection was found.

Level of physical activity and vitamin D status were not associated with risk of AANCCs.

Specific ART and the Risk of AANCCs

Current or cumulative use of abacavir, stavudine, and didanosine were not significantly associated with risk of AANCCs, whereas cumulative use of ritonavir was identified as an independent risk factor for AANCC (OR, 1.29 per 5 years of ritonavir use; 95% CI, 1.04–1.60; $P = .018$). Exploring this further, only cumulative duration of high (≥400 mg/24 hours) but not of lower doses of ritonavir remained borderline significantly associated with risk of AANCCs (OR, 1.08 per year high-dose ritonavir use; 95% CI, .99–1.18; $P = .083$; Table 4).

DISCUSSION

HIV-infected participants, compared with uninfected controls of similar age, had a significantly higher prevalence of AANCCs, both in terms of composite comorbidity burden, and more specifically of hypertension, cardiovascular and peripheral vascular disease, and impaired renal function.

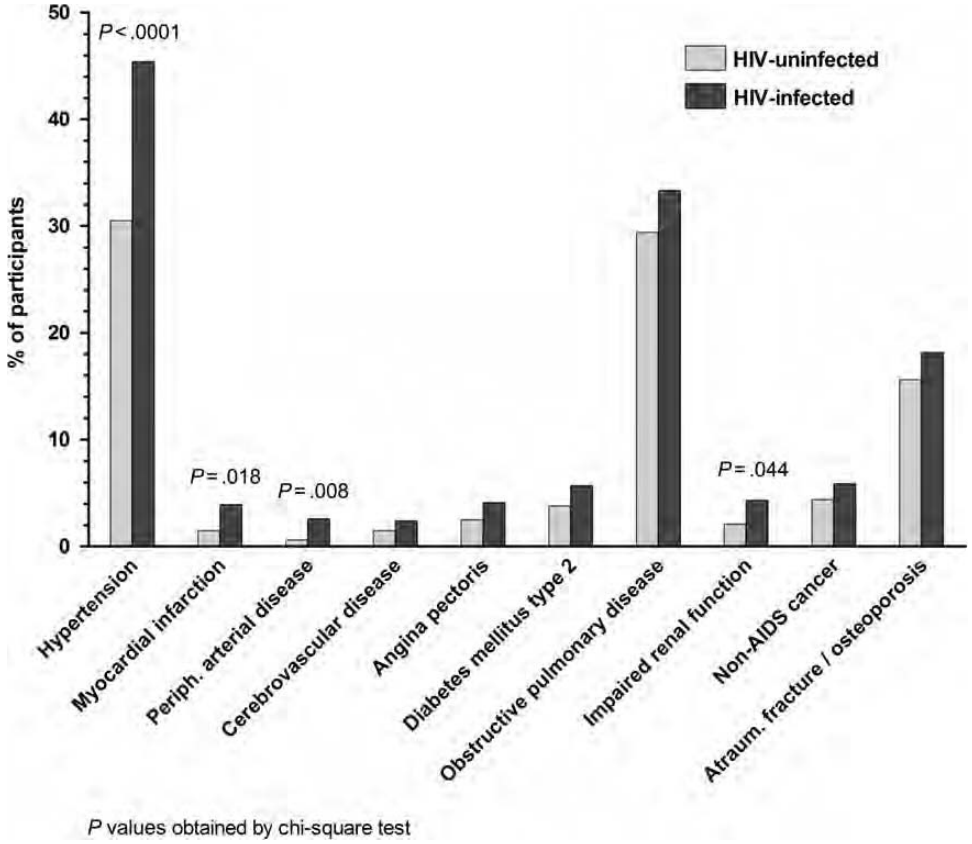

Figure 2. Prevalences of each of the different age-associated noncommunicable comorbidities over the 2 study groups. Abbreviation: HIV, human immunodeficiency virus.

HIV infection was independently associated with a higher total number of AANCCs, as were age, smoking, and positive family history for myocardial infarction, hypertension, diabetes mellitus type 2, or hypercholesterolemia. These traditional risk factors, as well as higher waist-to-hip ratio, independently also contributed to risk of AANCCs in each of the study groups. A borderline significant interaction between age and HIV infection suggested a stronger age effect among HIV-infected participants.

A longer time spent at a CD4 count <200 cells/μL and, to a lesser extent, more systemic inflammation and innate immune activation, as reflected in higher hs-CRP and sCD14 levels, as well as longer prior use of high-dose ritonavir (≥400 mg/24 hours), were additional factors contributing to AANCC burden.

Our finding that comorbidity was significantly more prevalent among HIV-infected individuals (the majority having sustained suppression of viremia on cART) compared with uninfected controls of similar age is compatible with earlier reports [8, 27–36]. Earlier studies, however, either did not include a comparable uninfected control group but used general population [8, 29–32, 35] or patient registry data for comparison [27, 28, 33, 34, 36], or were not designed a priori to prospectively capture data on comorbidity and comorbidity risk factors with similar detail and rigor [35]. To try and overcome these limitations, we purposely recruited our HIV-uninfected participants from a setting where they were expected to exhibit similar lifestyle and

Table 3. Values of Several Markers of Systemic Inflammation, Compared Between the 2 Study Groups

Marker	HIV-Uninfected Participants (n = 524)	HIV-Infected Participants (n = 540)	*P* Value
hs-CRP, mg/L	1.0 (0.6–1.9)	1.5 (0.7–3.5)	<.001*
hs-CRP >10 mg/L	1.6%	6.7%	<.001**
D-dimer, mg/L	0.24 (0.20–0.38)	0.23 (0.20–0.36)	.078*
D-dimer >0.5 mg/L	14.1%	13.2%	.659**
sCD14, ng/mL	1356 (1080–1738)	1576 (1305–2011)	<.001*
sCD163, ng/mL	252 (182–342)	289 (207–419)	<.001*

Data are presented as median (interquartile range) or percentage.

Abbreviations: HIV, human immunodeficiency virus; hs-CRP, high-sensitivity C-reactive protein; sCD14, soluble CD14; sCD163, soluble CD163.

* Wilcoxon rank-sum test.

** χ^2 test.

Table 4. Risk Factors for Age-Associated Noncommunicable Comorbidities, Multivariably Analyzed Using the HIV-Infected and HIV-Uninfected Study Groups Jointly

	Univariable Analysis			Multivariable Analysis		
Risk Factor	Odds Ratio	95% CI	*P* Value	Odds Ratio	95% CI	*P* Value
Age (per 5 years)	1.50	1.39–1.63	<.001	1.39	1.27–1.52	<.001
Smoking (per 5 pack-years)	1.10	1.07–1.13	<.001	1.08	1.05–1.12	<.001
Positive family history[a] (yes/no)	1.57	1.23–2.01	<.001	1.88	1.45–2.44	<.001
HIV infection (yes/no)	1.68	1.34–2.10	<.001	1.07	0.80–1.42	.661
Known cumulative duration of immunodeficiency (per year with a CD4 count <200 cells/μL)	1.33	1.20–1.48	<.001	1.23	1.10–1.38	<.001
Waist-to-hip ratio (per 0.1)	1.94	1.67–2.25	<.001	1.49	1.23–1.80	<.001
hs-CRP (per mg/mL)	1.06	1.03–1.09	<.001	1.03	0.99–1.06	.107
sCD14 (per 100 ng/mL)	1.02	1.01–1.04	.007	1.02	1.00–1.03	.074
Cumulative duration of ritonavir use in high dosages (≥400 mg/daily) (per year)	1.19	1.10–1.28	<.001	1.08	0.99–1.18	.083

The outcome variable is the number of age-associated noncommunicable comorbidities (AANCCs) per participant. Analyses were performed using ordinal logistic regression. This model was adjusted for sex, Dutch origin, sexual orientation, smoking, use of cocaine/ecstasy/cannabis, severe alcohol use, and hepatitis B/C coinfection (all of which were not significantly associated with risk for AANCC).

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; hs-CRP, high-sensitivity C-reactive protein; sCD14, soluble CD14.

[a] Positive family history: a first-degree family member suffering from myocardial infarction, hypertension, diabetes mellitus type 2, or hypercholesterolemia.

sexual risk-taking behavior as HIV-infected study participants. Although smoking and hepatitis B/C were more prevalent in HIV-infected participants and ecstasy use was more prevalent in controls (which also consisted of more native Dutch persons), overall the differences between both study groups were relatively minor. Our findings thus add robustness to the notion that AANCCs indeed are more prevalent among those living with HIV, including in those with a sustained response to ART.

Unraveling underlying mechanisms and risk factors for this increased comorbidity burden among HIV-infected individuals is the subject of ongoing research. A central question concerns the contributions of HIV infection itself (by viral- and immune-related mechanisms), coinfections (including cytomegalovirus and chronic viral hepatitis), and ART [18]. A study by Guaraldi et al identified longer ART exposure and lower nadir CD4 count as independent risk factors for non-AIDS comorbidities [8].

We found that although HIV infection status, duration of HIV infection, duration of ART use, and duration of immune deficiency (ie, duration of having CD4 counts <200 cells/μL) were each univariably associated with AANCCs, these associations were all confounded by duration of immunodeficiency.

HIV infection is associated with inflammation, innate immune activation, and altered coagulation [37–39], which are generally considered important drivers for comorbidity in both HIV-uninfected and HIV-infected individuals [15, 16, 40, 41]. Higher levels of sCD14 and hs-CRP, but not of sCD163 or D-dimer, were borderline significantly associated with increased risk for AANCCs. Additional work is needed to determine which specific inflammatory, innate and adaptive immune system, and coagulatory pathways are driving comorbidity risk, and to which extent this differs for individual comorbidities. Innate immune and particularly monocyte activation have recently been reported to be more relevant than T-cell activation in enhancing cardiovascular disease risk in HIV [42, 43].

Duration of exposure to high-dose ritonavir (≥400 mg/24 hours) in our analyses was borderline significantly associated with risk for AANCCs. Currently, ritonavir is almost exclusively used at lower doses, and exposure to higher doses in this cohort therefore occurred many years previously. Although identified in cross-sectional analyses and potentially driven by bias, plausible mechanisms by which ritonavir may contribute to AANCC risk include its known dose-dependent effect on lipids, induction of endothelial dysfunction [44, 45], and cellular accumulation of prelamin A, which may result in premature cellular senescence similar to what is observed in some genetically determined premature aging syndromes [46, 47].

Our results being those of cross-sectional analyses, we are merely able to demonstrate associations rather than causality. Of note, risk factors identified for the presence of the composite number of different AANCCs may differ in (the magnitude of) their effect when addressing specific comorbidities separately. Although the HIV-infected and HIV-uninfected study groups were largely comparable, differences in some demographic and lifestyle-related factors were present, which was addressed by adjusting all regression analyses for a broad range of demographic and lifestyle-related factors. Nonetheless, differences in

remaining unmeasured confounders potentially influencing our results cannot be excluded.

In conclusion, all AANCCs were numerically more prevalent, and peripheral arterial, cardiovascular disease, and impaired renal function significantly so, in this cohort of HIV-infected individuals with largely sustained suppressed viremia on cART. Besides cardiovascular risk factors, HIV infection and longer time spent with severe immunodeficiency increased the risk of higher AANCC burden. Less pronounced contributions were identified from residual inflammation, immune activation, and prior high-dose ritonavir use. The trend toward a stronger association between age and AANCC burden among HIV-infected participants might support the hypothesis of premature or accelerated aging in HIV [8–10]. Whether this reflects HIV acting as an additive risk factor for comorbidity development in conjunction with traditional risk factors, or includes HIV impacting on and accelerating the biology of aging itself, remains to be elucidated [18, 48].

Notes

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APPENDIX

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EXHIBIT D

Excess Risk for Atherosclerotic Cardiovascular Outcomes Among US Adults With HIV in the Current Era

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Background—In the 2000s, adults with HIV had a higher risk for atherosclerotic cardiovascular disease (ASCVD) compared with those without HIV. There is uncertainty if this excess risk still exists in the United States given changes in antiretroviral therapies and increased statin use.

Methods and Results—We compared the risk for ASCVD events between US adults aged ≥19 years with and without HIV who had commercial or supplemental Medicare health insurance between January 1, 2011, and December 31, 2016. Beneficiaries with HIV (n=82 426) were frequency matched 1:4 on age, sex, and calendar year to those without HIV (n=329 704). Beneficiaries with and without HIV were followed up through December 31, 2016, for ASCVD events, including myocardial infarction, stroke, and lower extremity artery disease hospitalizations. Most beneficiaries were aged <55 years (79%) and men (84%). Over a median follow-up of 1.6 years (maximum, 6 years), there were 3287 ASCVD events, 2190 myocardial infarctions, 891 strokes, and 322 lower extremity artery disease events. The rate per 1000 person-years among beneficiaries with and without HIV was 5.53 and 3.49 for ASCVD, respectively, 3.58 and 2.34 for myocardial infarction, respectively, 1.49 and 0.94 for stroke, respectively, and 0.65 and 0.31 for lower extremity artery disease hospitalizations, respectively. The multivariable-adjusted hazard ratio (95% CI) for ASCVD, myocardial infarction, stroke, and lower extremity artery disease hospitalizations comparing beneficiaries with versus without HIV was 1.29 (1.18–1.40), 1.26 (1.13–1.39), 1.30 (1.11–1.52), and 1.46 (1.11–1.92), respectively.

Conclusions—Adults with HIV in the United States continue to have a higher ASCVD risk compared with their counterparts without HIV. **(*J Am Heart Assoc*. 2020;9:e013744. DOI: 10.1161/JAHA.119.013744.)**

Key Words: HIV • myocardial infarction • peripheral artery disease • stroke

HIV infection is a global epidemic, and its prevalence continues to increase.[1] At the end of 2015, >1 million people had HIV in the United States.[1] Among US adults and adolescents diagnosed with HIV, 63% received some HIV medical care, 49% received continuous HIV care, and 51% achieved viral suppression.[1] The use of antiretroviral therapies (ARTs), in societies where they are widely available, has reduced deaths from opportunistic infections.[2,3] This has resulted in an increased proportion of deaths among people with HIV being attributed to atherosclerotic cardiovascular disease (ASCVD).[1,4–7]

Studies conducted in the United States from the 1990s and early 2000s reported that people with HIV infection have a higher risk for ASCVD events, including myocardial infarction (MI) and stroke, than their counterparts without HIV.[8–11] A retrospective cohort study using data from 1996 to 2011 on HIV-positive and HIV-negative members of Kaiser Permanente Southern California and Kaiser Permanente Northern California health plans reported that an excess risk for MI may no longer exist for US adults with HIV.[12] The authors hypothesized that the similar rates of MI in people with and without HIV may have resulted from increased awareness of HIV-associated cardiovascular risk and use of statins and antihypertensive agents, in addition to decreased use of hyperlipidemia-inducing protease inhibitors (PIs).[12,13] Other studies conducted during this time period suggest that HIV may still be associated with higher ASVD risk outside the United States.[6]

The main aim of the current study was to determine whether the risk for ASCVD, including MI, stroke, and lower

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Accompanying Tables S1 through S9 and Figure S1 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.119.013744

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Clinical Perspective

What Is New?

- The current analysis suggests that HIV is associated with a higher risk of atherosclerotic cardiovascular disease events, including myocardial infarction, stroke, and lower extremity arterial disease, in the contemporary era, despite more extensive use of antiretroviral therapy and increased use of statins.

What Are the Clinical Implications?

- Clinicians should be aware of the higher risk of atherosclerotic cardiovascular disease in people living with HIV and provide guideline-recommended therapy to lower their risk.

extremity artery disease (LEAD) events, is higher among patients with HIV compared with their counterparts without HIV in a contemporary cohort of US adults. We examined these associations overall and within subgroups of patients taking and not taking statins. To accomplish this aim, we conducted a retrospective cohort study using data from the MarketScan database (Truven Health Analytics, IBM Watson Health, Ann Arbor, MI).

Methods

Study Population

The MarketScan database contains administrative and claims data from individuals enrolled in various employer-sponsored healthcare plans and Medicare supplemental plans. We identified beneficiaries in the MarketScan database who had HIV infection, defined by meeting either of the following criteria between January 1, 2011, and December 31, 2016: (1) ≥1 hospitalization with a discharge diagnosis code for HIV in any position or (2) ≥2 pharmacy claims for ART. Discharge diagnosis codes for HIV included an *International Classification of Diseases, Ninth Revision* (*ICD-9*), code of 042.x to 044.x or V08 or an *ICD-10* code of B20.xx to B22.xx, B24.xx, or Z21.xx. Table S1 shows the list of ART medication by drug classes used in the current study. We restricted the study population to beneficiaries meeting the definition of HIV who were aged ≥19 years; had continuous health insurance coverage, including pharmacy benefits; and lived in the United States for the 365 days before being identified as having HIV in the MarketScan database. For each beneficiary, the index date was defined as the earliest date for which they had a diagnosis of HIV or at least 2 prescription fills for ART while meeting all of the criteria described above.

Beneficiaries without HIV were frequency matched to those with HIV. Specifically, for each beneficiary in the MarketScan database without HIV, we selected a random date between January 1, 2011, and December 31, 2016, and defined this as their index date. We further restricted the population to beneficiaries aged ≥19 years who had continuous health insurance coverage, including pharmacy benefits, and lived in the United States for the 365 days before their index date. For each beneficiary in the HIV cohort, we randomly selected 4 beneficiaries without HIV from the same age group (ie, 19–44, 45–54, 55–64, and ≥65 years), sex, and calendar year of their index date. The Institutional Review Board at the University of Alabama at Birmingham approved the current analysis and waived the requirement to obtain informed consent. Data used in the current study are available from Truven Health Analytics. Other study information is available from the corresponding author.

Beneficiary Characteristics

Beneficiary characteristics analyzed included age, sex, calendar year of their index date, and geographic region of residence. We used claims in the 365 days before each beneficiary's index date from the MarketScan database to identify the presence of comorbidities, including a history of coronary heart disease, stroke, peripheral artery disease, diabetes mellitus, heart failure, chronic kidney disease, liver disease, and depression. In addition, we used claims to identify receipt of cardiologist care, a hospitalization for any reason, tobacco use, use of antihypertensive medication, statin use and intensity, use of nonstatin lipid-lowering medication, and polypharmacy. Table S2 provides definitions for each of these variables. Use of ART medication was defined by ≥2 prescription fills for a nucleoside reverse transcriptase inhibitor, nonnucleoside reverse transcriptase inhibitor (NNRTI), PI, fusion inhibitor, entry inhibitor, integrase strand transfer inhibitor, or pharmacokinetic enhancer within 365 days before each beneficiary's index date, inclusive.

Cardiovascular Events During Follow-Up

Beneficiaries were followed up from their index date for the composite of ASCVD, including MI, stroke, and LEAD hospitalizations.[14,15] In addition, each component of the composite outcome was analyzed separately. Definitions of ASCVD, MI, stroke, and LEAD hospitalizations are shown in Table S3. ASCVD, MI, stroke, and LEAD hospitalizations were assessed through December 31, 2016, the last date for which we had outcome data available. For each outcome, beneficiaries were followed up until the earliest occurrence of their first event, loss of health insurance coverage, or December 31, 2016. Data on mortality are not available in the MarketScan database.

Statistical Analysis

We calculated baseline characteristics, the cumulative incidence of ASCVD using the Kaplan-Meier method, and the rate of ASCVD among beneficiaries with HIV and matched controls without HIV. Cox regression models were used to calculate hazard ratios for ASCVD comparing beneficiaries with versus without HIV. In addition to an unadjusted model, 2 models with progressive adjustment for covariates were used. Model 1 included adjustment for age, sex, calendar year, geographical region of residence, and history of coronary heart disease, diabetes mellitus, stroke, peripheral artery disease, and heart failure. Model 2 included adjustment for variables in model 1 and chronic kidney disease, liver disease, cardiologist care, a prior hospitalization for any reason, depression, tobacco use, polypharmacy, antihypertensive medication use, statin use and high versus low/moderate intensity, and nonstatin lipid-lowering medication use. We also calculated the cumulative incidence, event rate, and unadjusted and adjusted hazard ratios for an MI, stroke, and LEAD hospitalization, separately, comparing beneficiaries with versus without HIV.

We used Cox regression models to calculate multivariable-adjusted hazard ratios for an ASCVD, MI, stroke, and LEAD hospitalization comparing beneficiaries with versus without HIV within subgroups defined by each of the characteristics included in the multivariable-adjusted model. These models included adjustment for all variables in model 2 described above. To test whether hazard ratios for an ASCVD, MI, stroke, and LEAD hospitalization were different across subgroups defined by beneficiary characteristics, calculations were repeated in the overall population, including interaction terms between HIV infection status and each characteristic (eg, HIV×sex). We used the likelihood ratio test to calculate the *P* value for the interaction between HIV and characteristics with >2 levels (eg, age or calendar year). All the analyses described above were repeated stratified by statin use. Analyses were conducted in SAS, version 9.4 (SAS Institute Inc, Cary, NC) using a 2-sided level of significance $\alpha<0.05$.

Results

Baseline Characteristics of Study Population

There were 82 426 beneficiaries aged ≥19 years with commercial or supplemental Medicare health insurance in the MarketScan database with HIV who met the inclusion criteria for the current analysis (Figure S1). The characteristics of beneficiaries with HIV and matched controls without HIV (n=329 704) are shown in Table 1. Most beneficiaries with HIV and their matched controls were aged <55 years and men. There was a <1% difference in the percentage with diabetes mellitus, history of coronary heart disease, history of stroke, and history of peripheral artery disease between beneficiaries with and without HIV. A history of chronic kidney disease and liver disease, cardiologist care, a prior hospitalization for any reason, depression, tobacco use, polypharmacy, and antihypertensive medication use were more common among beneficiaries with HIV than their counterparts without HIV. Overall, 18.9% and 16.3% of beneficiaries with and without HIV were taking a statin, respectively. Most beneficiaries with HIV (96.0%) had ≥2 fills for ART medication within the 365 days before their index date, inclusive. Among beneficiaries with HIV, 50.2% were taking a nucleoside reverse transcriptase inhibitor, 44.2% were taking an NNRTI, 25.1% were taking a PI, and 21.7% were taking other ART classes.

Incidence of ASCVD Events

Over a median follow-up of 1.6 years (maximum, 6.0 years), there were 3287 ASCVD events, 2190 MIs, 891 strokes, and 322 LEAD events. Compared with beneficiaries without HIV, those with HIV had a higher cumulative incidence of ASCVD, MI, stroke, and LEAD hospitalizations (Figure 1). The risk for each outcome was higher among beneficiaries with versus without HIV in unadjusted and multivariable-adjusted models (Table 2). The multivariable-adjusted hazard ratio for ASCVD comparing beneficiaries with versus without HIV was 1.29 (95% CI, 1.18–1.40). The multivariable-adjusted hazard ratios (95% CIs) for an MI, stroke, and LEAD hospitalization in beneficiaries with versus without HIV were 1.26 (1.13–1.39), 1.30 (1.11–1.52), and 1.46 (1.11–1.92), respectively.

Beneficiaries with HIV had a higher risk for an ASCVD, MI, stroke, and LEAD hospitalization versus those without HIV within most subgroups defined by beneficiary characteristics (Figure 2 and Table S4). The hazard ratio for ASCVD was higher among beneficiaries aged 19 to 44 years versus those aged ≥45 years (*P*=0.04). Hazard ratios for ASCVD and stroke hospitalizations associated with HIV infection were statistically significantly higher among beneficiaries with versus without a prior hospitalization for any reason (each *P*-interaction<0.05). Hazard ratios for LEAD hospitalization were higher in beneficiaries without diabetes mellitus or liver disease. No other statistically significant differences were present when comparing hazard ratios for an ASCVD, MI, stroke, or LEAD hospitalization among beneficiaries with versus without HIV across subgroups.

Analyses Stratified by Statin Use

Characteristics of beneficiaries with and without HIV who were taking and not taking a statin, separately, are presented in Table S5. The rates of ASCVD, MI, stroke, and LEAD hospitalizations were higher in beneficiaries with versus without HIV among both those taking and not taking statins (Table 3). Among beneficiaries taking and not taking statins,

Table 1. Characteristic of Beneficiaries With HIV and Age-, Sex-, and Calendar Year–Matched Beneficiaries Without HIV in the MarketScan Database

Characteristics	Beneficiaries Without HIV (n=329 704)	Beneficiaries With HIV (n=82 426)
Calendar year		
2011	136 516 (41.4)	34 129 (41.4)
2012	53 244 (16.1)	13 311 (16.1)
2013	36 516 (11.1)	9129 (11.1)
2014	36 832 (11.2)	9208 (11.2)
2015	32 092 (9.7)	8023 (9.7)
2016	34 504 (10.5)	8626 (10.5)
Age, y		
19–44	140 600 (42.6)	35 150 (42.6)
45–54	118 472 (35.9)	29 618 (35.9)
55–64	60 328 (18.3)	15 082 (18.3)
≥65	10 304 (3.1)	2576 (3.1)
Male sex	276 548 (83.9)	69 137 (83.9)
Geographic region of residence		
Northeast	61 519 (18.7)	15 330 (18.6)
North central	75 444 (22.9)	11 186 (13.6)
South	126 531 (38.4)	38 255 (46.4)
West	62 749 (19.0)	16 557 (20.1)
Unknown	3461 (1.0)	1098 (1.3)
Diabetes mellitus	24 719 (7.5)	6664 (8.1)
History of CHD	9704 (2.9)	2815 (3.4)
History of stroke	907 (0.3)	596 (0.7)
History of peripheral artery disease	579 (0.2)	309 (0.4)
History of heart failure	1004 (0.3)	700 (0.8)
Chronic kidney disease	4629 (1.4)	3849 (4.7)
Liver disease	1295 (0.4)	2325 (2.8)
Cardiologist care	6627 (2.0)	2807 (3.4)
Any hospitalization	12 981 (3.9)	10 128 (12.3)
Depression	39 379 (11.9)	19 669 (23.9)
Tobacco use	9418 (2.9)	5640 (6.8)
Polypharmacy	32 883 (10.0)	27 602 (33.5)
Antihypertensive medication use	77 733 (23.6)	23 740 (28.8)
Statin use		
Overall	53 842 (16.3)	15 619 (18.9)
Low-/moderate-intensity statin use	44 421 (13.5)	12 049 (14.6)
High-intensity statin use	9421 (2.8)	3570 (4.3)
Nonstatin lipid-lowering medication use	14 744 (4.5)	6558 (8.0)
ART use	…	79 095 (96.0)
NRTIs	…	41 372 (50.2)
NNRTI	…	36 465 (44.2)

Continued

Table 1. Continued

Characteristics	Beneficiaries Without HIV (n=329 704)	Beneficiaries With HIV (n=82 426)
Protease inhibitors	…	20 713 (25.1)
Other*	…	17 890 (21.7)

Data are given as number (percentage) of each group. ART indicates antiretroviral therapy; CHD, coronary heart disease; NNRTI, non-NRTI; NRTI, nucleoside reverse transcriptase inhibitor.
*Other ART includes fusion inhibitors, entry inhibitors, integrase strand transfer inhibitors, and pharmacokinetic enhancers.

HIV was associated with an increased risk for ASCVD and MI after multivariable adjustment. After multivariable adjustment, the hazard ratio for stroke associated with HIV infection was 1.30 (95% CI, 1.07–1.58) among beneficiaries not taking statins and 1.25 (95% CI, 0.95–1.64) among their counterparts taking statins. The multivariable-adjusted hazard ratio for LEAD hospitalization associated with HIV infection was 1.62 (95% CI, 1.13–2.32) among beneficiaries not taking statins and 1.27 (95% CI, 0.83–1.94) among their counterparts taking statins. There was no evidence of effect

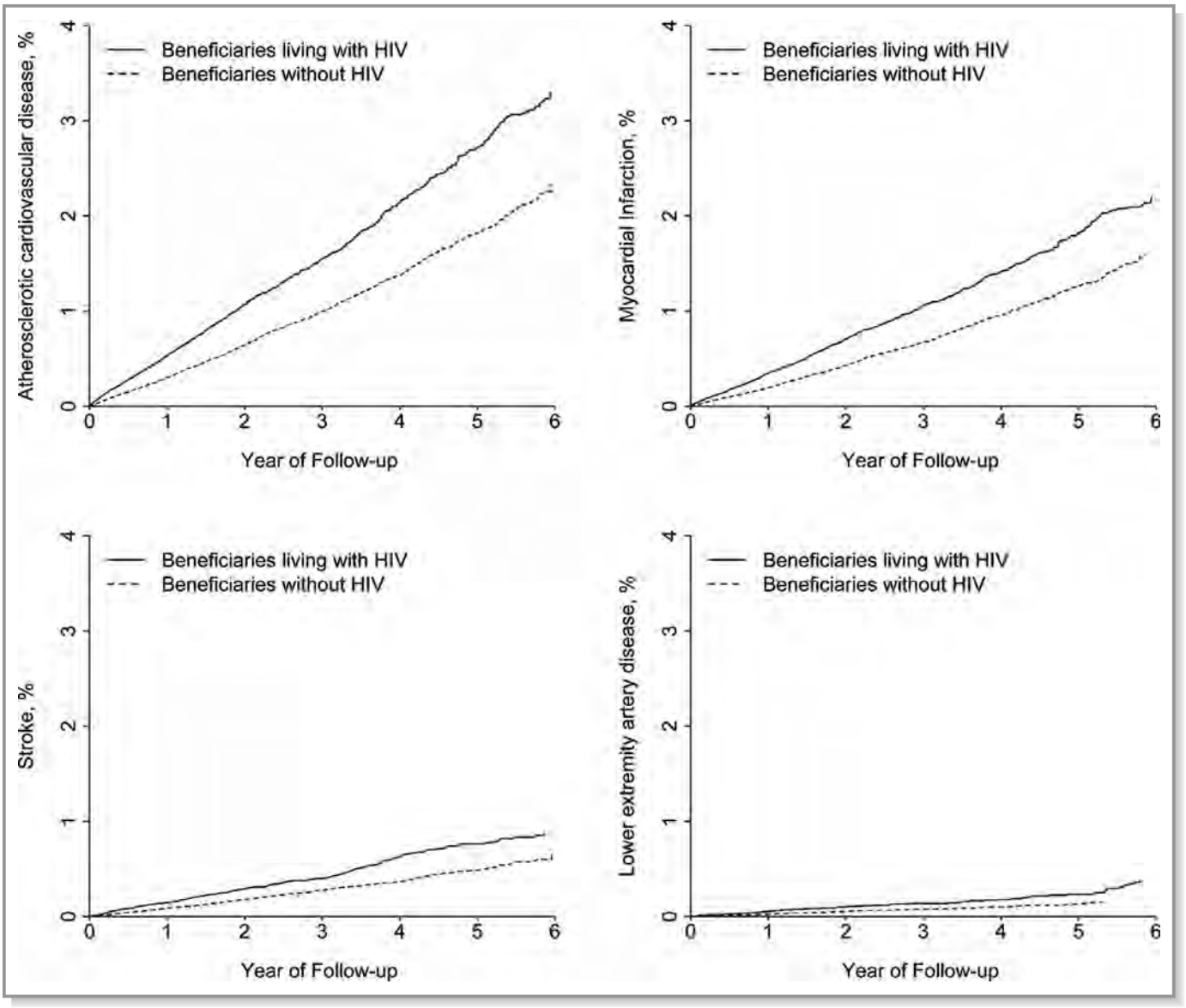

Figure 1. Cumulative incidence of atherosclerotic cardiovascular disease, myocardial infarction, stroke, and lower extremity artery disease hospitalizations among beneficiaries with HIV and age-, sex-, and calendar year–matched beneficiaries without HIV in the MarketScan database. Atherosclerotic cardiovascular disease includes myocardial infarction, stroke, and lower extremity artery disease hospitalizations.

Table 2. Rate and Hazard Ratios for ASCVD, MI, Stroke, and LEAD Hospitalizations Among Beneficiaries With HIV Versus Age-, Sex-, and Calendar Year–Matched Beneficiaries Without HIV in the MarketScan Database

Variables	Beneficiaries Without HIV (n=329 704)	Beneficiaries With HIV (n=82 426)
ASCVD		
Events	2356	931
Follow-up in person-years	675 955	168 294
Rate (95% CI), per 1000 person-years	3.49 (3.34–3.63)	5.53 (5.18–5.89)
Hazard ratio (95% CI)		
Unadjusted	1 (Reference)	1.57 (1.46–1.70)
Model 1	1 (Reference)	1.51 (1.40–1.64)
Model 2	1 (Reference)	1.29 (1.18–1.40)
MI		
Events	1586	604
Follow-up in person-years	677 211	168 780
Rate (95% CI), per 1000 person-years	2.34 (2.23–2.46)	3.58 (3.29–3.86)
Hazard ratio (95% CI)		
Unadjusted	1 (Reference)	1.53 (1.39–1.68)
Model 1	1 (Reference)	1.47 (1.34–1.62)
Model 2	1 (Reference)	1.26 (1.13–1.39)
Stroke		
Events	638	253
Follow-up in person-years	678 673	169 412
Rate (95% CI), per 1000 person-years	0.94 (0.87–1.01)	1.49 (1.31–1.68)
Hazard ratio (95% CI)		
Unadjusted	1 (Reference)	1.59 (1.37–1.84)
Model 1	1 (Reference)	1.50 (1.29–1.75)
Model 2	1 (Reference)	1.30 (1.11–1.52)
LEAD		
Events	212	110
Follow-up in person-years	679 271	169 547
Rate (95% CI), per 1000 person-years	0.31 (0.27–0.35)	0.65 (0.53–0.77)
Hazard ratio (95% CI)		
Unadjusted	1 (Reference)	1.96 (1.52–2.53)
Model 1	1 (Reference)	1.81 (1.40–2.35)
Model 2	1 (Reference)	1.46 (1.11–1.92)

ASCVD includes MI, stroke, and LEAD hospitalizations. The median (maximum) follow-up for all outcome events was 1.6 (6.0) years. Model 1 adjusts for age, sex, calendar year, geographic region of residence, history of coronary heart disease, diabetes mellitus, stroke, peripheral artery disease, and heart failure. Model 2 adjusts for variables in model 1 plus chronic kidney disease, liver disease, cardiologist care, any hospitalization, depression, tobacco use, polypharmacy, antihypertensive medication use, statin use and statin intensity, and nonstatin lipid-lowering medication use. ASCVD indicates atherosclerotic cardiovascular disease; LEAD, lower extremity artery disease; MI, myocardial infarction.

modification between statin use and HIV status on any of the outcomes after multivariable adjustment (all *P* values for interaction>0.10). Hazard ratios for ASCVD, MI, stroke, and LEAD hospitalization associated with HIV infection across subgroups defined by beneficiary characteristics stratified by statin use are shown in Tables S6 through S9.

Discussion

In the current analysis of US adults with commercial or Medicare health insurance, those with HIV had a higher risk for ASCVD events versus their counterparts without HIV. The risks for MI, stroke, and LEAD hospitalizations were each higher in

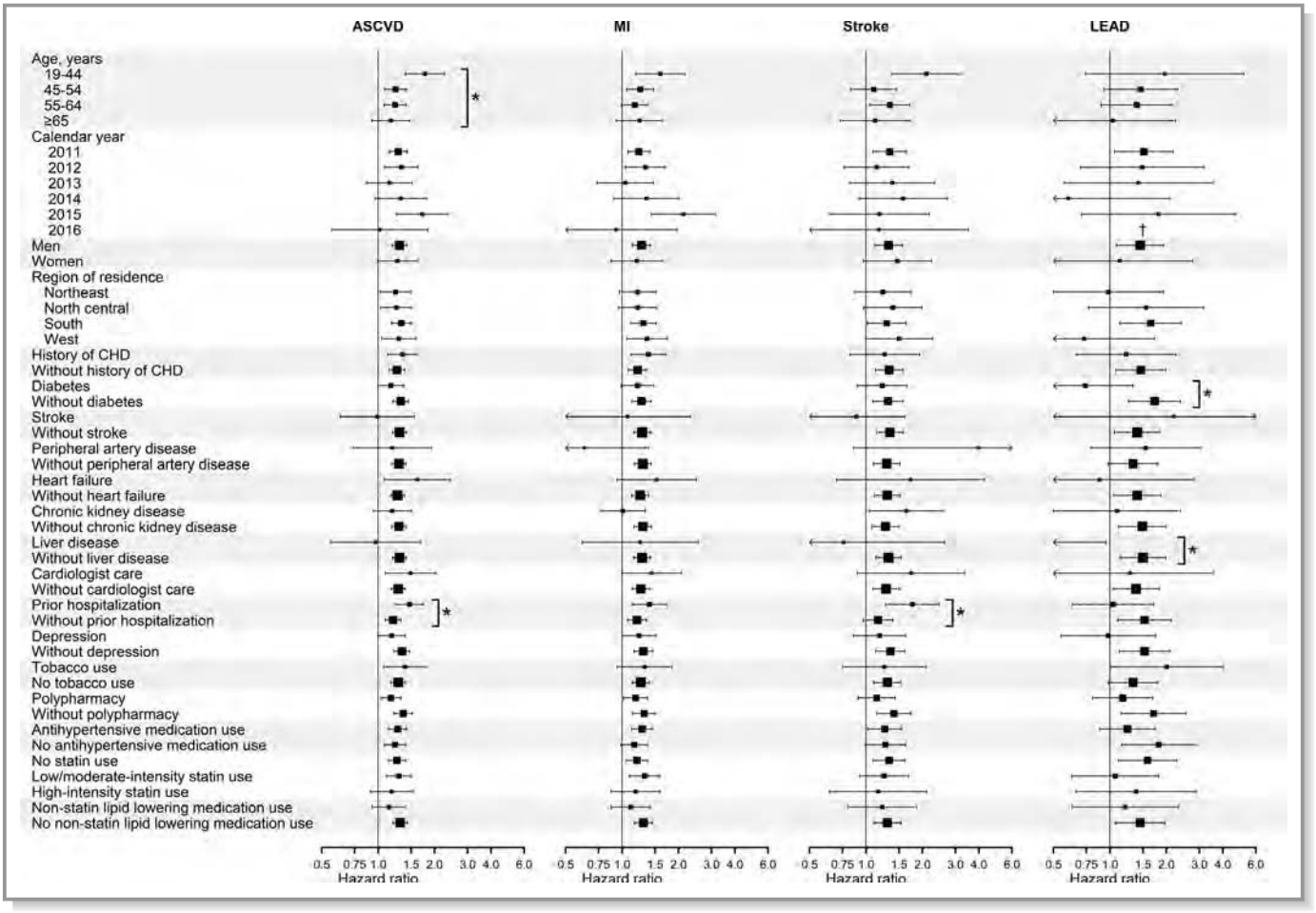

Figure 2. Hazard ratios (HRs) for atherosclerotic cardiovascular disease (ASCVD), myocardial infarction (MI), stroke, and lower extremity artery disease (LEAD) hospitalizations among beneficiaries with vs without HIV across subgroups defined by beneficiary characteristics. Squares represent mean point estimates for HRs, and horizontal bars represent 95% CIs. HRs and 95% CIs are shown in Table S4. HRs include adjustment for age, sex, calendar year, geographic region of residence, history of coronary heart disease (CHD), diabetes mellitus, stroke, peripheral artery disease, heart failure, chronic kidney disease, liver disease, cardiologist care, any hospitalization, depression, tobacco use, polypharmacy, antihypertensive medication use, statin use and statin intensity, and nonstatin lipid-lowering medication use. *$P<0.05$ comparing HRs for outcome events associated with HIV infection across subgroups. All other P values comparing HRs for outcome events associated with HIV infection across subgroups defined by beneficiary characteristics were ≥0.05. †Data not shown given the small number of events. Specifically, there were 6 LEAD hospitalizations during follow-up among beneficiaries in 2016.

beneficiaries with versus without HIV. The higher risk for ASCVD events associated with HIV was present within most subgroups and did not differ between people taking and not taking a statin. Results from the current analysis suggest that US adults with HIV continue to have a higher risk for ASCVD events versus those without HIV in the contemporary era, despite more extensive use of ART and increased use of statin therapy.

A meta-analysis of observational studies conducted between 1990 and 2015 found that the risk ratio associated with HIV infection was 1.79 (95% CI, 1.54–2.08) for MI and 2.56 (95% CI, 1.43–4.81) for stroke.[6] This meta-analysis included 4 studies from the United States that were all conducted before 2006. Data from a cohort study evaluating MI risk from 1996 to 2011 in Kaiser Permanente Southern California and Kaiser Permanente Northern California health plan members with and without HIV found that the multivariable-adjusted relative risk for MI associated with HIV infection declined over time, from 1.8 (95% CI, 1.3–2.6) in 1996 to 1999 to 1.0 (95% CI, 0.7–1.4) in 2010 to 2011.[12] Both Kaiser Permanente Southern California and Kaiser Permanente Northern California use a system-wide, integrated risk reduction strategy that may result in higher use of preventive interventions, lower viral loads, and higher CD4 levels versus patients receiving care elsewhere in the United States.[12,13] Therefore, these results may not be generalizable to all US adults with HIV. Results from the current analysis of a contemporary cohort of US adults with various employer-sponsored healthcare plans or Medicare supplemental health care suggest that US adults with HIV continue to have a higher risk for ASCVD, including MI, stroke, and LEAD

Table 3. Risk and Hazard Ratios for ASCVD, MI, Stroke, and LEAD Hospitalizations Among Beneficiaries With HIV Versus Age-, Sex-, and Calendar Year–Matched Beneficiaries Without HIV, Stratified by Statin Use in the MarketScan Database

Variables	Taking Statin Therapy		Not Taking Statin Therapy		*P* Value*
	Beneficiaries Without HIV (n=53 842)	Beneficiaries With HIV (n=15 619)	Beneficiaries Without HIV (n=275 862)	Beneficiaries With HIV (n=66 807)	
ASCVD					
Events	920	366	1436	565	. . .
Follow-up in person-years	118 585	35 211	557 370	133 083	. . .
Rate (95% CI), per 1000 person-years	7.76 (7.26–8.26)	10.39 (9.33–11.46)	2.58 (2.44–2.71)	4.25 (3.90–4.60)	. . .
Hazard ratio (95% CI)					
Unadjusted	1 (Reference)	1.33 (1.18–1.50)	1 (Reference)	1.63 (1.48–1.80)	0.01
Model 1	1 (Reference)	1.44 (1.27–1.63)	1 (Reference)	1.50 (1.36–1.66)	0.50
Model 2	1 (Reference)	1.28 (1.12–1.46)	1 (Reference)	1.24 (1.12–1.39)	0.90
MI					
Events	635	253	951	351	. . .
Follow-up in person-years	119 040	35 396	558 171	133 384	. . .
Rate (95% CI), per 1000 person-years	5.33 (4.92–5.75)	7.15 (6.27–8.03)	1.70 (1.60–1.81)	2.63 (2.36–2.91)	. . .
Hazard ratio (95% CI)					
Unadjusted	1 (Reference)	1.34 (1.16–1.55)	1 (Reference)	1.54 (1.37–1.74)	0.14
Model 1	1 (Reference)	1.44 (1.24–1.67)	1 (Reference)	1.42 (1.26–1.62)	>0.99
Model 2	1 (Reference)	1.29 (1.10–1.51)	1 (Reference)	1.18 (1.03–1.35)	0.47
Stroke					
Events	225	85	413	168	. . .
Follow-up in person-years	119 737	35 739	558 936	133 672	. . .
Rate (95% CI), per 1000 person-years	1.88 (1.63–2.12)	2.38 (1.87–2.88)	0.74 (0.67–0.81)	1.26 (1.07–1.45)	. . .
Hazard ratio (95% CI)					
Unadjusted	1 (Reference)	1.27 (0.99–1.63)	1 (Reference)	1.70 (1.42–2.03)	0.06
Model 1	1 (Reference)	1.40 (1.08–1.81)	1 (Reference)	1.55 (1.28–1.86)	0.38
Model 2	1 (Reference)	1.25 (0.95–1.64)	1 (Reference)	1.30 (1.07–1.58)	0.61
LEAD					
Events	102	45	110	65	. . .
Follow-up in person-years	119 898	35 749	559 373	133 798	. . .
Rate (95% CI), per 1000 person-years	0.85 (0.69–1.02)	1.26 (0.89–1.63)	0.20 (0.16–0.23)	0.49 (0.37–0.60)	. . .
Hazard ratio (95% CI)					
Unadjusted	1 (Reference)	1.36 (0.92–2.03)	1 (Reference)	2.35 (1.68–3.29)	0.04
Model 1	1 (Reference)	1.52 (1.01–2.27)	1 (Reference)	2.00 (1.42–2.80)	0.27
Model 2	1 (Reference)	1.27 (0.83–1.94)	1 (Reference)	1.62 (1.13–2.32)	0.33

ASCVD includes MI, stroke, and LEAD hospitalizations. Model 1 adjusts for age, sex, calendar year, geographic region of residence, history of coronary heart disease, diabetes mellitus, stroke, peripheral artery disease, and heart failure. Model 2 adjusts for variables in model 1 plus chronic kidney disease, liver disease, cardiologist care, any hospitalization, depression, tobacco use, polypharmacy, antihypertensive medication use, and nonstatin lipid-lowering medication use. ASCVD indicates atherosclerotic cardiovascular disease; LEAD, lower extremity artery disease; MI, myocardial infarction.

*Comparing hazard ratios associated with HIV infection among beneficiaries taking vs not taking statin therapy.

hospitalizations. These results are consistent with a prior publication of the VACS (Veterans Aging Cohort Study) conducted from 2003 to 2014, showing that people with HIV have a higher risk for LEAD events compared with their counterparts without HIV.[16]

The current results highlight the need for implementing interventions aimed to reducing the excess risk for ASCVD among individuals with HIV in the contemporary era. According to the 2018 American Heart Association and American College of Cardiology guideline on the management of blood cholesterol, HIV should be considered as a risk-enhancing factor for ASCVD when starting a clinician-patient discussion on statin therapy initiation.[17] The importance of ASCVD prevention and treatment in people with HIV was further stressed in the 2019 American Heart Association Scientific Statement "Characteristics, Prevention, and Management of Cardiovascular Disease in People Living With HIV."[7] In the current analysis, adults with HIV had a higher prevalence of chronic kidney disease, tobacco use, and use of antihypertensive medication in addition to a higher risk for ASCVD events versus their counterparts without HIV. Despite their higher prevalence of cardiovascular risk factors and higher risk for ASCVD events, the proportion of adults with HIV who were taking a statin was similar to those without HIV. Although we were not able to assess all indications for statin use (eg, low-density lipoprotein cholesterol [LDL-C] $\geq$190 mg/dL), only 16.3% of beneficiaries with HIV were taking a statin, suggesting that statin therapy may be underused in this high-risk population. A higher prevalence of comorbidities, including nonalcoholic fatty liver disease/lipodystrophy, viral hepatitis, chronic kidney disease, and polypharmacy, and concerns of drug interactions with certain classes of ART (eg, PIs and cobicistat) may contribute to an underuse of statin therapy in beneficiaries with HIV.[18]

Several factors may contribute to the higher risk for ASCVD events among beneficiaries with versus without HIV. The LDL-C reduction after the initiation of a statin may be smaller among patients with HIV.[19] NNRTIs reduce blood-statin levels and diminish the LDL-C response to statins.[20] As a result, high-risk HIV patients taking a statin who are treated with NNRTIs may have higher LDL-C levels than their counterparts without HIV. In the current analysis, 44.2% of beneficiaries with HIV were taking an NNRTI. It is possible that a small LDL-C reduction with statins among people with HIV may have contributed to the higher risk for ASCVD events associated with HIV infection in the current study. Other mechanisms that may contribute to the higher risk for ASCVD events associated with HIV infection include higher concentrations of atherogenic remnant lipoproteins,[21] impaired macrophage cholesterol efflux,[22,23] and a higher prevalence of nonlipid risk factors, such as smoking,[24] visceral adiposity,[25,26] insulin resistance,[26,27] chronic kidney disease,[28] chronic inflammation and immune activation,[29,30] and coagulation disorders.[29,31] In 3 large international HIV treatment trials, higher biomarkers of inflammation (interleukin-6 and high sensitivity CRP [C-reactive protein]) and coagulation (D-dimer) were associated with a greater risk of a fatal ASCVD event and all-cause mortality.[31]

Some ARTs can increase the risk for ASCVD events. Prior studies have reported that the PIs indinavir and ritonavir-boosted lopinavir increase the risk for cardiovascular events.[9,32] Currently, the most commonly used ritonavir-boosted PIs are darunavir and atazanavir.[4,33] In the prospective D:A:D (Data Collection on Adverse Events of Anti-HIV Drugs) study, the use of ritonavir-boosted darunavir but not ritonavir-boosted atazanavir was associated with a higher risk of cardiovascular events.[9,32] We have previously shown that the use of PI-based ART regimens declined from 50.8% in 2007 to 25.5% in 2015 among beneficiaries with HIV in the MarketScan database.[18] It is not known whether the reduction in PI-based ART among people with HIV will be accompanied by subsequent declines in ASCVD events.

Currently, there are no data from large clinical trials to guide interventions for the prevention of ASCVD in people with HIV. Despite the lack of data, statin therapy may have antiatherothrombotic properties in HIV-infected people, beyond LDL-C lowering, because of direct effects on reducing atherogenic lipoproteins and indirect effects on mitigating proinflammatory responses.[30,34] The contribution of statin therapy to reduce ASCVD events in people with HIV is being examined in the REPRIEVE (Randomized Trial to Prevent Vascular Events in HIV).[35] Specifically, REPRIEVE is testing whether pitavastatin reduces ASCVD risk among low- to moderate-risk patients with HIV who have LDL-C $<$130 mg/dL. Pitavastatin has no major drug interactions with ART regimens and is considered a safer agent in HIV-infected patients. Results from REPRIEVE may expand current indications for statin therapy among people with HIV.

The current analysis has several strengths, including using contemporary data from a large, nationwide cohort of US adults. Patients were followed for up to 6 years. Beneficiaries with HIV were matched by age, sex, and calendar year to controls without HIV selected from the same data source. The current analysis also has potential limitations. We analyzed data from beneficiaries with and without HIV who had commercial health insurance or Medicare supplemental health insurance. Therefore, results from the current study may not be generalizable to adults with HIV without health insurance. We used claims-based algorithms to identify cardiovascular risk factors that may result in some misclassification. The algorithm used to define tobacco use has high specificity, but low sensitivity.[36] Therefore, tobacco use was likely underestimated in the current study. Frazier and colleagues estimated that the prevalence of smoking in 2014 was 33.6% among adults with HIV in the Medical Monitoring Project, a surveillance system of US adults with HIV, and 16.8% among US adults from the

general population using data from the National Health Interview Survey.[37] Data on race and ethnicity, diet, exercise, illicit drug use, and mortality are not available in MarketScan. Data on LDL-C and other lipids and estimated glomerular filtration rate are not available for most beneficiaries in the MarketScan database and, therefore, were not analyzed. In addition, we did not have access to HIV viral load, CD4 count, and inflammatory markers to explore the potential contribution of immunodeficiency and residual inflammatory risk, which may play a role in the risk for ASCVD events among individuals with HIV. We defined statin use with pharmacy fills in the 365 days before each beneficiary's index date. Some beneficiaries may have stopped or started taking a statin after their index date, which may result in misclassification.

Conclusions

In the current analysis of contemporary data, US adults with health insurance and HIV had a higher risk for ASCVD compared with their counterparts without HIV. Beneficiaries with HIV also had a higher risk for MI, stroke, and LEAD hospitalizations versus their counterparts without HIV when each of these outcomes was analyzed separately. The higher risk of ASCVD among US adults with versus without HIV was present for those taking and not taking a statin. Clinicians should assess ASCVD risk for their patients with HIV and provide guideline-recommended treatment to lower this risk.

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SUPPLEMENTAL MATERIAL

Table S1. List of antiretroviral therapy included in the current analysis by drug classes.

Drug class	Medications
Nucleoside reverse transcriptase inhibitors	Abacavir Didanosine Stavudine Zidovudine
Non-nucleoside reverse transcriptase inhibitors	Delavirdine Efavirenz Etravirine Nevirapine Rilpivirine
Protease inhibitors	Atazanavir Darunavir Fosamprenavir Indinavir Lopinavir Nelfinavir Ritonavir (excluding combinations containing paritaprevir) Saquinavir Tipranavir
Fusion inhibitors	Enfuvirtide
Entry inhibitors	Maraviroc
Integrase strand transfer inhibitors	Dolutegravir Elvitegravir Raltegravir
Pharmacokinetic enhancers	Cobicistat

Lamivudine, tenofovir and emtricitabine were not included in the list of antiretroviral drugs as these medications are also used to treat hepatitis C infection or for human immunodeficiency virus pre-exposure prophylaxis.

Table S2. Definitions for beneficiary characteristics analyzed in the current study.

Characteristic	Definition
Age	Calculated on the index date using MarketScan beneficiary summary data.
Sex	Based on MarketScan beneficiary summary data.
Calendar year	Based on the index date.
Geographic region of residence	Based on MarketScan beneficiary summary data.
History of CHD[1]	Algorithm based on ICD-9 codes: Any of the following using claims within 365 days prior to or on the index date: (a) At least 1 inpatient claim with an ICD-9 diagnosis code of 410.xx-414.xx, V45.81 or V45.82. (b) At least 1 outpatient physician evaluation and management claim with an ICD-9 diagnosis code of 410.xx-414.xx, V45.81 or V45.82. (c) At least 1 inpatient or outpatient claim with an ICD-9 procedure code of 00.66, 36.0, 36.01-36.19, 36.2 or a current procedural terminology (CPT) code of 33510-33519, 33521-33523, 33530, 33533-33536, 92980-92982, 92984, 92995, 92996, 92920, 92921, 92924, 92925, 92928, 92929, 92933, 92934, 92937, 92938, 92941, 92943, 92944. Algorithm based on ICD-10 codes: Any of the following using claims within 365 days prior to or on the index date: (a) At least 1 inpatient claim with an ICD-10 diagnosis code of I21.09, I21.19, I21.11, I21.29, I21.4, I21.3, I25.10, I25.810, I25.811, I25.812, I25.3, I25.41, I25.42, Z95.1 or Z9861. (b) At least 1 outpatient physician evaluation and management claim with an ICD-10 diagnosis code of I21.09, I21.19, I21.11, I21.29, I21.4, I21.3, I25.10, I25.810, I25.811, I25.812, I25.3, I25.41, I25.42, Z95.1 or Z9861. (c) At least 1 inpatient or outpatient claim with an ICD-10 procedure code of 0210, 0211, 0212, 0213, 0270, 0271, 0272, 0273, 02C0, 02C1, 02C2, 02C3, 3E07 or a CPT code 33510-33519, 33521-33523, 33530, 33533-33536, 92980-92982, 92984, 92995, 92996, 92920, 92921, 92924, 92925, 92928, 92929, 92933, 92934, 92937, 92938, 92941, 92943, 92944.

Characteristic	Definition
Stroke[2]	Algorithm based on ICD-9 codes: Any of the following using claims within 365 days prior to or on the index date: (a) At least 1 inpatient ICD-9 diagnosis code (any position) of 430.xx, 431.xx, 433.x1, 434.x1 or 436.x. (b) At least 1 outpatient physician evaluation and management claim with ICD-9 diagnosis code (any position) of 430.xx, 431.xx, 433.x1, 434.x1 or 436.x. Algorithm based on ICD-10 codes: Any of the following using claims within 365 days prior to or on the index date, inclusive: (a) At least 1 inpatient ICD-10 diagnosis (primary or secondary position) of I60.xx, I61.xx, I63.xx, I67.89. (b) At least 1 outpatient physician evaluation and management claim with ICD-10 diagnoses (any position) of I60.xx, I61.xx, I63.xx, I67.89.
History of peripheral artery disease[3]	Algorithm based on ICD-9 codes: Any of the following using claims within 365 days prior to or on the index date: (a) At least 1 inpatient ICD-9 diagnosis code (any position) of 440.20-440.24, 440.31, 444.2x, 443.9, or 444.81. (b) At least 2 outpatient physician evaluation and management claims with an ICD-9 diagnosis code (any position) of 440.20-440.24, 440.31, 444.2x, 443.9, or 444.81, with the 2 claims on separate days. (c) At least 1 inpatient or outpatient claim with a CPT code 37205 or 75962. Algorithm based on ICD-10 codes: Any of the following using claims within 365 days prior to or on the index date: (a) At least 1 inpatient ICD-10 diagnosis code (any position) of I70.209, I70.219, I70.229, I70.25, I70.269, I70.499, I73.9. (b) At least 2 outpatient physician evaluation and management claims with an ICD-10 diagnosis code (any position) of I70.209, I70.219, I70.229, I70.25, I70.269, I70.499, I73.9, with the 2 claims on separate days (c) At least 1 inpatient or outpatient claim with a CPT code 37205 or 75962.

Characteristic	Definition
Diabetes[4-6]	Algorithm based on ICD-9 codes: Any of the following using claims within 365 days prior to or on the index date: (a) At least 1 inpatient claim with discharge ICD-9 diagnosis (any position) of 250.xx, 357.2, 362.0x, or 366.41. (b) At least 2 outpatient physician evaluation and management claims with ICD-9 diagnosis (any position) of 250.xx, 357.2, 362.0x, or 366.41, with the 2 claims occurring at least 7 days apart (c) At least 1 prescription drug event record for an oral antidiabetic drug fill or insulin. Algorithm based on ICD-10 codes: Any of the following using claims within 365 days prior to or on the index date: (a) At least 1 inpatient claim with a discharge ICD-10 diagnosis (any position) of E0836, E0842, E0936, E0942, E1010, E1011, E1029, E10311, E10319, E1036, E1039, E1040, E1042, E1051, E10618, E10620, E10621, E10622, E10628, E10630, E10638, E10641, E10649, E1065, E1069, E108, E109, E1100, E1101, E1129, E11311, E11319, E11329, E11339, E11349, E11359, E1136, E1139, E1140, E1142, E1151, E11618, E11620, E11621, E11622, E11628, E11630, E11638, E11641, E11649, E1165, E1169, E118, E119, E1310, E1336, E1342. (b) At least 2 carrier physician evaluation and management claims with ICD-10 diagnosis (any position) of E0836, E0842, E0936, E0942, E1010, E1011, E1029, E10311, E10319, E1036, E1039, E1040, E1042, E1051, E10618, E10620, E10621, E10622, E10628, E10630, E10638, E10641, E10649, E1065, E1069, E108, E109, E1100, E1101, E1129, E11311, E11319, E11329, E11339, E11349, E11359, E1136, E1139, E1140, E1142, E1151, E11618, E11620, E11621, E11622, E11628, E11630, E11638, E11641, E11649, E1165, E1169, E118, E119, E1310, E1336, E1342, with the 2 claims occurring at least 7 days apart. (c) At least 1 prescription drug event record for an oral antidiabetic drug fill or insulin.
History of heart failure[7]	Algorithm based on ICD-9 codes: Any of the following using claims within 365 days prior to or on the index date: (a) ≥1 inpatient claim with ICD-9 diagnosis code (any position) of 402.01, 402.11, 402.91, 404.01, 404.03, 404.11, 404.13, 404.91, 404.93, 428.X (b) ≥2 outpatient physician evaluation and management claims on separate calendar days with ICD-9 diagnosis code (any position) of 402.01, 402.11, 402.91, 404.01, 404.03, 404.11, 404.13, 404.91, 404.93, 428.x. Algorithm based on ICD-10 codes: Any of the following using claims within 365 days prior to or on the index date: (a) ≥1 inpatient claim with ICD-10 diagnoses (any position) of I110, I130, I132, I501, I5020, I5021, I5022, I5023, I5030, I5031, I5032, I5033, I5040, I5041, I5042, I5043, I509 (b) ≥2 outpatient physician evaluation and management claims on separate calendar days with ICD-10 diagnoses (any position) of I110, I130, I132, I501, I5020, I5021, I5022, I5023, I5030, I5031, I5032, I5033, I5040, I5041, I5042, I5043, I509.

Characteristic	Definition
History of chronic kidney disease[8]	Algorithm based on ICD-9 codes: Any of the following using claims within 365 days prior to or on the index date: (a) At least 1 inpatient claim with ICD-9 diagnosis code (any position) of 016.0, 095.4, 189.0, 189.9, 223.0, 236.91, 250.4, 271.4, 274.1, 283.11, 403.x1, 403.x0, 404.x2, 404.x3, 404.x0, 404.x1, 440.1, 442.1, 447.3, 572.4, 580–588, 591, 642.1, 646.2, 753.12–753.17, 753.19, 753.2, 794.4. (b) At least 1 outpatient physician evaluation and management claim with ICD-9 diagnosis code (any position) of 016.0, 095.4, 189.0, 189.9, 223.0, 236.91, 250.4, 271.4, 274.1, 283.11, 403.x1, 403.x0, 404.x2, 404.x3, 404.x0, 404.x1, 440.1, 442.1, 447.3, 572.4, 580–588, 591, 642.1, 646.2, 753.12–753.17, 753.19, 753.2, 794.4 Algorithm based on ICD-10 codes: Any of the following using claims within 365 days prior to or on the index date: (a) ≥1 inpatient claim with a discharge diagnosis code of chronic kidney disease (ICD-10 CM diagnosis code of A1811, A5275, C649, C689, D3000, D4100, D4120, D593, E1021, E1029, E1121, E1129, E748, I120, I129, I130, I1310, I1311, I132, I701, I722, K767, M1030, N003, N008, N009, N013, N022, N032, N033, N035, N038, N039, N040, N043, N044, N048, N049, N052, N055, N058, N059, N08, N1330, N170, N171, N172, N178, N179, N181, N182, N183, N184, N185, N186, N189, N19, N250, N251, N2581, N2589, N259, N269,Q6102, Q6119, Q612, Q613, Q614, Q615, Q618, Q6210, Q6211, Q6212, Q6231, Q6239, R944) in any discharge diagnosis position. (b) ≥1 physician evaluation and management claim with a diagnosis code of chronic kidney disease (ICD-10 diagnosis code of A1811, A5275, C649, C689, D3000, D4100, D4120, D593, E1021, E1029, E1121, E1129, E748, I120, I129, I130, I1310, I1311, I132, I701, I722, K767, M1030, N003, N008, N009, N013, N022, N032, N033, N035, N038, N039, N040, N043, N044, N048, N049, N052, N055, N058, N059, N08, N1330, N170, N171, N172, N178, N179, N181, N182, N183, N184, N185, N186, N189, N19, N250, N251, N2581, N2589, N259, N269,Q6102, Q6119, Q612, Q613, Q614, Q615, Q618, Q6210, Q6211, Q6212, Q6231, Q6239, R944) in any position.

Characteristic	Definition
Liver disease[4, 6]	Algorithm based on ICD-9 codes: Any of the following using claims within 365 days prior to or on the index date: (a) At least 1 inpatient claim with ICD-9 diagnosis code (any position) of 070.22, 070.23, 070.32, 070.33, 070.44, 070.54, 070.6, 070.9, 456.0x–456.2x, 570.xx, 571.xx, 572.2x–572.8, 573.3, 573.4, 573.8, 573.9, V42.7. (b) At least 2 outpatient physician evaluation and management claims with ICD-9 diagnosis code (any position) of 070.22, 070.23, 070.32, 070.33, 070.44, 070.54, 070.6, 070.9, 456.0x–456.2x, 570.xx, 571.xx, 572.2–572.8, 573.3, 573.4, 573.8, 573.9, V42.7. Algorithm based on ICD-10 codes: Any of the following using claims within 365 days prior to or on the index date: (a) At least 1 inpatient claim with ICD-10 diagnosis code (any position) of B18.x, I85.xx, I86.4, I98.2, K70.x, K71.1x, K71.3-K71.5x, K71.7, K72.xx-K74.xx, K76.0, K76.2x-K76.9x, Z94.4. (b) At least 2 outpatient physician evaluation and management claims with ICD-10 diagnosis code (any position) of B18.x, I85.xx, I86.4, I98.2, K70.x, K71.1x, K71.3-K71.5x, K71.7, K72.xx-K74.xx, K76.0, K76.2x-K76.9x, Z94.4.
Depression[7, 9]	Algorithm based on ICD-9 codes: defined by any of the following using claims within 365 days prior to or on the index date. (a) At least 1 inpatient claim with ICD-9 diagnosis code (any position) of 296.2x, 296.3x, 296.5x, 300.4x, 309.xx, 311.xx. (b) At least 1outpatient physician evaluation and management claim with ICD-9 diagnosis code (any position) of 296.2x, 296.3x, 296.5x, 300.4x, 309.xx, 311.xx. (c) At least 2 pharmacy claims for amitriptyline, amoxapine, bupropion, citalopram, clomipramine, desipramine, desvenlafaxine, doxepin, duloxetine, escitalopram, fluoxetine, fluvoxamine, imipramine, isocarboxazid, levomilnacipran, maprotiline, milnacipran, mirtazapine, nefazodone, nortriptyline, paroxetine, perphenazine, phenelzine, protriptyline, selegiline, sertraline, tranylcypromine, trazodone, trimipramine or venlafaxine. Algorithm based on ICD-10 codes: defined by any of the following using claims within 365 days prior to or on the index date. (a) At least 1 inpatient claim with ICD-10 diagnosis code (any position) of F20.4, F31.3x-F31.5x, F32.x, F33.xx, F34.1, F41.2, F43.2x. (b) At least 1 outpatient physician evaluation and management claim with ICD-10 diagnosis code (any position) of F20.4, F31.3x-F31.5x, F32.x, F33.xx, F34.1, F41.2, F43.2x. (c) At least 2 pharmacy claims for amitriptyline, amoxapine, bupropion, citalopram, clomipramine, desipramine, desvenlafaxine, doxepin, duloxetine, escitalopram, fluoxetine, fluvoxamine, imipramine, isocarboxazid, levomilnacipran, maprotiline, milnacipran, mirtazapine, nefazodone, nortriptyline, paroxetine, perphenazine, phenelzine, protriptyline, selegiline, sertraline, tranylcypromine, trazodone, trimipramine or venlafaxine.
Cardiologist care	Defined by ≥1 outpatient physician evaluation and management claim with provider type code 250 within 365 days prior to or on the index date.

Characteristic	Definition
Any hospitalization	Defined by ≥1 inpatient claim within 365 days prior to or on the index date.
Tobacco use[10]	Algorithm based on ICD-9 codes: Any of the following using claims within 365 days prior to or on the index date: (a) At least 1 inpatient claim with an ICD-9 diagnosis code of 305.1, 649.0x, 989.84, or V15.82 (b) At least 1 outpatient physician evaluation and management claim with an ICD-9 diagnosis code of 305.1, 649.0x, 989.84, or V15.82 (c) At least 1 inpatient or E/M outpatient claim with a CPT code of 99406, 99407, G0436, G0437, G9016, S9453, S4995, G9276, G9458, 1034F, 4004F, 4001F (d) At least 1 pharmacy claim for nicotine or varenicline Algorithm based on ICD-10 codes: Any of the following using claims within 365 days prior to or on the index date: (a) At least 1 inpatient claim with an ICD-10 diagnosis code of F17.200, O99.330-O99.335, T65.221A, T65.222A, T65.223A, T65.224A, T65.291A, T65.292A, T65.293A, T65.294A, Z72.0, Z87.891 (b) At least 1 outpatient physician evaluation and management claim with an ICD-10 diagnosis code of F17.200, O99.330-O99.335, T65.221A, T65.222A, T65.223A, T65.224A, T65.291A, T65.292A, T65.293A, T65.294A, Z72.0, Z87.891 (c) At least 1 inpatient or E/M outpatient claim with a CPT code of 99406, 99407, G0436, G0437, G9016, S9453, S4995, G9276, G9458, 1034F, 4004F, 4001F (d) At least 1 pharmacy claim for nicotine or varenicline
Antihypertensive medication use	Defined by ≥1 prescription fill for any thiazides, angiotensin-converting-enzyme inhibitors, angiotensin II receptor blockers, calcium channel blockers, diuretics, beta blockers, direct renin inhibitors, alpha-1 blockers, central $alpha_1$-agonists or direct vasodilators within 365 days prior to or on the index date.
Statin use and intensity	No statin use was defined by having no prescription fill for any statin dose and type within 365 days prior to or on the index date. Use of low/moderate-intensity statin was defined by ≥1 prescription fill for a statin but no high-intensity statin fills within 365 days prior to or on the index date. Use of high-intensity statin was defined by ≥1 prescription fill for any high-intensity statin in the 365 days prior to the MI hospital discharge date, inclusive. High-intensity statin includes atorvastatin 40-80 mg, rosuvastatin 20-40 mg and simvastatin 80 mg.
Non-statin lipid-lowering medication use	Defined by ≥1 prescription fill for ezetimibe, fibrates, niacin, or bile acid sequestrants, within 365 days prior to or on the index date.
Polypharmacy	Defined by having prescription fills for ≥10 different medications (based on the generic names) within 365 days prior to or on the index date. Fills for the same generic name were counted only once. For example, 10 prescription fills for atorvastatin were count as filling 1 medication.

CHD: coronary heart disease; CPT: current procedural terminology; ICD-9: international classification of diseases, ninth revision; ICD-10: international classification of diseases, tenth revision.

Table S3. Definition for outcomes events analyzed in the current study.

Outcome	Definition
Atherosclerotic cardiovascular disease hospitalization	Includes myocardial infarction, stroke, or lower extremity artery disease hospitalizations, as defined below
Myocardial infarction hospitalization	Overnight inpatient claim with an ICD-9 discharge diagnosis code of 410.xx except 410.x2 which represents a subsequent episode of care or an ICD-10 code I21.xx in any position.
Stroke hospitalization	Inpatient claim with an ICD-9 discharge diagnosis code of 430.xx, 431.xx, 433.x1, 434.x1 or 436.xx or an ICD-10 discharge diagnosis code of I60, I61 or I63 in the primary position.
Lower extremities artery disease hospitalization	The earliest of the following events: • An overnight inpatient claim with a discharge diagnosis code for acute limb ischemia in the primary discharge diagnosis position. • An overnight inpatient claim with a procedure code for embolectomy, thrombectomy or peripheral surgical revascularization in any position. • An overnight inpatient claim with a procedure code for thrombolysis in the absence of a discharge diagnosis code for acute myocardial infarction, ischemic stroke or pulmonary embolism in any position. • An overnight inpatient claim with a procedure code for lower extremity amputation above the ankle in any position, in the absence of a discharge diagnosis code for traumatic amputation of a leg on the same hospitalization. Amputations were counted as an event only if the patient had ≥1 inpatient or outpatient claim with a diagnosis code for peripheral artery disease in any position prior to or on the date of the amputation. *List of codes:* ICD9 diagnosis codes for acute limb ischemia: 444.0, 444.01, 444.09, 444.22, 444.81. ICD10 diagnosis codes for acute limb ischemia: I74.01, I74.09, I74.3, I74.5. CPT procedure codes for embolectomy or thrombectomy: 34201, 34203. ICD9 procedure codes for peripheral surgical revascularization: 38.08, 38.16, 38.18, 38.38, 38.48, 38.68, 38.88, 39.25, 39.29. ICD10 procedure codes for peripheral surgical revascularization: 0312096, 0312097, 0312098, 0312099, 031209B, 031209C, 03120A6, 03120A7, 03120A8, 03120A9, 03120AB, 03120AC, 03120J6, 03120J7, 03120J8, 03120J9, 03120JB, 03120JC, 03120K6, 03120K7, 03120K8, 03120K9, 03120KB, 03120KC, 03120Z6, 03120Z7, 03120Z8, 03120Z9, 03120ZB, 03120ZC, 031309B, 031309C, 03130A6, 03130A7, 03130A8, 03130A9, 03130AB, 03130AC, 03130J6, 03130J7, 03130J8, 03130J9, 03130JB, 03130JC, 03130K6, 03130K7, 03130K8, 03130K9, 03130KB,

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313099.

CPT procedure codes for peripheral surgical revascularization: 35302, 35303, 35304, 35305, 35351, 35355, 35361, 35363, 35371, 35372, 35381, 35480, 35481, 35482, 35483, 35485, 35521, 35537, 35538, 35539, 35540, 35541, 35546, 35548, 35549, 35551, 35556, 35558, 35563, 35565, 35566, 35570, 35571, 35583, 35585, 35587, 35621, 35623, 35641, 35646, 35647, 35651, 35654, 35656, 35661, 35663, 35665, 35666, 35671, 35875, 35876.

ICD9 procedure codes for thrombolysis: 99.10.

ICD10 procedure codes for thrombolysis: 3E03317, 3E04317, 3E05317, 3E06317, 3E08317.

CPT procedure codes for thrombolysis: 37184, 37211, 37213.

ICD9 diagnosis codes for acute myocardial infarction: 410.x0, 410.x1.

ICD10 diagnosis codes for acute myocardial infarction: I21.x, I21.xx.

ICD9 diagnosis codes for ischemic stroke: 433.x1, 434.x1.

ICD10 diagnosis codes for ischemic stroke: I63, I63.x, I63.xx, I63.xxx.

ICD9 diagnosis code for pulmonary embolism: 415.1x.

ICD10 diagnosis codes for pulmonary embolism: T80.0XXA, T81.718A, T81.72XA, T82.817A, T82.818A, I26.90, I26.99.

ICD9 procedure codes for lower extremity amputation above the ankle: 84.13, 84.14, 84.15, 84.16, 84.17.

ICD10 procedure codes for lower extremity amputation above the ankle: 0Y6M0Z0, 0Y6N0Z0, 0Y6H0Z3, 0Y6J0Z3, 0Y670ZZ, 0Y680ZZ, 0Y6C0Z1, 0Y6C0Z3, 0Y6D0Z1, 0Y6D0Z2, 0Y6D0Z3, 0Y6F0ZZ, 0Y6G0ZZ, 0Y6H0Z1, 0Y6H0Z2, 0Y6J0Z1, 0Y6J0Z2, 0Y620ZZ, 0Y630ZZ, 0Y640ZZ.

CPT procedure codes for lower extremity amputation above the ankle: 27590, 27591, 27592, 27598, 27880, 27881, 27882, 27888.

ICD9 diagnosis code for traumatic amputation of a leg: 897.x.

ICD10 diagnosis codes for traumatic amputation of a leg: S78.xxxA, S88.xxxA, where xxx can be any 3-digit number.

ICD9 diagnosis codes for peripheral artery disease: 440.2, 440.20, 440.21, 440.22, 440.23, 440.24, 440.29, 440.3, 440.30, 440.31, 440.32, 440.4, 443.9.

ICD10 diagnosis codes for peripheral artery disease: I70.2, I70.20, I70.201, I70.202, I70.203, I70.208, I70.209, I70.21, I70.211, I70.212, I70.213, I70.218, I70.219, I70.22, I70.221, I70.222, I70.223, I70.228, I70.229, I70.23, I70.231, I70.232, I70.233, I70.234, I70.235, I70.238, I70.239, I70.24, I70.241, I70.242, I70.243, I70.244, I70.245, I70.248,

I70.249, I70.25, I70.26, I70.261, I70.262, I70.263, I70.268, I70.269, I70.29, I70.291, I70.292, I70.293, I70.298, I70.299, I70.3, I70.30, I70.301, I70.302, I70.303, I70.308, I70.309, I70.31, I70.311, I70.312, I70.313, I70.318, I70.319, I70.32, I70.321, I70.322, I70.323, I70.328, I70.329, I70.33, I70.331, I70.332, I70.333, I70.334, I70.335, I70.338, I70.339, I70.34, I70.341, I70.342, I70.343, I70.344, I70.345, I70.348, I70.349, I70.35, I70.36, I70.361, I70.362, I70.363, I70.368, I70.369, I70.39, I70.391, I70.392, I70.393, I70.398, I70.399, I70.4, I70.40, I70.401, I70.402, I70.403, I70.408, I70.409, I70.41, I70.411, I70.412, I70.413, I70.418, I70.419, I70.42, I70.421, I70.422, I70.423, I70.428, I70.429, I70.43, I70.431, I70.432, I70.433, I70.434, I70.435, I70.438, I70.439, I70.44, I70.441, I70.442, I70.443, I70.444, I70.445, I70.448, I70.449, I70.45, I70.46, I70.461, I70.462, I70.463, I70.468, I70.469, I70.49, I70.491, I70.492, I70.493, I70.498, I70.499, I70.5, I70.50, I70.501, I70.502, I70.503, I70.508, I70.509, I70.51, I70.511, I70.512, I70.513, I70.518, I70.519, I70.52, I70.521, I70.522, I70.523, I70.528, I70.529, I70.53, I70.531, I70.532, I70.533, I70.534, I70.535, I70.538, I70.539, I70.54, I70.541, I70.542, I70.543, I70.544, I70.545, I70.548, I70.549, I70.55, I70.56, I70.561, I70.56, I70.562, I70.563, I70.568, I70.569, I70.59, I70.591, I70.592, I70.593, I70.598, I70.599, I70.6, I70.60, I70.601, I70.602, I70.603, I70.608, I70.609, I70.61, I70.611, I70.612, I70.613, I70.618, I70.619, I70.62, I70.621, I70.622, I70.623, I70.628, I70.629, I70.63, I70.631, I70.632, I70.633, I70.634, I70.635, I70.638, I70.639, I70.64, I70.641, I70.642, I70.643, I70.644, I70.645, I70.648, I70.649, I70.65, I70.66, I70.661, I70.662, I70.663, I70.668, I70.669, I70.69, I70.691, I70.692, I70.693, I70.698, I70.699, I70.7, I70.70, I70.701, I70.702, I70.703, I70.708, I70.709, I70.71, I70.711, I70.712, I70.713, I70.718, I70.719, I70.72, I70.721, I70.722, I70.723, I70.728, I70.729, I70.73, I70.731, I70.732, I70.733, I70.734, I70.735, I70.738, I70.739, I70.74, I70.741, I70.742, I70.743, I70.744, I70.745, I70.748, I70.749, I70.75, I70.76, I70.761, I70.762, I70.763, I70.768, I70.769, I70.79, I70.791, I70.792, I70.793, I70.798, I70.799, I70.92, I73.9.

CPT: current procedural terminology; ICD-9: international classification of diseases, ninth revision; ICD-10: international classification of diseases, tenth revision.

Table S4. Hazard ratios for atherosclerotic cardiovascular disease, myocardial infarction, stroke and lower extremity artery disease hospitalizations among beneficiaries with versus without HIV across subgroups defined by beneficiary characteristics in the MarketScan database.

	ASCVD		Myocardial infarction		Stroke		LEAD	
	HR (95% CI)	p-value*	HR (95% CI)	p-value*	HR (95% CI)	p-value*	HR (95% CI)	p-value*
Age, years								
19-44	1.78 (1.40, 2.26)		1.60 (1.18, 2.16)		2.10 (1.37, 3.21)		1.96 (0.74, 5.18)	
45-54	1.24 (1.08, 1.42)	0.04	1.25 (1.06, 1.47)	0.36	1.10 (0.83, 1.46)	0.08	1.46 (0.93, 2.30)	0.80
55-64	1.23 (1.07, 1.40)		1.17 (0.99, 1.38)		1.34 (1.05, 1.72)		1.39 (0.90, 2.16)	
≥65	1.17 (0.93, 1.48)		1.24 (0.94, 1.66)		1.12 (0.73, 1.73)		1.04 (0.48, 2.23)	
Calendar year								
2011	1.28 (1.15, 1.43)		1.23 (1.08, 1.41)		1.34 (1.09, 1.65)		1.52 (1.06, 2.18)	
2012	1.33 (1.08, 1.63)		1.33 (1.04, 1.70)		1.14 (0.76, 1.69)		1.49 (0.70, 3.19)	
2013	1.15 (0.87, 1.52)	0.44	1.04 (0.74, 1.48)	0.10	1.38 (0.82, 2.32)	0.94	1.42 (0.57, 3.53)	†
2014	1.32 (0.96, 1.81)		1.35 (0.91, 2.03)		1.58 (0.92, 2.72)		0.60 (0.17, 2.07)	
2015	1.72 (1.25, 2.36)		2.12 (1.43, 3.14)		1.18 (0.64, 2.17)		1.82 (0.71, 4.70)	
2016	1.02 (0.57, 1.85)		0.93 (0.44, 1.97)		1.17 (0.39, 3.51)		†	
Men	1.30 (1.19, 1.42)	0.89	1.27 (1.14, 1.42)	0.74	1.32 (1.11, 1.56)	0.62	1.45 (1.08, 1.93)	0.58
Women	1.27 (0.99, 1.62)		1.20 (0.87, 1.64)		1.47 (0.97, 2.25)		1.13 (0.46, 2.75)	
Region of residence								
Northeast	1.24 (1.03, 1.49)		1.21 (0.97, 1.52)		1.23 (0.87, 1.74)		0.98 (0.50, 1.92)	
North central	1.25 (1.03, 1.51)	0.97	1.21 (0.96, 1.53)	0.81	1.39 (0.97, 1.98)	0.82	1.56 (0.77, 3.16)	0.39
South	1.33 (1.18, 1.51)		1.30 (1.11, 1.52)		1.29 (1.01, 1.63)		1.65 (1.13, 2.41)	
West	1.29 (1.04, 1.59)		1.36 (1.06, 1.74)		1.50 (0.99, 2.26)		0.73 (0.31, 1.74)	
History of CHD	1.35 (1.13, 1.63)	0.56	1.38 (1.12, 1.71)	0.33	1.26 (0.82, 1.93)	0.81	1.22 (0.66, 2.25)	0.57
Without history of CHD	1.26 (1.15, 1.39)		1.21 (1.08, 1.36)		1.33 (1.12, 1.57)		1.46 (1.07, 1.98)	
Diabetes	1.17 (1.00, 1.38)	0.26	1.21 (0.99, 1.48)	0.69	1.22 (0.90, 1.65)	0.70	0.74 (0.41, 1.32)	0.01
Without diabetes	1.32 (1.20, 1.45)		1.27 (1.13, 1.43)		1.31 (1.09, 1.58)		1.73 (1.26, 2.38)	
Stroke	0.99 (0.63, 1.56)	0.27	1.07 (0.49, 2.31)	0.62	0.89 (0.49, 1.61)	0.20	2.19 (0.47, 10.15)	0.53
Without stroke	1.30 (1.20, 1.42)		1.28 (1.16, 1.42)		1.34 (1.14, 1.58)		1.40 (1.06, 1.86)	
Peripheral artery disease	1.19 (0.74, 1.93)	0.71	0.63 (0.29, 1.39)	0.08	3.97 (0.86, 18.27)	0.19	1.55 (0.79, 3.04)	0.71
Without peripheral artery disease	1.29 (1.19, 1.41)		1.29 (1.16, 1.42)		1.29 (1.10, 1.52)		1.33 (0.98, 1.80)	
Heart failure	1.62 (1.08, 2.43)	0.25	1.53 (0.94, 2.49)	0.44	1.73 (0.70, 4.29)	0.53	0.88 (0.28, 2.81)	0.44
Without heart failure	1.27 (1.17, 1.38)		1.25 (1.13, 1.39)		1.30 (1.10, 1.52)		1.40 (1.05, 1.87)	
Chronic kidney disease	1.19 (0.94, 1.50)	0.50	1.01 (0.77, 1.33)	0.11	1.64 (1.04, 2.58)	0.33	1.09 (0.50, 2.37)	0.48
Without chronic kidney disease	1.29 (1.18, 1.41)		1.29 (1.16, 1.44)		1.27 (1.08, 1.51)		1.49 (1.11, 1.99)	
Liver disease	0.97 (0.56, 1.69)	0.27	1.25 (0.61, 2.54)	0.93	0.76 (0.22, 2.62)	0.40	0.18 (0.03, 0.98)	0.01
Without liver disease	1.30 (1.20, 1.42)		1.28 (1.15, 1.41)		1.32 (1.13, 1.54)		1.50 (1.13, 1.98)	
Cardiologist care	1.49 (1.09, 2.03)	0.36	1.44 (1.00, 2.08)	0.47	1.74 (0.90, 3.37)	0.39	1.28 (0.46, 3.56)	0.90
Without cardiologist care	1.28 (1.17, 1.39)		1.26 (1.13, 1.40)		1.28 (1.09, 1.50)		1.38 (1.04, 1.84)	

Prior hospitalization	1.47 (1.24, 1.75)	0.04	1.40 (1.13, 1.73)	0.24	1.77 (1.27, 2.46)	0.02	1.04 (0.63, 1.71)	0.23
Without prior hospitalization	1.20 (1.09, 1.32)		1.20 (1.07, 1.35)		1.16 (0.97, 1.40)		1.53 (1.10, 2.12)	
Depression	1.18 (1.00, 1.40)	0.18	1.23 (1.00, 1.51)	0.63	1.18 (0.86, 1.63)	0.42	0.98 (0.55, 1.76)	0.18
Without depression	1.34 (1.22, 1.47)		1.30 (1.16, 1.46)		1.35 (1.13, 1.62)		1.53 (1.12, 2.09)	
Tobacco use	1.39 (1.07, 1.80)	0.57	1.32 (0.94, 1.85)	0.83	1.25 (0.75, 2.10)	0.92	2.01 (1.01, 4.00)	0.29
No tobacco use	1.28 (1.17, 1.39)		1.26 (1.14, 1.40)		1.30 (1.10, 1.53)		1.33 (0.98, 1.80)	
Polypharmacy	1.17 (1.03, 1.32)	0.06	1.18 (1.02, 1.37)	0.25	1.14 (0.91, 1.44)	0.21	1.17 (0.81, 1.69)	0.18
Without polypharmacy	1.36 (1.22, 1.52)		1.31 (1.14, 1.51)		1.41 (1.14, 1.74)		1.71 (1.15, 2.55)	
Antihypertensive medication use	1.27 (1.14, 1.42)	0.63	1.28 (1.13, 1.46)	0.34	1.26 (1.02, 1.55)	0.99	1.24 (0.88, 1.74)	0.18
No antihypertensive medication use	1.23 (1.08, 1.40)		1.16 (0.99, 1.37)		1.27 (0.99, 1.62)		1.82 (1.16, 2.87)	
No statin use	1.26 (1.13, 1.40)		1.20 (1.05, 1.37)		1.33 (1.09, 1.61)		1.59 (1.11, 2.28)	
Low/moderate-intensity statin use	1.29 (1.11, 1.51)	0.84	1.32 (1.10, 1.59)	0.66	1.25 (0.92, 1.68)	0.88	1.07 (0.63, 1.83)	0.48
High-intensity statin use	1.18 (0.91, 1.53)		1.18 (0.88, 1.60)		1.16 (0.64, 2.11)		1.38 (0.66, 2.89)	
Non-statin lipid lowering medication use	1.14 (0.93, 1.40)	0.23	1.11 (0.87, 1.41)	0.24	1.22 (0.80, 1.86)	0.76	1.21 (0.63, 2.32)	0.60
No non-statin lipid lowering medication use	1.31 (1.20, 1.43)		1.30 (1.16, 1.45)		1.30 (1.10, 1.54)		1.45 (1.07, 1.97)	

ASCVD: atherosclerotic cardiovascular disease; CHD: coronary heart disease; CI: confidence interval; HIV: human immunodeficiency virus; HR: hazard ratio; LEAD: lower extremity artery disease.

HRs include adjustment for age, sex, calendar year, geographic region of residence, history of CHD, diabetes, stroke, peripheral artery disease, heart failure, chronic kidney disease, liver disease, cardiologist care, any hospitalization, depression, tobacco use, polypharmacy, antihypertensive medication use, statin use and statin intensity, and non-statin lipid-lowering medication use.

* Comparing HR for outcome events associated with HIV infection across subgroups defined by beneficiary characteristics.
† Data not shown given the small number of events. Specifically, there were 6 LEAD hospitalizations during follow-up among beneficiaries in 2016.

Table S5. Characteristics of beneficiaries with HIV and age, sex and calendar year-matched beneficiaries without HIV stratified by statin use in the MarketScan database.

	Taking statins		Not taking statins	
	Beneficiaries without HIV (n=53,842)	Beneficiaries with HIV (n=15,619)	Beneficiaries without HIV (n=275,862)	Beneficiaries with HIV (n=66,807)
Age, years, n (%)				
19-44	5,223 (9.7%)	2,190 (14.0%)	135,377 (49.1%)	32,960 (49.3%)
45-54	22,741 (42.2%)	6,742 (43.2%)	95,731 (34.7%)	22,876 (34.2%)
55-64	20,732 (38.5%)	5,454 (34.9%)	39,596 (14.4%)	9,628 (14.4%)
≥65	5,146 (9.6%)	1,233 (7.9%)	5,158 (1.9%)	1,343 (2.0%)
Calendar year, n (%)				
2011	24,412 (45.3%)	7,558 (48.4%)	112,104 (40.6%)	26,571 (39.8%)
2012	8,423 (15.6%)	2,366 (15.1%)	44,821 (16.2%)	10,945 (16.4%)
2013	5,467 (10.2%)	1,509 (9.7%)	31,049 (11.3%)	7,620 (11.4%)
2014	5,521 (10.3%)	1,442 (9.2%)	31,311 (11.4%)	7,766 (11.6%)
2015	4,706 (8.7%)	1,303 (8.3%)	27,386 (9.9%)	6,720 (10.1%)
2016	5,313 (9.9%)	1,441 (9.2%)	29,191 (10.6%)	7,185 (10.8%)
Male sex, n (%)	47,810 (88.8%)	13,650 (87.4%)	228,738 (82.9%)	55,487 (83.1%)
Geographic region of residence, n (%)				
Northeast	10,114 (18.8%)	3,027 (19.4%)	51,405 (18.6%)	12,303 (18.4%)
North central	12,756 (23.7%)	1,990 (12.7%)	62,688 (22.7%)	9,196 (13.8%)
South	21,658 (40.2%)	6,700 (42.9%)	10,4873 (38.0%)	31,555 (47.2%)
West	8,746 (16.2%)	3,671 (23.5%)	54,003 (19.6%)	12,886 (19.3%)
Unknown	568 (1.1%)	231 (1.5%)	2,893 (1.0%)	867 (1.3%)
History of CHD, n (%)	7,370 (13.7%)	1,905 (12.2%)	2,334 (0.8%)	910 (1.4%)
Diabetes, n (%)	14,571 (27.1%)	3,253 (20.8%)	10,148 (3.7%)	3,411 (5.1%)
History of stroke, %	547 (1.0%)	265 (1.7%)	360 (0.1%)	331 (0.5%)
History of peripheral artery disease, n (%)	353 (0.7%)	153 (1.0%)	226 (0.1%)	156 (0.2%)
History of heart failure, n (%)	585 (1.1%)	273 (1.7%)	419 (0.2%)	427 (0.6%)
Chronic kidney disease, n (%)	2,245 (4.2%)	1,218 (7.8%)	2,384 (0.9%)	2,631 (3.9%)
Liver disease, n (%)	267 (0.5%)	340 (2.2%)	1,028 (0.4%)	1,985 (3.0%)
Cardiologist care, n (%)	2,728 (5.1%)	1,229 (7.9%)	3,899 (1.4%)	1,578 (2.4%)
Any hospitalization, n (%)	4,054 (7.5%)	1,884 (12.1%)	8,927 (3.2%)	8,244 (12.3%)
Depression, n (%)	10,635 (19.8%)	4,949 (31.7%)	28,744 (10.4%)	14,720 (22.0%)
Tobacco use, n (%)	2,577 (4.8%)	1,038 (6.6%)	6,841 (2.5%)	4,602 (6.9%)
Polypharmacy, n (%)	15,515 (28.8%)	8,611 (55.1%)	17,368 (6.3%)	18,991 (28.4%)
Antihypertensive medication use, n (%)	35,045 (65.1%)	8,762 (56.1%)	42,688 (15.5%)	14,978 (22.4%)
Statin use, n (%)				
No statin use	-	-	275,862 (100%)	66,807 (100%)
Low/moderate-intensity statin use	44,421 (82.5%)	12,049 (77.1%)	-	-
High-intensity statin use	9,421 (17.5%)	3,570 (22.9%)	-	-
Non-statin lipid-lowering medication use, n (%)	9,266 (17.2%)	3,599 (23.0%)	5,478 (2.0%)	2,959 (4.4%)
ART use, n (%)	-	15,243 (97.6%)	-	63,852 (95.6%)
NRTIs	-	6,672 (42.7%)	-	34,700 (51.9%)
NNRTI	-	6,768 (43.3%)	-	29,697 (44.5%)
Protease inhibitors	-	4,228 (27.1%)	-	16,485 (24.7%)
Other	-	3,043 (19.5%)	-	14,847 (22.2%)

ART: antiretroviral therapy; CHD: coronary heart disease; HIV: human immunodeficiency virus; NNRTI: non-nucleoside reverse transcriptase inhibitors; NRTI: nucleoside reverse transcriptase inhibitors.
Other ART includes fusion inhibitors, entry inhibitors, integrase strand transfer inhibitors, and pharmacokinetic enhancers.

Table S6. Hazard ratios for an atherosclerotic cardiovascular disease hospitalization among beneficiaries with versus without HIV across subgroups defined by beneficiary characteristics stratified by statin use.

	Taking a statin		Not taking a statin	
	HR (95% CI)	p-value*	HR (95% CI)	p-value*
Age, years				
19-44	1.36 (0.79, 2.33)		1.80 (1.38, 2.36)	
45-54	1.43 (1.13, 1.80)	0.76	1.14 (0.97, 1.35)	0.03
55-64	1.20 (0.99, 1.47)		1.23 (1.03, 1.48)	
≥65	1.26 (0.91, 1.74)		1.06 (0.75, 1.50)	
Calendar year				
2011	1.29 (1.09, 1.53)		1.23 (1.07, 1.42)	
2012	1.24 (0.89, 1.72)		1.30 (1.00, 1.69)	
2013	1.38 (0.87, 2.19)	>0.99	0.92 (0.64, 1.33)	0.08
2014	1.17 (0.70, 1.97)		1.36 (0.90, 2.04)	
2015	1.24 (0.65, 2.35)		1.93 (1.33, 2.80)	
2016	1.29 (0.49, 3.36)		0.74 (0.33, 1.63)	
Men	1.28 (1.12, 1.48)	0.82	1.27 (1.13, 1.42)	0.99
Women	1.22 (0.82, 1.82)		1.26 (0.92, 1.73)	
Region of residence				
Northeast	1.33 (1.00, 1.77)		1.12 (0.88, 1.42)	
North central	1.28 (0.94, 1.74)	0.57	1.21 (0.94, 1.55)	0.61
South	1.19 (0.97, 1.47)		1.40 (1.19, 1.64)	
West	1.43 (1.03, 1.97)		1.17 (0.88, 1.55)	
History of CHD	1.29 (1.04, 1.61)	0.96	1.55 (1.09, 2.21)	0.24
Without history of CHD	1.28 (1.08, 1.50)		1.22 (1.09, 1.37)	
Diabetes	1.11 (0.89, 1.38)	0.14	1.24 (0.96, 1.60)	0.99
Without diabetes	1.38 (1.17, 1.63)		1.24 (1.10, 1.39)	
Stroke	0.67 (0.35, 1.30)	0.06	1.41 (0.71, 2.79)	0.66
Without stroke	1.32 (1.15, 1.50)		1.25 (1.12, 1.39)	
Peripheral artery disease	0.75 (0.36, 1.55)	0.13	2.15 (1.03, 4.49)	0.11
Without peripheral artery disease	1.31 (1.14, 1.49)		1.23 (1.11, 1.38)	
Heart failure	2.43 (1.40, 4.20)	0.02	1.18 (0.64, 2.17)	0.87
Without heart failure	1.24 (1.08, 1.42)		1.25 (1.12, 1.39)	
Chronic kidney disease	1.28 (0.93, 1.77)	0.97	1.07 (0.77, 1.49)	0.32
Without chronic kidney disease	1.28 (1.11, 1.48)		1.26 (1.12, 1.41)	
Liver disease	4.07 (1.24, 13.32)	0.07	0.52 (0.26, 1.02)	0.01
Without liver disease	1.25 (1.10, 1.43)		1.28 (1.15, 1.43)	
Cardiologist care	1.53 (1.04, 2.24)	0.31	1.52 (0.90, 2.59)	0.49
Without cardiologist care	1.24 (1.08, 1.43)		1.25 (1.12, 1.40)	
Prior hospitalization	1.58 (1.23, 2.04)	0.05	1.37 (1.09, 1.73)	0.25
Without prior hospitalization	1.18 (1.01, 1.38)		1.18 (1.04, 1.33)	
Depression	1.30 (1.02, 1.65)	0.89	1.04 (0.82, 1.32)	0.07
Without depression	1.27 (1.09, 1.49)		1.32 (1.17, 1.49)	
Tobacco use	1.33 (0.88, 2.01)	0.85	1.40 (0.99, 1.98)	0.45
No tobacco use	1.26 (1.10, 1.45)		1.23 (1.10, 1.38)	
Polypharmacy	1.17 (1.00, 1.38)	0.05	1.14 (0.95, 1.37)	0.33
Without polypharmacy	1.55 (1.24, 1.93)		1.27 (1.11, 1.45)	
Antihypertensive medication use	1.27 (1.10, 1.47)	0.97	1.25 (1.07, 1.47)	0.65
No antihypertensive medication use	1.28 (0.95, 1.72)		1.20 (1.03, 1.38)	
Non-statin lipid lowering medication use	1.04 (0.81, 1.34)	0.08	1.40 (0.97, 2.01)	0.59
No non-statin lipid lowering medication use	1.37 (1.18, 1.60)		1.24 (1.11, 1.38)	

CHD: coronary heart disease; CI: confidence interval; HIV: human immunodeficiency virus; HR: hazard ratio.

Hazard ratios include adjustment for age, sex, calendar year, geographic region of residence, history of CHD, diabetes, stroke, peripheral artery disease, heart failure, chronic kidney disease, liver disease, cardiologist care, any hospitalization, depression, tobacco use, polypharmacy, antihypertensive medication use, and non-statin lipid-lowering medication use.

* Comparing HR for atherosclerotic cardiovascular disease hospitalizations associated with HIV infection across subgroups defined by beneficiary characteristics.

Table S7. Hazard ratios for a myocardial infarction hospitalization among beneficiaries with versus without HIV across subgroups defined by beneficiary characteristics stratified by statin use.

	Taking a statin		Not taking a statin	
	HR (95% CI)	p-value*	HR (95% CI)	p-value*
Age, years				
19-44	1.14 (0.60, 2.18)		1.66 (1.18, 2.35)	
45-54	1.50 (1.15, 1.96)	0.57	1.10 (0.90, 1.35)	0.19
55-64	1.17 (0.92, 1.50)		1.14 (0.90, 1.44)	
≥65	1.34 (0.90, 1.98)		1.08 (0.71, 1.66)	
Calendar year				
2011	1.28 (1.05, 1.56)		1.16 (0.97, 1.39)	
2012	1.21 (0.81, 1.80)		1.32 (0.96, 1.81)	
2013	1.48 (0.86, 2.53)	0.92	0.71 (0.44, 1.14)	0.01
2014	1.14 (0.58, 2.24)		1.41 (0.84, 2.36)	
2015	1.84 (0.86, 3.93)		2.14 (1.34, 3.42)	
2016	1.93 (0.62, 5.99)		0.47 (0.15, 1.47)	
Men	1.32 (1.12, 1.56)	0.41	1.19 (1.04, 1.37)	0.67
Women	1.05 (0.65, 1.70)		1.30 (0.85, 1.98)	
Geographic region of residence				
Northeast	1.38 (0.98, 1.94)		1.01 (0.74, 1.37)	
North central	1.46 (1.02, 2.07)	0.75	1.04 (0.75, 1.42)	0.46
South	1.15 (0.89, 1.48)		1.37 (1.13, 1.67)	
West	1.52 (1.05, 2.20)		1.21 (0.86, 1.70)	
History of CHD	1.32 (1.03, 1.69)	0.93	1.60 (1.06, 2.41)	0.15
Without history of CHD	1.28 (1.05, 1.57)		1.15 (1.00, 1.32)	
Diabetes	1.16 (0.89, 1.50)	0.32	1.25 (0.91, 1.72)	0.74
Without diabetes	1.39 (1.14, 1.69)		1.16 (1.01, 1.35)	
Stroke	0.68 (0.22, 2.15)	0.25	1.36 (0.39, 4.78)	0.91
Without stroke	1.32 (1.13, 1.55)		1.20 (1.05, 1.37)	
Peripheral artery disease	0.40 (0.11, 1.49)	0.07	0.93 (0.29, 2.98)	0.62
Without peripheral artery disease	1.33 (1.14, 1.56)		1.20 (1.05, 1.37)	
Heart failure	2.35 (1.19, 4.65)	0.10	1.12 (0.55, 2.30)	0.93
Without heart failure	1.27 (1.08, 1.49)		1.18 (1.03, 1.35)	
Chronic kidney disease	1.17 (0.79, 1.72)	0.58	0.85 (0.57, 1.27)	0.09
Without chronic kidney disease	1.32 (1.11, 1.57)		1.21 (1.05, 1.40)	
Liver disease	5.63 (0.97, 32.61)	0.14	0.67 (0.29, 1.57)	0.17
Without liver disease	1.28 (1.09, 1.50)		1.21 (1.06, 1.39)	
Cardiologist care	1.60 (1.02, 2.50)	0.31	1.26 (0.67, 2.39)	0.88
Without cardiologist care	1.26 (1.06, 1.49)		1.20 (1.05, 1.38)	
Prior hospitalization	1.68 (1.23, 2.29)	0.08	1.17 (0.88, 1.56)	>0.99
Without prior hospitalization	1.20 (1.00, 1.44)		1.16 (1.00, 1.35)	
Depression	1.44 (1.08, 1.93)	0.41	1.01 (0.76, 1.36)	0.17
Without depression	1.26 (1.04, 1.52)		1.26 (1.09, 1.46)	
Tobacco use	1.54 (0.91, 2.62)	0.51	1.11 (0.71, 1.73)	0.76
No tobacco use	1.28 (1.08, 1.51)		1.19 (1.04, 1.37)	
Polypharmacy	1.20 (0.99, 1.46)	0.10	1.12 (0.89, 1.41)	0.64
Without polypharmacy	1.56 (1.20, 2.02)		1.18 (1.00, 1.40)	
Antihypertensive medication use	1.33 (1.12, 1.58)	0.46	1.20 (0.98, 1.46)	0.70
No antihypertensive medication use	1.16 (0.80, 1.67)		1.13 (0.95, 1.36)	
Non-statin lipid lowering medication use	1.04 (0.77, 1.40)	0.09	1.27 (0.81, 1.98)	0.77
No non-statin lipid lowering medication use	1.42 (1.18, 1.70)		1.18 (1.02, 1.35)	

CHD: coronary heart disease; CI: confidence interval; HIV: human immunodeficiency virus; HR: hazard ratio.

Hazard ratios include adjustment for age, sex, calendar year, geographic region of residence, history of CHD, diabetes, stroke, peripheral artery disease, heart failure, chronic kidney disease, liver disease, cardiologist care, any hospitalization, depression, tobacco use, polypharmacy, antihypertensive medication use, and non-statin lipid-lowering medication use.

* Comparing HR for myocardial infarction hospitalizations associated with HIV infection across subgroups defined by beneficiary characteristics.

Table S8. Hazard ratios for a stroke hospitalization among beneficiaries with versus without HIV across subgroups defined by beneficiary characteristics stratified by statin use.

	Taking a statin		Not taking a statin	
	HR (95% CI)	p-value*	HR (95% CI)	p-value*
Age, years				
19-44	1.67 (0.58, 4.82)		2.14 (1.34, 3.41)	
45-54	1.28 (0.75, 2.20)	0.93	1.04 (0.74, 1.45)	0.09
55-64	1.28 (0.87, 1.89)		1.39 (1.00, 1.92)	
≥65	1.10 (0.59, 2.04)		1.12 (0.61, 2.06)	
Calendar year				
2011	1.20 (0.84, 1.73)		1.38 (1.07, 1.78)	
2012	1.29 (0.69, 2.38)		1.00 (0.58, 1.73)	
2013	1.70 (0.65, 4.42)	†	1.28 (0.68, 2.42)	0.84
2014	1.28 (0.51, 3.22)		1.72 (0.86, 3.45)	
2015	0.59 (0.15, 2.37)		1.50 (0.75, 2.99)	
2016	†		0.92 (0.23, 3.73)	
Men	1.19 (0.89, 1.59)	0.27	1.37 (1.11, 1.69)	0.83
Women	1.87 (0.92, 3.82)		1.26 (0.74, 2.15)	
Geographic region of residence				
Northeast	1.20 (0.66, 2.17)		1.21 (0.78, 1.87)	
North central	0.82 (0.40, 1.69)	0.75	1.72 (1.13, 2.61)	0.53
South	1.46 (0.98, 2.18)		1.16 (0.86, 1.58)	
West	1.25 (0.60, 2.60)		1.61 (0.98, 2.66)	
History of CHD	1.17 (0.71, 1.95)	0.81	1.53 (0.66, 3.52)	0.83
Without history of CHD	1.29 (0.94, 1.77)		1.32 (1.08, 1.61)	
Diabetes	1.08 (0.72, 1.63)	0.48	1.39 (0.88, 2.18)	0.78
Without diabetes	1.32 (0.92, 1.90)		1.30 (1.04, 1.61)	
Stroke	0.62 (0.24, 1.60)	0.13	1.26 (0.52, 3.04)	>0.99
Without stroke	1.34 (1.01, 1.77)		1.32 (1.08, 1.61)	
Peripheral artery disease	†	†	†	†
Without peripheral artery disease	1.23 (0.93, 1.61)		1.31 (1.07, 1.59)	
Heart failure	1.55 (0.47, 5.17)	0.58	2.72 (0.51, 14.45)	0.36
Without heart failure	1.21 (0.91, 1.59)		1.32 (1.08, 1.60)	
Chronic kidney disease	1.42 (0.74, 2.72)	0.70	1.84 (0.95, 3.59)	0.35
Without chronic kidney disease	1.22 (0.91, 1.64)		1.29 (1.05, 1.58)	
Liver disease	†	†	†	†
Without liver disease	1.20 (0.91, 1.57)		1.36 (1.12, 1.65)	
Cardiologist care	1.52 (0.68, 3.42)	0.60	3.05 (0.88, 10.55)	0.20
Without cardiologist care	1.21 (0.91, 1.61)		1.30 (1.06, 1.58)	
Prior hospitalization	1.40 (0.87, 2.27)	0.49	2.09 (1.30, 3.33)	0.02
Without prior hospitalization	1.16 (0.84, 1.61)		1.14 (0.91, 1.43)	
Depression	0.94 (0.58, 1.52)	0.17	1.39 (0.89, 2.16)	0.86
Without depression	1.39 (1.01, 1.92)		1.31 (1.05, 1.63)	
Tobacco use	0.97 (0.41, 2.29)	0.56	1.64 (0.83, 3.25)	0.46
No tobacco use	1.25 (0.94, 1.67)		1.29 (1.05, 1.58)	
Polypharmacy	1.16 (0.84, 1.60)	0.47	1.10 (0.79, 1.54)	0.26
Without polypharmacy	1.47 (0.92, 2.33)		1.40 (1.10, 1.78)	
Antihypertensive medication use	1.14 (0.84, 1.54)	0.28	1.38 (1.04, 1.84)	0.49
No antihypertensive medication use	1.65 (0.92, 2.95)		1.20 (0.92, 1.58)	
Non-statin lipid lowering medication use	0.99 (0.58, 1.70)	0.41	1.80 (0.88, 3.68)	0.43
No non-statin lipid lowering medication use	1.32 (0.97, 1.79)		1.29 (1.06, 1.59)	

CHD: coronary heart disease; CI: confidence interval; HIV: human immunodeficiency virus; HR: hazard ratio.

Hazard ratios include adjustment for age, sex, calendar year, geographic region of residence, history of CHD, diabetes, stroke, peripheral artery disease, heart failure, chronic kidney disease, liver disease, cardiologist care, any hospitalization, depression, tobacco use, polypharmacy, antihypertensive medication use, and non-statin lipid-lowering medication use.

* Comparing HR for stroke hospitalizations associated with HIV infection across subgroups defined by beneficiary characteristics.
† Data not shown given the small number of events. Specifically, among beneficiaries taking a statin, there were 6 stroke events in those in 2016, 9 stroke events in those with a history of peripheral artery disease, and 4 stroke events in those with a history of liver disease. Among beneficiaries not taking a statin, there were 3 stroke events in those with a history of peripheral artery disease and 9 stroke events in those with a history of liver disease.

Table S9. Hazard ratios for a lower extremity artery disease hospitalization among beneficiaries with versus without HIV across subgroups defined by beneficiary characteristics stratified by statin use.

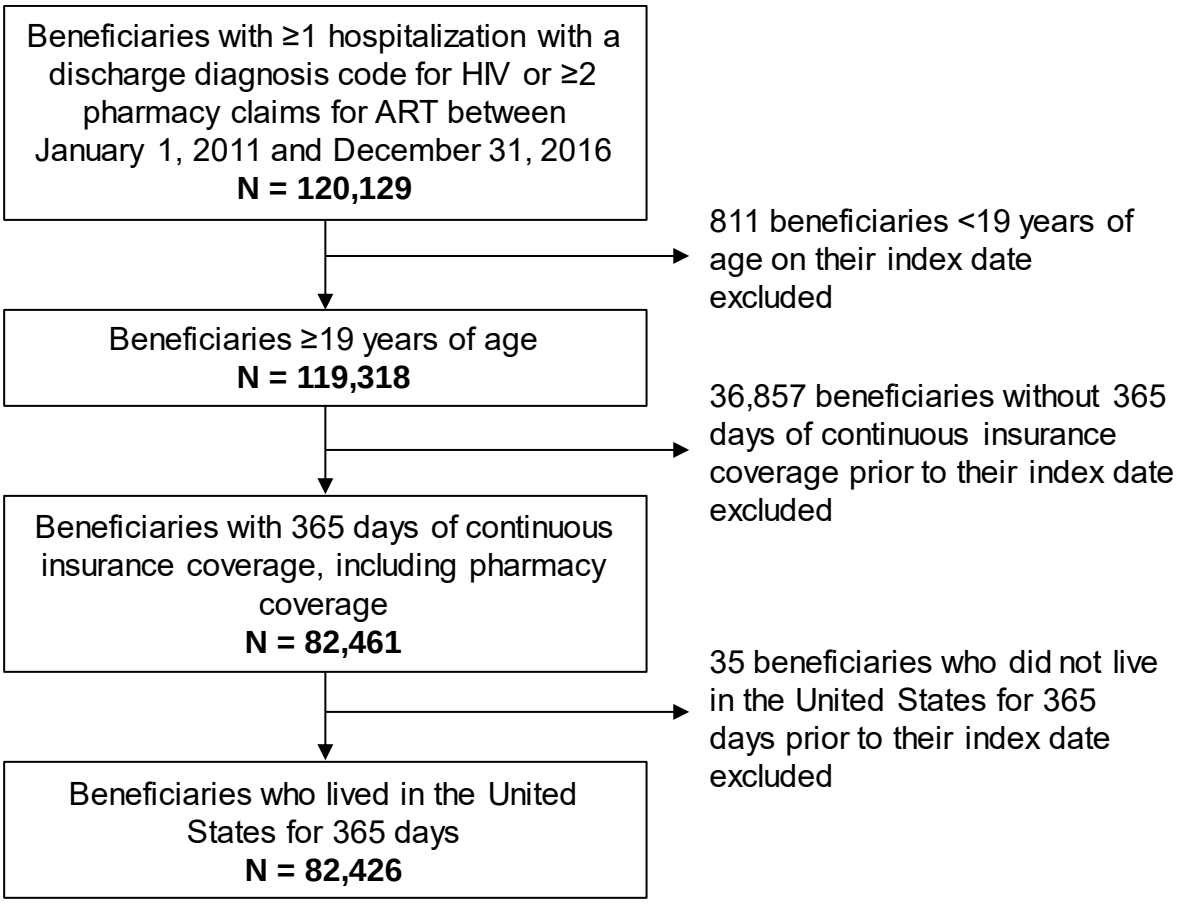
	Taking a statin		Not taking a statin	
	HR (95% CI)	p-value*	HR (95% CI)	p-value*
Age, years				
19-44	1.16 (0.11, 11.82)		2.11 (0.72, 6.19)	
45-54	0.64 (0.24, 1.71)	0.31	1.95 (1.16, 3.30)	0.56
55-64	1.66 (0.94, 2.94)		1.11 (0.56, 2.20)	
≥65	0.91 (0.31, 2.64)		1.23 (0.39, 3.90)	
Calendar year				
2011	1.55 (0.90, 2.68)		1.47 (0.91, 2.39)	
2012	0.82 (0.20, 3.42)		2.70 (1.08, 6.77)	
2013	1.10 (0.28, 4.29)	†	1.43 (0.37, 5.58)	†
2014	0.71 (0.12, 4.37)		0.27 (0.03, 2.82)	
2015	†		1.78 (0.57, 5.56)	
2016	†		†	
Men	1.28 (0.83, 1.97)	0.35	1.60 (1.09, 2.37)	0.79
Women	0.43 (0.05, 3.90)		1.41 (0.51, 3.93)	
Region of residence				
Northeast	0.41 (0.13, 1.33)		1.70 (0.71, 4.06)	
North central	1.42 (0.50, 4.01)	0.41	1.91 (0.69, 5.29)	0.10
South	1.19 (0.62, 2.26)		2.04 (1.26, 3.28)	
West	2.25 (0.64, 7.95)		0.20 (0.04, 0.99)	
History of CHD	1.49 (0.72, 3.06)	0.52	0.87 (0.27, 2.77)	0.32
Without history of CHD	1.08 (0.63, 1.83)		1.65 (1.12, 2.43)	
Diabetes	0.73 (0.33, 1.63)	0.12	0.77 (0.33, 1.81)	0.09
Without diabetes	1.56 (0.93, 2.62)		1.76 (1.17, 2.65)	
Stroke	†	†	†	†
Without stroke	1.22 (0.78, 1.91)		1.53 (1.06, 2.21)	
Peripheral artery disease	1.08 (0.39, 2.95)	0.92	2.96 (0.96, 9.10)	0.17
Without peripheral artery disease	1.21 (0.75, 1.95)		1.38 (0.93, 2.05)	
Heart failure	1.62 (0.36, 7.20)	0.73	0.12 (0.00, 3.85)	0.13
Without heart failure	1.14 (0.72, 1.78)		1.62 (1.11, 2.34)	
Chronic kidney disease	1.18 (0.41, 3.36)	0.90	1.19 (0.33, 4.33)	0.63
Without chronic kidney disease	1.27 (0.79, 2.04)		1.66 (1.14, 2.42)	
Liver disease	†	†	†	†
Without liver disease	1.24 (0.81, 1.90)		1.73 (1.20, 2.49)	
Cardiologist care	1.41 (0.40, 4.97)	0.66	0.97 (0.13, 7.41)	0.62
Without cardiologist care	1.11 (0.70, 1.75)		1.57 (1.08, 2.28)	
Prior hospitalization	0.93 (0.42, 2.03)	0.43	1.22 (0.62, 2.38)	0.56
Without prior hospitalization	1.33 (0.80, 2.22)		1.66 (1.07, 2.56)	
Depression	1.38 (0.63, 3.00)	0.67	0.61 (0.25, 1.46)	0.03
Without depression	1.10 (0.66, 1.85)		1.85 (1.24, 2.75)	
Tobacco use	1.40 (0.49, 4.01)	0.80	2.43 (0.89, 6.61)	0.31
No tobacco use	1.18 (0.73, 1.89)		1.45 (0.97, 2.17)	
Polypharmacy	1.00 (0.60, 1.64)	0.20	1.50 (0.84, 2.67)	0.86
Without polypharmacy	1.80 (0.85, 3.80)		1.64 (1.02, 2.64)	
Antihypertensive medication use	1.15 (0.72, 1.83)	0.47	1.40 (0.85, 2.31)	0.44
No antihypertensive medication use	1.72 (0.64, 4.63)		1.87 (1.12, 3.13)	
Non-statin lipid lowering medication use	1.04 (0.47, 2.30)	0.74	2.88 (0.86, 9.69)	0.40
No non-statin lipid lowering medication use	1.24 (0.75, 2.06)		1.55 (1.05, 2.27)	

CHD: coronary heart disease; CI: confidence interval; HIV: human immunodeficiency virus; HR: hazard ratio.

Hazard ratios include adjustment for age, sex, calendar year, geographic region of residence, history of CHD, diabetes, stroke, peripheral artery disease, heart failure, chronic kidney disease, liver disease, cardiologist care, any hospitalization, depression, tobacco use, polypharmacy, antihypertensive medication use, and non-statin lipid-lowering medication use.

* Comparing HR for lower extremity artery disease hospitalizations associated with HIV infection across subgroups defined by beneficiary characteristics.
† Data not shown given the small number of lower extremity artery disease hospitalizations (n<10) in each cell.

Figure S1. Flow-chart of beneficiaries with HIV in the MarketScan database included in and excluded from the current analysis.



ART: antiretroviral therapy; HIV: human immunodeficiency virus.
Persons without HIV were matched 4:1 on age, sex and calendar year with persons with HIV in the MarketScan database.

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EXHIBIT E

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Antiretroviral neurotoxicity

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Abstract

Combination antiretroviral therapy (CART) has proven to effectively suppress systemic HIV burden, however, poor penetration into the central nervous system (CNS) provides incomplete protection. Although the severity of HIV-associated neurocognitive disorders (HAND) has been reduced, neurological disease is expected to exert an increasing burden as HIV-infected patients live longer. Strategies to enhance penetration of antiretroviral compounds into the CNS could help to control HIV replication in this reservoir but also carries an increased risk of neurotoxicity. Efforts to target antiretroviral compounds to the CNS will have to balance these risks against the potential gain. Unfortunately, little information is available on the actions of antiretroviral compounds in the CNS, particularly at concentrations that provide effective virus suppression. The current studies evaluated the direct effects of 15 anti-retroviral compounds on neurons to begin to provide basic neurotoxicity data that will serve as a foundation for the development of dosing and drug selection guidelines. Using sensitive indices of neural damage, we found a wide range of toxicities, with median toxic concentrations ranging from 2 to 10,000 ng/ml. Some toxic concentrations overlapped concentrations currently seen in the CSF but the level of toxicity was generally modest at clinically relevant concentrations. Highest neurotoxicities were associated with abacavir, efavarenz, etravirine, nevaripine, and atazanavir, while the lowest were with darunavir, emtracitabine, tenofovir, and maraviroc. No additive effects were seen with combinations used clinically. These data provide initial evidence useful for the development of treatment strategies that might reduce the risk of antiretroviral neurotoxicity.

Keywords

HIV; Dementia; Neurons; Therapy; Treatment

Introduction

HIV-1 rapidly enters the central nervous system (CNS) after infection and establishes a persistent viral reservoir. CNS HIV infection frequently results in neurological disease marked by a set of cognitive, motor, and behavioral symptoms known as HIV-associated neurocognitive disorders (HAND) (Antinori et al. 2007). Potent combination antiretroviral (ARV) therapies have been shown to improve cognition and reduce the prevalence of HIV-associated Dementia, the most severe form of HAND (Dilley et al. 2005; Sacktor et al. 2001; Saksena and Smit 2005). Recent studies have shown that mild–moderate neurocognitive manifestations of HIV infection persist in about 40 % of patients on treatment (Sacktor et al. 2002; Villa et al. 1996). In addition, studies indicate that the prevalence of neurocognitive disorders is increasing as patients live longer (Heaton et al. 2011; Robertson et al. 2007). To control viral replication in the brain, strategies are under development to increase the penetration of ARV compounds across the blood–brain barrier

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(Bressani et al. 2010; Mahajan et al. 2010; Manda et al. 2010; Prabhakar et al. 2011; Saiyed et al. 2010). Although these compounds have well described toxic actions systemically and in the peripheral nervous system (Bartlett and Lane 2012), little is known about the toxicity of the compounds to neurons in the CNS. One recent study reported that cognition improved for up to 96 weeks in a group of immunologically and virologically stable patients who elected to come off of treatment (Robertson et al. 2010). These results raised the possibility that even low concentrations of ARVs that penetrate the brain may have some detrimental effects. If this is true, future efforts at delivering higher concentrations of ARVs to the CNS will have to take into consideration the potential adverse effects. Careful studies of the effects of ARV compounds at concentrations required to suppress viral replication are needed to evaluate this possibility and to guide the use of compounds that will minimize CNS complications. To provide a comparative analysis of the neurotoxicity of ARV compounds, we evaluated the direct effects of 15 different ARV compounds and six different combinations of ARV compounds on primary cultures of rat neurons.

Methods

Primary cultures of rat forebrain

Fetuses were harvested at E17 from pregnant female Long–Evans rats, washed with ice cold HEPES-buffered Hank's balanced salt solution (HBSS) and the brain removed. The euthanasia and tissue harvest protocols were done in accordance with NIH and institutional guidelines and were approved by the Institutional Animal Care and Use Committee. The cortex/hippocampus was dissected from the brain and cleaned of duraarachnoid membrane and visible vessels. The tissue was transferred to a 15-ml tube containing 5 ml calcium–magnesium free-HBSS + 2.4 U/ml dispase + 2 U/ml DNase I and incubated for 20–30 min at 37 °C. Tissue was triturated and allowed to settle for 2 min, and the suspended cells were transferred to a 50-ml culture tube containing 25 ml of complete medium (Minimum Essential Medium [MEM] + 10 % fetal bovine serum [Invitrogen, Certified FBS] + 20 μg/ml gentamicin). The trituration/collection cycle was repeated until most of the tissue was dispersed. Dissociated cells were seeded at a density of 20,000–100,000 cells/cm^2 on poly-D-lysine-coated coverslips or 100,000 cells/cm^2 in poly-D-lysine-coated 96-well plates. Cultures were >90 % neurons 3 days after seeding based on morphology and stain for microtubule-associated protein-2 (MAP-2). Cultures were fed by 50 % medium exchange three times a week. Cultures were allowed to mature normally for 6 days without the use of mitotic inhibitors.

Antiretroviral compounds

ARV compounds were obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID. Concentrated (1,000×–10,000×) stocks were made in water (ddC, ddI, AZT, FTC, TDF, 3TC, ATV, MVC), DMSO (ABC, EFV, NVP, APV, DRV, RTV), or ethanol (ETV) and diluted in culture medium for use (plasma therapeutic concentrations in Table 1 were designated as 1X). Compounds were added to the cultures in concentrations ranging from 0.01 to 300 μg/ml to investigate the dose response of each individual compound. Combinations of compounds were tested using the reported plasma levels for each drug in the combination (see Table 1).

ARV challenge

At 6 days in culture, the rat cortical neurons were challenged for 1 week with one of 15 ARVs at concentrations ranging from 0.01 to 300 μg/ml to determine single drug toxicities. At 6 days neurons express extensive synaptophysin immunoreactivity and excellent responsiveness to glutamate. The period from 6 to 13 days represents a time in which the neurons are healthy and stable providing a solid baseline for the toxicity studies.

Concentrations were chosen to represent a range of at least one order of magnitude above and below the therapeutic plasma concentrations used in patients to suppress HIV replication. A summary of this information is provided in Table 1. In addition, six combinations of ARVs currently in the DHHS guidelines were tested to determine the impact of multiple drugs.

MAP-2 immunostaining for assessment of neuronal loss and damage

Neurons were identified by MAP-2 immunostaining. Cells were fixed in ice cold 97 % methanol, 3 % acetic for 10 min at room temperature and washed in 0.01 M phosphate-buffered saline (PBS, 3×5 min). Cells were incubated in blocking buffer containing 3 % normal goat serum in 0.01 PBS for 60 min at room temp. Polyclonal rabbit anti-MAP-2 (Chemicon/Millipore, Bilerica, MA) was then applied at a dilution of 1:500 in blocking buffer and incubated overnight at 4 °C. The cells were washed three times in PBS and incubated in goat anti-rabbit Alexa488 or mouse anti-rabbit Alexa568 (Molecular Probes/ Invitrogen, Carlsbad, CA) at a dilution of 1:500 for 1 h at room temperature. Cells were then washed 3×5 min, counterstained with bis-benzimide (0.5 μM; Sigma, St. Louis, MO) for 20 min in PBS and washed 2×5 min in PBS. Coverslips were mounted onto slides with Fluoromount (Southern Biotech, Birmingham AL) and 96-well plates were filled with 50 % glycerol solution in PBS.

MAP-2 data analysis

Three or four images were digitally captured from each well at a magnification of 193× using the MetaMorph™ System. An intensity threshold was set to highlight the MAP-2 stained neurons and proximal dendrites within each image while limiting background. The thresholded area was then measured as a percent of total area. Neuronal damage typically included beading and pruning of dendrites with a corresponding decrease in MAP-2 staining. The decrease in MAP-2+ processes was apparent in intact neurons and proved to be a very sensitive index of neuronal damage. However, a caveat of this approach is that the use of sensitive measures also introduces greater error in the assessments which must be taken into consideration in the interpretation of the results. The accuracy of estimates from the data was therefore calculated based on the variation seen. Data from each image were averaged for each well within a 96-well plate and then across three to four replicate experiments from different cultures. Concentration–effect curves and an estimate of the median toxic concentration (TC_{50}) were generated from the average data using Graphpad Prism software. Since some damage (e.g., beading of dendrites) may not correlate with decreases in MAP-2 stain intensity, we also confirmed damage by quantifying the extent of beading and by rating the quality of the neurons on a scale of 1 to 10 (10 being very healthy with abundant neurons and intact processes, 5 representing abundant, well stained neurons with moderate beading and/or loss of dendrites and 1 representing an almost complete loss of MAP-2+ neurons). The bead density correlated well with the MAP-2 stain intensity values (r=0.534±0.036) indicating that the beading paralleled changes in the density of the processes. Thus, the independent influence of beading in the analysis was small. In addition, the qualitative ratings verified that the MAP-2 quantification matched the visible damage to the neurons. Over the concentrations studied, no treatments resulted in complete loss of MAP-2 staining. Thus, the TC_{50} value reflects the relative potency of the compound but not the total amount of damage. The maximum amount of MAP-2 loss or damage was recorded separately to provide an estimate of the extent of damage.

Correction for DMSO damage

Several of the drugs used were not soluble in aqueous solutions and were therefore dissolved in DMSO to make the stock solutions. These stocks were prepared at concentrations 1,000 to 10,000 times the therapeutic concentration to minimize toxicity of the vehicle. However,

to provide an accurate correction for toxicity of the DMSO, a dose response curve was run on each plate. The dose–response curve for both the Metamorph MAP-2 stain measurements (% threshold) and visual damage assessments are illustrated in Fig. 1. Values represent mean ± SEM. In addition, the DMSO dose–response curve for loss of mitochondrial activity based on conversion of MTT is shown. In each case, there was no consistent DMSO toxicity until concentrations exceeded 0.44 % (log % = −0.356, the point at which the best-fit curve begins to drop). By 4 % there was extensive loss of MAP-2 stain (52–88 %), neuron integrity and MTT conversion. Toxicity due to DMSO was subtracted from each compound at the matched concentration to provide the best estimate of the toxicity of the compound. Concentrations with DMSO greater than 1 % (0 value for log DMSO) were not included in the TC_{50} calculations.

Calculation of the toxicity index and toxicity risk

To provide a relevant endpoint, we asked whether the toxic effects seen in vitro occurred at ARV concentrations achieved in patients on ARV therapy. The toxicity index was calculated as the log of the reported therapeutic plasma concentration divided by the estimated TC_{10} value from the data in Fig. 3. A concentration one log lower than the TC_{50} was used to provide an estimate of the toxicity threshold and corresponded to about 10 % loss of the MAP-2 stain (TC_{10}). A value of 0 was obtained when the TC_{10} was equal to the therapeutic plasma concentration in Table 1. Negative values reflect favorable toxicity profiles while positive values reflect less favorable profiles. Toxicity risk was estimated in the same fashion from current estimates of ARV concentrations in the CSF. Values greater than or equal to 0 indicate that concentrations in the CSF under current treatment conditions are in the toxic range.

Antiretroviral compounds and glutamate-induced changes in intracellular calcium

Neuronal cultures were incubated for 2 days in medium containing ARV compounds at concentrations that matched the therapeutic concentrations in plasma. Cells were then transferred to HEPES-buffered artificial CSF (aCSF: NaCl 137 mM, KCl 5.0 mM, $CaCl_2$ 2.3 mM, $MgCl_2$ 1.3 mM, glucose 20 mM, HEPES 10 mM, adjusted pH 7.4 with NaOH) and pre-loaded with the calcium indicator dye Fluo-4 NW (1:4 dilution; Molecular Probes/Invitrogen) in serum-free medium at 37 °C. After 30 min, the coverslip was transferred to a specialized stage for imaging (Warner Instruments). Time lapse digital images were captured automatically by the Metamorph system. Three pre-stimulation images were taken to establish baseline calcium in each cell. In some experiments, the acute and delayed response to glutamate was then measured after the application of 10 μM glutamate to the chamber. The increase in fluorescence intensity was then calculated relative to the baseline fluorescence for each neuron within the field to correct for any differences in dye loading or intrinsic fluorescence. Data from several runs was consolidated and the average cellular response and standard error calculated.

Estimates of mitochondrial membrane potential with TMRM and MTT

To image changes in mitochondrial membrane potential at high resolution, neuronal cultures on coverslips at 20,000 cells/cm^2 were incubated for 2 days with ARV compounds and then 100 nM tetramethylrhodamine methyl ester (TMRM) was added to the medium. Cultures were incubated for 10 min at 37 °C and the neuronal mitochondria imaged at 1680× in aCSF using the MetaMorph™ System. The intensity of stain was measured, averaged across cells and compared between compounds. Images of the stained mitochondria were taken to provide an indication of changes in morphology. Higher throughput analyses for dose–response studies were conducted using MTT conversion in 96 well plates at a neuron density of 10^5 cells/cm^2. Briefly, 10 μl of a 12 mM stock of MTT in PBS was added to 100 μl of culture medium and the cells were incubated at 37 °C for 2 h. The medium was then

removed and 50 μl of DMSO added to each well. After mixing, the plate was incubated at 37 °C for 10 min. The plate was again mixed and the OD of the solution measured on a plate reader at 540 nm.

Results

Antiretroviral compounds have a wide range of neurotoxic potencies

Examples of the types of damage seen in neural cultures treated for 7 days with ARV compounds are illustrated in Fig. 2. Untreated cultures have large, well stained MAP-2+ neurons (green) with elaborate outgrowth of processes (Fig. 2a). In general, the ARVs were not highly toxic. Typical damage included beading (Fig. 2b), simplification of the dendritic processes (Fig. 2c), and neuronal shrinkage. The images illustrate changes in response to efavirenz (Fig. 2b, d) or atazanavir (Fig. 2c) but are representative of all ARVs. Extensive cell death and damage was only seen at the highest concentrations of some compounds (Fig. 2d; efavarenz, 300 μg/ml) and was confounded by the toxicity of DMSO. Most cultures showed a combination of effects (e.g., beading and pruning) which resulted in a loss of MAP-2 stained processes which could be measured by a decrease in the area occupied by MAP-2+ cells and processes. The ability of the measures of MAP-2 staining to track with damage to the neurons was verified using blinded ratings of the quality of the neural cultures on a scale of 1–10 (1 = extensive damage and loss of MAP-2 stain, 10 = no damage, excellent stain). The damage ratings closely paralleled the MAP-2 analysis but were generally less sensitive than the quantitative measures of MAP-2 density.

The curves in Fig. 3 illustrate the change in total area of MAP-2+ cells and processes relative to the vehicle-treated control cultures with increasing concentrations of each compound. To provide a comparison to concentrations present in vivo, we have included a solid vertical line to indicate the concentration reported in plasma of patients on therapy. Concentrations measured in CSF are indicated by the dashed vertical line. The median toxic concentration (TC_{50}) calculated from these curves for each compound is summarized in Table 1. The maximum loss of MAP-2 immunoreactivity is also included to indicate the extent of damage since each compound induced only a partial loss of neuron staining over the concentrations tested. The NRTI's ddC, DDI, FTC and AZT induced the greatest amount of damage (42–52 % loss of MAP-2). The NNRTI's and the protease inhibitors generally produced less total loss of MAP-2 (17–36 %). DRV, MVC and TDF produced the least amount of damage (17–22 %). Cell death was assessed by a semi-automated count of small condensed bis-benzimide stained nuclei using Metamorph integrated morphometry. The relative number of nuclei consistent with dead or apoptotic cells ranged from 0.4 % to 2.5 % for the 15 compounds compared to 1.6±0.9 % for vehicle controls. The highest values (ABC 1.4 %, 3TC 1.7 %, EFV 1.9 % and ATV 2.5 %) were generally consistent with the MAP-2 results but no changes were significantly different from controls and all were in the range of normal cell death typically seen in these cultures. Therefore, cell death contributes very little if at all to the neurotoxicity.

Therapeutic concentrations of most ARVs would be predicted to have moderate neurotoxic activity

To generate a profile of relative toxicities, a toxicity index was calculated for each compound. To provide a conservative estimate of potential toxicity, we elected to base the toxicity index on the lowest concentration at which toxicity might begin to develop. A threshold concentration one log lower than the TC_{50} was chosen to represent approximately a 10 % drop in the MAP-2 intensity (TC_{10}). The log transform of the plasma concentration seen in patients on therapy divided by the TC_{10} is illustrated in Fig. 4a. A score of 0 on this scale is obtained when the therapeutic plasma concentration is equal to the TC_{10} (the toxic

threshold). Negative scores reflect less neurotoxicity and positive scores greater neurotoxicity. Based on this analysis, eight compounds (ABC, DDI, 3TC, EFV, ETR, NVP, APV and ATV) have a relatively high risk of neurotoxic effects (index = 1.79–3.11) assuming plasma levels were achieved in CNS. TDF, AZT, DRV and RTV have a lower risk of toxicity (index = 0.58–1.07) and ddC, FTC and MVC would be predicted to have no significant toxic effects (index ≤0). To provide an estimate of the potential risk of toxicity given current values for CSF penetration of each compound, the TC_{10} was divided by the reported CSF concentration for each drug. In this case, values greater than or equal to 0 indicate that CSF concentrations are potentially high enough to produce neuronal damage. Based on this assessment, three compounds (ABC, DDI, NVP) have a relatively high potential risk (index = 1.34–2.76). 3TC, ETR, APV and ATV have some low risk (index = 0.12–0.81), whereas ddC, FTC, TDF, EFV, DRV, RTV and MVC have no predicted risk (index <0).

Combinations of antiretroviral drugs did not have additive neurotoxic effects

To determine if combination ARV therapy poses an increased risk of neurotoxicity, we challenged neurons with six different combinations. The plasma concentration from Table 1 was used for each compound. The loss of MAP-2 immunoreactivity after 7 days is summarized in Fig. 5 relative to matched, untreated cultures. The amount of damage at therapeutic concentrations was not extensive suggesting that the combinations did not have additive neurotoxic effects. The greatest loss of MAP-2 immunoreactivity was seen for the combination, ATV, RTV, TDF, FTC (28.8 %), followed by DRV, ABC, 3TC (24.6 %), EFV, ABC, 3TC (17.1 %) and NVP, TDF, FTC (12.5 %). The combinations EFV, TDF, FTC and DRV, TDF, FTC did not produce significant damage. The density of neurons/mm^2 did not change (range = 216±8 to 241±12/mm^2) indicating that there was no significant neuronal loss under these conditions. The low level of cell death was verified by an analysis of condensed and fragmented bisbenzimide stained nuclei indicative of apoptotic cells. The relative number of nuclei with apoptotic profiles ranged from 1.55± 0.79 % to 3.01±0.35 % versus 1.15±0.60 % for untreated cultures. Although no changes were significantly different from controls, an inverse correlation was seen between MAP-2 density and apoptotic nuclei (r=−0.766), suggesting a possible small effect on cell viability.

Different ARV combinations may either sensitize or desensitize neurons to the toxic actions of glutamate

To evaluate the functional response of neurons after exposure to ARV combinations for 2 days, we challenged the neurons with a moderate concentation of glutamate (10 μM) and measured both the acute and delayed intra-cellular calcium response (Fig. 6). The mean peak calcium response to glutamate was 508±51 brightness units above baseline. Drug combinations that produced the greatest damage (DRV, ABC, 3TC) showed larger acute and delayed increases in intracellular calcium in response to glutamate. Combinations with more moderate toxicity (ATV, RTV, TDF, FTC) showed unaltered acute responses to glutamate but a significant delayed response. A less toxic combination (EFV, TDF, FTC) showed a decreased acute response to glutamate followed by a small delayed increase. When neural cultures were challenged acutely with the most toxic combination, DRV, ABC, 3TC, in the absence of glutamate no change in intracellular calcium was seen relative to the aCSF controls. Thus, although the ARVs alter neuronal responsiveness to excitatory neurotransmission, they have no direct effect on calcium accumulation.

Toxicity did not correlate with changes in the mitochondrial membrane potential

Since damage to mitochondria is a known effect of NRTIs (Kline et al. 2009; Lewis et al. 2006; Moyle 2005; Saitoh et al. 2007, 2008; Venhoff et al. 2007), we assessed changes in the mitochondrial membrane potential in response to selected ARVs using the dye TMRM

and MTT assays of mitochondrial viability. Two NRTIs (DDI, TDF) with high toxic potential, one NNRTI (EFV) with moderate toxic activity, one PI with high toxicity (ATV) and one PI with low toxicity (RTV) were selected for a high resolution analysis of mitochondria after acute exposure for 2 days. Examples of the staining with the membrane potential probe, TMRM, are shown in Fig. 7a. TMRM intensity was variable, as illustrated by the mean values in Fig. 7b and the distribution of intensities in Fig. 7c, but no clear relationship was seen to neurotoxic activity. The least toxic compound tested (RTV) had the lowest TMRM staining whereas NRTIs with relatively high toxicity displayed either increased (+74.2 %, DDI) or decreased (−15.6 %, ATV) staining. Changes in mitochondrial morphology were seen under all treatment conditions. The morphology changed from the typical long fingerlike structures to slightly more rounded structures, particularly within the cell body. Again there was no clear relationship between mitochondrial morphology and TMRM intensity or neurotoxicity.

To further evaluate if mitochondrial changes might emerge with longer treatments, five NRTIs (ddC, ddI, FTC, TDF, AZT) expected to have effects on mitochondria, one NNRTI (EFV) and two protease inhibitors (ATV, RTV) were added to neural cultures at eight different concentrations and incubated for a period of 1 week. The cells were then tested using the MTT assay as a quantifiable index of mitochondrial activity and the dose–response relationship evaluated. None of the conditions showed changes in MTT conversion in response to ARV treatment at doses up to 10 μg/ml (not shown). MTT tetrazolium product OD values ranged from 0.185 to 0.203 versus 0.178 to 0.204 for vehicle-treated cultures and the dose–response curve was flat (not shown) with the exception of decreases at high concentrations which could be attributed to the effects of DMSO (e.g., see Fig. 1).

Discussion

Eradication of HIV from the CNS reservoir has been difficult due, in part, to the poor penetration of ARV compounds across the blood–brain barrier. HIV within the CNS continues to produce damage and is a source of infectious virus (Schnell et al. 2009, 2010; Smit et al. 2004). Protection of the brain and elimination of the CNS viral reservoir are high priorities in the treatment of HIV. As newer compounds and methods are developed that improve delivery across the blood–brain barrier, more consideration will have to be given to potential neurotoxicity. Numerous studies indicate that long-term HAART treatment is associated with a range of adverse effects including hepatic steatosis, neuropathy, cardiomyopathy, pancreatitis, lactic acidosis, ototoxicity, retinal lesions and possibly lipodystrophy (Bartlett and Lane 2012). Many of these effects are thought to be associated with the loss of mitochondrial DNA resulting from NRTI inhibition of mitochondrial DNA polymerase gamma (Kakuda 2000). However the mechanisms that give rise to the damage are not well understood and in some cases damage to cells does not correlate with mitochondrial DNA depletion (Kline et al. 2009; Maagaard et al. 2006; Maagaard and Kvale 2009). Substantial differences in NRTI toxicity have been noted between different tissues reinforcing the need to examine the direct effects on neural tissue. Our in vitro studies provide a comparison of the relative neurotoxicity of different ARV compounds using measures of neuronal dysfunction that are sensitive and designed to reflect the earliest forms of HIV-associated damage. The studies were optimized to measure direct effects of the compounds on neurons. However, it is important to note that this is but one of many possible endpoints that may be relevant. These studies cannot address the potential impact of long-term exposure (months to years) or potential interactions in vivo that may affect neurotoxicity. Nevertheless, these studies begin to provide information on potential risks associated with CNS penetration of ARVs.

Neurotoxicity of individual ARVs ranged from undetectable to moderate and was not due to neuron death

No compound was highly toxic but neural damage in the form of dendritic beading and pruning was a common observation. This pathology was reflected in the loss of MAP-2 staining. Since beading and pruning have been documented under a variety of pathological conditions (Bellizzi et al. 2005; Greenwood et al. 2007; Takeuchi et al. 2005), they most likely reflect a general endpoint associated with neuronal dysfunction (Greenwood et al. 2007; Kim et al. 2010; Masliah et al. 1992). Under these in vitro test conditions, a wide range of median toxic concentrations (TC_{50}) was seen with a low value of 2.2 ng/ml and high values of >5 μg/ml (Table 1). The TC_{50} of ABC, ddI, and NVP fell within the range of concentrations seen in CSF of patients on ARV therapy. The median toxic concentration of ABC, APV, ATV, ddI, EFV, ETR, NVP, RTV, TDF and 3TC, fell within the range of concentrations seen in the plasma suggesting that they may have significant neurotoxic effects if present in CNS at therapeutic concentrations. DRV, FTC, and MVC produced little toxicity at relevant plasma and CSF concentrations. The ratio of the therapeutic plasma concentration to the TC_{10} provided an index of the potential toxicity at concentrations that would suppress HIV replication. The TC_{10} value was used to reflect the earliest drop in the dose–response curve (approximately a 10 % decrease) in an effort to provide a conservative estimate of the concentration at which toxicity might begin. Twelve of the compounds were above this theoretical threshold and some exceeded the threshold by two logs. The range was greater than three logs indicating considerable variation in the relative risk. The lower toxicity indices for ddC, FTC, DRV, RTV and MVC indicate that it may be possible to deliver therapeutic concentrations of some drugs into the CNS with minimal relative risk.

The ratio of the CSF concentration of each drug to the TC_{10} provided an index of the risk of toxicity based on current use. The relatively high penetration of ABC and NVP yielded toxicity risk values much greater than zero indicating that they may be present in CSF at levels capable of producing neurotoxicity. DDI, 3TC, APV and ATV also had scores greater than zero but the predicted amount of toxicity would be minimal. Even in cases where higher levels of toxicity were seen, it is noteworthy that the toxic effects were not due to cell death. The toxic effects associated with dendrite beading and possibly pruning are likely to be reversible, raising the possibility that neuroprotective treatments may be effective in preventing or reversing ARV-associated damage.

Acute effects of the ARVs on neurons may be unrelated to effects on mitochondria

Of the few studies that have looked at the effects of ART on neural tissue. A recent study by Divi et al. (2010) in year-old patas monkey offspring treated in utero with various NRTIs showed a 28.8–51.8 % depletion of mitochondrial DNA. Similar depletion was seen in the liver, suggesting a similar susceptibility between brain and liver. Most toxicity studies have focused on the ability of NRTIs to cause a long-term suppression of mitochondrial DNA through the inhibition of mitochondrial DNA polymerase gamma. However, these compounds also have effects on the ADP/ATP translocator and adenylate kinase, suggesting that other effects may contribute to toxicity (Ciccosanti et al. 2010; Kakuda 2000). Primary neural cultures limit the ability to do long-term studies but clearly demonstrate that damage can be seen with exposures of 2–7 days. Some studies in cell lines have shown that long-term exposure to NRTIs was necessary to deplete mitochondrial DNA and damage cells (Kline et al. 2009), whereas other studies have shown significant depletion of mitochondrial DNA in myoblasts and myotubes by ddI (Saitoh et al. 2008) and human hepatoma cells by various NRTIs (Venhoff et al. 2007) with more modest exposures of 5–25 days. Large tissue differences in the sensitivity to NRTIs have been reported. Based on the slow turnover of mitochondrial DNA in neurons one might expect that they would be less sensitive to the effects of the NRTIs (Wang et al. 1997). The relatively rapid appearance of damage within 7

days in our primary neural cultures and minimal changes in markers of mitochondrial function suggests that other factors may contribute to toxicity. This possibility is consistent with in vitro studies of synaptosomes and isolated mitochondria incubated with CSF achievable concentrations of ddC (6–11 ng/ml) (Opii et al. 2007). Signs of oxidative stress, release of cytochrome *C* and a reduction in anti-apoptotic proteins were apparent within 6 h. Thus, further analyses of ARV toxicity should explore various potential mechanisms of action of the ARV compounds.

Damage was not restricted to the NRTIs as the NNRTIs and PIs also produced damage equal to or greater than the NRTIs. Less is known about the potential neurotoxicity of NNRTIs and PIs although both are associated with significant side effects. All NNRTIs tested in our studies had neurotoxic potencies that were comparable to the NRTIs. This translated to a high toxicity index for all NNRTIs. The PIs had a wider range of potencies with DRV showing very low neurotoxic potency relative to APV and ATV. This was associated with a favorable toxicity index for DRV. RTV was also less toxic than APV and ATV but slightly more toxic than DRV. The fusion inhibitor, maraviroc (MVC) had a relatively low neurotoxic potency and the lowest toxicity index of all drugs tested. Distinctive differences in the potential neurotoxicity of various PIs and entry inhibitors indicate that a rational selection of ARVs may minimize CNS side effects.

Antiretroviral combinations did not have additive toxic effects

ARV combinations at estimated plasma concentrations produced similar types of damage as seen with individual compounds with little indication of strong additive effects. In some combinations, compounds with a favorable toxicity index appeared to offset the deleterious effects of compounds with a high toxicity index. This was seen not only in the neuronal damage but also in the calcium signaling of neurons in response to glutamate. This observation is similar to studies by Venhoff et al. (2007), where TDF and 3TC attenuated ddI cytotoxicity in human hepatoma cells. These observations indicate that neurotoxicity may be controlled not only by selecting compounds based on their individual properties but also based on interactions between compounds. The ability of the combination of EFV, TDF, FTC to suppress calcium signaling in response to a glutamate challenge raises the possibility that particular combinations could offer some level of protection by reducing the delayed accumulation of calcium. However, the potential impact of the acute “protective” effects will not be entirely clear until we have a better understanding of the toxic mechanisms.

Comparison of ART toxicity to HIV-associated neuronal damage

While the intent of these studies was to identify potential toxic interactions of ARVs with neurons using sensitive measures of neuronal dysfunction, it is important to weigh the potential benefit against the risks. When compared to conditions that recapitulate the toxic effects of HIV under the same culture conditions, the effects of the ARVs are small. For example, direct effects of the ARVs on calcium homeostasis are small relative to the large changes following exposure of the neurons to conditioned medium from HIV-treated human monocyte-derived macrophages.

Estimates of the loss of MAP-2 immunoreactivity at plasma concentrations ranged from approximately 9 % to 28 %. This contrasts with losses of approximately 70 % under conditions that mimic HIV infection in vitro (unpublished data). Based on these in vitro studies, the risk of ARV-associated damage in the CNS relative to the risks of HIV infection would appear to be low.

Conclusions

As we develop new antiviral approaches to target HIV in the CNS greater attention to strategies that minimize the risk to neurons will be needed. Our studies indicate that there is a risk of direct neurotoxic interactions that varies substantially between compounds and that informed selection of ARVs and ARV combinations used to target the CNS may minimize adverse effects. While these data begin to identify potential risks, they should not be viewed as guidelines for the clinical use of ARVs. Similar studies will need to correlate in vitro measures to clinical observations to establish the validity of the in vitro toxicity measures. In addition, long-term effects of the compounds may give rise to other forms of damage and will need to be evaluated. Our ability to make rational choices for therapies directed to the CNS is currently limited by a paucity of data on the complex interactions of ARV compounds with neural tissue. Our studies presented here illustrate that the potential for neural damage and dysfunction is significant and highlight the need for further exploration of the mechanism of neurotoxicity of these compounds individually and in combinations.

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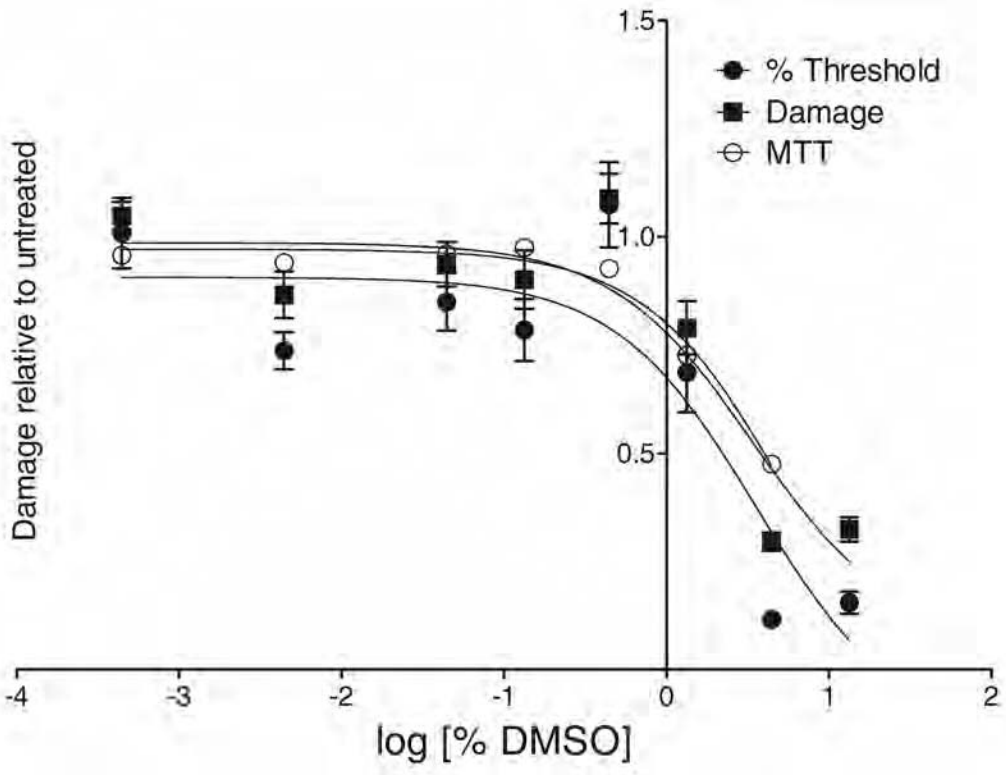

Fig 1.

DMSO toxicity dose–response curve. Neuronal toxicity due to DMSO relative to untreated controls was similar in three different analyses which measured the area occupied by MAP-2 stained cells (% threshold), damage scores based on morphology of MAP-2 stained cells (damage) and results from an MTT assay for mitochondrial activity. In each case, damage was first apparent after reaching a concentration of 1 % DMSO (log DMSO=0). Values are mean ± SEM

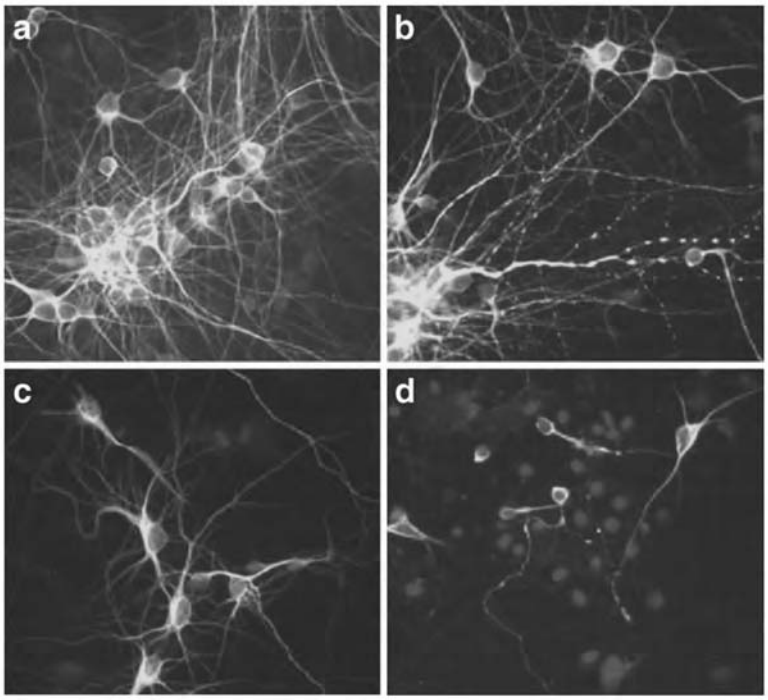

Fig 2.

Examples of the types of damage seen and measured in neural cultures treated for 1 week with efavirenz or atazanavir. Neurons were stained for MAP-2 (*green*) and counterstained with the nuclear stain, bisbenzimide (*blue*). The images illustrate the types of damage that contribute to toxicity but were not matched for neuron density and do not necessarily reflect the average extent of damage for the compounds illustrated since cultures often contained a mix of each type of damage. **a** Untreated cultures contained healthy neurons with extensive outgrowth of processes. **b** Beading of the dendrites was an early sign of damage and often appeared adjacent to other normal looking neurons (7 days efavirenz). **c** Some neurons showed normal cell bodies but had less extensive elaboration of dendrites relative to matched untreated cultures (7 days atazanavir). **d** The most severe damage such as shrinkage of the neuropil and an extensive loss of dendrites was seen only at the highest concentrations of some drugs (efavarenz at 30 μg/ml) and was generally confounded by the presence of high concentrations of DMSO. However, similar elements could be seen in many cultures with less severe overall damage. In each case, the damage causes a loss of area occupied by MAP-2 stained neurons which could be quantified to provide a measure of toxic damage in live neurons

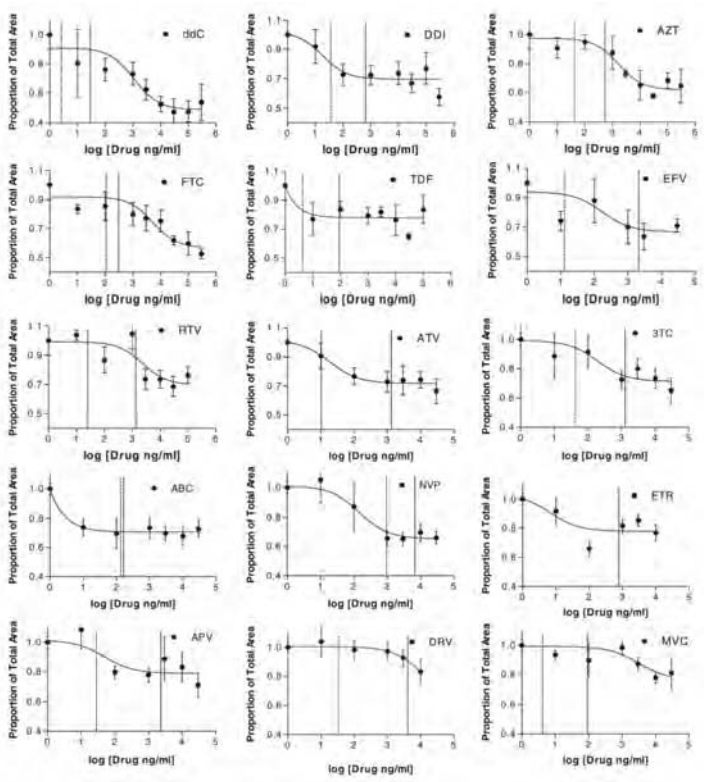

Fig 3.

Dose–response curves for damage induced by each of 15 different antiretroviral compounds incubated in neural cultures for 1 week (day 6 to day 13). The proportion of total area occupied by MAP-2 stained neurons and processes relative to untreated control cultures is plotted versus the log of the drug concentration (ng/ml). The best-fit sigmoidal curve was determined using Graphpad Prism software and was used to calculate the median toxic concentration (TC_{50}) for each compound which is summarized in Table 1. The shape and midpoint of the curves varied widely between compounds indicating variable toxic properties. No compound caused a complete loss of MAP-2 immunoreactivity at the concentrations tested with the loss ranging from 17 % to 52 %. *Solid lines* indicate the therapeutic concentration reported in plasma of HIV patients. *Dashed lines* indicate the reported concentration in the CSF. Values are mean ± SEM

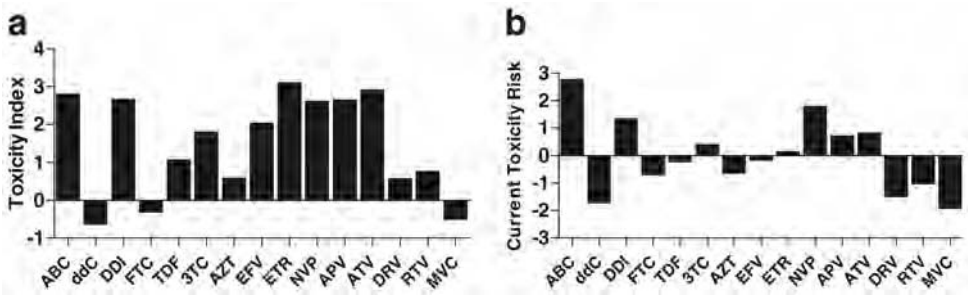

Fig 4.

Illustration of the estimated toxicity index and toxicity risk for each of 15 antiretroviral compounds. To reflect the threshold concentration at which toxicity might first begin to develop, the toxicity index was calculated as the log of the reported therapeutic plasma concentration divided by the estimated TC_{10} value from the data in Fig. 3. **a** Six compounds had a toxicity index >2 and three more an index >1 indicating a relatively high risk of toxic effects from most compounds. AZT, DRV and RTV had a modest index between 0 and 1 while ddC, FTC and MVC had negative values indicating a relatively low risk of neural damage at therapeutic concentrations. **b** Toxicity risk was estimated in the same fashion from the current estimates of ARV concentrations in the CSF. ABC, DDI, and NVP had values >1 (1.34–2.76), indicating significant current risk of toxicity. APV (0.71) and ATV (0.81) had a low risk of toxicity, whereas all other compounds had negligible risk of toxicity based on this assessment

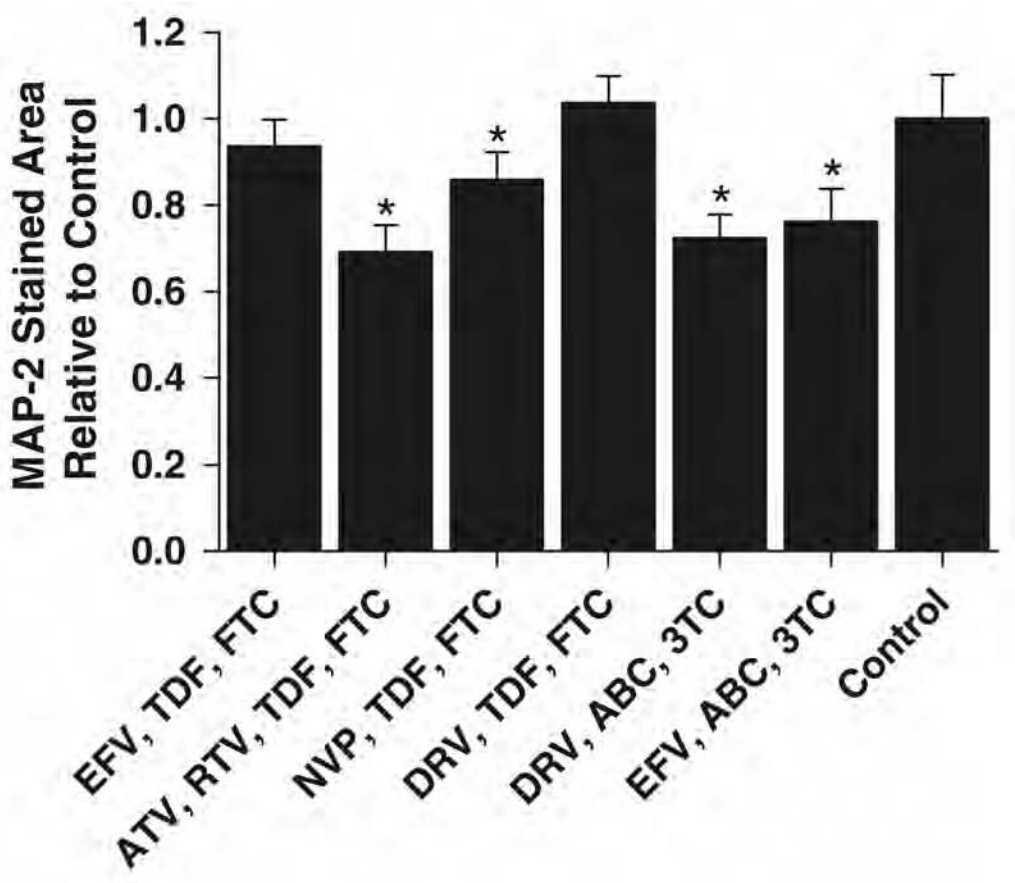

Fig 5.

Loss of MAP-2 stain in neural cultures treated for 7 days with one of five different antiretroviral combinations relative to untreated cultures. The concentration of each drug was matched to the therapeutic plasma concentration in Table 1. Four combinations significantly decreased the MAP-2 stained area (*p<0.05), although the extent of damage was moderate with a maximum loss of 31 % of the MAP-2 stained area. Values are mean ± SEM

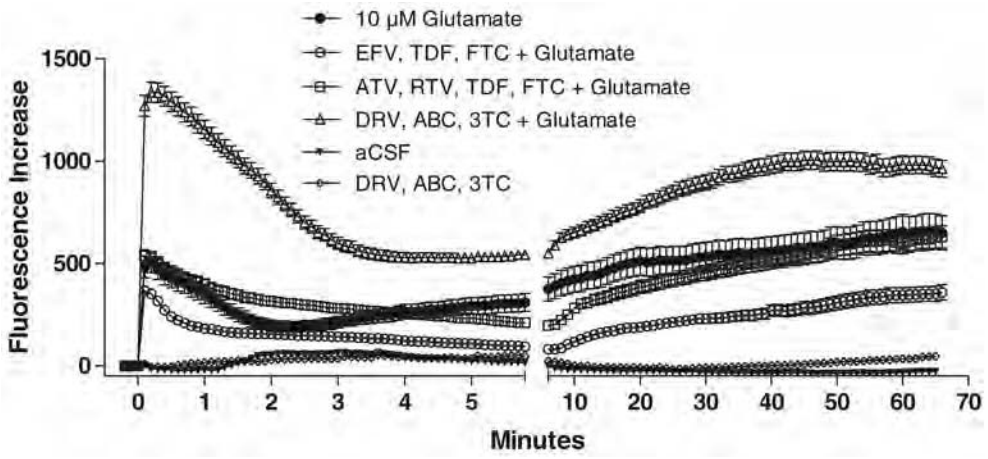

Fig 6.

Neuronal calcium responses to a 10 μM glutamate challenge after incubation in the indicated antiretroviral cocktail for 2 days. The mean fluorescence of neurons loaded with the calcium indicator dye Fluo-4 was measured over time. Fluo-4 fluorescence is proportional to the intracellular calcium concentration. A robust acute response to glutamate was seen in the control neurons (*filled circles*) receiving no antiretroviral treatment followed by a slight rise in the delayed phase. Pre-treatment of the neurons with the ARV combination ATV, RTV, TDF, FTC (*open squares*) resulted in a similar acute and delayed response. Pre-treatment with the more toxic combination of DRV, ABC, 3TC (*open triangles*) resulted in a much greater acute response followed by a larger delayed accumulation of calcium. The less toxic combination of EFV, TDF, FTC (*open circles*) reduced the magnitude of the acute response to glutamate. Although the net accumulation of calcium was lower, the rate of accumulation in the delayed phase was similar to the other ARV combinations. Neurons directly challenged with the ARV cocktail (DRV, ABC, 3TC; *open diamonds*) in the absence of glutamate had no effect on intracellular calcium and were indistinguishable from aCSF controls

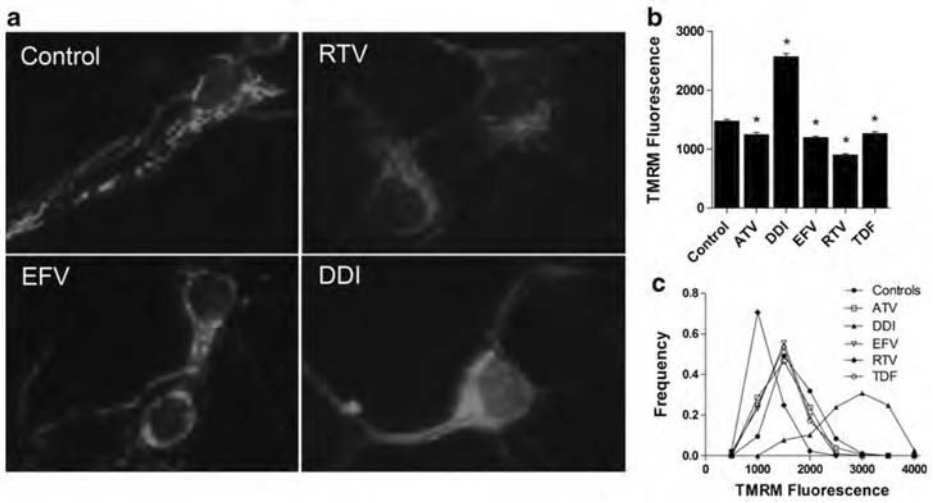

Fig 7.

a Examples of cultured neurons stained with the mitochondrial potential sensitive dye TMRM. Control mitochondria were seen as brightly stained, long fingerlike structures in the neurons. Treatment with RTV depleted the mitochondrial membrane potential as indicated by the low intensity of the TMRM stain. EFV had a small effect on the intensity of TMRM. DDI induced an increase in the TMRM fluorescence indicating an increase in the mitochondrial membrane potential. Each compound tested caused a slight rounding of the mitochondria. **b** Quantification of the average TMRM fluorescence in neurons treated with different ARVs. All ARVs tested resulted in a significant decrease in the TMRM fluorescence with the exception of DDI which increased the fluorescence (*p<0.05). **c** A frequency analysis illustrating the shift in the distribution of TMRM intensities for each ARV. Most ARVs induced a small shift to the left (decrease in mitochondrial membrane potential). RTV induced the largest shift to the left, whereas DDI produced a notable shift to the right

Table 1

Summary of ARV concentrations in plasma and CSF relative to toxic activity in vitro

Drug	Molecular weight	Max % MAP-2 loss	% Threshold TC50	Plasma concentration (ng/ml)	CSF concentration (ng/ml)	Reference
Abacavir (ABC)	286.3	27.2	2.2	139 (median)	128 (median)	Caparelli et al. 2005
2′,3′-Dideoxycytidine (ddC)	211.2	52.5	1065	25.3 (Cmax)	2.1 (Cmax)	ddC package insert
2′,3′-Dideoxyinosine (DDI)	236	42.5	18.4	840 (Cmax)	40 (mean	Burger et al. 1995; Hoetelmans et al. 1998
Emtricitabine [(−) FTC]	247.2	47.3	5287	261 (median)	109 (median)	Best et al. 2009a
Tenofovir (TDF)	305.2	18	80.9	96 (median)	5 (median)	Best et al. 2008
Lamivudine (3TC)	229.3	34.9	193.4	1195 (Cmax)	46 (median)	van Praag 2002
Zidovudine (AZT)	267	42.4	1638	635 (Cmax)	38 (median)	van Praag 2002
Efavirenz (EFV)	315.7	36.5	199.5	2145 (median)	13.9 (median)	Best et al. 2009a
Etravirine (ETR)	435.3	23.1	6.8	875.7 (Cmax)	0.9 (Cmax * 0.001)	Kakuda et al 2008
Nevirapine (NVP)	266.3	34.7	151.2	6199 (Cmax)	932 (median)	van Praag 2002
Amprenavir (APV)	505.6	29.1	49	2150 (median	25 (median)	Letendre et al. 2008
Atazanavir Sulfate (ATV)	802.9	33.5	15.8	1278 (median)	10.3 (median)	Best et al. 2009b
Darunavir (DRV)	593.7	17	10452	3930 (median)	34.2 (median)	Yilmaz et al. 2009
Ritonavir (RTV)	721	31.4	2375	1400 (Cmax)	23	Liu et al. 2007; Kravcik et al. 1999
Maraviroc (MVC)	513.7	22	2978	94.9 (median)	3.6 (median)	Yilmaz et al. 2009

NRTI nucleoside reverse transcriptase inhibitor, *NNRTI* non-nucleoside reverse transcriptase inhibitor, *PI* protease inhibitor, *EI* entry inhibitor, *MAP-2* microtubule associated protein-2, *TC50* median toxic concentration, *ND* no damage

EXHIBIT F

MAJOR ARTICLE HIV/AIDS

Neurocognitive Change in the Era of HIV Combination Antiretroviral Therapy: The Longitudinal CHARTER Study

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(See the Editorial Commentary by Cysique on pages 481–2.)

Background. Human immunodeficiency virus (HIV)-associated neurocognitive disorders (HAND) can show variable clinical trajectories. Previous longitudinal studies of HAND typically have been brief, did not use adequate normative standards, or were conducted in the context of a clinical trial, thereby limiting our understanding of incident neurocognitive (NC) decline and recovery.

Methods. We investigated the incidence and predictors of NC change over 16–72 (mean, 35) months in 436 HIV-infected participants in the CNS HIV Anti-Retroviral Therapy Effects Research cohort. Comprehensive laboratory, neuromedical, and NC assessments were obtained every 6 months. Published, regression-based norms for NC change were used to generate overall change status (decline vs stable vs improved) at each study visit. Survival analysis was used to examine the predictors of time to NC change.

Results. Ninety-nine participants (22.7%) declined, 265 (60.8%) remained stable, and 72 (16.5%) improved. In multivariable analyses, predictors of NC improvements or declines included time-dependent treatment status and indicators of disease severity (current hematocrit, albumin, total protein, aspartate aminotransferase), and baseline demographics and estimated premorbid intelligence quotient, non-HIV-related comorbidities, current depressive symptoms, and lifetime psychiatric diagnoses (overall model $P < .0001$).

Conclusions. NC change is common in HIV infection and appears to be driven by a complex set of risk factors involving HIV disease, its treatment, and comorbid conditions.

Keywords. cognitive change; HIV; antiretroviral therapy; comorbidities.

Availability of combination antiretroviral therapy (cART) has substantially improved medical morbidity and life expectancy in human immunodeficiency virus (HIV)-infected (HIV^+) individuals. Nevertheless, HIV-associated neurocognitive disorders (HAND) remain common [1–3]. Although the prevalence of the most severe form of HAND, HIV-associated dementia, has declined since the introduction of cART, milder forms of HAND persist and may be more prevalent in "earlier" disease stages that are maintained much longer than in the pre-cART era [2, 4, 5]. Recent reviews suggest modest neurocognitive (NC) improvement in HIV^+ groups after beginning cART [6–8], but little is known about the incidence and predictors of NC change over time.

So far only 1 study has used formal NC "norms for change" to classify significant NC improvement or

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[a]The CHARTER Group members are listed in the Appendix.

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decline in individual participants [9]. One hundred ninety-two HIV^+ and 101 HIV-uninfected (HIV^-) Chinese former plasma donors were followed for 1 year, and results of the HIV^- group were used to develop regression-based norms for change that adjusted for known factors that may affect follow-up results of medically stable people (eg, baseline level of performance, normal variability, and practice effects). Twenty-seven percent of the HIV^+ group evidenced NC decline over 1 year; this change was predicted by baseline AIDS and lower CD4, and at follow-up was associated with lack of viral suppression on cART and lower current CD4.

Here we present the longitudinal CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) findings, including both baseline and time-dependent predictors of NC change. We used recently published regression-based norms for NC change, developed with a large US sample that was administered the CHARTER test battery over multiple visits [10].

METHODS

Subjects

Four hundred thirty-six CHARTER participants who underwent 4–7 study visits (16–72 months of follow-up; mean, 35 months) were identified from the longitudinal cohort (n = 699). This group had a total of 2680 visits, and its demographic, HIV disease, and treatment characteristics at baseline are summarized in Table 1.

Table 1. Baseline Demographic, HIV Disease, and Treatment Characteristics of CHARTER Sample With 4–7 Study Visits (n = 436)

Characteristic	Mean (SD), Median (IQR), or %
Age, y, mean (SD)	43.9 (8.4)
Education, y, mean (SD)	12.9 (2.5)
Sex, male	80%
Race/ethnicity	
Non-Hispanic white	43%
Non-Hispanic black	44%
Hispanic	11%
Other	2%
Comorbidity status	
Incidental	59%
Contributing	29%
Confounding	12%
AIDS	60%
Nadir CD4 count, cells/μL, median (IQR)	184 (49–320)
Current CD4 count, cells/μL, median (IQR)	459 (289–644)
Currently on cART	70%
Duration current regimen, mo, mean (SD)	18.0 (21.2)
Prior cART only	12%
ART naive	18%
Undetectable HIV in plasma (n = 436)	41% (58% if on ART)
Undetectable HIV in CSF (n = 395)	66% (85% if on ART)
Neurocognitive impairment	46%

Abbreviations: ART, antiretroviral therapy; cART, combination antiretroviral therapy; CHARTER, CNS HIV Anti-Retroviral Therapy Effects Research; CNS, central nervous system; CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; IQR, interquartile range; SD, standard deviation.

Procedures

At all study visits, subjects completed a venipuncture, neuromedical assessment, comprehensive NC testing, detailed substance use history, a fully structured psychiatric interview for lifetime and current (30-day) diagnoses of major depression and alcohol and other psychoactive substance use disorders, and a measure of mood symptoms in the previous 14 days. At visits where the participant consented (n = 2408), lumbar puncture was performed. Details of the CHARTER assessments, including assessment of comorbidities, are provided in prior publications [1, 2]. All procedures were approved by the human subjects protection committees of each participating institution. Written informed consent was obtained from all study participants.

Determination of Overall NC Change

To determine NC change, we generated a *z* score for each of 15 neuropsychological variables based on published normative data [10]. These *z* scores reflect how well or poorly the person performed at follow-up, relative to normal expectation for someone with the same baseline NC and other relevant characteristics (eg, age, education). The *z* scores were then averaged to provide a summary regression change score (sRCS). The top 5% of the sRCS distribution of the normative sample defined the "improve" range and the bottom 5% defined the "decline" range [10]. The remaining middle 90% was defined as "stable." NC change status from baseline was generated for each follow-up visit. The individual visit change status for each participant was then merged into an overall change status: (1) decline: if a participant had at least 1 "decline" status and no "improve" status across visits; (2) improve: if a participant had at least 1 "improve" status and no "decline" status; (3) stable: if a participant had no "decline" or "improve" NC change status (all visits "stable"). Two participants out of an original cohort of 438 met criteria for both "improve" and "decline" during the follow-up period (at different visits) and were thus excluded from analyses.

The published normative standards for detecting NC change were derived from 172 HIV^- controls and 124 HIV^+ individuals who were selected based on strict criteria for clinical stability [10]. This provided a broader range of baseline NC performance in the total normative sample. The sRCS results were virtually identical for the HIV^- and stable HIV^+ subgroups (means across visits in *z* score units were 0.00 [standard deviation (SD), 0.32] for HIV^+ and 0.01 [SD, 0.34] for HIV^-; $P = .93$).

Statistical Methods

Participants classified by overall change status were compared on baseline characteristics using analysis of variance and Tukey honestly significant difference tests or χ^2 tests. No other adjustments for multiple testing were applied. Initially, potential time-varying and static predictors of the first time when any NC change occurred were screened individually using Cox proportional hazards modeling. Variables univariably significant at a liberal 10% level were then combined into a Cox time-dependent multivariable model, and backward elimination with a minimal Akaike information criterion (AIC) was used to reduce the model. Because the resultant model was based only on visits having nonmissing data for all variables in the initial full model, the AIC-based modeling was repeated on reduced models, thus allowing inclusion of more visits. The procedure continued until no further variable reduction occurred. This method identifies a concise combination of predictors based on maximal available data and after eliminating variables that fail to contribute to a better fit if included in the model. The procedure includes variables contributing important information without regard to *P* values.

RESULTS

Over the total follow-up period, 99 participants (22.7%) were defined as NC "decliners," 72 (16.5%) were "improvers," and 265 (60.8%) were "stable" (Supplementary Digital Data Figure 1).

Decliners had a mean sRCS (*z* score units) of −0.52 compared with −0.01 for stable participants and 0.42 for improvers ($P < .001$); these mean *z* score differences approximate medium effect sizes. At the last visit, 61% of decliners were NC impaired, compared with 37% of the stable participants and 24% of the improvers (decline > stable > improve; $P < .0001$). Of the 99 decliners, 66 (66.7%) met criteria for symptomatic NC impairment (impairment with documented functional decline [1]) during their follow-up. This contrasts with 37.5% with symptomatic impairment for the improvers and 40.4% for stable participants ($\chi^2_{df=2} = 22.5$, $P < .0001$). Figure 1 shows the 3 groups' mean changes in average, demographically corrected NC standard scores (*T* scores) from baseline to last visit. This demonstrates that, even though the visit where a change occurred usually was well before visit 7 (62% were before visit 4), the groups evidence very different trajectories across the entire follow-up period: Although NC performance was quite comparable at baseline, the 3 groups were very different at the final visit ($F = 42.98$; $P < .0001$). Importantly, however, although individuals within the total CHARTER group showed variable NC trajectories, the overall prevalence of HAND did not increase: 45.9% had NC impairment at baseline and 40.1% were impaired at their final visit.

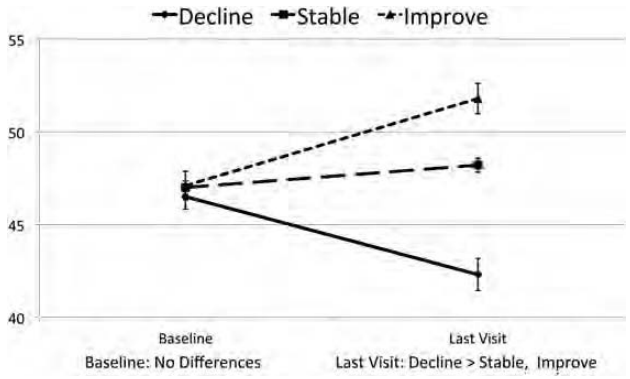

Figure 1. Mean neurocognitive *T* score at baseline and last follow-up for 3 change groups.

Baseline Predictors of NC Change

Although decliners, improvers, and stable participants did not differ on most baseline demographics, disease, or treatment variables, there were a few differences (Table 2). Decliners were more likely than improvers to be female, Hispanic, and to have detectable virus in plasma and cerebrospinal fluid (CSF) (overall, and if on antiretroviral therapy [ART]); in all of these respects, the stable group was intermediate between decliners and improvers. Although decliners tended to have more severe non-HIV comorbidities than both other groups, the comparison was statistically significant only vs the stable group for confounding conditions.

Stability of Viral Suppression and NC Change

We classified participants as being "always undetectable" (plasma HIV RNA at all visits ≤50 copies/mL), "always detectable" (plasma HIV RNA at all visits >50 copies/mL), and "sometimes detectable" (plasma HIV RNA at least 1 visit ≤50 copies/mL, and at least 1 visit >50 copies/mL). We then compared these groups on their average sRCS across all visits. The "always detectable" group had a lower average sRCS score than both the "always undetectable" and "sometimes detectable" groups (−0.26 vs −0.06 and 0.003, respectively; $P = .002$), indicating greater NC decline in the "always detectable" group compared with the other groups (see Figure 2).

Predictors of Earlier NC Decline or Improvement

Univariable survival analyses using a combination of both time-invariant and time-dependent predictors were run to explore the effects of these predictors on incident NC change. "Time dependent" in this context means that the values of these variables are subject to change from visit to visit. Potential predictors univariably screened for subsequent multivariable analysis included demographic, disease, treatment, laboratory, and psychiatric variables. Table 3 displays the predictors identified as candidates for multivariable modeling ($P < .10$).

Table 2. Comparison of Neurocognitive Change Groups on Baseline Demographic, Treatment, and Disease Characteristics

Characteristic	Decline	Stable	Improve	Group Differences[a] $P < .05$
No. of visits, mean (SD)	6.2 (1.1)	6.1 (1.2)	6.2 (1.2)	
Age, y, mean (SD)	42.7 (8.4)	44.2 (8.7)	43.8 (7.5)	
Education, mean (SD)	12.5 (2.6)	12.9 (2.4)	13.3 (2.4)	
Male sex	72%	81%	86%	D < I
Race/ethnicity				
Non-Hispanic white	36%	44%	50%	
Non-Hispanic black	39%	46%	44%	
Hispanic	20%	8%	6%	D > I, S
Other	5%	2%	0%	
WRAT-3 Reading subtest, mean (SD)	92.8 (15.2)	93.8 (14.8)	96.6 (15.0)	
Comorbidity				
Incidental	48%	65%	58%	
Contributing	32%	27%	31%	
Confounding	20%	8%	11%	D > S
NC impaired	51%	45%	42%	
AIDS	55%	62%	61%	
Nadir CD4, median (IQR)	206 (60–354)	180 (41–327)	171 (26–303)	
Current CD4, median (IQR)	432 (264–617)	469 (295–659)	432 (245–588)	
ART	68%	70%	72%	
CPE score[b]	7.8 (2.2)	7.6 (1.9)	7.8 (2.0)	
Detectable plasma	66%	59%	50%	I < D
Detectable on ART	50%	44%	31%	I < D
Detectable CSF	47%	40%	28%	I < D, S
Detectable on ART	30%	24%	8%	I < D, S
PI-based regimen	60%	56%	46%	
HCV infected	31%	24%	25%	

Abbreviations: ART, antiretroviral therapy; CPE, central nervous system penetration effectiveness; CSF, cerebrospinal fluid; HCV, hepatitis C virus; IQR, interquartile range; NC, neurocognitive; PI, protease inhibitor; SD, standard deviation; WRAT-3, Wide Range Achievement Test, 3rd ed.

[a] D, decliner; I, improver; S, stable.

[b] Letendre et al [11].

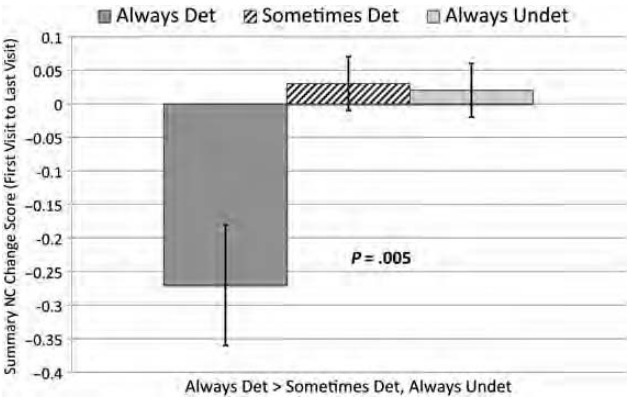

Figure 2. Plasma viral load over time vs average neurocognitive (NC) change score. Abbreviations: Det, detectable; Undet, undetectable.

Candidate predictors for multivariable modeling of either decline or improvement included time-dependent HIV treatment status (on/off ART) and disease severity indicators (degree of immunosuppression, HIV RNA load in plasma and CSF, serum total protein, albumin, hematocrit, high-density lipoprotein [HDL], and aspartate aminotransferase [AST]), all in the "expected" direction of worse medical management/status being associated with decline, and better management/status with improvement. In addition, overall severity of non-HIV comorbidities [2] and some specific (substance and mood related) psychiatric diagnoses as well as higher depressive symptoms were associated with earlier NC decline, whereas absence of lifetime major depression and substance use diagnoses were predictors of earlier improvement.

Table 3. Univariable Predictors of Time to Neurocognitive Change (Decline or Improvement)

	Decline				Improvement			
Predictor	Risk	Reference	RR	*P* Value	Risk	Reference	RR	*P* Value*
Age	Younger	1 y[a]	1.02	.0937				
Sex	Female	Male	1.76	.0153				
Ethnicity	Hispanic	Non-Hispanic	2.35	.0018				
Education					Higher	1 y[b]	1.10	.0534
Premorbid IQ[c]					Higher	1 unit[b]	1.02	.0473
ART status[d]	Off ART	On ART	1.91	.0038				
CD4[d]	Lower	100 cells[a]	1.14	.0024				
Nadir CD4	Higher	100 cells[b]	1.09	.0833				
Plasma VL[d]	Higher	1 $\log_{10}$[b]	1.26	.0026	Lower	1 $\log_{10}$[a]	1.27	.0295
Det/Undet[d]					Undet	Det	1.53	.0876
CSF VL[d]	Higher	1 $\log_{10}$[b]	1.26	.0552	Lower	1 $\log_{10}$[a]	1.47	.0476
Det/Undet[d]	Det	Undet	1.50	.0790	Undet	Det	1.73	.0952
AST[d]					Lower	1 unit[a]	1.01	.0172
Protein total[d]					Lower	1 unit[a]	1.96	<.0001
Albumin[d]	Lower	1 unit[a]	2.36	<.0001				
HDL[d]	Lower	1 unit[a]	1.01	.0367				
HCT[d]	Lower	1 unit[a]	1.10	<.0001	Higher	1 unit[b]	1.06	.0244
Comorbidity[e]	Severe	Minimal	2.47	.0007				
Utox[d]	Positive	Negative	1.58	.0497				
LT cannabis Dx[d]					No	Yes	1.58	.0863
LT methamphetamine Dx[d]	Yes	No	1.81	.0148				
LT any substance Dx[d]					No	Yes	1.63	.0576
MDD (last 30 d)[d]	Yes	No	1.68	.0659				
LT MDD[d]	Yes	No	1.71	.0118	No	Yes	1.63	.0396
Beck (total)[d]	Higher	1 unit[b]	1.03	.0051				

Abbreviations: ART, antiretroviral therapy; AST, aspartate aminotransferase; Beck, Beck Depression Inventory II; CSF, cerebrospinal fluid; Det, detectable viral load; Dx, history of abuse or dependence diagnosis; HCT, hematocrit; HDL, high-density lipoprotein; IQ, intelligence quotient; LT, lifetime; MDD, major depressive disorder; RR, relative risk; SD, standard deviation; Undet, undetectable viral load; Utox, urine toxicology for drugs with central nervous system effects; VL, viral load.

[a] Higher/older.

[b] Lower.

[c] Measured using Wide Range Achievement Test, 3rd ed, Reading Standard Score (population mean = 100, SD = 15).

[d] Variable modeled in a time-dependent manner.

[e] Comorbidity rating [1, 2]: minimal/incidental, moderate/contributing, severe/confounded.

*$P < .10$ was considered significant.

Finally, younger age, female sex, and Hispanic ethnicity increased risk for decline, whereas higher education level and premorbid intelligence quotient (IQ) estimate (reading level) were associated with improvement.

In the final multivariable model, Hispanic ethnicity (vs non-Hispanic: relative risk [RR], 2.16 [95% confidence interval {CI}, 1.29–3.61]); confounded comorbidity status (vs incidental: RR, 2.12 [95% CI, 1.22–3.67]); being off ART (vs on ART: RR, 1.94 [95% CI, 1.26–3.00]); having low albumin (vs 1 unit higher: RR, 1.58 [95% CI, .99–2.52]) and low hematocrit (vs 1 unit higher: RR, 1.08 [95% CI, 1.03–1.13]); and having a lifetime methamphetamine use diagnosis (vs none: RR, 1.87 [95% CI, 1.16–3.02]) and more depressive symptoms (vs 1 unit lower: RR, 1.02 [95% CI, 1.00–1.04]) were associated with earlier time to NC decline (model $P < .0001$). The multivariable combination of predictors of improvement included higher premorbid IQ estimate (vs 1 unit less: RR, 1.02 [95% CI, 1.00–1.04]); lower total protein (vs 1 unit higher: RR, 1.85 [95% CI, 1.30–2.63]); lower AST (vs 1 unit higher: RR, 1.01 [95% CI, 1.00–1.03]); and no lifetime major depressive disorder (vs positive history: RR, 2.09 [95% CI, 1.27–3.45]) (model $P < .0001$).

DISCUSSION

Whereas previous cross-sectional and longitudinal research assessed clinical correlates of NC impairment in HIV-infected

individuals (ie, HAND), our focus was on the incidence, nature, and predictors of NC change. Importantly, we employed recently published, regression-based norms that allowed us to detect significant NC change in individual participants, while controlling for normal test–retest variability, practice effects, and statistical artifacts. We found that almost 40% of subjects showed NC change, with 23% declining and 17% improving. The clinical significance of "decliner" status, in particular, is supported by significantly higher rates of symptomatic NC impairment in this group compared with those who were stable or improved.

We identified many univariable candidate predictors of NC decline or improvement when time-dependent clinical and laboratory findings were considered. These included several predictors specific to HIV and its treatment (ART status, immunosuppression, and plasma and CSF HIV RNA load), as well as others reflecting more general health status (AST, serum protein, albumin, HDL, and hematocrit). The multivariable model predicting time to decline reflected significant combined effects of 5 time-dependent variables (being off ART and having a lower hematocrit, lower albumin, a lifetime methamphetamine use diagnosis, and more depressive symptoms) and 2 static predictors (more significant non-HIV risks for NC impairment and Hispanic ethnicity). By contrast, a significant combination of multivariable predictors of NC improvement included 3 time-dependent variables (lower serum protein, lower AST, and absence of any lifetime history of major depressive disorder) and 1 static predictor (higher estimated premorbid IQ).

As an observational study, CHARTER is unable to demonstrate ART effects. Nevertheless, beneficial effects of cART on VL and immune function are well established [6–8], so the joint, time-dependent links of ART status and associated HIV biomarkers with both positive and negative NC outcomes are potentially important. Specifically, our findings suggest that being off ART uniquely increases risk for NC decline, and current virologic control and degree of immunocompetence were univariable predictors of both types of NC change. Also consistent with the CHARTER findings, in the 1 prior observational study that used regression-based NC norms for change to identify significant NC decline, such decline was associated with lower follow-up CD4 and lack of viral suppression in a large HIV^+ Chinese sample [9].

Considered together, the current and previously published findings suggest that protection of the central nervous system (CNS) and favorable NC outcomes may be achieved by instituting cART early [12] and monitoring patients carefully to ensure maintenance of viral suppression and immunocompetence. In practice, however, these treatment goals often are not fully realized: Only 79 of the 436 (18.1%) longitudinal participants in CHARTER had undetectable virus in plasma during all study visits. Furthermore, sustained viral suppression does not in itself preclude persisting or even incident HAND [7]. The mechanisms of NC decline in virally suppressed patients are uncertain, but ART toxicity [13] and other non-HIV-related factors may be involved in some cases.

In addition to HIV disease and treatment predictors, we identified several other participant characteristics that may influence NC outcomes over time (Table 3). Factors that appeared beneficial include the combination of higher education level and reading-based estimate of premorbid IQ. These 2 variables are indicators of "cognitive reserve" [14], a concept used to explain individual differences in the threshold of CNS insult required to produce symptomatic neurologic disease. In particular, our finding that higher premorbid IQ was a unique predictor of NC improvement extends prior cross-sectional [15, 16] and longitudinal [17] studies supporting positive effects of cognitive reserve in HIV.

As noted in the CHARTER baseline report, this HIV^+ population had many and diverse non-HIV-related comorbid conditions that may confer increased risks for NC impairment [2]. All participants were classified at baseline into 1 of the 3 specified comorbidity levels [1], and each successive level had more of such comorbidities (averaging 1.5, 3.2, and 4.2 conditions, respectively; $P < .01$ for all comparisons). In addition, successive groups had more severe comorbidities, as well as higher rates of NC impairment (40%, 59%, 83%). Past research has tended to exclude people with the highest level of comorbidities (those that represent "confounds"), and we are not aware of any previous, systematic attempt to relate comorbidity level to NC change over time. Here we found that the rate of NC decline was much higher in the participants who were classified as confounded at baseline (38.5%, vs 24.6% for those with "contributing" and 18.3% for those with "incidental" comorbidities). The specific reasons for these differences undoubtedly are as varied and complex as the patterns of comorbid conditions involved, and could not be established here with any certainty. Nevertheless, the published comorbidity classification system [1] has shown good interrater reliability in CHARTER [2], and successive levels of comorbidity appear to confer increased risk not only for cross-sectional NC impairment, but also for NC decline over time. These associations may justify more frequent or intensive medical monitoring and support for HIV^+ patients who have higher comorbidity burdens.

We propose that, in univariable analyses, lower age and higher nadir CD4 appeared as marginal ($P < .10$) predictors of time to NC decline only because of their associations with other participant characteristics in this cohort. In fact, among our NC decliners, lower age was significantly associated not only with shorter duration of HIV infection and higher nadir CD4 counts, but also with higher likelihood of coinfection with hepatitis C virus, higher AST, and increased likelihood of a lifetime methamphetamine use diagnosis. As noted, neither age nor nadir CD4 cell count was identified as a contributor to the multivariable prediction models.

We did not anticipate a higher rate of NC decline to be associated with Hispanic ethnicity, but there are multiple factors

that may be contributing to worse outcomes in this group. In general, Hispanics in the United States tend to have lower access to healthcare than non-Hispanic whites [18, 19]. They are much less likely to have health insurance coverage [18] or a usual source of healthcare [19]. HIV$^+$ Hispanics tend to be late to be tested for the virus [20, 21], to obtain medical care, and to initiate therapy after diagnosis [22]. They also may be more likely to receive suboptimal HIV care [23]. Thus, not surprisingly, they have been observed to have worse HIV disease characteristics, including lower CD4 counts, higher plasma viral loads, more opportunistic infections, and higher rates of AIDS [24, 25]. Among HIV$^+$ adults, reduced life expectancy from late initiation of therapy and from early discontinuation of therapy are greatest for Hispanics [26]. Few studies have examined the prevalence and pattern of NC impairment among HIV$^+$ Hispanics. The limited data available are cross-sectional and typically include selected groups with small numbers [27–30]. Although there have been inconsistent findings [22], most studies have found Hispanic ethnicity to be associated with worse NC status [27, 29, 30].

Future research should attempt to clarify factors within the HIV$^+$ Hispanic population that may influence disease outcomes in general, and NC decline in particular. CHARTER was not designed for this goal. Even though all CHARTER participants were receiving care during this study, it is of interest that, compared to non-Hispanic whites, Hispanics in CHARTER had a higher rate of AIDS (74% vs 58%, $P = .03$) and lower nadir CD4 cell count (median of 96 vs 190, $P = .04$). Other factors that may be relevant to consider in future research include immigration status, country of origin, time living in the United States, housing, employment, nutrition, acculturation, language barriers, and health literacy, to name a few (see [31]).

A major limitation of the current study is that it is observational, without control (other than statistical adjustments) over the many treatments, diseases, and other factors that may affect NC outcomes over time. Causation is difficult to assign in observational research. However, our finding of significant, time-dependent clinical and biological predictors of NC change, and the fact that many such associations are consistent with those in prior longitudinal studies, suggests that the observed associations may be clinically meaningful. Furthermore, they suggest that consistent use of ART to maintain virologic control and avoid serious immunosuppression may have beneficial long-term effects in protecting the CNS and improving NC outcomes. Finally, increased comorbidity burden and factors associated with Hispanic ethnicity deserve more attention in clinical care of HIV$^+$ individuals.

Notes

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Disclaimer. The views expressed in this article are those of the authors and do not reflect the official policy or position of the United States government.

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

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APPENDIX

The CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) group is affiliated with the Johns Hopkins University; Mount Sinai School of Medicine; University of California, San Diego; University of Texas Medical Branch, Galveston; University of Washington, Seattle; and Washington University, St Louis; and is headquartered at the University of California, San Diego. Members are as follows. Director: Igor Grant, MD; Co-directors: J. Allen McCutchan, MD, Ronald J. Ellis, MD, PhD, Thomas D. Marcotte, PhD; Center Manager: Donald Franklin Jr; Neuromedical Component: Ronald J. Ellis, MD, PhD (Principal Investigator [PI]), J. Allen McCutchan, MD, Terry Alexander, RN; Laboratory, Pharmacology and Immunology Component: Scott Letendre, MD (PI), Edmund Capparelli, PharmD; Neurobehavioral Component: Robert K. Heaton, PhD (PI), J. Hampton Atkinson, MD, Steven Paul Woods, PsyD, Matthew Dawson; Virology Component: David M. Smith, MD (PI); Imaging Component: Christine Fennema-Notestine, PhD (Co-PI), Michael J. Taylor, PhD (Co-PI), Rebecca Theilmann, PhD; Data Management Unit: Anthony C. Gamst, PhD (PI), Clint Cushman; Statistics Unit: Ian Abramson, PhD (PI), Florin Vaida, PhD; Protocol Coordinating Component: Thomas D. Marcotte, PhD (PI), Jennifer Marquie-Beck, MPH; Johns Hopkins University site: Justin McArthur (PI), Vincent Rogalski, RN; Icahn School of Medicine at Mount Sinai site: Susan Morgello, MD (Co-PI) and David Simpson, MD (Co-PI), Letty Mintz, NP; University of California, San Diego site: J. Allen McCutchan, MD (PI), Will Toperoff, NP; University of Washington site: Ann Collier, MD (Co-PI) and Christina Marra, MD (Co-PI), Trudy Jones, MN, ARNP; University of Texas site: Benjamin Gelman, MD, PhD (PI), Eleanor Head, RN, BSN; and Washington University site: David Clifford, MD (PI), Muhammad Al-Lozi, MD, Mengesha Teshome, MD.

EXHIBIT G

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MAJOR ARTICLE

Changes in Body Mass Index and Atherosclerotic Disease Risk Score After Switching From Tenofovir Disoproxil Fumarate to Tenofovir Alafenamide

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Background. Switching from tenofovir disoproxil fumarate (TDF) to tenofovir alafenamide (TAF)-containing antiretroviral therapy (ART) can improve renal function and bone mineral density in people with human immunodeficiency virus (PWH). The switch can also negatively influence cholesterol, but changes in body mass index (BMI) and atherosclerotic cardiovascular disease (ASCVD) risk are unknown.

Methods. This retrospective observational study evaluated BMI and ASCVD risk score changes in virologically suppressed PWH who switched from TDF to TAF without switching other ART regimen components. Adults on TDF for ≥1 year with 2 consecutive HIV ribonucleic acid values <200 copies/mL before a TAF switch were included. Body weight, BMI, cholesterol, and ASCVD risk score were collected for the year before and after the switch. Pre- and postswitch values were compared with the Wilcoxon signed-rank test. Changes in BMI and ASCVD scores were modeled using generalized estimating equations regression.

Results. One hundred ten patients were included. In unadjusted analyses, there were significant increases in weight, BMI, total cholesterol, LDL, HDL, and ASCVD risk score in the year after switching from TDF to TAF (each $P \leq .01$). In regression models, switching from TDF to TAF was associated with a 0.45 kg/m^2 increase in BMI (95% confidence interval [CI], 0.14–0.76) and a 13% increase in ASCVD risk score (95% CI, 4%–23%).

Conclusions. We observed significant BMI and ASCVD score increases in PWH 1 year after switching from TDF to TAF. The mechanism of changes is unclear and requires additional study.

Keywords: BMI; cardiovascular disease risk; HIV; tenofovir alafenamide; weight gain.

Switching from tenofovir disoproxil fumarate (TDF) to tenofovir alafenamide (TAF)-containing antiretroviral therapy (ART) can maintain virologic efficacy, while preserving or improving renal function and bone mineral density in people with human immunodeficiency virus (PWH) [1–3]. The switch may also negatively influence cholesterol. Patients that switched to TAF-containing ART in clinical trials had significant increases in total (Tchol) high-density lipoprotein (HDL-C) and low-density lipoprotein (LDL-C) cholesterol levels [3–5]. The impact of these changes on atherosclerotic cardiovascular disease (ASCVD) risk is unclear [6]. In addition, recent studies suggest that initiating TAF alongside an integrase inhibitor in treatment-naive patients or switching from TDF to TAF in treatment-experienced patients can lead to weight gain [7, 8]. Patients in the switch study often had changes in other ART regimen components, thereby confounding the ability to analyze TAF alone [7]. The purpose of this study was to determine whether changes in weight, body mass index (BMI), and ASCVD risk score occur after switching from TDF to TAF, without switching other ART regimen components.

METHODS

This was a retrospective, observational study involving virally suppressed PWH who switched from TDF to TAF-containing ART between January 2016 and March 2018 at an urban, academic medical center. Institutional review board approval was obtained before data collection. Adult patients on TDF-containing ART for at least 1 year were included if they had evidence of persistent viral suppression. This was defined as having at least 2 consecutive human immunodeficiency virus (HIV) viral load values <200 copies/mL and no values >200

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copies/mL in the year before the TAF switch. Patients were excluded if any other component of their ART regimen changed in the year before or after the TAF switch.

Demographic data including age, sex, and race were extracted from medical records of eligible patients along with preswitch CD4 cell counts, concomitant ART agents, duration of HIV infection, and total years on ART. Any non-ART medications prescribed for chronic use in the year after the TDF to TAF switch were also collected. Each non-ART medication was labeled as being associated with weight gain, weight loss, or no weight change according to package labeling.

To assess the study endpoints, body weight, BMI, Tchol, LDL-C, HDL-C, and triglyceride values were collected for the year before and after the switch. Pre- and postswitch median values for each of these parameters were then calculated.

To determine pre- and postswitch ASCVD risk scores, the 2018 American College of Cardiology/American Heart Association guidelines on treating blood cholesterol were used [6]. According to these guidelines, ASCVD scores can estimate the 10-year risk of a cardiovascular event in patients between 40 and 75 years old. As a result, only patients between these ages with all other necessary data points to perform the ACSVD calculation were assessed. Using age, sex, race, cholesterol, blood pressure, diabetes, and smoking status, ASCVD risk scores were generated for all eligible patients at the time of the ART switch and between 6 and 12 months thereafter for comparison.

Statistical Analysis

Patient demographics were summarized with counts and percentages for categorical variables and summarized with means and standard deviations or medians and interquartile ranges (IQRs) for numeric variables. Unadjusted distributions of the pre- and postswitch values for all study endpoints were summarized with medians and IQRs and compared with Wilcoxon signed-rank tests. To further investigate the association of switching from TDF to TAF with BMI and ASCVD risk score, 2 separate generalized estimating equation regression models were constructed. The covariates selected as candidates for each model included the following: a pre- versus post-TAF switch indicator, age, sex, race, concomitant medications that can cause weight gain, concomitant medications that can cause weight loss, and time since HIV diagnosis. Two-way interactions with covariates and the pre- versus post-TAF indicator were also assessed. Interaction and main effect terms were removed from the model one at a time using a hierarchical variable selection procedure (ie, never removing a main term that is part of an interaction term in the model) with a final retention criterion of 0.05. The ASCVD risk scores were highly right-skewed, so those data were log-transformed before modeling. All the analyses and visualizations were performed and created with SAS 9.4 (SAS Institute Inc., Cary, NC). The significance level of each test was $\alpha = 0.05$.

RESULTS

A total of 110 patients met study criteria and were included in the analysis (Table 1). The majority were African American (58.2%) and male (72.7%) with a mean age of 50 years old. Patients had been living with HIV and receiving ART for a median of 12 and 8 years, respectively, with persistent viral suppression and immunologic recovery in response to ART. Approximately half of the patients were receiving integrase inhibitor-based regimens before their TAF switch.

The majority of subjects (65.5%) were either overweight or obese at the time they switched to TAF. The median patient weight was more than 185 pounds with a BMI of 28 kg/m^2. In the year after their switch to TAF, patients had significant unadjusted increases in both weight and BMI. On average, patients gained 3 pounds and their BMI increased by 0.5 kg/m^2 (each $P \leq .01$) (Table 2). In the regression model for BMI, only sex was retained as a covariate. The results of the model were consistent with the unadjusted analysis, suggesting that switching from TDF to TAF was associated with a 0.45 kg/m^2 mean increase in BMI (95% confidence interval [CI], 0.14–0.76).

Unadjusted analyses also showed significant increases in Tchol, LDL-C, and HDL-C in the year after patients switched from TDF to TAF (each $P \leq .01$) (Table 2). It is notable that, although the total cholesterol to HDL-C ratio did not change significantly after the switch, significant changes in ASCVD risk scores were observed. A total of 91 of 110 patients were between the ages of 40 and 75 and were eligible for ASCVD score calculations. Of these, 68 had all other data necessary to perform the calculations both before and after the switch. For these patients, the median ASCVD score rose from 6.9% to 8.1% after switching to TAF ($P < .01$). Our regression model, adjusting for age, sex, race, concomitant medications that can cause weight gain, and time with HIV, suggested that switching from TDF to TAF was associated with a 13% average increase in ASCVD risk score (95% CI, 4%–23%).

DISCUSSION

We observed significant increases in both BMI and ASCVD risk in PWH who switched from TDF to TAF without changing any other ART regimen components. Importantly, patients in these analyses had longstanding HIV infection that was persistently controlled by ART. Patients were also at or above a normal BMI when switching to TAF. Therefore, it is unlikely that the weight gain patients experienced in this study represented a return to health, which commonly occurs after individuals initiate ART for the first time [9, 10].

In patients with advanced HIV who are underweight before starting ART, weight gain can prolong survival [11, 12]. In contrast, being overweight when initiating treatment or becoming obese on ART can increase a patient's risk for dyslipidemia, diabetes, hypertension, and cardiovascular disease (CVD) [13–15].

Table 1. Demographics and Other Patient Characteristics Summary

Characteristic	All Patients (n = 110)
Age, mean (SD)	50 (11.7)
Sex, n (%)	
Male	80 (72.7)
Female	30 (27.3)
Race, n (%)	
African American	64 (58.2)
White	38 (34.5)
Hispanic	6 (5.5)
Asian	2 (1.8)
Years since HIV diagnosis, median (IQR)	12.0 (11.0)
Years on ART, median (IQR)	8.0 (8.0)
Preswitch CD4 count (cell/mm^3), median (IQR)	627.5 (381.0)
Preswitch BMI category, n (%)	
Underweight	4 (3.6)
Normal weight	34 (30.9)
Overweight	31 (28.2)
Obese	41 (37.3)
Other ART agent, n (%)	
Integrase inhibitor	54 (49.1)
Protease inhibitor	18 (16.4)
Nonnucleoside reverse-transcriptase inhibitor	32 (29.1)
Other	6 (5.4)
Concomitant medication cause weight gain, n (%)	34 (30.9)
Concomitant medication cause weight loss, n (%)	29 (26.4)

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; HIV, human immunodeficiency virus; IQR, interquartile range; SD, standard deviation.

This is concerning because obesity is becoming increasingly prevalent in PWH, and these individuals already carry a disproportionate risk for CVD [16–18]. More than half of the patients in this analysis were overweight or obese at baseline and experienced modest increases in BMI 1 year after switching to TAF. Importantly even modest increases in BMI may be clinically relevant. In a recent analysis of over 9000 PWH enrolled in the Data-collection on Adverse Effects of Anti-HIV Drugs (D:A:D) study, the risk of diabetes and CVD increased by approximately 20% per unit of BMI gained in the year after starting ART [13]. The extent to which the BMI changes we observed after switching ART could influence a patient's risk of developing diabetes or CVD is unclear.

In terms of dyslipidemia, we observed significant increases in Tchol, LDL-C, and HDL-C in our patients after switching to TAF. These findings are consistent with observations from clinical trials. Also consistent with previous trials, we did not observe changes in Tchol/HDL-C ratios. Current guidelines on treating blood cholesterol recommend calculating an ASCVD risk score using cholesterol data in addition to a person's age, sex, race, blood pressure, and smoking status [6]. When performing this assessment for our patients, we observed a 13% increase in ASCVD risk scores after switching to TAF. This resulted in many patients becoming eligible for treatment with a statin medication for ASCVD risk reduction. More specifically, before TAF, the median ASCVD risk score in our sample was 6.9%, indicating that over half (53.3%) of the sample was below the threshold of 7.5% to meet statin eligibility criterion. After switching to TAF, the average 13% increase in ASCVD risk scores shifted 50.7% of our sample over the 7.5% statin criterion. Taken together with the increases in BMI and cholesterol, the changes in ASCVD risk score may indicate an increased risk for CVD in PWH after switching from TDF to TAF.

This study has several limitations. First, as an observational study, the results cannot establish causal relationships between TAF and increases in BMI or ASCVD risk. Second, as a retrospective chart review, we relied on the accuracy and completeness of medical records, but omissions or inaccuracies that influenced the results were possible. In addition, although we made every attempt to control for confounding variables with restrictive inclusion criteria and statistical analyses, it remains possible that data not measured or collected could have influenced our results. For example, we were unable to collect data on patients' caloric intake and physical activity. Finally, our cohort was predominantly African American and male from a single academic center in the Northeastern United States. As a result, the findings may not be generalizable to other populations. Given these limitations, additional investigations will be necessary to determine whether there are causal relationships

Table 2. Unadjusted Outcomes Summary

Outcome Variable	Preswitch (TDF)	Postswitch (TAF)	Change (Post–Pre)	*P* Value
Weight (lbs), median (IQR)	185.4 (55.8)	190.5 (60.5)	3.0 (9.2)	<.01
BMI (kg/m^2), median (IQR)	28.0 (10.8)	28.2 (10.0)	0.5 (1.4)	<.01
Total cholesterol, median (IQR)	173.8 (44.0)	195.0 (42.0)	12.5 (32.3)	<.01
LDL cholesterol, median (IQR)	98.6 (40.2)	112.1 (46.6)	8.2 (21.0)	<.01
HDL cholesterol, median (IQR)	51.0 (19.0)	55.8 (24.0)	3.0 (12.0)	<.01
Total to HDL cholesterol ratio, median (IQR)	3.5 (1.6)	3.5 (1.7)	0.1 (0.6)	.25
Triglyceride levels, median (IQR)	103.5 (68.0)	109.5 (93.0)	4.0 (64.0)	.28
Atherosclerotic CVD risk score, median (IQR)	6.9 (8.1)	8.1 (10.9)	0.4 (1.9)	<.01
Creatinine clearance, median (IQR)	104.0 (38.0)	102.5 (42.0)	−1.0 (17.0)	.82

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

between TAF exposure and increases in BMI or ASCVD risk. If relationships do exist, the associated mechanisms and any additional risk factors should be determined to optimize treatment for all PWH.

CONCLUSIONS

We observed significant increases in BMI and ASCVD risk in PWH 1 year after a switch from TDF to TAF without changes in other ART regimen components. The mechanisms associated with these metabolic changes are unclear and require additional study.

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Author contributions. J. J. S. and K. N. S. contributed to study concept and design. J. J. S., K. N. S., J. R. O., and J. A. D. contributed to acquisition, analysis, or interpretation of data. A. S. and S. W. K. performed statistical analysis. J. J. S. and J. R. O. drafted the manuscript. J. J. S., K. N. S., J. R. O., A. S., S. W. K., and J. A. D. critically reviewed and revised the manuscript.

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EXHIBIT H

Open Forum Infectious Diseases

MAJOR ARTICLE

Atherosclerotic Cardiovascular Disease Risk Profile of Tenofovir Alafenamide Versus Tenofovir Disoproxil Fumarate

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Background. In human immunodeficiency virus (HIV) treatment, tenofovir alafenamide (TAF) is associated with greater increases in all fasting cholesterol subgroups compared with tenofovir disoproxil fumarate (TDF). Because lipid abnormalities may contribute to cardiovascular morbidity and mortality, cardiovascular risk assessment is integral to routine HIV care. This post hoc study evaluates the impact of lipid changes on predicted atherosclerotic cardiovascular disease (ASCVD) risk and statin eligibility in treatment-naive adults living with HIV treated with TAF or TDF.

Methods. Participants (N = 1744) were randomized (1:1) to initiate TAF or TDF, each coformulated with elvitegravir/cobicistat/emtricitabine (studies GS-US-292-0104 and GS-US-292-0111). Eligibility for statin therapy and estimated 10-year ASCVD risk among adults aged 40–79 years treated with TAF or TDF for 96 weeks (W96) were analyzed based on American College of Cardiology/American Heart Association Pooled Cohort Equations. Categorical shifts in 10-year ASCVD risk from <7.5% to ≥7.5% by W96 on TAF versus TDF were calculated.

Results. Participants initiating TAF versus TDF in the overall study population showed small but significant increases in median fasting lipid parameters at W96, including total cholesterol (191 vs 177 mg/dL; $P < .001$), low-density lipoprotein ([LDL] 119 vs 112 mg/dL; $P < .001$), and high-density lipoprotein ([HDL] 51 vs 48 mg/dL; $P < .001$), respectively. At baseline, 18% and 23% on TAF versus TDF had a 10-year ASCVD risk score ≥7.5%, with mean risk scores low overall for TAF versus TDF at baseline (4.9% vs 5.4%; $P = .35$) and W96 (6.1% vs 6.2%; $P = .04$). Increases in ASCVD risk from baseline to W96 were driven by both increasing age and changes in total cholesterol (TC) and HDL cholesterol. At W96, TC/HDL ratios (median) were 3.7 for both groups ($P = .69$). There was no difference between shifts in categorical risk for TAF versus TDF (9% vs 5%; $P = .19$). Eligibility for high-intensity statin therapy were similar for TAF versus TDF groups (19% vs 21%; $P = .47$).

Conclusions. Lipid changes with TAF as part of coformulated regimens do not substantively affect CVD risk profiles compared with TDF.

Keywords. atherosclerosis; cardiovascular disease; HIV; tenofovir alafenamide; tenofovir disoproxil fumarate.

Advances in antiretroviral therapy (ART) have significantly reduced mortality from human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS), but a gap in survival remains between people with HIV (PWH) and HIV-uninfected individuals. The leading causes of death in PWH have shifted from AIDS-related events to serious non-AIDS events, including cardiovascular disease (CVD), liver disease, kidney disease, and non-AIDS-related cancer [1]. Multiple studies have shown that the risk of CVD is ~2.0-fold higher in PWH versus HIV-uninfected individuals, even after controlling for traditional CVD risk factors, although the underlying mechanisms are controversial [2, 3]. Methods for assessing cardiovascular risk in the setting of HIV are needed as well as strategies to reduce risk.

Cardiovascular disease risk assessment tools conjoined from traditional CVD risk factors exist for the general population and have largely been derived from longitudinal cohorts [4]. These CVD risk prediction tools when applied to PWH consistently underestimate their risk of CVD [5, 6]. Independent HIV-specific factors, such as exposure to certain ART and inflammatory responses, that likely play a key role in HIV-associated atherosclerosis are not captured in these assessments.

The effect of ART on cardiovascular (CV) risk is complex with different risks associated with short-term and long-term

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ART and also risks attributable to different agents. Evidence from clinical trials and cohorts demonstrate that there appears to be a significant benefit of ART associated with reduction in morbidity and mortality from CVD, particularly in the short term [7]. However, despite the benefits of ART, use of some ART agents has been associated with increased risk of CVD, including some ritonavir-boosted protease inhibitors (lopinavir, indinavir, darunavir) and some nucleoside reverse-transcriptase inhibitors (NRTIs) (didanosine and abacavir [ABC]) [8, 9]. Other ART agents (efavirenz), although not specifically shown to increase CVD risk, have been associated with dyslipidemia [10].

The tenofovir (TFV) prodrug tenofovir disoproxil fumarate (TDF) has not been associated with increased risk of CVD in multiple cohort studies [11]. Treatment with TDF has consistently been associated with lower lipids compared with other NRTI-containing regimens in either ART-naive or virologically suppressed individuals [12]. Tenofovir disoproxil fumarate has a lipid-lowering effect that involves all lipid fractions, believed to be associated with the plasma levels of TFV [11], although the mechanism by which the lowering of lipids occurs is not well understood, and the degree of lipid lowering is lower compared with traditional lipid-lowering agents such as statins. However, TDF has been associated with declines in renal function and bone mineral density (BMD). These renal and bone associations prompted the development of tenofovir alafenamide (TAF), a novel prodrug of TFV, which enables a lower dose of TFV to be used (either 10 or 25 mg TFV, depending on the regimen, vs 300 mg of TFV in TDF). Tenofovir alafenamide has shown less impact on kidney function and BMD decline than TDF in Phase 3 clinical trials and similar impact compared with ABC [13–16].

The fasting lipid profiles of ART-naive adults treated with elvitegravir (EVG, E) 150 mg, cobicistat (COBI, C) 150 mg, and emtricitabine (FTC, F) 200 mg coformulated with either TAF 10 mg or TDF 300 mg for 144 weeks have been reported [16]. Namely, TAF was associated with a larger median increase in low-density lipoprotein (LDL) from baseline (18 vs 8 mg/dL, $P < .001$). The purpose of this post hoc study was to evaluate the impact of lipid changes on predicted atherosclerotic CVD (ASCVD) risk and statin eligibility in ART-naive adults with HIV treated with either E/C/F/TAF or E/C/F/TDF [17].

METHODS

Study Population

Studies GS-US-292-0104 and GS-US-292-0111 were 2 randomized, double-blind, placebo-controlled, international trials comparing initiation of ART with TAF 10 mg versus TDF 300 mg, both of which were coformulated with E/C/F in single-tablet regimens (STRs) [13–16]. Antiretroviral therapy-naive adults (N = 1733) with HIV-1 ribonucleic acid (RNA) ≥1000 copies/mL, estimated glomerular filtration rate by Cockcroft-Gault ($eGFR_{CG}$) ≥50 mL/minute, and genotypic sensitivity to all components of the 2 STRs were randomized 1:1 to initiate E/C/F/TAF or E/C/F/TDF. As previously described, the primary endpoint of the study was achievement of virologic success (HIV-1 RNA <50 copies/mL) at Week 48; subjects continued through secondary endpoints at Week 96 and 144 [13–16].

These studies were done according to protocol without significant deviations and are registered with ClinicalTrials.gov, numbers NCT01780506 and NCT01797445.

Cardiovascular Risk Prediction Equations

The American College of Cardiology/American Heart Association (ACC/AHA) 2013 Pooled Cohort Risk Equations were used to estimate the 10-year risk for a first-hard atherosclerotic cardiovascular event in individuals enrolled in the study who were aged ≥40 years without evidence of pre-existing ASCVD (Figure 1) [3]. Patients in this analysis ranged in age from 40 to 79 years old [17] (Table 1) and included those with data at baseline and at least 1 post-baseline visit to calculate the ASCVD risk score. The choice of the ACC/AHA 2013 Pooled Cohort Risk Equation was guided by the fact that this equation has been previously shown to be the most accurate of the 4 CVD risk equations (also including Framingham, ATPIII, and Data Collection on Adverse events of Anti-HIV Drugs [D:A:D] CVD risk equations) at discerning Type 1 versus Type 2 myocardial infarction (MI) and predicting observed MI rate in PWH from the CFAR Network of Integrated Clinical Systems (CNICS) Cohort [19].

Outcome Measures

The primary endpoint used to characterize the CVD risk profile of fasting lipid changes measured in adults treated with either E/C/F/TAF or E/C/F/TDF from baseline to Week 96 was the mean estimated 10-year ASCVD risk score in participants aged 40 to 79 years derived from the Pooled Cohort Risk Equations. (Adults <40 years of age are excluded from this analysis because the Pooled Cohort Risk Equation is not validated for this population. Only participants with baseline data and data from at least 1 post-baseline visit used to calculate changes in ASCVD risk were included in this analysis.)

Secondary endpoints included the following: (1) proportion of subjects with virologic suppression in the overall population; (2) proportion of participants with high-density lipoprotein (HDL) <40 mg/dL and HDL ≥60 mg/dL; (3) proportion of participants with an estimated 10-year ASCVD risk of ≥7.5%; (4) proportion of participants eligible for high-intensity statin therapy based on any 1 of 4 criteria proposed by the ACC/AHA 2013 Cholesterol Treatment Guidelines [4]: and (5) CV adverse events (AEs) and discontinuation due to CV AEs.

Statistical Analyses

Rates of virologic suppression were reported as a proportion who achieved HIV-1 RNA <50 copies/mL at Week 48 and Week

Table 1. Baseline Characteristics

Characteristics	Statin Eligibility Analysis Population		ASVCD Risk Analysis Population	
	E/C/F/TAF N = 866	E/C/F/TDF N = 867	E/C/F/TAF N = 219[c]	E/C/F/TDF N = 272[c]
Age, median years	33	35	47	47
Male	85%	85%	81%	79%
Race and Ethnicity				
White	56%	57%	65%	68%
Black or African descent	26%	25%	22%	19%
Hispanic or Latino	19%	19%	16%	12%
BMI, median kg/m^2	24.4	24.5	26.5	26.4
Estimated GFR by Cockcroft-Gault, median mL/min	117	114	108	106
Diabetes mellitus[a]	3%	5%	7%	9%
Hypertension[a]	14%	17%	27%	31%
Hyperlipidemia[a]	11%	12%	23%	21%
HIV-1 RNA >100 000 copies/mL	23%	23%	23%	20%
CD4 cell count <200 cells/μL	13%	14%	17%	14%
Symptomatic HIV infection or AIDS diagnosis	10%	7%	9%	4%
Smoker[b]	30.6%	29.3%	21.9%	25.0%

Abbreviations: AIDS, acquired immune deficiency syndrome; ASCVD, atherosclerotic cardiovascular disease; BMI, body mass index; C, cobicistat; E, elvitegravir; F, emtricitabine; GFR, glomerular filtration rate; HIV, human immunodeficiency virus; RNA, ribonucleic acid; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

NOTE: $P > .05$ for all differences between groups.

[a]Based on medical history.

[b]Based on patient report at Week 48.

[c]The number of participants age 40 years to 79 years with data at baseline and at least 1 post-baseline visit to calculate the ASCVD risk score.

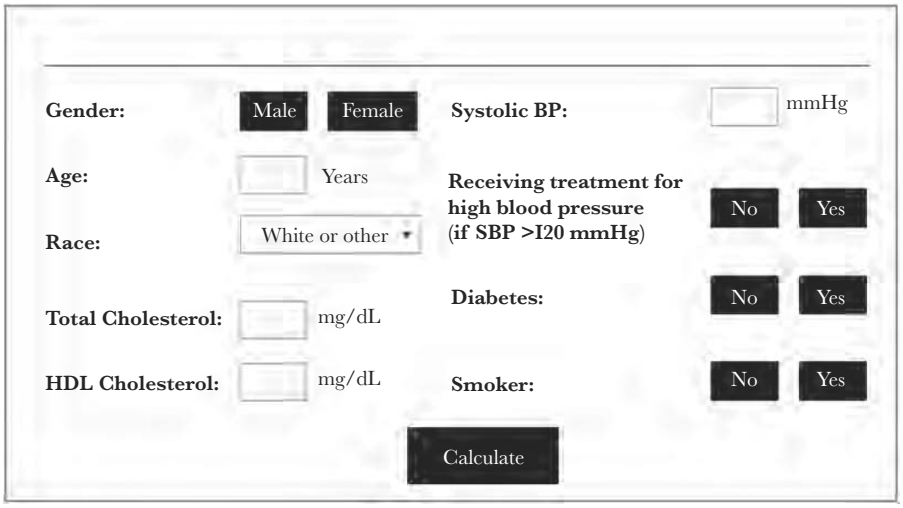
96 by the US Food and Drug Administration Snapshot algorithm; for comparison of efficacy by treatment, a noninferiority margin of 12% was used. Statistical analysis of the efficacy endpoints has previously been described [15]. Data for fasting lipids at baseline and Week 96 were reported as median values (mg/dL). Data for CVD risk by HDL were reported as proportions of 1 of 3 categories: (1) HDL ≥60 mg/dL, (2) HDL 40–59 mg/dL, and (3) HDL <40 mg/dL. The analysis of statin eligibility is based on all participants in the safety analysis set, thus the participants who initiated statin therapy were not excluded. The proportion eligible for high-intensity statin was described as a percentage of the population eligible based on each of the 4 ACC/AHA 2013 criteria. A subject may have been eligible for statin therapy based on any of the 4 criteria and was counted in each individual criteria. When calculating the overall proportion of subjects eligible for statin therapy based on any of these criteria, each subject was counted only once.

The mean estimated 10-year ASCVD risk score was calculated as a mean percentage at all time points. The proportion of participants with an estimated 10-year ASCVD risk of <7.5% or ≥7.5% was described as a percentage of the study population. In the ASCVD analysis, baseline differences were analyzed using an analysis of variance model with treatment as a fixed effect. For post-baseline differences in ASCVD risk, a covariance effect model, with treatment as a fixed effect and baseline ASCVD risk score as a covariate, was used. The CV AE data were described as a percentage experiencing 3 defined CV endpoints: rate of CV AEs, rate of serious CV AEs, and discontinuation due to CV AEs.

RESULTS

Of 1744 randomized subjects, 1733 were treated with at least 1 dose of study drug: N = 866 E/C/F/TAF and N = 867 E/C/F/TDF. Baseline characteristics, efficacy, and safety through Weeks 48, 96, and 144 have been reported previously [13–16]. All subjects treated were assessed for statin eligibility, and 491 (N = 219 E/C/F/TAF and N = 272 E/C/F/TDF) subjects met the required criteria for the ASCVD risk subanalysis. Baseline characteristics for subjects in the ASCVD analysis were well balanced ($P > .05$ for baseline parameters). For those on E/C/F/TAF, baseline characteristics were as follows: median age, 47 years old; male, 81%; white, 65%; median $eGFR_{CG}$, 108 mL/minutes; 7% diabetes mellitus; 27% hypertension; 23% hyperlipidemia; and 21.9% cigarette smoking (Table 1). Small and not significantly different proportions of subjects at baseline in both groups had documented CVD in their medical histories (TAF 10 of 866, 1.1%; TDF 14 of 867, 1.6%).

Elvitegravir/C/F/TAF was noninferior in virologic efficacy to E/C/F/TDF at Weeks 48 and 96 [13–15]. In the overall study population, those initiating TAF versus TDF showed small but significant increases in median fasting lipid parameters at Week 96, including total cholesterol ([TC] 191 vs 177 mg/dL; $P < .001$), LDL (119 vs 112 mg/dL; $P < .001$), and HDL (51 vs 48 mg/dL; $P < .001$), respectively (Figure 2). Nevertheless, the



The Pooled Cohort Risk Equations estimate the 10-year risk for a first atherosclerotic cardiovascular disease (ASCVD) event among patients age 40 to 79 years without pre-existing cardiovascular disease. Approximately 32.9% of the ASCVD-free, non-pregnant U.S. population between 40 and 79 years of age have a 10-year risk of a first hard ASCVD of at least 7.5%. Therefore, an estimated 10-year ASCVD risk score of ≥7.5% is considered high risk for ASCVD.[3,50]

Figure 1. American College of Cardiology/American Heart Association (ACC/AHA) 2013 Pooled Cohort Risk Equations. BP, blood pressure; HDL, high-density lipoprotein; SBP, systolic BP.

TC/HDL ratios, often viewed as a clinically relevant surrogate for prediction of future CVD events [20], were identical between the TAF and TDF arms at Week 96 (3.7 in both, $P = .69$). When considering those subjects with HDL cholesterol data at both baseline and Week 96 (TAF, n = 741; TDF, n = 731), the baseline proportions with high risk HDL (<40 mg/dL) were well balanced (33% each). At Week 96, a smaller proportion on TAF versus TDF had high-risk HDL (16% vs 24%, respectively) (Figure 3).

At baseline, a small proportion of subjects were on a lipid-modifying agent (TAF, 21 of 866, 2.4%; TDF 27 of 867, 3.1%). Using the ACC/AHA 2013 Guidelines on the treatment of cholesterol (Appendix 1), the proportions of participants eligible for high-intensity statin therapy based on any 1 of 4 criteria were similar for subjects treated with TAF versus TDF (19% vs 21%, $P = .47$). The 4 individual criteria for high-intensity statin eligibility were generally well balanced between the 2 groups (Figure 4). During the study, additional subjects in each group initiated lipid-modifying agents with no difference in proportion between the 2 groups by Week 96 (TAF, 3.8% vs TDF, 4.4%; $P = .63$). There were 2 key criteria that drove statin eligibility through Week 96: criteria no. 3 in the E/C/F/TDF arm had smaller increases in HDL, and criteria no. 4 in the E/C/F/TAF arm had larger increases in LDL (Figure 5).

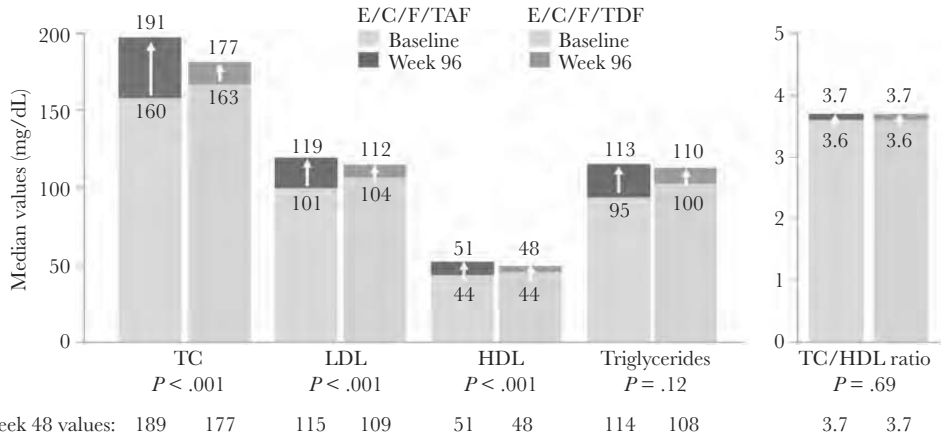
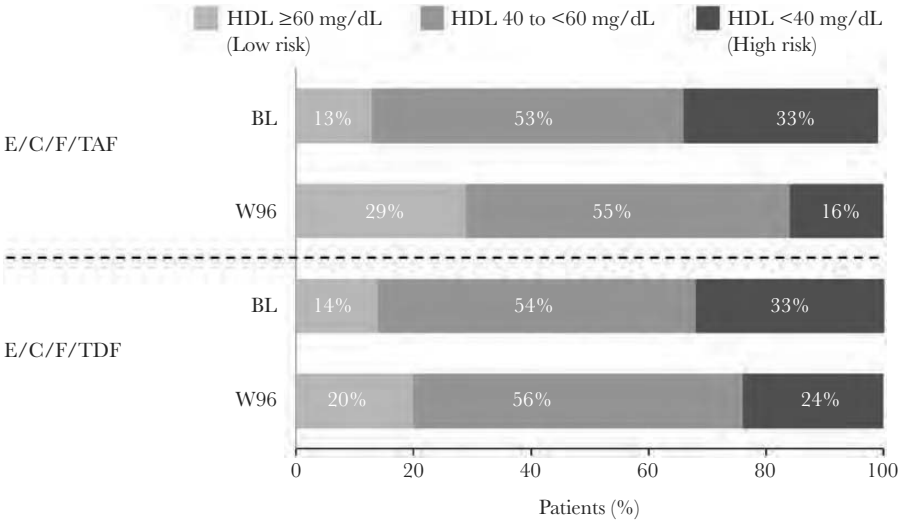


Figure 2. Fasting lipids at baseline and Week 96 results. C, cobicistat; E, elvitegravir; F, emtricitabine; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TAF, tenofovir alafenamide; TC, total cholesterol; TDF, tenofovir disoproxil fumarate.



*Only participants with HDL cholesterol data at baseline and week 96 are included in this analysis (N = 741 TAF; N = 731 TDF). BL, baseline; W, week.

Figure 3. Cardiovascular disease risk by high-density lipoprotein (HDL) category at baseline (BL) and Week 96 (W96) results. C, cobicistat; E, elvitegravir; F, emtricitabine; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

Compared with the overall study, the subpopulation in the analysis of 10-year ASCVD risk, representing 491 subjects of 1733 enrolled subjects (TAF, n = 219; TDF, n = 272), was older (approximately a decade) and had higher rates of diabetes, hypertension, and hyperlipidemia (Table 1). The overall cigarette smoking rate within the ASCVD risk group (23.6%) was significantly lower compared with individuals aged <40 years (33.3%; $P = .0001$). At baseline, 18% and 23% of individuals on TAF or TDF, respectively, had a 10-year ASCVD risk score ≥7.5%. Overall, the estimated mean 10-year ASCVD risk scores for the TAF or TDF groups were low (<7.5%) at all time points analyzed through Week 96 (baseline [4.9% vs 5.4%; $P = .35$], Week 48 [5.9% for both arms; $P = .075$], and Week 96 [6.1% vs 6.2%; $P = .04$], respectively) (Figure 5). Although a statistical difference was noted by Week 96, the increases in ASCVD risk from baseline to Week 96 were driven by both increasing age and changes in TC and HDL cholesterol. Consequently, by Week 96, the proportions with ASCVD risk ≥7.5% had increased in both the TAF and TDF groups with similar proportions in both (27% and 28%, respectively). Within the subpopulation analyzed

	E/C/F/TAF (N = 866)	**E/C/F/TDF (N = 867)**
Proportion eligible for statin based on each criteria		
1. Diabetes & LDL of 70–189 mg/dL *	3%	3%
2. Clinical atherosclerotic CVD *	3%	5%
3. Estimated ASCVD risk of ≥7.5% & LDL of 70–189 mg/dL*	7%	9%
4. LDL ≥190 mg/dL*	9%	6%
Proportion eligible for statin based on any criteria above[⊥]	19%	21%

Eligibility for statin can occur at any visits from baseling to Week 96.
* A subject may be eligible for statin based on multiple criteria and is counted in each eligible criteria.
[⊥] Participants eligible for statin based on any one criteria ie those eligible based on multiple criteria are counted only once. $P = .47$.

Key criteria that drove statin eligibility through Week 96
- Criteria #3 (for TDF group): Due to smaller increase in HDL
- Criteria #4 (for TAF group): Due to larger increase in LDL

Figure 4. Proportion eligible for high-intensity statin. ASCVD, atherosclerotic cardiovascular disease; C, cobicistat; CVD, cardiovascular disease; E, elvitegravir; F, emtricitabine; LDL, low-density lipoprotein; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

Mean % (standard deviation)	E/C/F/TAF	E/C/F/TDF
Baseline ⊥	4.9% (4.91%)	5.4% (5.52%)
Week 48 +	5.9% (6.20%)	5.9% (6.53%)
Week 96 §	6.1% (5.70%)	6.2% (6.27%)

⊥P = .35 +P = .075 §P = .04
P value for baseline difference (analysis of variance model with treatment as a fixed effect).
P values for post-baseline differences (covariance effect model with treatment as a fixed effect and basline ASCVD risk score as a covariate).

- Overall ASCVD risk at Week 96 were low (<7.5%) for both groups
- Increases in ASCVD risk from baseline to Week 96 were driven by increasing age and changes in total and HDL cholesterol

Figure 5. Mean estimated 10-year atherosclerotic cardiovascular disease (ASCVD) risk score. C, cobicistat; E, elvitegravir; F, emtricitabine; HDL, high-density lipoprotein; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

for ASVD risk, categorical shifts in 10-year ASCVD risk from <7.5% to ≥7.5% or vice versa were also defined. Of subjects on TAF versus TDF, there was no statistical difference between the groups in shifts in their categorical risk (9% and 5%, respectively) (P = .19).

In the overall study, the occurrence of CVD AEs in either group was infrequent (TAF or TDF, 2% vs 3%; P = .46). Similar discontinuation due to CV AEs rates were observed in subjects treated with TAF or TDF (0.6% vs 0.5%; P = .75) (Figure 6). There were 2 deaths in the TAF arm due to stroke and alcohol intoxication, 1 each, and 3 deaths in the TDF arm due to MI (2) and alcohol and drug intoxication (1).

DISCUSSION

In 2 large Phase 3 clinical trials of HIV treatment-naive adults, rates of virologic suppression at 96 weeks were high for participants randomized to TAF versus TDF, each coformulated with E/C/F. Rates of CV AEs were low and similar for participants treated with TAF and TDF, with few serious CVD events occurring in either arm. Using a prediction equation optimized for PWH, the 10-year risk for ASCVD incorporating fasting lipid changes was low in antiretroviral (ARV)-naive PWH aged ≥40 years treated with TAF or TDF [19]. There was no difference in the number of participants taking TAF or TDF eligible for high-intensity statin therapy, with approximately 1 in 5 meeting at least 1 category by current ACC/AHA guidelines, a proportion consistent with trends for increasing rates of hyperlipidemia in North American HIV cohorts [21]. Although 20% of subjects overall were eligible to use statin medications, lipid-modifying agents were initiated at a rate of only 4% over 96 weeks during this study. Similar trends have been observed in large clinical cohorts in Western countries [22] despite multiple studies having documented that PWH are at increased risk of CVD. More initiatives are needed to reinforce the necessity of ASCVD risk assessment and implementation of primary interventions, namely, statin therapy, to reduce CVD risk in PWH.

In both groups, the mean 10-year ASCVD risk scores at Week 96 remained below 7.5%, the threshold above which identifies persons with high ASCVD risk. The overall cigarette smoking rate at 23.6% among individuals included in the ASCVD risk analysis was significantly lower compared with individuals aged <40 years (33.3%), a rate that is more than double the overall proportion of smokers (14%) in the United States [23]. There were no significant differences between smoking rates among the TAF and TDF groups within the ASCVD risk cohort. Small increases in scores from baseline in both groups were driven by increasing age and shifts in lipid parameters, notably larger increases in LDL with TAF, and smaller increases in HDL with

	E/C/F/TAF n = 866	E/C/F/TDF n = 867
Rate of cardiovascular AEs*	2%	3%
Rate of serious cardiovascular AEs+	0.6%	0.5%
Discontinuation due to Cardiovascular AEs, n	0	1⊥

* P = .46 +P = .75 ⊥Cardiac arrest, not considered related to study drug

Figure 6. Cardiovascular adverse events (AEs) results. C, cobicistat; E, elvitegravir; F, emtricitabine; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

TDF. These mean lipid changes were primarily established by Week 48, with minimal changes from Week 48 to 96, suggesting static ARV contributions after the first year of ART initiation. Tenofovir disoproxil fumarate's lipid-lowering attributes, the mechanism of which is unknown, have been consistently observed in studies among virologically suppressed patients, including a randomized double-blind, crossover, placebo-controlled study in subjects with hypercholesterolemia on stable protease inhibitor monotherapy that showed significant decreases in TC and LDL at 12 weeks after adding TDF/FTC [11, 24–26]. With TAF-containing regimens, the lower dose of TFV delivered abrogates the lipid-lowering effect, and small increases in lipid fractions are observed in PWH initiating TAF-containing regimens. The scale of lipid changes observed on TAF is similar to those observed on non-TDF regimens.

Congruent to the LDL increases observed with TAF, HDL elevations also occurred at a higher magnitude with TAF compared with TDF, a potentially important phenomenon because HDL levels >60 mg/dL independently reduce risk of CVD [27]. High-density lipoprotein is generally accepted to have anti-inflammatory and antioxidant properties [28]. Human immunodeficiency virus viremia may directly affect HDL metabolism by upregulating cholesteryl ester transfer protein activity, which enhances transfer of cholesterol to apoB lipoproteins that promote atherogenesis [29]. The capacity of HDL to increase cholesterol efflux from macrophages is a critical function of HDL and may restrict development of atherosclerosis [30]. A greater proportion of participants taking TAF, approximately 30%, shifted from a high risk to low risk, or protective stratum of HDL compared with TDF (20%). As a result, the TC/HDL ratios remained virtually unchanged and matched for participants taking either TAF or TDF at 96 weeks. The TC/HDL ratio represents a cumulative index of metabolic abnormalities of an atherogenic dyslipidemic profile that predicts ischemic CVD risk more accurately than others ratios, such as LDL/HDL [31]. The Framingham Heart Study indicates that for men, a TC/HDL ratio of 5 signifies average risk for CVD, with 3.4 indicating approximately half the average risk. Women tend to have higher HDL levels, with a ratio of 4.4 signifying average risk, and 3.3 denoting approximately half the average risk [32]. For both TAF and TDF, the TC/HDL ratios in our study suggest a similar, lower-than-average, 10-year risk for CVD, with the higher TC balanced by higher HDL in participants taking TAF. However, there is clinical equipoise regarding the impact of therapeutically raising HDL to avert cardiovascular events [33–37]. Lipid-lowering drug trial data support the approach that LDL cholesterol lowering should be pursued more aggressively when HDL cholesterol is low [38–42]. In our study, although rates of statin introduction were low overall, there were 2 main criteria that drove statin eligibility through Week 96: the TDF arm had smaller increases in HDL, and the TAF arm had larger increases in LDL.

The relative paucity of lipid-modifying agents initiated relative to participants on TAF or TDF eligible for high-intensity therapy underscores the imperative for greater scrutiny towards the "statin gap" in the comprehensive care of PWH in the United States. In the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) and HIV Outpatient Study (HOPS) cohorts, this gap—the difference between patients indicated for statin therapy by ATP III criteria and actual number of patients prescribed statins—stands at 46%–53% [5, 22]. In our study, which enrolled participants contemporaneously with the NA-ACCORD and HOPS data, the gap was 80% and 79% for participants taking TAF and TDF, respectively. Modeling future CVD burden for PWH from the ATHENA cohort using the D.A.D. CVD risk equation, it has been proposed that CVD incidence will increase by 55% between 2015 and 2030. Monitoring and treatment of dyslipidemia and hypertension will have the greatest impact on averting CVD events (17%–20%) along with benefits associated with HIV-specific interventions, including early HIV diagnosis and treatment, and avoiding antiretrovirals associated with increased CVD risk [43]. This model only accounts for the LDL-lowering effects of statins, and it does not consider the immunologic mechanisms of statins hypothesized to attenuate inflammation and immune activation that may further reduce CVD risk in PWH [44–50].

We acknowledge several limitations in our study. This was a post hoc analysis, and the study was not powered to determine differences in CVD risk by randomized drug assignment. Likewise, the actual number of CVD occurrences were minimal in each group, and a 2- to 3-year follow-up may be too short to see any difference in ASCVD. The ACC/AHA 2013 Pooled Cohort Risk Equation is validated for adults aged 40 to 79 years; ASCVD risk was only assessed for approximately one quarter of the study population. As such, TC and HDL values used to calculate risk prediction scores may not be fully representative of comparative mean fasting lipid changes occurring among participants exposed to TAF and TDF. The performance of predictive equations may vary with the primary prevention interventions, and none of the equations accounted for documented statin therapy, although overall statin use was minimal in both TAF and TDF groups.

CONCLUSIONS

In summary, lipid changes in treatment-naive patients taking TAF as part of coformulated single tablet regimens do not substantively affect the CVD risk profile in comparison to TDF. Although predicted CVD risk was low over 96 weeks, approximately 1 in 5 participants overall irrespective of randomized ART met criteria for statin therapy, yet only 1 in 5 of those eligible were prescribed lipid-modifying agents. Because prediction equations may underestimate CVD risk in PWH, and further research is investigating immunomodulatory features of

statins, intensified primary care strategies are required to appropriately identify PWH that could potentially benefit from preventative CVD interventions.

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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APPENDIX 1: ACC/AHA 2013 Guidelines on the Treatment of Cholesterol

Four groups that may benefit from high-intensity statin therapy

1. Diabetics aged 40 to 75 years; LDL 70 to 189 mg/dL; without clinical ASCVD
2. Individuals with clinical ASCVD*
3. Individuals with estimated 10-year ASCVD risk of ≥7.5%[‡]; LDL 70 to 189 mg/dL; without clinical ASCVD or diabetes
4. Individuals with primary elevations of LDL ≥ 190 mg/dL

*Clinical ASCVD is defined as acute coronary syndrome or a history of myocardial infarction (MI), stable or unstable angina, coronary or other arterial revascularization, stroke, transient ischemic attack, or peripheral arterial disease presumed to be of atherosclerotic origin.[3]

[†]The estimated 10-year ASCVD risk (for nonfatal MI, coronary heart disease death, nonfatal and fatal stroke) is determined using the Pooled Cohort Risk Equations.[3]

[‡]An estimated 10-year ASCVD risk of ≥7.5% is considered high.[3]

EXHIBIT I

OPEN

Renal safety of tenofovir alafenamide vs. tenofovir disoproxil fumarate: a pooled analysis of 26 clinical trials

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Objective: Compared with tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF) has been associated with improvement in markers of renal dysfunction in individual randomized trials; however, the comparative incidence of clinically significant renal events remains unclear.

Design: We used a pooled data approach to increase the person-years of drug exposure analysed, maximizing our ability to detect differences in clinically significant outcomes.

Methods: We pooled clinical renal safety data across 26 treatment-naive and antiretroviral switch studies to compare the incidence of proximal renal tubulopathy and discontinuation due to renal adverse events between participants taking TAF-containing regimens vs. those taking TDF-containing regimens. We performed secondary analyses from seven large randomized studies (two treatment-naive and five switch studies) to compare incidence of renal adverse events, treatment-emergent proteinuria, changes in serum creatinine, creatinine clearance, and urinary biomarkers (albumin, beta-2-microglobulin, and retinol binding protein-to-creatinine ratios).

Results: Our integrated analysis included 9322 adults and children with HIV ($n = 6360$ TAF, $n = 2962$ TDF) with exposure of 12 519 person-years to TAF and 5947 to TDF. There were no cases of proximal renal tubulopathy in participants receiving TAF vs. 10 cases in those receiving TDF ($P < 0.001$), and fewer individuals on TAF (3/6360) vs. TDF (14/2962) ($P < 0.001$) discontinued due to a renal adverse event. Participants initiating

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TAF-based vs. TDF-based regimens had more favourable changes in renal biomarkers through 96 weeks of therapy.

Conclusion: These pooled data from 26 studies, with over 12 500 person-years of follow-up in children and adults, support the comparative renal safety of TAF over TDF.

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Keywords: adverse drug event, drug safety biomarkers, HAART, HIV, proximal renal tubular dysfunction, renal fanconi syndrome, tenofovir disoproxil fumarate

Introduction

Tenofovir (TFV) disoproxil fumarate (TDF) is a nucleotide reverse transcriptase inhibitor that is highly efficacious and generally well tolerated. However, TDF is associated with renal adverse events, including proximal renal tubulopathy (PRT), which occurs in less than 1% of individuals [1,2]. TFV alafenamide (TAF), a TFV prodrug, is associated with a mean 91% lower plasma TFV exposure compared with TDF [3]. As higher plasma TFV levels have been associated with nephrotoxicity [4,5], reduced circulating TFV levels are hypothesized to result in fewer renal adverse events. In phases 2 and 3 clinical trials of both treatment-naive and virologically suppressed adults and children [3,6–35], TAF-containing regimens have demonstrated high efficacy and favorable changes in renal biomarkers including creatinine clearance (CrCl), total and tubular proteinuria, and albuminuria compared with a variety of unboosted and ritonavir (RTV)-boosted or cobicistat (COBI)-boosted TDF-containing regimens. It has been more challenging to determine whether the favourable biomarker profile of TAF translates into improved renal clinical outcomes, due to the low rates of renal events in individual trials, although the 144 week follow-up of the pooled pivotal trials for elvitegravir (EVG)/COBI/emtricitabine (FTC)/TAF had zero cases of PRT and zero renal discontinuations compared with four cases of PRT and 12 renal discontinuations in the EVG/COBI/FTC/TDF group [8]. To better understand the renal clinical outcomes in TAF vs. TDF-containing HIV regimens, we conducted a large integrated analysis of people living with HIV (PLH) from 26 TAF clinical trials. These trials included cumulative exposures of 12 519 person-years to TAF and 5947 person-years to TDF, thereby providing increased statistical power to evaluate the comparative impact on renal adverse events and renal function over time.

Methods

Study design and participants

We included 26 phases 2 and 3 multicenter, multinational, clinical studies of TAF-containing regimens in PLH including adults, adolescents, and children (aged ≥6 years) who were either ART-naive or virologically suppressed on a stable ART regimens containing TDF. These studies were conducted between 28 December 2011 and 4 December 2017. Study design and inclusion criteria, including minimum renal function, of each trial are described in Appendix Table 1, http://links.lww.com/QAD/B470. Of the 26 studies, 14 were double blinded and randomized, six were open label and randomized, and six were single arm. All trials were undertaken in accordance with the Declaration of Helsinki and approved by central or site-specific review boards or ethics committees. All participants or their legal guardians (if minors) provided written, informed consent.

Procedures

Postbaseline study visits were conducted at weeks 4, 8, 12, 24, 36, and 48 and every 12 weeks thereafter until week 96. Renal laboratory tests included serum creatinine (SCr), CrCl by Cockcroft–Gault, treatment-emergent proteinuria by dipstick, urine albumin-to-creatinine ratio (UACR), and tubular proteinuria [urine retinol binding protein-to-creatinine ratio (RBP : Cr) and β2-microglobulin-to-creatinine ratio (β2M : Cr)] (Covance Laboratories, Indianapolis, Indiana, USA).

Renal safety was assessed by recording of adverse events, which were coded using the Medical Dictionary for Regulatory Activities (MedDRA, version 18.1–19.1) (Appendix Table 2, http://links.lww.com/QAD/B470).

Analysis of primary renal safety outcomes

The primary renal safety outcomes were incidence of PRT events, and study drug renal discontinuation events. For primary outcomes analysis, we pooled all participants from the 26 available trials who received at least one dose of study drug (safety analysis set). We derived safety measures data using all data collected on or after study drug was first given up to either the data cut date for participants still on study drug or up to 30 days after the last dose of study drug for participants who permanently discontinued treatment early. We summarized baseline demographics and characteristics of the included participants with descriptive statistics.

We defined 'renal discontinuation events' as investigator-reported discontinuation events for which the attributable MedDRA code exists in selected renal preferred terms from the 'renal and urinary disorders' System Organ Class (Appendix Table 2, http://links.lww.com/QAD/B470). Similarly, PRT cases were defined as investigator-reported adverse events indicative of tubular disorders, including reported terms of PRT and Fanconi syndrome (preferred terms are provided in Appendix Table 3, http://links.lww.com/QAD/B470), regardless of study drug relatedness. The cumulative incidence rates of investigator-reported cases of PRT and renal adverse events leading to study drug discontinuation were calculated as the number of events divided by the total numbers of participants pooled from the 26 trials treated with TAF-containing or TDF-containing regimens, respectively. The differences in the cumulative incidence rates between treatment groups were compared using Fisher's exact test. To minimize type I error resulting from multiple hypothesis testing, we performed primary endpoint analysis in a predetermined sequence, only proceeding to the second endpoint (renal discontinuation events) if the first endpoint (PRT events) analysis demonstrated statistical significance with $\alpha = 0.05$.

Analysis of secondary renal outcomes

We assessed secondary renal outcomes including treatment-emergent renal adverse events, SCr, CrCl, treatment-emergent gross proteinuria (by dipstick), UACR, and tubular proteinuria (urine RBP : Cr and β2M : Cr). Treatment-emergent proteinuria was defined as 1+ or greater proteinuria by dipstick on any occasion during trial follow-up, regardless of persistence. Urine protein-to-creatinine ratio was monitored during the trials, but a change in assay methodology occurring partway through several trials resulted in data unsuitable for integrated analysis. For the analysis of these secondary renal outcomes, we selected a subset of trials that satisfied the following predetermined criteria: randomized design; TAF and TDF arms; and at least 48 weeks of follow-up. Based on these criteria, a total of seven trials were selected, including two treatment-naive studies and five virologically suppressed studies (referred to as switch studies) (Fig. 1). To facilitate accurate assessment of CrCl changes in study participants, we excluded participants who switched from an ART regimen lacking a known creatinine transport inhibitor to a regimen containing a known creatinine transport inhibitor (rilpivirine, dolutegravir, bictegravir, COBI, or RTV) [36–41]. This approach allowed us to reduce confounding caused by SCr increases attributable to initiation of a creatinine transport inhibitor.

Study Population	Study no.	Study design	N	Treatment
TN adults (n=7)	292-0102	DB, R	170	E/C/F/TAF vs E/C/F/TDF
	141-1475	DB, R	98	BIC+F/TAF vs DTG+F/TAF
	380-1490	DB, R	645	B/F/TAF vs DTG+F/TAF
	299-0102	DB, R	153	DRV/COBI/FTC/TAF vs DRV+COBI+FTC/TDF
	380-1489	DB, R	629	B/F/TAF vs ABC/DTG/3TC
	292-0104	DB, R	867	E/C/F/TAF vs E/C/F/TDF
	292-0111	DB, R	866	E/C/F/TAF vs E/C/F/TDF
VS adults (n=12)	366-1160	DB, R	875	EFV/FTC/TDF vs FTC/RPV/TAF
	366-1216	DB, R	630	FTC/RPV/TAF vs FTC/RPV/TDF
	311-1089	DB, R	663	F/TAF+3rd agent vs F/TDF+3rd agent
	292-0109	OL, R	1436	E/C/F/TAF vs TDF-containing regimens
	380-1878	OL, R	577	B/F/TAF vs boosted PI-regimens
	380-1844	DB, R	563	B/F/TAF vs ABC/DTG/3TC
	311-1717	DB, R	556	F/TAF+3rd agent vs ABC/3TC+3rd agent
	292-1823	OL, R	274	E/C/F/TAF vs ABC/3TC+3rd agent
	366-1992	OL, R	148	E/C/F/TAF vs R/F//TAF
	380-1961	OL, R	470	B/F/TAF vs E/C/F/TAF, E/C/F/TDF or ATV+RTV+F/TDF
	236-0128	OL, R	212	E/C/F/TAF vs ATV/r + FTC/TDF
	292-1824	Single arm	37	E/C/F/TAF
TN & VS adults (n=1)	292-1249	Single arm	77	E/C/F/TAF
TE adults (n=2)	292-0117	DB, R	37	TAF+failing regimen vs placebo+failing regimen
	292-0119	OL, R	133	E/C/F/TAF+DRV vs pre-existing regimen
TN & VS children (n=1)	292-0106	Single arm	102	E/C/F/TAF
VS adolescents (n=1)	292-1515	Single arm	60	E/C/F/TAF
TN & VS children & adolescents (n=2)	311-1269	Single arm	28	F/TAF
	380-1474	Single arm	24	B/F/TAF

Primary outcomes
(N=26 trials, 9,322 participants)
1) PRT events
2) Discontinuations due to renal AEs

Secondary outcomes
(N=7 trials; n=2 naïve [1733 participants], n=5 suppressed [4092 participants])
1) Treatment-emergent renal AEs
2) SCr (mg/dL)
3) CrCl (mL/min)
4) Treatment-emergent proteinuria (dipstick)
5) UACR
6) Tubular proteinuria (urine RBP:Cr and β2M:Cr)

Fig. 1. Characteristics of studies included in the integrated analysis. Treatment-naive studies included in the secondary analysis are highlighted in blue, virologically suppressed people living with HIV studies are highlighted in green. 3TC, lamivudine; ATV, atazanavir; AE, adverse event; B, BIC, bictegravir; C, COBI, cobicistat; DRV, darunavir; DTG, dolutegravir; DB, double blind; E, elvitegravir; FTC, emtricitabine; OL, open label; PI, protease inhibitor; R, randomized; R, RPV, rilpivirine; RTV, ritonavir; STR, single tablet regimen; TE, treatment-experienced; TN, treatment-naive; VS, virologically suppressed.

Using these data, we evaluated the incidence rates of treatment-emergent renal adverse events (Appendix Table 2, http://links.lww.com/QAD/B470) and of proteinuria by dipstick. We also summarized change from baseline in SCr and CrCl and percentage change from baseline in UACR, RBP : Cr, and β2M : Cr. We used logistic regression models to compare the differences in incidence rates between treatment groups and linear regression and rank analysis of covariance (adjusted for baseline demographics and disease characteristics selected from step-wise procedure) for change and percentage change from baseline in renal parameters, respectively.

To control for type I error in the testing of multiple secondary renal outcomes hypotheses, we employed the following testing strategies. First, the primary comparisons of PRT and renal discontinuation events in all 26 studies were analyzed using a predefined sequence as described above. Subsequently, hypothesis testing for secondary outcomes was performed using the Holm–Bonferroni method; *P* values reported in the text are Holm–Bonferroni adjusted [42,43]. We used SAS Software Version 9.4 (SAS Institute Inc., Cary, North Carolina, USA) for all analyses. All studies were conducted according to protocol without substantial deviations

Results

We included a collective 9322 individuals across 26 studies (Appendix Table 1, http://links.lww.com/QAD/B470). Participants either initiated or switched to regimens containing TAF ($n = 6360$) or initiated or continued on regimens containing TDF ($n = 2962$) (Table 1). Baseline median age was 42 years, 21% were women, and 27% were of black race. Pooled data included exposure of 12 519 person-years to TAF and 5947 person-years to TDF.

Primary analyses

Incidence of proximal renal tubulopathy events

In the dataset including all 26 studies, 14 of which were double blinded, there were no cases of PRT or Fanconi syndrome reported in the TAF group (Fig. 2). Ten cases of PRT, including Fanconi syndrome, were reported by site investigators for the TDF group (0.34% of participants, $P < 0.001$ vs. TAF). Of the PRT cases, nine of 10 were investigator reported as study drug related, nine of 10 occurred during blinded therapy, and eight of 10 resulted in study drug discontinuation. Appendix Fig. 1, http://links.lww.com/QAD/B470 shows the specific ART regimens, duration of study drug exposure relative to onset of PRT and relatedness to study drug as determined by the site investigator. The timing of PRT development was variable but often occurred well into therapy, including three of 10 cases developing in participants who were virologically suppressed on TDF for at least 6 months at the time of enrolment (Appendix Fig. 1, http://links.lww.com/QAD/B470).

Discontinuations due to renal adverse events

In the dataset including all 26 studies, three of 6360 individuals (0.05%) who received TAF discontinued study drug due to renal adverse events compared with 14 of 2962 (0.47%) participants in the TDF group ($P < 0.001$) (Fig. 2). Of the 14 participants in the TDF group, four were in open-label studies and the remainder were in double-blinded studies; 12 of 14 discontinuations were reported as study drug-related. All three participants in the TAF group were enrolled in open-label studies, and no discontinuations were reported as study-drug related. Appendix Fig. 2, http://links.lww.com/QAD/B470 shows the specific ART regimens, duration of study drug exposure relative to onset of the renal adverse event, as well as relatedness to the study drug as determined by the investigator. Appendix Table 4, http://links.lww.com/QAD/B470 provides clinical narratives describing the renal discontinuation events.

Table 1. Baseline demographic and clinical characteristics.

Characteristic		TAF, $N = 6360$	TDF, $N = 2962$	Total, $N = 9322$
Age (years)		41 (7, 80)	42 (18, 79)	42 (7, 80)
Sex	Male	4966 (78%)	2436 (82%)	7402 (79%)
	Female	1394 (22%)	526 (18%)	1920 (21%)
Race	White	3796 (60%)	1884 (64%)	5680 (61%)
	Black	1799 (28%)	739 (25%)	2538 (27%)
	Asian	373 (6%)	181 (6%)	554 (6%)
	Other	376 (6%)	153 (5%)	529 (6%)
	Declined to respond	16 (<1%)	5 (<1%)	21 (<1%)
Ethnicity	Hispanic or Latino	1188 (19%)	537 (18%)	1725 (19%)
Treatment status	Naive	2191 (34%)	975 (33%)	3166 (34%)
	Experienced	4169 (66%)	1987 (67%)	6156 (66%)
CrCl (ml/min)		108.8 (91.2, 129.6)	107.7 (90.9, 128.4)	108.6 (91.1, 129.3)

Data are median (IQR) or *n* (%), except for age, which is median (range). CrCl, creatinine clearance; IQR, interquartile range; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

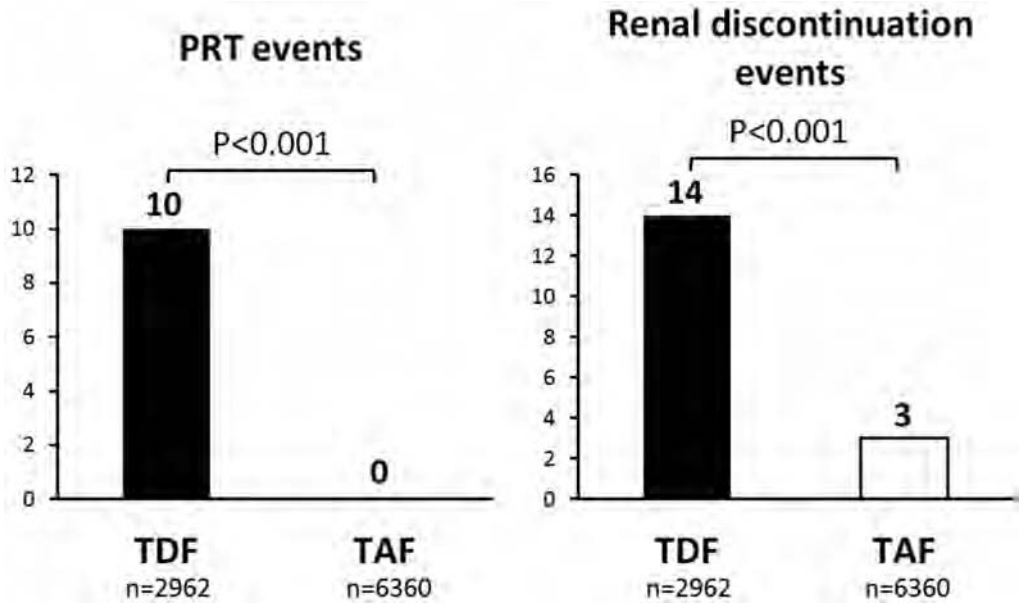

Fig. 2. Cases of proximal renal tubulopathy and renal adverse events leading to study drug discontinuation across 26 clinical studies. The incidence of proximal renal tubulopathy and renal discontinuation events were determined using pooled data from 26 studies as described in the Methods section. Differences between treatment groups compared using Fisher exact test.

Secondary analyses

We next sought to compare secondary renal outcomes between TAF-based and TDF-based regimens both in the settings of treatment-naive ART initiation and regimen switch in virologically suppressed PLH. To this end, we identified two ART-naive studies and five switch studies that were randomized, included both TAF and TDF arms, and included at least 48 weeks of follow-up (Fig. 1).

Total of all renal adverse events in antiretroviral therapy-naive people living with HIV

Based on pooled data from two randomized, double-blinded studies of treatment-naive PLH, clinical renal adverse events through week 96 were reported significantly less frequently in the TAF group than in the TDF group [47/866 (5.4%) vs. 74/867 (8.5%), $P = 0.042$].

Changes in renal laboratory parameters and biomarkers in antiretroviral therapy-naive people living with HIV

In treatment-naive PLH, median change from baseline at weeks 48 and 96 in SCr was significantly lower in the TAF group compared with TDF group [difference in least squares mean (LSM) −0.03 mg/dl, $P \leq 0.001$ at week 96] (Fig. 3a). Similarly, we noted that median CrCl had declined less in the TAF group compared with the TDF group (difference in LSM 6.0 ml/min, $P \leq 0.001$ for week 96) (Fig. 3b).

In treatment-naive PLH, we observed that treatment-emergent proteinuria at week 96 (defined as 1+ or greater proteinuria by dipstick on any occasion) was reported for fewer people in the TAF group compared with those in the TDF group [307/862; (36%) vs. 354/865 (41%); $P = 0.034$].

Treatment-naive PLH initiating TAF-based regimens had greater decreases or smaller increases from baseline through week 96 in median urinary biomarkers (UACR, RBP : Cr, β2M : Cr) compared with TDF (Fig. 4). At week 96, median UACR decreased by 5.2% with TAF vs. an increase of 4.9% with TDF ($P \leq 0.001$) (Fig. 4a). Median RBP : Cr increased by 13.8% with TAF compared with an increase of 74.2% on TDF ($P \leq 0.001$) (Fig. 4b). Median β2M : Cr declined by 32.1% with TAF compared with an increase of 33.5% on TDF ($P \leq 0.001$) (Fig. 4c).

Total of all renal adverse events in virologically suppressed people living with HIV

We evaluated pooled data from five randomized studies (two open-label, three blinded) of virologically suppressed PLH who switched from TDF-containing to TAF-containing regimens or continued their baseline TDF-based regimen. We observed no difference in the rate of reported clinical renal adverse events in these switch studies [114/2291 (5%) vs. 89/1801 (5%), $P = 1.00$].

Changes in renal biomarkers in virologically suppressed people living with HIV

For virologically suppressed PLH, there was a greater reduction in median SCr from baseline in the TAF group compared with the TDF group (difference in LSM −0.03 mg/dl, $P \leq 0.001$ for week 96) (Fig. 3a). Median

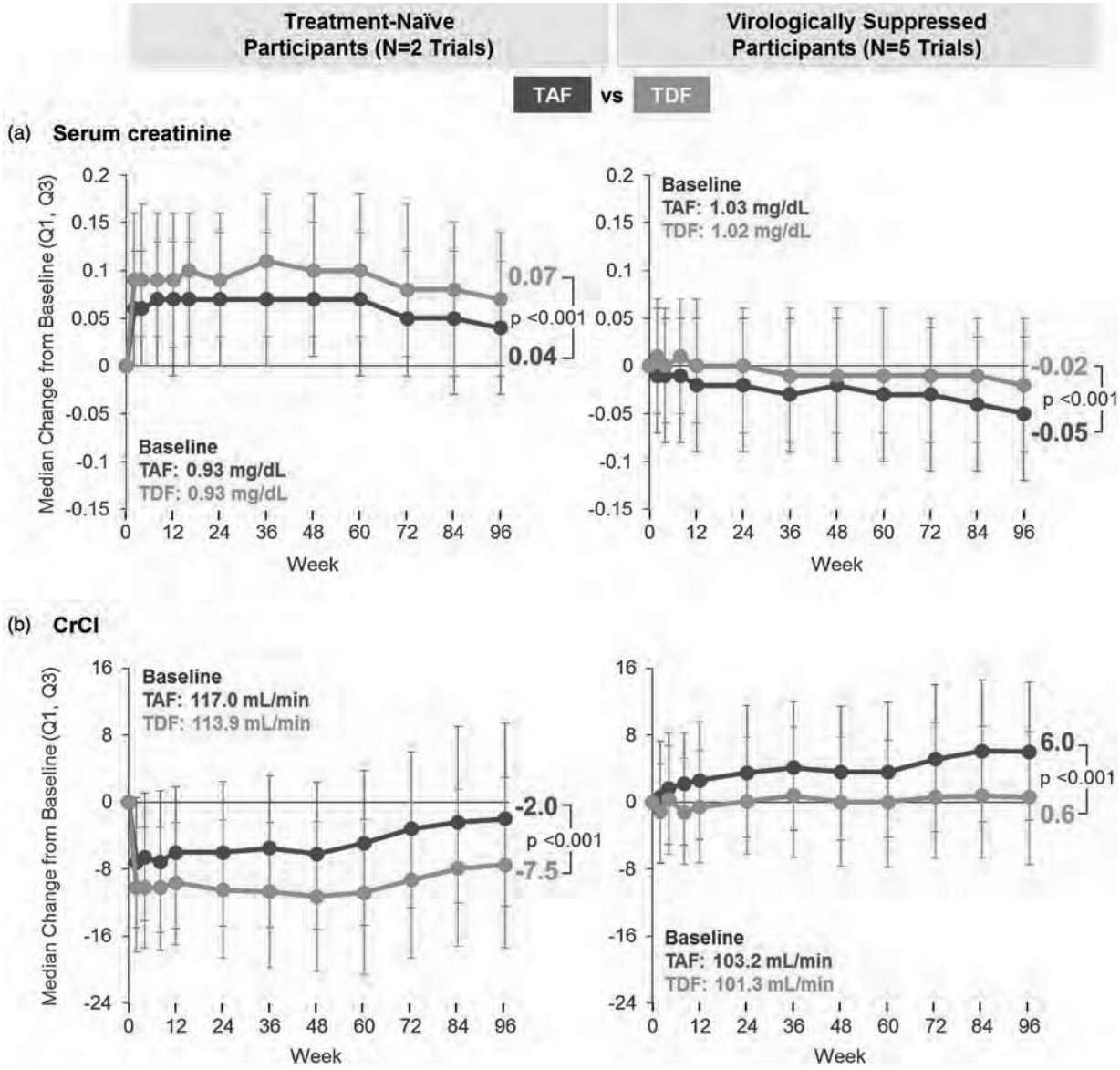

Fig. 3. Longitudinal changes in renal laboratory parameters. Serum creatinine (a) and creatinine clearance (b) were determined longitudinally as described in the Methods section, and are depicted as median change from baseline (purple = tenofovir alafenamide, orange = tenofovir disoproxil fumarate). In each panel, the first plot depicts pooled data from two treatment-naive studies, and the second plot depicts data from five virologically suppressed studies. Differences between treatment groups in changes from baseline were compared using linear regression (baseline demographics and disease characteristics selected from step-wise procedure adjusted).

CrCl increased in the TAF group while no change was seen in the TDF group (difference in LSM 5.2 ml/min, $P \leq 0.001$ for week 96) (Fig. 3b).

In virologically suppressed PLH, we observed that treatment-emergent proteinuria at week 96 (defined as 1+ or greater proteinuria by dipstick on any occasion) was reported for fewer people in the TAF group compared with those in the TDF group [636/2287 (28%) vs. 561/1794 (31%); $P = 0.04$].

In virologically suppressed participants switching from TDF to TAF, TAF-based regimens had greater decreases or smaller increases from baseline through week 96 in median renal biomarkers (UACR, RBP : Cr, β2M : Cr) compared with TDF (Fig. 4). Median UACR decreased by 5.4% on TAF and increased by 27.0% on TDF ($P \leq 0.001$) (Fig. 4a). Median RBP : Cr decreased by 2.3% on TAF and increased 61.2% on TDF ($P \leq 0.001$) (Fig. 4b). Median β2M : Cr decreased by 25.8% with TAF and increased by 53.0% on TDF ($P \leq 0.001$) (Fig. 4c).

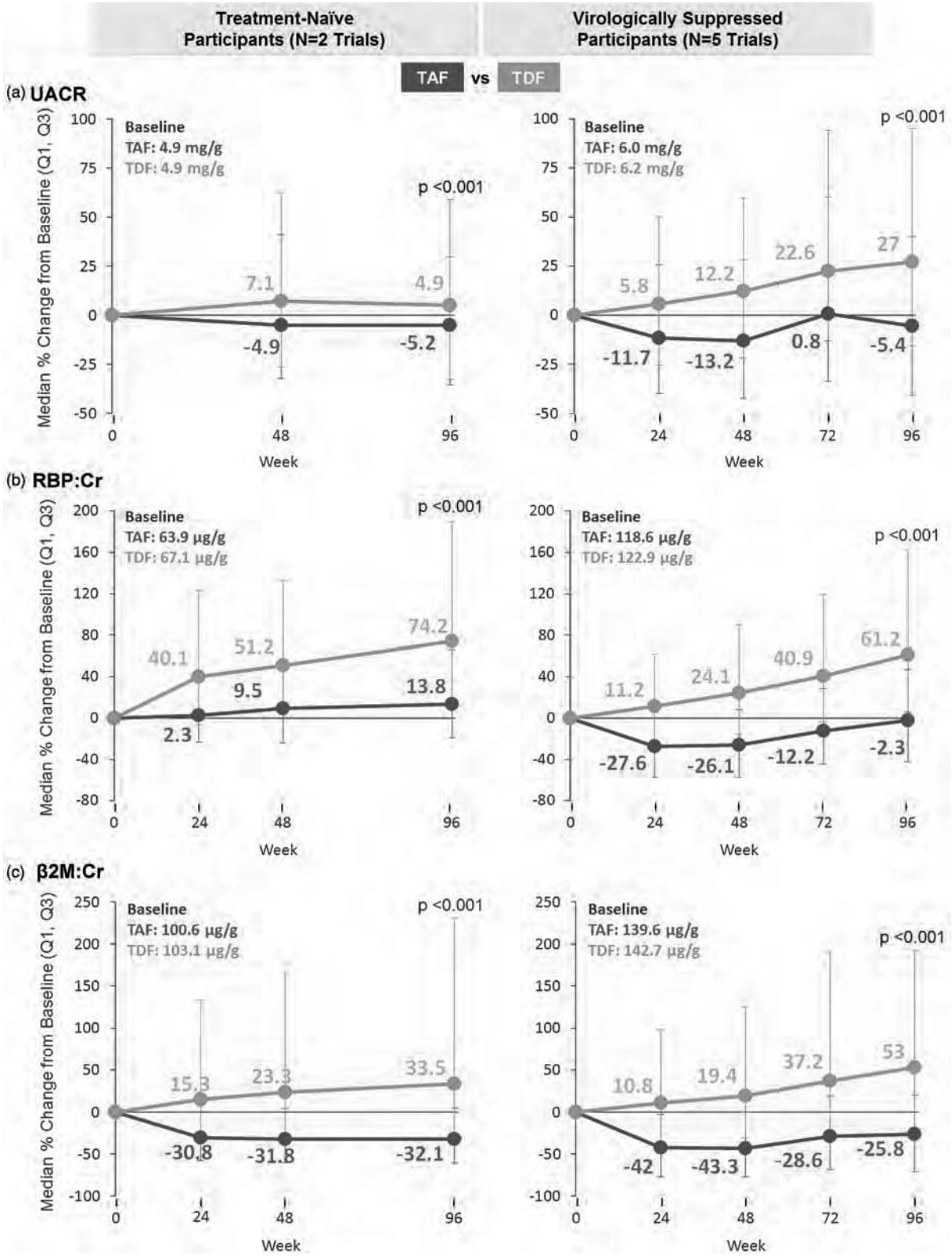

Fig. 4. Longitudinal changes in renal biomarkers. Urine albumin to creatinine ratio (a), retinol binding protein-to-creatinine ratio (b), and β2-microglobulin-to-creatinine ratio (c) were determined longitudinally as described in the Methods section and are depicted as median percentage change from baseline (purple = tenofovir alafenamide, orange = tenofovir disoproxil fumarate). In each panel, the first plot depicts pooled data from two treatment-naive studies, and the second plot depicts data from five virologically suppressed studies. Differences between treatment groups in changes from baseline were compared using linear regression (baseline demographics and disease characteristics selected from step-wise procedure adjusted).

Discussion

Previous studies have demonstrated more favourable renal biomarker profiles in TAF-containing regimens compared with TDF-containing regimens; however, the sample sizes of individual trials and the overall low rate of clinically significant renal adverse events in these trials limited the ability to detect differences in the rates of these events with the exception of the pooled pivotal EVG trials. In the present analysis, we integrated data from 26 individual trials and were able to demonstrate the renal safety of TAF over TDF across a broad range of PLH, including those who were treatment naive and those who were virologically suppressed at switch. After 12 519 person-years of exposure to TAF, there were no cases of PRT or Fanconi syndrome (identified objectively and independently by the primary investigator caring for the participant) and significantly fewer discontinuations due to renal adverse events in the TAF group compared with the TDF group. Notably, only three (0.02%) renal discontinuation events were reported in participants on TAF; none of these were reported as study drug-related by the investigators, and all had plausible alternative causes.

In treatment-naive participants, we observed fewer overall renal adverse events in participants taking TAF-containing regimens compared with those taking TDF-containing regimens. No difference in overall renal adverse events was observed in participants enrolled in switch studies; this may be explained by the fact that participants in those studies were already maintained on TDF at the time of enrolment, and thus self-selected as less likely to develop renal adverse events.

By using an integrated analysis, we were able to demonstrate favourable changes in renal biomarkers in participants taking TAF-containing regiments compared with those taking TDF, both in treatment-naive and treatment-experienced patients who switched to TAF-containing regimens. Our findings demonstrate favourable changes in CrCl as well as in proximal tubule function (RBP and β2M ratios). We also observed a lower incidence of treatment-emergent proteinuria in participants taking TAF-containing regimens. The observed incidences of proteinuria were high, but notably these are cumulative incidences over 96 weeks of follow-up, and are consistent with previously reported incidences of proteinuria in PLH [44]. These biomarker findings in combination with the clinical outcomes suggest that TAF does not induce proximal tubule dysfunction.

The mechanism for the improved renal safety profile of TAF is likely related to the approximately 90% lower plasma levels of TFV seen in participants receiving TAF compared with those receiving TDF. This mechanism is supported by the reported association between declines in renal tubular function and higher TFV plasma concentrations [45–47].

Conversely, the use of boosting agents such as RTV and COBI increase TFV exposure, and accordingly the use of boosting agents has been associated with an increased risk of renal adverse events [2,48]. A recent meta-analysis sought to compare the renal safety profiles of TDF-containing regimens in the presence and absence of boosting agents, and suggested that unboosted TDF could have a similar renal safety profile as TAF [48]. However, the aforementioned meta-analysis is limited by a relatively small number of participants and short duration of follow-up. In the findings presented here, nine out of 10 PRT cases occurred in participants receiving boosted regimens; however, one severe case of PRT occurred in a participant receiving TDF without a boosting agent. Our data support the principle that boosting agents increase the risk of TFV-associated renal adverse events; however, our ability to make robust conclusions about the renal safety of unboosted TDF is limited by the comparatively small number of participants taking such regimens (of 9322 total participants, 2962 were on TDF, and of those 1101 were on TDF without a boosting agent). Although the question of renal safety of TDF in unboosted regimens warrants more evaluation, the available data indicate that TAF can be safely used with boosted as well as unboosted third agents with a very low incidence of clinically significant renal events.

We note several limitations to our analyses. It is challenging to diagnose PRT, and no commonly accepted single diagnostic exists in the clinic to confirm PRT. As such, we utilized investigator-reported events to document PRT, which may have underestimated the number of PRT cases. A reporting bias is possible given the use of investigator reported events, but is unlikely to have affected our findings as most of the included trials were double-blinded, and the majority of reported renal discontinuation events and PRT cases were reported during blinded trial phases. Our clinical trial participants may have been healthier than the general population of PLH due to the presence of inclusion and exclusion criteria in the trials, although TAF was found to safe in patients with impaired renal function (CrCl 30–70 ml/min, many of whom with diabetes mellitus, hypertension, and proteinuria), with no reported cases of PRT and overall stable renal function through 96 weeks of follow-up [49]. We also acknowledge that we did not have individual level data on the duration of prior TDF therapy in our trials and therefore could not adjust the rates accordingly.

Despite these limitations, the integrated analysis presented here is based on the large cumulative exposure in person-years to TAF, both in antiretroviral naïve and virally suppressed populations. Furthermore, the pooled data used for analysis includes a demographically diverse population with a wide age range, a large number of women, and diverse ethnic background. It is also notable that a proportion of participants had relatively low CrCl,

with variable CrCl eligibility cut-offs of 30, 50, or 70 ml/min in the trials included in this analysis (Appendix Table 1, http://links.lww.com/QAD/B470). The clinical trial data are supported by experience from the postapproval use in PLH in which currently there has been no renal safety signal with 1.1 million cumulative person-years exposure to TAF.

In conclusion, the pooled data from 26 clinical studies, representing over 12 500 patient-years of follow-up in children and adults on TAF, suggests that the favourable renal biomarker profile observed with TAF vs. TDF in the individual trials translates into a lower rate of clinically significant renal events. These data support a comparative renal safety advantage of TAF over TDF in a broad range of PLH.

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Conflicts of interest

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EXHIBIT J

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Bone Loss in HIV Infection

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Opinion Statement

Human immunodeficiency virus (HIV) infection is an established risk factor for low bone mineral density (BMD) and subsequent fracture, and treatment with combination antiretroviral therapy (cART) leads to additional BMD loss, particularly in the first 1–2 years of therapy. The prevalence of low BMD and fragility fracture is expected to increase as the HIV-infected population ages with successful treatment with cART. Mechanisms of bone loss in the setting of HIV infection are likely multifactorial, and include viral, host, and immune effects, as well as direct and indirect effects of cART, particularly tenofovir disoproxil fumarate (TDF) and the protease inhibitors (PIs). Emerging data indicate that BMD loss following cART initiation can be mitigated by prophylaxis with either long-acting bisphosphonates or vitamin D and calcium supplementation. In addition, newer antiretrovirals, particularly the integrase strand transfer inhibitors and tenofovir alafenamide (TAF), are associated with less intense bone loss than PIs and TDF. However, further studies are needed to establish optimal bone sparing cART regimens, appropriate screening intervals, and preventive measures to address the rising prevalence of fragility bone disease in the HIV population.

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Conflicts of Interest
Dr. Caitlin A. Moran, Dr.› M. Neale Weitzmann, and Dr. Ighovwerha Ofotokun declare no conflicts of interest.

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This article does not contain any new studies with human or animal involvement performed by the authors.

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Keywords

HIV; osteopenia; osteoporosis; combination antiretroviral therapy; immune reconstitution; bisphosphonates

I. Introduction

HIV-infected individuals treated with combination antiretroviral therapy (cART) can expect to attain a near-normal life expectancy [1]; however, they will experience age-related comorbidities including cardiovascular disease, musculoskeletal abnormalities, and renal impairment at rates that exceed those of the general population [2, 3]. As the HIV population ages, the prevalence of these comorbidities in this population is expected to increase significantly [3]. Therefore, it is becoming increasingly important for clinicians caring for HIV-infected patients to be aware of the risks of noninfectious comorbidities in this population, as well as strategies to mitigate these risks.

Osteopenia and osteoporosis as defined by the World Health Organization criteria [femoral neck or lumbar spine T-score as measured by dual energy X-ray absorptiometry (DXA) between −1.0 and −2.5 (osteopenia) and less than or equal to −2.5 (osteoporosis)] [4] are associated with HIV infection itself, as well as with cART [5, 6]. Indeed, cART further aggravates the bone mineral density (BMD) loss associated with HIV infection: patients experience an additional 2% to 6% BMD loss in the first 1–2 years of cART, a rate of bone loss similar to that seen in postmenopausal osteoporosis [7, 8]. Because this higher prevalence of bone disease in the HIV population is accompanied by a clinically significant increased rate of bone fractures [9, 10], it is important to understand the mechanisms underlying HIV-associated bone loss, as well as current options to prevent further bone loss in this population. This review will focus on new evidence regarding the pathogenesis of HIV-associated bone loss due to both HIV infection itself and the effects of cART, as well as strategies for mitigating metabolic bone disease and fragility fractures in the HIV-infected population.

II. The scope and burden of HIV-associated bone loss

Current estimates suggest that the prevalence of osteopenia and osteoporosis in the setting of HIV infection ranges from 48% to 55% and 10% to 34%, respectively [11–13], and is expected to increase as the HIV population ages [3]. This high prevalence of bone disease is associated with an elevated rate of bone fractures despite the relatively young age of the HIV population [14]. The landmark population-based study by Triant *et al.*, which involved 8,525 HIV-infected patients and 2,208,792 HIV-uninfected controls, found that fracture prevalence among HIV-infected patients of both sexes was two- to four-fold higher than among the HIV-uninfected controls [9]. These findings were confirmed in more recent studies of fracture prevalence involving patients in the United States (U.S.) in the HIV Outpatient Study (HOPS) cohort [15], the Veterans Aging Cohort Study Virtual Cohort (VASC-VC) [16], and the Women's Interagency HIV Study (WIHS) cohort [17]. Internationally, a more recent Spanish population-based cohort study of 2,489 HIV-infected and 1,115,667 HIV-uninfected participants over the age of 40 demonstrated an overall age- and sex-adjusted hip

fracture hazard ratio (HR) for HIV infection of 4.7, with a stronger association in older age groups [10]. In Denmark, a case-control study using nationwide health registry data analyzed 124,655 non-traumatic fracture cases and 373,962 age- and sex-matched controls, and found an almost 9-fold increased risk for hip fracture among HIV-infected patients compared to their HIV-uninfected counterparts [18]. Taken together, these population-based studies demonstrate the high burden of fragility fractures in the HIV population, which is projected to increase as the HIV population ages.

III. Proposed mechanisms of HIV-associated bone loss

The maintenance of skeletal health involves ongoing bone remodeling that requires a balance between bone deposition mediated by mesenchymal stem cell (MSC)-derived osteoblasts and bone resorption mediated by monocyte-macrophage-derived osteoclasts [19]. The osteoblast-osteoclast balance is mediated by cross talk between these bone cells and immune cells [6]. Osteoclast differentiation is stimulated by the binding of receptor activator of nuclear factor kappa-B (RANK) ligand (RANKL) to RANK expressed on cells of the monocytic lineage. In physiological conditions RANKL is secreted predominantly by cells of the osteoblast lineage including osteoblast progenetors (MSCs), osteoblasts [19], and osteocytes, as well as by hypertrophic chondrocytes [20, 21]. The activity of RANKL is inhibited by osteoprotegerin (OPG), a decoy receptor of RANKL, which, under normal conditions, is produced predominantly by B cells in the bone marrow in response to T cell signaling, as well as by MSCs and osteoblasts. However, under inflammatory conditions, T and B cells are further activated to produce RANKL instead of OPG [22•, 23]. This production of RANKL and OPG by immune cells suggests a link between the skeletal and immune systems, the “immunoskeletal interface”. Furthermore, pro-inflammatory cytokines including interleukin (IL)-1, IL-6, IL-7, IL-17, macrophage-colony stimulating factor (M-CSF) and tumor necrosis factor (TNF)-α stimulate osteoclast differentiation either by promoting RANKL, stimulating expression of RANK, or by suppressing production of OPG, favoring enhanced bone loss. TNF-α further amplifies RANKL activity while interferon (IFN)-γ has direct inhibitory effects on osteoclast differentiation [19, 24••], but can potently stimulate osteoclastogensis through immune mediated signals. Many of these cytokines including IL-7 and TNF-α further disregulate bone turnover by suppressing osteoblast differentiation [24••]. Therefore, any perturbations in this complex “immunoskeletal interface” [25] that result in an increase in osteoclastic bone resorption relative to osteoblastic bone formation will lead to BMD loss.

The development of metabolic bone disease in the setting of HIV infection is likely multifactorial. Traditional risk factors for low BMD, including low body mass index (BMI), smoking, hypogonadism or menopause, and corticosteroid use are highly prevalent among HIV-infected individuals [12, 26–29]. However, individuals with HIV experience BMD loss beyond what would be expected from these traditional risk factors alone [30], and there is evidence that both the HIV virus itself, and treatment with cART, contribute to ongoing bone loss in the HIV population.

III.A Mechanisms of virus-associated bone damage

Data suggest that HIV has both direct and indirect effects on osteoblasts, osteoclasts, and their cross-talk regulation [31]. In a prospective cohort study by Hileman *et al.*, treatment-naïve, HIV-infected individuals experienced a greater rate of BMD loss than age, race, and sex matched HIV-uninfected controls over 48 weeks [32]. And studies of cART-naïve, HIV-infected individuals have shown that longer duration of HIV infection [26, 33], more advanced WHO HIV stage [34], and greater levels of HIV viremia [35] are all associated with greater BMD loss, suggesting that the virus itself and the inflammation associated with HIV infection have an effect on BMD.

III.A.i Effects of inflammation and adaptive immune dysregulation on bone— Markers of inflammation and immune activation are associated with BMD loss in HIV-infected individuals. Hileman *et al.* found that higher plasma levels of IL-6 were associated with greater BMD loss among HIV-infected, but not HIV-uninfected, participants [32]. In addition, in a cross-sectional analysis of 457 Tanner stage 5 behaviorally HIV-infected males and females aged 14–25 and seronegative controls, soluble CD14 (sCD14), a marker of macrophage activation, was greater in HIV-infected males than in HIV-uninfected males [36], and a negative correlation between bone mass and sCD14 was seen in both sexes [36]. Taken together, these results suggest that inflammation and innate immune activation play a role in HIV-induced bone loss.

HIV infection also causes dysfunction in adaptive immunity that results in bone loss. In HIV-uninfected persons, activated T cells have been shown to produce RANKL and stimulate osteoclastogenesis in a number of inflammatory conditions including rheumatoid arthritis [37] and postmenopausal osteoporosis [38]. In a cross-sectional study of 78 HIV-infected patients who underwent DXA screening, patients with low BMD (osteopenia or osteoporosis) had a greater frequency of activated $CD4^+$ ($CD4^+HLA\text{-}DR^+$) and activated $CD8^+$ ($CD8^+HLA\text{-}DR^+$) T cells; in a subset of 57 patients virologically suppressed on cART, those with low BMD continued to display a greater frequency of activated $CD8^+$, but not activated $CD4^+$, T cells, suggesting that some immune activation leading to decreased BMD persists despite virologic suppression [39]. However, the clinical significance of these findings is unclear. In a retrospective analysis of the AIDS Clinical Trials Group (ACTG) Longitudinal-Linked Randomized Trial (ALLRT), a longitudinal cohort of participants enrolled in other ACTG studies, markers of T cell activation ($CD8^+CD38^+HLA\text{-}DR^+$) were not associated with an increased incidence of fracture, although this study had low power to detect associations [40].

B cells are also affected by HIV infection. Our group has shown that B cells switch from OPG production to RANKL production in animal models of HIV infection [41], and that B cells isolated from cART-naïve HIV-infected individuals displayed increased RANKL production and decreased OPG production compared to B cells isolated from HIV-uninfected controls [22•]. Furthermore, these changes were associated with an increase in bone turnover markers and a decrease in BMD in HIV-infected individuals compared with HIV-uninfected controls [22•].

III.A.ii Direct effects of HIV on bone—There is also *in vitro* evidence that HIV directly affects bone remodeling. Human osteoblasts exposed to HIV protein p55-gag and envelope glycoprotein gp120 had decreased alkaline phosphatase activity, calcium deposition, and cell proliferation and viability [42, 43], while exposure of $CD3^+$ T cells to gp120 resulted in a significant increase of RANKL production and subsequent osteoclast differentiation [44, 45]. Furthermore, MSCs chronically exposed over 20 days to HIV proteins Tat and Nef *in vitro* exhibited premature senescence, increased oxidative stress, and mitochondrial dysfunction resulting in decreased osteoblastic differentiation [46]. These data suggest that the effect of HIV on BMD may be partially mediated by a range of HIV proteins; however, additional studies are needed to confirm these findings *in vivo*.

III.B Mechanisms of antiretroviral therapy-associated bone damage

There is compelling evidence that HIV-associated bone loss is paradoxically worsened by cART. In a meta-analysis by Brown and Qaqish, HIV-infected individuals on cART had a 2.5-fold increased odds of prevalent low BMD compared with those who were cART-naïve [47]. This effect is universal across all antiretroviral drug classes, although the magnitude of BMD loss may vary by drug regimen [5, 48–50]. Of particular interest is the observation that the vast majority of bone loss occurs within the first 1–2 years after cART initiation, with subsequent stabilization of BMD thereafter [51–53]. It also has been observed that a lower baseline CD4 count is associated with a greater degree of BMD loss after cART initiation [54•]. These data suggest that, in addition to the direct effects of specific antiretrovirals on bone, some of the bone loss seen immediately after cART initiation may be due to HIV disease reversal and immune reconstitution.

III.B.i Role of T cell restoration in cART-associated bone damage—Recently, our group has explored the role of immune reconstitution and T cell repopulation in BMD loss after cART initiation. Our group created an animal model of immune reconstitution by adoptive transfer of T cells into T cell knock-out mice to mimic cART-induced T cell re-expansion, and observed an increase in the osteoclastogenic cytokines RANKL and TNF-α, along with corresponding decreases in cortical and trabecular bone mass 12 weeks after adoptive transfer of T cells [24••]. These findings were confirmed in part in humans in a prospective cohort study of 20 cART-naïve HIV-infected participants initiating cART. A rapid increase in plasma bone turnover markers was observed as early as two weeks after cART initiation, and lasted through 24 weeks [55••]. Peak bone resorption was noted at around 12 weeks: the time at which T cell recovery after cART initiation reaches a significant level [56]. Therefore, it seems likely that in humans, as was described in the mouse model, T cell repopulation plays a role in cART-induced bone loss.

III.B.ii Tenofovir disoproxil fumarate-associated bone loss—Tenofovir disoproxil fumarate (TDF) is a prodrug of the nucleotide reverse transcriptase inhibitor (NtRTI) tenofovir (TFV). Both cART-naïve and virologically suppressed HIV-infected patients exposed to TDF experience 1–3% more bone loss than those exposed to other NRTIs in [57–61]. Additionally, reversible TDF-associated bone loss has been observed in healthy, HIV-uninfected men and women taking TDF for pre-exposure prophylaxis (PrEP) [62–64], thus confirming the effects of TDF on bone beyond what can be attributed to viral or immune

factors. Although there is some *in vitro* evidence that TDF directly affects osteoblast and osteoclast gene expression [65, 66], the putative mechanism of TDF-associated bone loss is phosphate wasting caused by proximal renal tubular dysfunction. TDF is metabolized to TFV in the plasma. In the kidney, TFV is taken up from the plasma by the organic anion transporter at the proximal tubular cells and is then excreted into urine in the tubular space at a slower rate than it is taken up [67, 68]. Accumulation of TFV in the proximal tubular cells can lead to proximal renal tubular dysfunction, the most severe form of which is a Fanconi-like syndrome (hyperphosphaturia, hyperaminoaciduria, and glucosuria) that can result in osteomalacia (poorly mineralized bone matrix), even with preserved glomerular function [67, 69, 70]. Milder TDF-associated renal tubular dysfunction and alterations in phosphate metabolism can still result in a reduction in BMD [71, 72]. Indeed, hyperphosphaturia has been correlated with BMD loss even in the setting of normal phosphatemia [73].

In contrast to TDF, tenofovir alafenamide (TAF) is an alanine ester prodrug of TFV whose pharmacokinetic properties result in greater concentrations of TFV in HIV-target cells with approximately 90% lower TFV plasma concentrations than are seen with TDF [68, 74, 75]. This lower plasma concentration results in less TFV uptake by the kidney and lower proximal tubule TFV concentrations, which in turn leads to less proximal tubule dysfunction [75] and subsequent BMD loss. In clinical trials, cART-naïve HIV-infected patients experienced less BMD loss when started on a TAF-containing regimen compared with a TDF-containing regimen [76, 77], and HIV-infected patients who were virologically suppressed on a TDF-containing regimen saw improvements in their renal function and BMD when switched to a TAF-containing regimen [78]. Whether the long-term bone effects of TAF are similar to those of other non-TDF NRTIs remains to be seen.

III.B.iii Protease inhibitor associated bone loss—The effects of protease inhibitors (PIs) on BMD are somewhat controversial: most, but not all, clinical studies point to an association between PI use and BMD loss [50, 79–83]. In randomized clinical trials, PI-based regimens are associated with a greater degree of BMD loss than either non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens [58, 84] or integrase strand transfer inhibitor (INSTI)-based regimens [85, 86]. The mechanisms behind PI-associated bone loss are unclear, and *in vitro* data are conflicting and vary by drug. Gibellini *et al.* demonstrated that treatment of osteoblasts with fosamprenavir (FPV), but not indinavir (IDV), saquinavir (SQV), atazanavir (ATV), tipranavir (TPV) or darunavir (DRV) results in an increase in OPG expression and subsequent decrease in RANKL production [87], conditions unfavorable for osteoclastic bone resorption. In contrast, Fakruddin *et al.* showed that, in human peripheral blood mononuclear cells (PBMCs), physiologic blocks to osteoclastogenesis are inhibited by ritonavir (RTV) and SQV, two PIs that have been associated with osteopenia, but not IDV and nelfinavir (NFV) [44]. Other *in vitro* studies have demonstrated that PIs can alter osteoblast gene expression [88], decrease osteoblast differentiation by MSCs [89], and increase osteoclast differentiation [45, 90, 91]. Given that the effects observed *in vitro* vary by specific drug, and are not all consistent with clinical observations, there are likely additional or alternative mechanisms by which PIs affect BMD.

In addition to the direct effects of PIs on osteoblasts and osteoclasts, there are data to suggest that a portion of the bone loss associated with PIs can be attributed to concomitant TDF use. In clinical trials of viremic and virologically suppressed HIV-infected patients on standard PI-based cART regimens, maintaining PIs while removing the TDF-containing NRTI backbone results in less BMD loss compared with continuing an NRTI-containing regimen [92–94]. Indeed, RTV has been shown to inhibit active TFV secretion by the proximal tubule, resulting in an increase of plasma TFV concentrations by 25–35% [95–97]. However, compared with other drug classes, PIs are associated with decreased BMD regardless of NRTI backbone [58, 84], suggesting that the mechanism of their effects go beyond alterations in TFV metabolism.

III.C Role of altered vitamin D metabolism in HIV-associated bone loss

Vitamin D is important for maintaining adequate serum calcium levels. Inadequate vitamin D (insufficiency and deficiency) can lead to secondary hyperparathyroidism, which in turn stimulates RANKL production that results in osteoclastogenesis and subsequent bone loss [98]. Likely due in part to chronic inflammation and viral replication [99], inadequate vitamin D is highly prevalent in the HIV-infected population, with estimates ranging from 24–87% in different cohorts and geographic locations [100–103]. cART can lead to further reduction of serum 25-hydroxyvitamin D [25-(OH)D] levels, with the greatest decrease seen with the NNRTI efavirenz (EFV) [101]. In addition, PIs have been shown to inhibit conversion of 25-(OH)D to the active metabolite 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D] *in vitro* [104], but the clinical importance of these cART-associated alterations in vitamin D metabolism is unclear.

IV. Management of HIV-associated bone loss

Due to the complex pathogenesis of bone loss in the setting of HIV infection, screening, risk factor modification, prophylaxis, and treatment continue to present clinical challenges. Fortunately, there is emerging evidence that HIV-associated bone loss can be attenuated by a variety of interventions including the use of newer bone sparing cART regimens, calcium and vitamin D supplementation, and prophylaxis with bisphosphonates.

IV.A Screening for low BMD in HIV infection

Screening for osteopenia and osteoporosis in HIV-infected individuals remains challenging because current screening modalities, including the Fracture Risk Assessment Tool (FRAX) and DXA, are not validated in the relatively young HIV population. Current recommendations from the Osteo Renal Exchange Program (OREP) suggest that all HIV-infected men between the ages of 40–49 and all HIV-infected premenopausal women 40 years of age or older should undergo screening for 10-year fragility fracture risk with a FRAX assessment every 2–3 years, and that men 50 years of age and older, postmenopausal women, and others at high risk such as those with a history of fragility fracture or who are on chronic glucocorticoid therapy should undergo DXA screening, if available [105•]. European AIDS Clinical Society (EACS) guidelines are generally similar [106]. While these recommendations provide some guidance to practicing clinicians, they are limited by the availability of evidence. DXA and FRAX are each well validated in the general population

[107]; however, their utility in the HIV-infected population is less clear. As the OREP guidelines note, the WHO diagnostic T-score criteria for DXA were designed for postmenopausal women, and men 50 years of age and older, and may not be applicable to a younger, HIV-infected population [4, 105•]. Additionally, studies involving the VACS-VC show that each modality underestimates fracture risk in the setting of HIV infection, although FRAX improves when HIV is included as a cause of secondary osteoporosis [108, 109]. In HIV-infected men in the United Kingdom with a median age of 45 years, FRAX had a poor sensitivity and specificity for osteoporosis compared with DXA [110]. However, alternative methods of screening, such as bone turnover markers, are not practical in the clinical setting, and DXA is often not available in resource-limited settings. The development of a more accurate screening tool for fragility fracture in the setting of HIV infection is needed.

IV.B Prophylaxis and treatment

Several strategies exist for the prevention and management of osteoporosis in the setting of HIV infection. These include selection of cART regimens associated with less BMD loss, calcium and vitamin D supplementation, and potentially, prophylaxis with long-acting bisphosphonates.

IV.B.i Selection of cART regimen—It should first be noted that the benefits of cART far outweigh any risks of future bone disease, and current WHO guidelines recommend initiation of therapy in all HIV-infected persons regardless of CD4 count [111]. The selection of any cART regimen is complex and involves a number of viral and host factors. TDF and PIs, both of which are known to carry a greater risk of bone loss, remain important components of recommended first-line and salvage regimens [111, 112]. However, for HIV-infected patients at particularly high risk for fragility bone disease, it is reasonable to consider cART regimens that are not associated with a higher risk of bone loss, such as abacavir (ABC)-containing NRTI backbones and INSTI-based regimens [105•], or to avoid regimens containing both TDF and a PI. Furthermore, the development of TAF has allowed for the use of a TFV prodrug in some patients for whom TDF has been contraindicated, and may offer an additional option for those at high risk for bone disease. While its long-term effects on fracture are not known, TAF in the short-term has been associated with the same degree of bone loss as other TDF-sparing regimens [76], and is an attractive option for those in whom either ABC is contraindicated, or who have another indication for TFV, such as chronic hepatitis B infection. Although much of the cART-associated bone loss occurs in the first 1–2 years of therapy, there does appear to be some benefit in switching from TDF to TAF [78], and this strategy may be beneficial in those who develop additional risk factors for fragility bone disease while on cART.

IV.B.ii Calcium and vitamin D supplementation—In HIV-infected patients with vitamin D deficiency, supplementation with vitamin D has been shown to improve BMD and reduce PTH levels [113]. Furthermore, Overton *et al.*, in a prospective randomized, double-blind, placebo-controlled trial of cART-naïve HIV-infected individuals initiating TDF/emtricitabine (FTC)/EFV, who had baseline 25-(OH)D levels above 25 nmol/L, showed that calcium and vitamin D3 (cholecalciferol) supplementation attenuates BMD loss by about

50% at 48 weeks [114•]. Therefore, although there is little evidence for widespread use of vitamin D supplementation in the general population to prevent osteoporosis [98], it may be beneficial to prevent bone loss in HIV-infected patients who are initiating cART even in the absence of vitamin D deficiency. Further data on adequate dosing and duration of vitamin D supplementation are lacking and require further study as some data suggest that while a low concentration of vitamin D is needed to prevent osteomalacia, higher doses may block bone formation [115]. Besides, emerging data also seem to suggest that calcium supplementation may be associated with increase risk of CVD [116].

IV.B.iii Bisphosphonates—Bisphosphonates inhibit bone resorption, are the most widely used drugs in the treatment of postmenopausal osteoporosis, and are associated with a decreased risk of fracture in that population [117]. Data regarding their use in the HIV-infected population are also promising: bisphosphonates have been shown to improve BMD in HIV-infected men and women in a number of clinical trials [118–120]. In a meta-analysis of the effects of bisphosphonates in HIV-infected patients with low BMD, a mean increase in BMD of 2.85% at the lumbar spine, 1.18% at the femoral neck, and 2.12% at the total hip was seen at 48 weeks [121]. Interestingly, in a phase IIb trial of viremic, cART-naïve, HIV-infected patients without osteoporosis, our group demonstrated that a single infusion of zoledronic acid at the start of therapy with TDF/FTC + ATV/RTV prevented BMD loss at the lumbar spine, hip and femoral neck at 48 weeks [122••]. Although the long-term effects of bisphosphonates in the HIV population on clinically important outcomes such as fracture remain to be determined, these data are promising. Given the challenge of screening all HIV patients initiating cART, the inaccuracy of current screening tools, and the magnitude of bone loss associated with some of the cART regimens, this is a simple, safe and practical step to completely prevent cART-induced bone loss, especially for patients who are being treated with regimens associated with greater bone loss including TDF- and PI-containing regimens. Further study regarding the use of bisphosphonates for either prevention or treatment of HIV-associated bone loss is warranted.

IV.B.iv Other treatment modalities—There are fewer data regarding the use of other pharmacologic agents for the treatment of low BMD in the setting of HIV infection. Teriparatide is a human recombinant PTH used to treat severe osteoporosis in the general population, but its efficacy in HIV-infected individuals is unknown. There is one case report of successful teriparatide use in an HIV-infected man with severe osteoporosis [123] although robust data regarding its use in HIV-infected patients are lacking. In addition, the 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitor (statin) simvastatin has been shown to increase osteoblast activity and decrease osteoclastogenesis, and is associated with increased fracture healing in *in vivo* animal models [124], while pravastatin has been shown to prevent PI-induced senescence in MSCs and restore osteoblastic potential *in vitro* [89]. Clinically however, in HIV-infected individuals on stable cART who had heightened immune activation, treatment with rosuvastatin for 96 weeks was not associated with any changes in BMD, although it was associated with improvement in lean body mass [125]. More data on the BMD effects of these medications in HIV-infected patients are needed.

V. Conclusions

HIV infection is associated with a clinically important loss of BMD that is compounded by treatment with cART. This problem is likely to increase as the HIV-infected population ages. Although much remains unknown about the pathogenesis of low BMD in HIV-infected individuals, current research suggests that immune reconstitution is responsible for a significant proportion of the bone loss seen after cART initiation, and that this effect can be mitigated by bisphosphonate therapy. Further research regarding optimal screening modalities and intervals, cART regimens, and treatment modalities are needed to stem the tide of bone loss and fragility fracture in the aging HIV population.

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EXHIBIT K

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SHORT COMMUNICATION

The rate of bone loss slows after 1–2 years of initial antiretroviral therapy: final results of the Strategic Timing of Antiretroviral Therapy (START) bone mineral density substudy

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Objectives

Initial antiretroviral therapy (ART) causes loss of bone mineral density (BMD) over the first 1–2 years. Whether this loss continues with longer therapy is unclear. We determined changes in bone and spine BMD over 5 years in adults receiving immediate or deferred initial ART.

Methods

In the Strategic Timing of Antiretroviral Therapy (START) BMD substudy, ART-naïve adults with CD4 counts > 500 cells/μL were randomized to immediate or deferred ART. Deferred group participants not yet on ART were offered ART after May 2015. Mean per cent changes in total hip and lumbar spine BMD (measured annually by dual-energy X-ray absorptiometry) were compared between groups using longitudinal mixed models. Fracture rates were also compared between groups for all START participants.

Results

Substudy participants (immediate group, n = 201; deferred group, n = 210; median age 32 years; 80% non-white; 24% female) were followed for a mean 4.5 years until December 2016. In the immediate group, > 96% used ART throughout. In the deferred group, 16%, 58% and 94% used ART at years 1, 3 and 5, respectively. BMD decreased more in the immediate group initially; groups had converged by year 3 at the spine and year 4 at the hip by intent-to-treat (ITT). BMD changes after year 1 were similar in the immediate group and in those off ART in the deferred group [mean difference: spine, 0.03% per year; 95% confidence interval (CI) −0.4, 0.4; P = 0.88; hip, −0.2% per year; 95% CI −0.7, 0.3; P = 0.37]. Fracture incidence did not differ significantly between groups (immediate group, 0.86/100 person-years versus deferred group, 0.85/100 person-years; hazard ratio 1.01; 95% CI 0.76, 1.35; P = 0.98).

Conclusions

Significant ART-induced bone loss slowed after the first year of ART and became similar to that in untreated HIV infection.

Keywords: antiretroviral therapy, bone mineral density, clinical trial, HIV

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Introduction

Bone mineral density (BMD) declines in the first 1 to 2 years of initial antiretroviral therapy (ART) [1]. This decline is greater in those receiving tenofovir disoproxil fumarate (TDF) and/or a boosted protease inhibitor. But even in those not receiving either of these medications, there appears to be a decline of about 1% over 1–2 years [2–5], a decline that is greater than would be expected in a young adult population and has been attributed to altered immune function following suppression of HIV replication [6,7].

Whether BMD continues to decline after the first year of initial ART is less clear. Some studies have reported stable BMD after year 1 [8], in some cases up to year 5 [9]. But other studies have reported ongoing decline [10]. Unfortunately, most studies only have follow-up of 1 or 2 years.

Data on the impact of treated versus untreated HIV infection are sparse. The International Network for Strategic Initiatives in Global HIV Trials Strategic Timing of Antiretroviral Therapy (START) study randomized ART-naïve adults with CD4 lymphocyte counts > 500 cells/μL to immediate ART versus deferring ART until CD4 count < 350 cells/μL; participants in the deferred group were advised to commence ART, after a planned analysis in May 2015 found that immediate ART decreased the risk of clinical events. At that time, we reported that immediate ART in the START BMD substudy resulted in steeper BMD decline (1–2%) relative to deferred ART after a mean follow-up of 2.2 years [11]. After May 2015, all participants were offered ART. We now report final START BMD data collected up to December 2016 after a mean follow-up of 4.5 years, complemented with fracture data collected for all participants in the parent START study.

Methods

Study design and participants

Details regarding the START trial's design, participants and assessments have been published previously [12], as have the baseline characteristics, assessments, endpoints, sample size calculations and statistical plan of its BMD substudy [13].

START randomized 4684 HIV-positive, ART-naïve adults to immediate ART initiation versus deferring ART. ART regimens were not protocol-specified, but were selected pre-randomization. All remaining untreated participants in the deferred group were offered ART in May 2015 after mean follow-up of 3.0 years as a consequence of demonstration of because significant clinical benefit was demonstrated for immediate ART [12].

The START BMD substudy co-enrolled 424 START participants at 33 clinical sites in 11 countries between June 2011 and June 2013, with follow-up completed on 31 December 2016. Participants underwent annual dual-energy X-ray absorptiometry (DXA) on a single Hologic (Marlborough, MA, USA) or Lunar scanner (GE Healthcare, Chicago, IL, USA) to measure BMD at the hip and lumbar spine (L1–L4); all scans were performed using a standardized protocol and were centrally analysed [11].

The substudy was approved by the institutional review board at each participating site and performed in compliance with the Declaration of Helsinki and local regulatory requirements. All participants provided written, informed consent prior to enrolment.

Study outcomes

The co-primary outcomes were the per cent changes from baseline in total hip BMD and lumbar spine BMD. Prespecified secondary outcomes included change in femoral neck BMD, and rates of BMD loss upon ART initiation in the immediate ART group and among participants in the deferred group prior to ART start (patients with untreated HIV infection). Evaluation of clinical parameters associated with rates of BMD change was also planned. Fractures were reported for all participants in the parent START study at baseline and annually.

Statistical analyses

Changes in BMD from baseline were expressed as per cent of baseline BMD. The primary analysis was the intent-to-treat (ITT) comparison between the immediate and deferred ART groups for per cent change in BMD using longitudinal mixed models, adjusted for baseline BMD and visit. Groups were compared for changes in BMD to each year of follow-up using analysis of covariance (ANCOVA) models adjusted for baseline BMD. We also compared the immediate group (excluding those who did not start ART during year 1) versus the deferred group censored at ART start. Methods to estimate annual rates of BMD change, for subgroup analyses, and to estimate associations of baseline factors with changes in BMD were described previously [11].

Fracture incidence rates were estimated in the parent START population using data acquired up to 31 December 2016. Treatment groups were compared by ITT using a Cox proportional hazards model, stratified by region.

Analyses were performed with SAS version 9.3 (SAS Institute, Cary, NC) and R version 3 [14].

Results

Participant characteristics

The present analysis included 411 (96.9%) of the 424 BMD substudy participants (immediate group, $n = 201$;

deferred group, $n = 210$); we excluded 13 participants with no analysable DXA scan at baseline or during follow-up (Fig. S1). Baseline characteristics of these two groups were well matched (Table S1). The racially diverse population had a median age of 32 years; 26% were female and 80% non-Caucasian.

Participants were followed for a mean 4.5 [standard deviation (SD) 0.5] years; data completeness was > 90% at each visit (Table S2). In the immediate group, > 96% used ART each year up to year 5. In the deferred group, 16%, 29%, 58%, 84% and 94% used ART at years 1–5, respectively (Fig. S2). Initial ART contained TDF for 82.8% of participants, efavirenz for 78.1% and a protease inhibitor for 13.0%. No tenofovir alafenamide (TAF) was used.

Changes in BMD

Mean per cent changes in BMD from baseline and annual rates of BMD change at the spine and total hip are summarized in Table 1. BMD had declined more in the immediate than in the deferred group by year 1 [estimated difference −1.7% (95% confidence interval (CI) −2.3, −1.2) at the spine and −1.6% (95% CI −2.2, −0.9) at the total hip; Fig. 1a,b). BMD values in the immediate and deferred groups had largely converged by years 3–4 as deferred group participants progressively initiated ART. In the immediate group after year 1, rates of change in BMD were stable at the spine, but continued to decline by about 0.5% per year at the total hip.

When follow-up was censored at ART start in the deferred group, the immediate minus deferred treatment difference at year 1 was −2.0% (95% CI −2.6, −1.3) at the spine, and −2.0% (95% CI −2.6, −1.4) at the hip. After year 1, the annual rates of BMD change in the immediate group and those remaining off ART in the deferred group were not significantly different (Fig. 1d–f).

In subgroup analyses, the treatment difference in spine BMD was larger among those with baseline CD4 counts ≥ 650 cells/μL compared with those with lower CD4 counts (−1.7% and −0.3%, respectively; $P = 0.008$ for heterogeneity). BMD losses were less steep among smokers than among nonsmokers (spine −0.7% and −2.2%, respectively; hip −0.7% and −2.8%, respectively; $P = 0.05$ for heterogeneity at both the hip and spine) (Fig. S3). There was no evidence for heterogeneity of the treatment effect among subgroups defined by age, sex, race, geographical region, baseline HIV RNA level and TDF in the pre-specified ART regimen.

Predictors of BMD decline

In the immediate ART group, no baseline variable consistently predicted greater loss of BMD at both the hip and spine (Table S3). Higher HIV viral load was associated with steeper decline of total hip BMD. A higher CD8 count was associated with steeper decline of spine BMD, and smoking was associated with steeper BMD loss at the hip. Within the deferred group (censored at ART start), a lower CD4 count was associated with steeper BMD decline at the spine and femoral neck (Table S4).

Incidence of fractures

In the parent START study, 182 (3.9%) of the 4684 participants experienced a fracture: 91 (0.86 per 100 person-years) in the immediate group and 91 (0.85 per 100 person-years) in the deferred group (hazard ratio 1.01; 95% CI 0.76, 1.35; $P = 0.98$) (Table S5, Fig. S4).

Discussion

These final data from the START BMD substudy confirm that initial ART significantly accelerates BMD loss over the first 1–2 years in young adults. With up to 5 years of follow-up, there was evidence for ongoing BMD loss in both groups, but the between-group difference in later years was modest and not significant.

Our data provide reassurance that the steep rate of BMD loss in the first year of ART is ameliorated in subsequent years, as observed in other cohorts [15–17]. Indeed, after year 1, the rate of loss in the immediate ART group was similar to that in patients who remained ART naïve in the deferred group. This finding supports similar data comparing adults on stable ART and matched HIV-negative controls [15]. However, the rate of decline after year 1 remained greater than might be expected in a population of this age [18]. The normal rate of decline in BMD in later years is influenced by race/ethnicity and age [19,20]. Our participants were racially diverse, so a comparison with a cohort such as National Health and Nutrition Examination Survey III may not be appropriate. Longitudinal data from a Canadian study suggest that there is virtually no loss of BMD at either hip or spine until after the age of 40 years [18].

There was no consistent signal for CD4 count, CD8 count or CD4:CD8 ratio as a predictor of greater bone loss at both the hip or the spine. Our results are in agreement with those of others who reported that a higher pre-ART viral load predicted greater bone loss with initiation of ART [2,17]. Our study is also in agreement with another randomized trial of ART in which a lower CD4 count did not predict greater bone loss on initiation of ART [4], in contrast to other studies [17]. The finding that higher CD8 counts were associated with greater bone loss in those initiating ART is

Table 1 Mean per cent changes in bone mineral density (BMD) from baseline, and annual rates of change, by treatment group

	Immediate ART group versus deferred ART group by intention to treat						Immediate group versus deferred ART group participants prior to ART start*					
	Immediate ART		Deferred ART				Immediate ART		Deferred ART			
	n	Mean change (95% CI)	*n*	Mean change (95% CI)	Difference (95% CI)	*P*	*n*	Mean change (95% CI)	*n*	Mean change (95% CI)	Difference (95% CI)	*P*
Spine												
Year 1	194	−2.1 (−2.5, −1.6)	197	−0.3 (−0.7, 0.1)	−1.7 (−2.3, −1.2)	< 0.001	189	−2.1 (−2.5, −1.6)	171	−0.04 (−0.5, 0.4)	−2.0 (−2.6, −1.4)	< 0.001
Year 2	189	−1.5 (−2.1, −1.0)	195	−0.4 (−0.9, 0.1)	−1.1 (−1.9, −0.4)	< 0.01	184	−1.5 (−2.1, −0.9)	134	0.5 (−0.1, 1.0)	−2.0 (−2.8, −1.2)	< 0.001
Year 3	184	−1.6 (−2.2, −1.0)	194	−1.1 (−1.7, −0.5)	−0.5 (−1.4, 0.4)	0.26	180	−1.6 (−2.2, −0.9)	79	0.2 (−0.6, 1.0)	−1.8 (−2.9, −0.7)	< 0.01
Year 4	146	−1.8 (−2.6, −1.0)	147	−1.4 (−2.1, −0.7)	−0.4 (−1.4, 0.7)	0.52	142	−1.7 (−2.5, −1.0)	27	−0.6 (−2.3, 1.0)	−1.1 (−3.0, 0.9)	0.27
Year 5	27	−4.1 (−5.7, −2.6)	32	−1.7 (−3.4, 0.02)	−2.5 (−4.8, −0.1)	0.05	27	−4.1 (−5.7, −2.6)	2	4.9 (1.7, 8.0)	−9.0 (−15.1, −3.0)	< 0.01
Overall	201	−1.8 (−2.3, −1.4)	210	−0.9 (−1.3, −0.4)	−1.0 (−1.6, −0.3)	< 0.01	196	−1.8 (−2.2, −1.4)	176	0.2 (−0.3, 0.7)	−2.0 (−2.7, −1.3)	< 0.001
Rate of change												
Year 1 to 2	184	0.5 (0.03, 0.9)	185	−0.1 (−0.5, 0.3)	0.6 (−0.1, 1.2)	0.08	179	0.5 (0.1, 0.9)	130	0.5 (0.1, 1.0)	−0.02 (−0.6, 0.6)	0.95
Year 2 to 3	177	0.02 (−0.4, 0.5)	185	−0.8 (−1.2, −0.3)	0.8 (0.1, 1.4)	0.02	173	−0.01 (−0.5, 0.4)	75	−0.5 (−1.2, 0.1)	0.5 (−0.3, 1.3)	0.20
Year 3 to 4	143	0.2 (−0.4, 0.7)	145	−0.7 (−1.3, −0.2)	0.9 (0.1, 1.7)	0.02	139	0.2 (−0.3, 0.8)	25	0.1 (−1.2, 1.4)	0.12 (−1.3, 1.5)	0.86
Year 4 to 5	26	−0.9 (−2.2, 0.4)	32	−0.4 (−1.6, 0.7)	−0.5 (−2.3, 1.2)	0.55	26	−0.9 (−1.8, −0.1)	2	2.2 (−0.9, 5.2)	−3.1 (−6.5, 0.4)	0.08
From 1 year overall	197	0.1 (−0.1, 0.3)	204	−0.5 (−0.7, −0.3)	0.5 (0.3, 0.8)	< 0.001	192	0.1 (−0.1, 0.3)	140	0.2 (−0.1, 0.5)	0.03 (−0.4, 0.4)	0.88
Total hip												
Year 1	193	−2.0 (−2.6, −1.5)	197	−0.5 (−0.9, −0.1)	−1.6 (−2.2, −0.9)	< 0.001	188	−2.2 (−2.7, −1.6)	171	−0.2 (−0.6, 0.2)	−2.0 (−2.6, −1.3)	< 0.001
Year 2	188	−2.8 (−3.5, −2.1)	195	−1.4 (−2.0, −0.8)	−1.3 (−2.2, −0.4)	< 0.01	183	−2.9 (−3.5, −2.2)	133	−0.7 (−1.2, −0.1)	−2.2 (−3.1, −1.3)	< 0.001
Year 3	183	−3.2 (−3.9, −2.5)	193	−2.1 (−2.9, −1.4)	−1.0 (−2.0, 0.0)	0.05	179	−3.2 (−3.9, −2.5)	79	−0.6 (−1.3, 0.1)	−2.6 (−3.8, −1.5)	< 0.001
Year 4	146	−3.5 (−4.4, −2.6)	148	−3.2 (−4.0, −2.4)	−0.2 (−1.4, 0.9)	0.68	142	−3.6 (−4.5, −2.7)	27	−1.2 (−3.1, 0.6)	−2.4 (−4.6, −0.3)	0.03
Year 5	27	−5.6 (−7.6, −3.6)	32	−3.8 (−5.8, −1.8)	−2.0 (−4.9, 1.0)	0.20	27	−5.6 (−7.6, −3.6)	2	5.7 (−1.9, 13.2)	−11.3 (−19.0, −3.5)	< 0.01
Overall	200	−2.8 (−3.3, −2.2)	210	−1.7 (−2.2, −1.1)	−1.1 (−1.9, −0.3)	< 0.01	195	−2.9 (−3.3, −2.4)	176	−0.5 (−1.0, 0.1)	−2.1 (−2.9, −1.4)	< 0.001
Rate of change												
Year 1 to 2	183	−0.8 (−1.3, −0.4)	185	−0.8 (−1.2, −0.3)	0.0 (−0.7, 0.7)	> 0.99	178	−0.8 (−1.2, −0.4)	129	−0.3 (−0.8, 0.2)	−0.5 (−1.1, 0.2)	0.18
Year 2 to 3	176	−0.2 (−0.7, 0.4)	184	−0.8 (−1.3, −0.2)	0.6 (−0.2, 1.4)	0.12	172	−0.1 (−0.7, 0.5)	75	−0.2 (−1.1, 0.7)	0.1 (−0.9, 1.2)	0.84
Year 3 to 4	143	−0.2 (−0.7, 0.3)	145	−1.1 (−1.6, −0.6)	0.9 (0.2, 1.6)	0.02	139	−0.2 (−0.7, 0.2)	25	−0.1 (−1.1, 1.0)	−0.1 (−1.3, 1.1)	0.88
Year 4 to 5	26	−1.3 (−2.0, −0.5)	32	−0.6 (−1.2, 0.11)	−0.8 (−1.8, 0.3)	0.13	26	−1.3 (−2.2, −0.4)	2	0.6 (−2.7, 3.8)	−1.8 (−5.4, 1.8)	0.31
From 1 year overall	196	−0.5 (−0.7, −0.2)	204	−0.9 (−1.1, −0.6)	0.4 (0.02, 0.7)	0.04	191	−0.4 (−0.7, −0.2)	139	−0.3 (−0.6, 0.1)	−0.2 (−0.7, 0.3)	0.37

*Participants in the immediate ART group who did not start ART within the first year are excluded, and follow-up in the deferred ART group is censored at ART start. ART, antiretroviral therapy; CI, confidence interval.

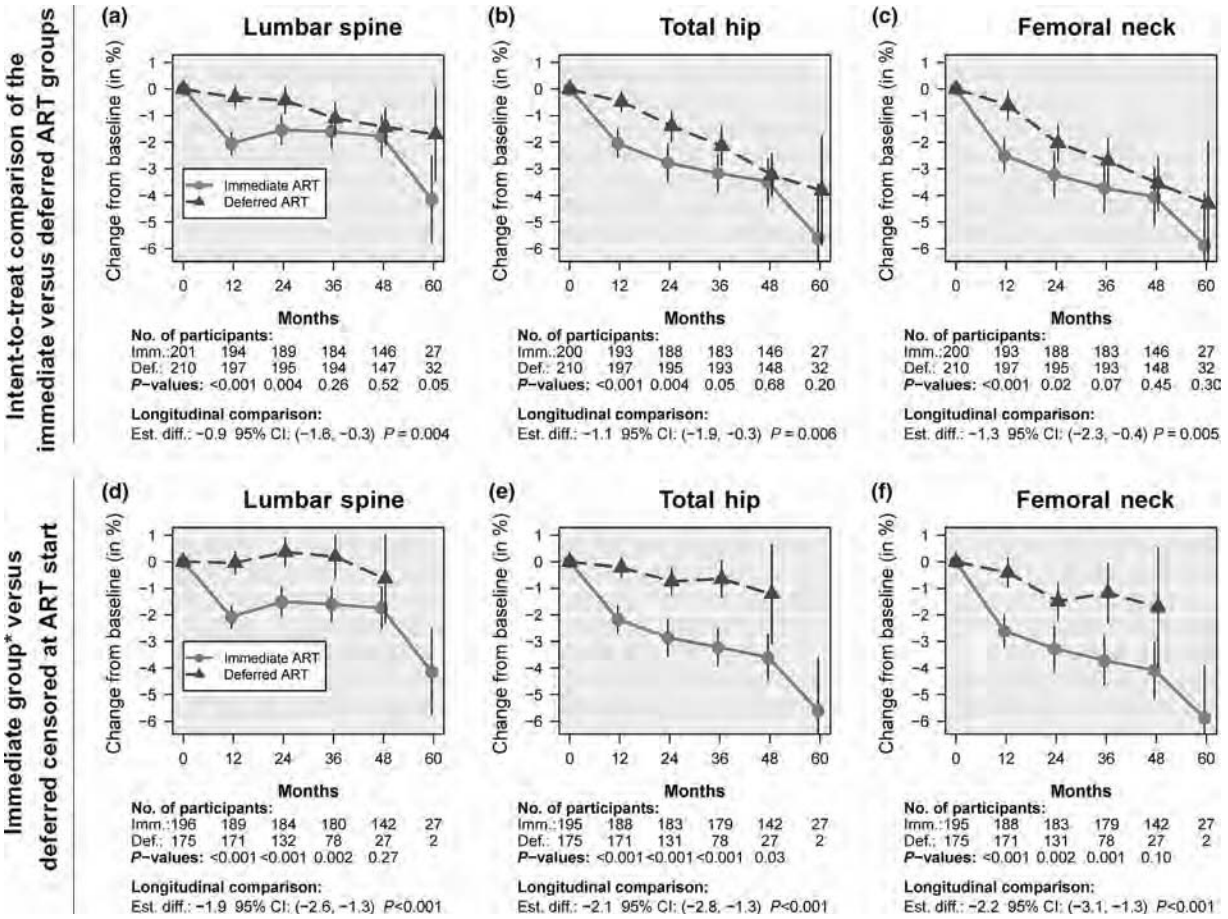

Fig. 1 Mean per cent change in bone mineral density (BMD) with 95% confidence intervals (CIs) from baseline to follow-up. Panels (a)–(c) show the intent-to-treat comparison between the immediate and deferred antiretroviral therapy (ART) groups. In panels (d)–(f), follow-up in the deferred group was censored at ART start, and in the immediate group, participants who did not start ART in the first year were excluded. (a, d) Lumbar spine; (b, e) total hip; (c, f) femoral neck. *In the immediate group, five participant who did not start ART in the first year were excluded.

novel and not previously reported. The finding that none of the traditional risk factors for bone loss predicted change in BMD may have been attributable to the young age of our study population.

Our analysis has limitations. Most participants in the deferred group had initiated ART by year 4. Therefore, the ITT comparison between the immediate and deferred groups does not accurately quantify the effect of ART versus untreated HIV infection in the later years, while comparison of the immediate group versus patients with untreated HIV infection in the deferred group is not protected by randomization. However, given that immediate ART is now standard-of-care, it is highly unlikely that another study will ever evaluate the long-term effects of ART relative to no ART. Most ART regimens contained TDF, which is a well-known cause of BMD loss; TDF remains a common backbone, in part because of its preferred status within the World Health Organization (WHO) guidelines [21].

In summary, bone loss with initial ART slowed after the first year of ART, and the rates of change in BMD after the first year were similar with and without ART. In this relatively young population, ART-related BMD loss did not translate into a greater incidence of fractures.

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Conflicts of interest: AC has received research funding from Bristol-Myers Squibb, Gilead Sciences, and ViiV Healthcare; in-kind research support from Novartis; conference travel sponsorships from Bristol-Myers Squibb, Gilead Sciences, and ViiV Healthcare; and has served on advisory boards for Gilead Sciences, MSD and ViiV Healthcare. KE received travel support from Merck Sharp & Dohme for attendance at DMC meetings. AL received honoraria and salary from MSD and Janssen. AS has received a grant from Hologic Inc. JH's institution received funding for her participation in advisory boards for Gilead Sciences, AbbVie and ViiV Healthcare. BG, NWE, AVS, AA, SBF, JIB, VE, ALR, PWGM, SP and DW declare no conflict of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Baseline characteristics
Table S2. Participant disposition at years 1–5
Table S3. Predictors of per cent change in BMD in the immediate ART group, excluding participants who did not start ART within the first year
Table S4. Predictors of per cent change in BMD in the deferred ART group, censored at ART start date
Table S5. Sites of fractures, with numbers and rates per 100 person-years, for all fractures and those that occurred with minimal trauma
Figure S1. CONSORT diagram
Figure S2. Per cent of participants on ART and per cent with HIV RNA < 200 copies/mL
Figure S3. Subgroup analyses for changes in BMD
Figure S4. Kaplan–Meier curves for the cumulative percentage of participants with fractures over time, by treatment group

EXHIBIT L

Morbidity and Mortality Weekly Report

Antiretroviral Therapy and Viral Suppression Among Active Duty Service Members with Incident HIV Infection — United States, January 2012–June 2018

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Human immunodeficiency virus (HIV) infection is a deployment-limiting medical condition for U.S. armed forces in the Department of Defense (DoD) (*1*). HIV management using contemporary antiretroviral therapy (ART) regimens permits effective suppression of viremia among persons in clinical care. Although service members with HIV infection can remain in military service, treatment outcomes have not been fully described. Data from the Defense Medical Surveillance System (DMSS) were analyzed to estimate ART use and viral suppression among DoD service members with diagnosed HIV infection during January 2012–June 2018 (*2*). Among 1,050 service members newly diagnosed with HIV infection during January 1, 2012–December 31, 2017, 89.4% received ART within 6 months of HIV diagnosis, 95.4% within 12 months, and 98.7% by the end of the surveillance period on June 30, 2018. Analyses determined that, among 793 persons who initiated ART and remained in military service for ≥1 year, 93.8% received continuous ART, 99.0% achieved viral suppression within 1 year after ART initiation, and 96.8% were virally suppressed at receipt of their last viral load test. The DoD model of HIV care demonstrates that service members with HIV infection who remain in care receive timely ART and can achieve both early and sustained viral suppression.

DoD routinely screens its service members for HIV infection to ensure force health protection and to protect the battlefield blood supply (*1*). All active duty service members with HIV infection receive care through the Military Health System and can be retained in service if they can perform their duties. Clinical evaluations are performed by military infectious disease physicians following diagnosis of HIV infection and at least every 6 to 12 months thereafter.

Demographic information, military service personnel records, and laboratory data were extracted from the DMSS, which maintains longitudinal service-related and clinical surveillance data for all personnel throughout their military service. All cases of incident HIV infection occurring among active duty service members during January 1, 2012–December 31, 2017, were identified from surveillance data validated against HIV case lists maintained by each military service. Activated reservists and National Guard members were excluded because DMSS does not record accurate follow-up time for reserve or National Guard members. Pharmacy records for dispensed ART prescriptions were obtained from the DoD Pharmacy Data Transaction Service. This analysis was conducted by the Armed Forces Health Surveillance Branch as part of routine medical surveillance efforts on the health outcomes of service members living with HIV infection. Because the branch was conducting this analysis in its capacity as a public health authority providing medical surveillance support to DoD policymakers, institutional review board approval was not required.

ART initiation was assessed for 1,050 service members with incident HIV infection who remained in service for ≥6 months after diagnosis. ART initiation was defined as dispensation of an initial ART prescription during a specified time frame following diagnosis of HIV infection (within 6 months, within 12 months, or by the end of the study period).

Among 1,050 service members with incident HIV infection, 243 (23.1%) were excluded from analysis of continuous ART and viral suppression because of inadequate follow-up time (206; 19.6%) or incomplete viral load testing (37; 3.5%) and an additional 14 (1.3%) because ART history was missing, leaving 793 (75.5%) service members with incident HIV infection and at least 1 year of follow-up for analysis.* The 243 service members who initiated ART but were not included in additional analysis for continuous ART and viral suppression were similar demographically to the 793 who were included and had no evidence of being immunocompromised (median baseline CD4 count = 513 [interquartile range (IQR) = 386–659] cells/μL).

Continuous receipt of ART and viral suppression were assessed among the 793 persons who remained in service for at least 1 year after ART initiation and who had documented viral suppression within 6 months of ART initiation or a viral load test 6–12 months after ART initiation. Continuous ART was defined as dispensation of at least a 6 months' supply of ART within 6 months of initiating ART. Viral suppression was defined as a viral load measurement of <200 copies of HIV RNA per mL within 1 year of ART initiation. Viral suppression was also reported at the last viral load test during follow-up 1 year after ART initiation and at the last viral load

* Chart review determined that among the 14 persons without documentation of ART receipt, four received ART through civilian care, three were "elite controllers" who had spontaneous viral suppression without ART, three refused ART, three started ART after the end of the surveillance period, and one had provider documentation stating "no indication" because of a CD4 count >500 cells/μL.

test of the surveillance period. In addition, viral suppression was calculated for each year of follow-up after HIV diagnosis, as the percentage of service members whose last viral load test during each year of follow-up was <200 copies of HIV RNA per mL, among service members with at least one viral load test during that follow-up year.

The median interval from diagnosis of HIV infection to the first viral load test indicating viral suppression was also calculated overall and stratified by year of HIV diagnosis. In addition, the overall median interval from HIV diagnosis to the last viral load test in the surveillance period was calculated. Median CD4 counts were calculated at baseline and at the last CD4 test during the surveillance period. SAS statistical software (version 9.4; SAS Institute) was used for all analyses.

Among 1,050 service members with incident HIV infection, 939 (89.4%) initiated ART within 6 months of diagnosis, 1,002 (95.4%) within 12 months, and 1,036 (98.7%) by the end of the surveillance period (Table 1). ART initiation within 6 months of diagnosis was more common among older service members, males, and those in the Air Force (Table 1). Initial ART regimens were anchored by integrase strand transfer inhibitors (63.0%), nonnucleoside reverse transcriptase inhibitors (28.2%), protease inhibitors (6.2%), or other combinations of these agents with or without nucleoside reverse transcriptase inhibitors (2.6%). After exclusion of the 243 service members with inadequate follow-up or viral load testing and the 14 with missing history of ART, among the remaining 793 service members, 744 (93.8%) received continuous ART, and 785 (99.0%) had at least one viral load result indicating viral suppression within 1 year after ART initiation (Table 2). Continuous receipt of ART was more prevalent among older service members, non-Hispanic whites, non-Hispanic blacks, males, officers, and pilot/aircrew personnel, compared with their respective counterparts. A high percentage of viral load suppression within 1 year after ART initiation (>96%) was achieved among all demographic subgroups. A total of 772 (97.4%) service members were virally suppressed at their last viral load test during follow-up 1 year after ART initiation and 768 (96.8%) were virally suppressed at their last viral load test of the surveillance period (Table 2). The percentage of service members with HIV infection who achieved viral suppression ranged from 91.6% of 787 persons in the first year of follow-up to 100% of 15 persons in the seventh year (Table 3). The interval from HIV diagnosis to first viral load test indicating viral suppression ranged from 6.9 months (IQR = 4.9–10.9) in 2012 to 2.9 months (IQR = 2.5–4.3) in 2017 (median = 4.6 months ([IQR = 2.9–7.2]). The median CD4 count at baseline was 486 cells/μL (IQR = 342–625) and 717 (IQR = 565–909) at the last test during the surveillance period.

Discussion

In 2014, based on surveillance data, CDC indicated that 96% of adults with HIV infection in the United States receiving outpatient medical care self-reported currently taking ART, and 98% reported ever taking ART (*3*). In addition, national data indicate that 81.5% to 85.9% of persons engaged in HIV clinical care during 2016–2017 were virally suppressed at their last test (*4*,*5*). Findings from the current analysis suggest that a high percentage of active duty service members receive ART and achieve viral suppression. The Military Health System permits free universal access for active duty service members throughout all aspects of the HIV care continuum, such as routine testing, specialty care evaluations, laboratory monitoring, and ART. The DoD model of HIV care demonstrates that ART and viral suppression goals can be achieved among a segment of the U.S. population who receive clinical care in a large health care system, despite high mobility and geographic dispersal.

Viral suppression among U.S. service members with HIV infection has increased over time. A study of Air Force service members with HIV infection found that 93% attained viral suppression 1 year after ART initiation during 2006–2011, an increase from 78.6% during 2000–2005 (*6*). The U.S. Military HIV Natural History Study, an observational study of military service members and beneficiaries with HIV infection, determined that viral suppression at 1 year after diagnosis among active duty patients who initiated ART during 2000–2007 was 84%, compared with 64% during 1996–1999 (*7*). Since the 1990s, duration of military service after diagnosis of HIV infection has increased substantially, and the number of AIDS-defining illnesses has decreased (*8*,*9*). The combination of more potent ART with fewer adverse effects and the increased availability of single-tablet regimens have likely contributed to improved outcomes, including the high ART uptake and levels of viral suppression noted in this analysis. In addition, the U.S. military mandates periodic evaluations for service members with HIV infection. DoD and service-specific HIV-related policies stipulate that progressive clinical illness or immune deficiency necessitates duty restrictions and, potentially, a referral for medical evaluation for continued service.[†,§,¶,**] Cumulatively, these policies likely enhance adherence to ART among service members with HIV infection. Viral suppression also has population-level benefits; a recent CDC study of HIV transmission along the continuum of care in 2016 reported that the Treatment as Prevention[††] strategy can effectively

† https://www.esd.whs.mil/Portals/54/Documents/DD/issuances/dodi/648501p.pdf.
§ https://www.med.navy.mil/sites/nmcphc/Documents/nbimc/648501p.pdf.
¶ https://static.e-publishing.af.mil/production/1/af_sg/publication/afi44-178/afi44-178.pdf.
** https://www.army.mil/e2/downloads/rv7/r2/policydocs/r600_110.pdf.
†† https://www.hiv.gov/tasp.

TABLE 1. Service members* who initiated antiretroviral therapy (ART) within 6 months, 12 months, or by the end of the surveillance period after diagnosis of incident human immunodeficiency virus (HIV) infection — U.S. Armed Forces, January 2012–June 2018

	Time of ART initiation after HIV diagnosis no. (%)		
Characteristic† (total no.)	6 mos	12 mos	Ever§
Total (1,050)	**939 (89.4)**	**1,002 (95.4)**	**1,036 (98.7)**
Sex			
Male (1,023)	916 (89.5)	976 (95.4)	1,009 (98.6)
Female (27)	23 (85.2)	26 (96.3)	27 (100.0)
Age group, yrs			
<20 (31)	27 (87.1)	29 (93.5)	30 (96.8)
20–29 (744)	659 (88.6)	709 (95.3)	733 (98.5)
30–39 (224)	204 (91.1)	215 (96.0)	222 (99.1)
40–49 (44)	42 (95.5)	42 (95.5)	44 (100.0)
≥50 (7)	7 (100.0)	7 (100.0)	7 (100.0)
Race/Ethnicity			
White, non-Hispanic (296)	271 (91.6)	283 (95.6)	293 (99.0)
Black, non-Hispanic (483)	418 (86.5)	459 (95.0)	475 (98.3)
Hispanic (160)	150 (93.8)	155 (96.9)	159 (99.4)
Asian/Pacific Islander (30)	27 (90.0)	30 (100.0)	30 (100.0)
Other/Unknown (81)	73 (90.1)	75 (92.6)	79 (97.5)
Marital status			
Married (352)	318 (90.3)	338 (96.0)	349 (99.1)
Single (659)	587 (89.1)	627 (95.1)	648 (98.3)
Other (39)	34 (87.2)	37 (94.9)	39 (100.0)
Service			
Army (422)	348 (82.5)	394 (93.4)	414 (98.1)
Navy (345)	322 (93.3)	335 (97.1)	343 (99.4)
Air Force (190)	187 (98.4)	187 (98.4)	188 (98.9)
Marine Corps (93)	82 (88.2)	86 (92.5)	91 (97.8)
Rank			
Enlisted (965)	861 (89.2)	920 (95.3)	951 (98.5)
Officer (85)	78 (91.8)	82 (96.5)	85 (100.0)
Occupation			
Combat-specific (105)	91 (86.7)	99 (94.3)	103 (98.1)
Motor transport (51)	46 (90.2)	49 (96.1)	50 (98.0)
Pilot/Aircrew (16)	14 (87.5)	14 (87.5)	15 (93.8)
Repair/Engineer (264)	244 (92.4)	256 (97.0)	261 (98.9)
Communications/Intelligence (305)	273 (89.5)	290 (95.1)	300 (98.4)
Health care (127)	111 (87.4)	121 (95.3)	127 (100.0)
Other (182)	160 (87.9)	173 (95.1)	180 (98.9)

* Service members were required to have at least 6 months follow-up time after diagnosis of incident HIV infection.
† All demographic and military characteristics ascertained at the time of incident HIV infection diagnosis.
§ By June 30, 2018.

eliminate secondary sexual transmission of HIV from persons virally suppressed on ART (*10*).

The findings in this report are subject to at least two limitations. First, records of dispensed ART medications were used to estimate ART initiation and continued use; no data on adherence were available. However, viral load determinations following ART dispensation suggest a high level of adherence. Second, DoD service members constitute an open population with varying entry and exit dates; therefore, rates of ART use and viral suppression could only be assessed for persons who remained in service during specified periods.

DoD embodies a contemporary national model of successful HIV care, given the high uptake of HIV treatment and achievement of viral suppression by its service members. DoD will continue to review its policies and the scientific literature and report findings of health outcomes among service members living with HIV infection.

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TABLE 2. Continuous antiretroviral therapy (ART)*,† and viral suppression within 1 year after ART initiation and at last viral load test during the surveillance period,§ among active duty service members in military human immunodeficiency virus (HIV) care¶ — U.S. Armed Forces, January 2012–June 2018

Characteristic (total no.)	No. (%)		
	Continuous ART	Viral suppression within 1 year	Viral suppression, last test
Total (793)	**744 (93.8)**	**785 (99.0)**	**768 (96.8)**
Sex			
Male (771)	728 (94.4)	763 (99.0)	746 (96.8)
Female (22)	16 (72.7)	22 (100.0)	22 (100.0)
Age group, yrs			
<20 (23)	22 (95.7)	23 (100.0)	20 (87.0)
20–29 (553)	512 (92.6)	547 (98.9)	534 (96.6)
30–39 (178)	171 (96.1)	176 (98.9)	175 (98.3)
40–49 (35)	35 (100.0)	35 (100.0)	35 (100.0)
≥50 (4)	4 (100.0)	4 (100.0)	4 (100.0)
Race/Ethnicity			
White, non-Hispanic (207)	199 (96.1)	206 (99.5)	203 (98.1)
Black, non-Hispanic (370)	355 (95.9)	365 (98.6)	357 (96.5)
Hispanic (137)	118 (86.1)	135 (98.5)	132 (96.4)
Asian/Pacific Islander (23)	19 (82.6)	23 (100.0)	23 (100.0)
Other/Unknown (56)	53 (94.6)	56 (100.0)	53 (94.6)
Marital status			
Married (257)	241 (93.8)	256 (99.6)	254 (98.8)
Single (507)	474 (93.5)	501 (98.8)	485 (95.7)
Other (29)	29 (96.6)	28 (96.6)	29 (100.0)
Service			
Army (300)	278 (92.7)	295 (98.3)	292 (97.3)
Navy (277)	257 (92.8)	274 (98.9)	266 (96.0)
Air Force (149)	144 (96.6)	149 (100.0)	143 (96.0)
Marine Corps (67)	65 (97.0)	67 (100.0)	67 (100.0)
Rank			
Enlisted (724)	675 (93.2)	716 (98.9)	699 (96.5)
Officer (69)	69 (100.0)	69 (100.0)	69 (100.0)
Occupation			
Combat-specific (74)	72 (97.3)	73 (98.6)	73 (98.6)
Motor transport (37)	34 (91.9)	36 (97.3)	36 (97.3)
Pilot/Aircrew (11)	11 (100.0)	11 (100.0)	11 (100.0)
Repair/Engineer (213)	202 (94.8)	212 (99.5)	209 (98.1)
Communications/Intelligence (231)	212 (91.8)	227 (98.3)	222 (96.1)
Health care (103)	95 (92.2)	102 (99.0)	99 (96.1)
Other (124)	118 (95.2)	124 (100.0)	118 (95.2)

* Continuous ART was defined as having been dispensed at least 180 days' supply of ART medications within 6 months of initiating ART.
† Service members were required to have at least 1-year follow-up time after ART initiation. In addition, they must have been virally suppressed within 6 months of ART initiation or have a viral load test on file from 6 to 12 months after ART initiation.
§ Viral suppression was defined as having a viral load <200 copies of HIV RNA per mL according to any viral load test that was performed within 1 year after ART initiation.
¶ All demographic/military characteristics measured at the time of incident HIV diagnosis.

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All authors have completed and submitted the International Committee of Medical Journal Editors form for disclosure of potential conflicts of interest. No potential conflicts of interest were disclosed.

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TABLE 3. Viral suppression among active duty service members in military human immunodeficiency virus (HIV) care (N = 793),* by year of follow-up — U.S. Armed Forces, January 1, 2012–June 30, 2018

No. of follow-up† yrs	No. with ≥1 viral load test	No. (%) virally suppressed§
1	787¶	721 (91.6)
2	727	705 (97.0)
3	511	500 (97.8)
4	315	305 (96.8)
5	182	177 (97.3)
6	78	76 (97.4)
7	15	15 (100.0)

* Service members were required to have at least 1 year of follow-up after ART initiation and to have been virally suppressed within 6 months of ART initiation or have a viral load test on file from 6 to 12 months after ART initiation.
† After diagnosis of HIV infection.
§ Last viral load of each follow-up year <200 copies of HIV RNA per mL.
¶ No. of persons who had a viral load test within 1 year of HIV diagnosis = 787 of 793.

Summary

What is already known about this topic?

U.S. Department of Defense (DoD) service members with human immunodeficiency virus (HIV) infection can remain in military service; however, treatment outcomes have not been fully described.

What is added by this report?

During January 2012–June 2018, 93.8% of service members with HIV infection who remained in care received continuous antiretroviral therapy (ART). Viral suppression was achieved in 99.0% within 1 year of ART initiation and in 96.8% at the last test during the surveillance period.

What are the implications for public health practice?

The DoD model of HIV care demonstrates that the goals of high ART uptake and viral suppression can be achieved and maintained in a large health care system.

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EXHIBIT M

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Effect of HIV Antibody VRC01 on Viral Rebound after Treatment Interruption

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Abstract

BACKGROUND—The discovery of potent and broadly neutralizing antibodies (bNAbs) against human immunodeficiency virus (HIV) has made passive immunization a potential strategy for the prevention and treatment of HIV infection. We sought to determine whether passive administration of VRC01, a bNAb targeting the HIV CD4-binding site, can safely prevent or delay plasma viral rebound after the discontinuation of antiretroviral therapy (ART).

METHODS—We conducted two open-label trials (AIDS Clinical Trials Group [ACTG] A5340 and National Institutes of Health [NIH] 15-I-0140) of the safety, side-effect profile, pharmacokinetic properties, and antiviral activity of VRC01 in persons with HIV infection who were undergoing interruption of ART.

RESULTS—A total of 24 participants were enrolled, and one serious alcohol-related adverse event occurred. Viral rebound occurred despite plasma VRC01 concentrations greater than 50 μg per milliliter. The median time to rebound was 4 weeks in the A5340 trial and 5.6 weeks in the NIH trial. Study participants were more likely than historical controls to have viral suppression at week 4 (38% vs. 13%, P = 0.04 by a two-sided Fisher's exact test in the A5340 trial; and 80% vs. 13%, P<0.001 by a two-sided Fisher's exact test in the NIH trial) but the difference was not significant at week 8. Analyses of virus populations before ART as well as before and after ART interruption showed that VRC01 exerted pressure on rebounding virus, resulting in restriction of recrudescent viruses and selection for preexisting and emerging antibody neutralization–resistant virus.

CONCLUSIONS—VRC01 slightly delayed plasma viral rebound in the trial participants, as compared with historical controls, but it did not maintain viral suppression by week 8. In the small number of participants enrolled in these trials, no safety concerns were identified with passive

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

immunization with a single bNAb (VRC01). (Funded by the National Institute of Allergy and Infectious Diseases and others; ACTG A5340 and NIH 15-I-0140 ClinicalTrials.gov numbers, NCT02463227 and NCT02471326.)

Therapeutic administration of monoclonal antibodies has revolutionized treatment options in oncology, rheumatology, endocrinology, gastroenterology, neurology, and the field of infectious diseases.[1,2] The use of broadly neutralizing antibodies (bNAbs) against human immunodeficiency virus (HIV) is a potential approach to the prevention of HIV infection and its therapy and cure.[3,4] VRC01 is a bNAb that targets the CD4-binding site of the HIV envelope glycoprotein. VRC01 has been shown to neutralize approximately 90% of a broad panel of 190 group M HIV envelope pseudotyped viruses with a mean 50% inhibitory concentration (IC_{50}) of 0.33 μg per milliliter.[5] Passive administration of bNAbs, including VRC01, has been shown to prevent HIV transmission in animal models[6–9] and is now being tested in clinical trials of vertical and horizontal transmission in humans.

Combination antiretroviral therapy (ART) potently suppresses HIV replication; however, it does not eradicate the persistent viral reservoir.[10] In most HIV-infected persons, plasma viral rebound predictably occurs within days after treatment interruption.[11–14] HIV-specific bNAbs hold potential advantages over current ART. First, bNAbs can be administered as long-acting agents by means of antibody engineering[6] or vectored delivery. [15,16] Second, unlike classic ART, antibody Fc effector functions enable the killing of HIV-infected cells, which may assist in the clearance of the persistent viral reservoir.[4,17] Finally, bNAbs engage the host immune system and may augment antiviral responses.[18,19]

Preclinical studies of single and combination bNAbs in animal models have shown virus suppression, enhanced viral killing, augmented anti-HIV immune responses, and reduction of the cellular reservoir.[18,20,21] In clinical trials involving humans, no safety concerns have been identified thus far with passive administration of bNAbs targeting the CD4-binding site in healthy uninfected persons and in participants with chronic HIV infection who have either viremia or viral suppression.[22–24] Passive administration of VRC01 to HIV viremic persons led to a reduction of 1.1 to 1.8 $\log_{10}$ in plasma HIV viremia, although it was ineffective in the quarter of study participants who had baseline resistance to the antibody.[24] In addition, we previously found that HIV isolated from the latent viral reservoir of many, but not all, infected persons was inhibited by VRC01 ex vivo.[17] Collectively, these findings suggest that passive immunotherapy with VRC01 could potentially prevent plasma viral rebound in HIV-infected persons after analytic treatment interruption.

Here, we report the results of two phase 1 clinical trials designed to investigate the feasibility of achieving sustained suppression of plasma viremia (virologic remission) in HIV-infected persons by means of multiple infusions of VRC01 after the discontinuation of ART that had been successfully suppressing plasma viremia below the detectable concentration. Our goals were to ascertain whether the passive administration of VRC01 is safe and has an acceptable adverse-event and side-effect profile, leads to high VRC01 plasma concentrations, can suppress plasma viremia after the discontinuation of ART, and could inform our understanding of recrudescent viruses after immunologic intervention.

METHODS

STUDY DESIGN AND OVERSIGHT

We conducted two clinical trials with similar designs to evaluate the safety, adverse-event and side-effect profile, pharmacokinetic characteristics, and antiviral activity of the human monoclonal antibody VRC-HIVMAB060-00-AB (VRC01) in HIV-infected persons who were undergoing analytic treatment interruption. The first trial, AIDS Clinical Trials Group (ACTG) A5340, was conducted at the clinical research sites of the University of Pennsylvania and University of Alabama (see the protocol, available with the full text of this article at NEJM.org). The second trial (NIH 15-I-0140, hereafter referred to as the National Institutes of Health [NIH] trial) was conducted at the National Institute of Allergy and Infectious Diseases, NIH, in Bethesda, Maryland (see the protocol).

Both trials had similar entry criteria and recruited participants who had chronic HIV infection with fully suppressed plasma viremia while receiving ART (details are provided in the Supplementary Methods section in the Supplementary Appendix, available at NEJM.org). Study participants were not prescreened for sensitivity of the virus to neutralization by VRC01 in either trial.

The trials were reviewed by the institutional review boards of each participating institution. All participants provided written informed consent. All the authors vouch for the accuracy and completeness of the data and analyses and the fidelity of the trial to the respective protocol. There was no commercial support for these trials.

TREATMENT PROCEDURES, STUDY OBJECTIVES, AND STUDY OUTCOMES

In step 1 of the A5340 trial, 14 participants received up to three doses of VRC01 (40 mg per kilogram of body weight administered intravenously) at 3-week intervals (Fig. 1). One week after the first dose of VRC01, participants discontinued ART and were followed at weekly intervals until they had a confirmed CD4 T-cell count of less than 350 cells per cubic millimeter or a return of HIV viremia, which was defined as an HIV RNA level of 200 copies or more per milliliter followed by a confirmation level of 1000 copies or more per milliliter or three consecutive measurements of 200 copies or more per milliliter. On confirmation of viral rebound or a decrease in CD4 T cells, participants entered step 2, at which point ART was reinitiated and participants were followed until the HIV viral load was less than 50 copies per milliliter.

The primary objectives of the A5340 trial were to assess the safety and side-effect profile of multiple doses of VRC01 administered to persons with plasma viremia suppressed to below detectable levels and to estimate the proportion of participants with a return of viremia in the presence of high plasma levels of VRC01 at 8 weeks of analytic treatment interruption. Secondary objectives were the frequency of rebound viremia at 4 weeks and the evaluation of the pharmacokinetic characteristics of the product. Key exploratory objectives were the frequency of development of antibodies against VRC01 and the genetic and phenotypic characterization of the rebound virus. We calculated that 13 participants with data that could be evaluated would be required to provide this trial with 95% power to detect a difference of

40 percentage points (i.e., a return of viremia by 8 weeks in 50% of trial participants vs. 90% of historical controls)[14] at a two-sided alpha level of 0.10.

In the NIH trial, 10 participants received infusions of VRC01 (at a dose of 40 mg per kilogram) intravenously 3 days before and 14 and 28 days after the discontinuation of ART and monthly thereafter (Fig. 1). Plasma viremia and CD4 T-cell counts were measured at baseline (day −3) and subsequently (Fig. 1). Participants who met any of the following criteria discontinued VRC01 infusions and resumed ART: a decrease of more than 30% in the baseline CD4 T-cell count or an absolute CD4 T-cell count below 350 cells per cubic millimeter, a sustained (≥2 weeks) HIV plasma viremia greater than 1000 copies per milliliter, any HIV-related symptoms, or pregnancy.

The primary end point of the NIH trial was safety, as defined according to the rate of occurrence of grade 3 or higher adverse events, including serious adverse events, that were possibly related to infusion of VRC01. The secondary end point was virologic efficacy, as defined according to the number of participants who met protocol-defined, virologic, immunologic, or clinical criteria to discontinue VRC01 infusions and restart ART. Post hoc analyses of the sequence diversity at the time of plasma viral rebound and the neutralization capacity of VRC01 and other bNAbs against autologous HIV before and after antibody infusions were performed.

STATISTICAL ANALYSIS

Statistical analysis of the time to the first confirmed HIV RNA level of 200 copies or more per milliliter during the analytic treatment interruption was performed in both trials (post hoc in the NIH trial). Measurements of HIV RNA levels that were taken closest to each scheduled week were obtained, and the cumulative probability of continued virologic suppression (i.e., no confirmed HIV RNA level ≥200 copies per milliliter) was calculated by means of Kaplan–Meier methods.

In both trials, a two-sided Fisher's exact test was used to compare the percentage of trial participants with viral rebound at 4 and 8 weeks (post hoc in the NIH trial) with the percentage of historical controls with viral rebound in previous treatment-interruption trials conducted by the ACTG.[14] In the A5340 trial, the proportion of participants with adverse events was estimated with an exact 95% confidence interval. The proportion of participants who had return of viremia at 8 weeks of analytic treatment interruption and who had data that could be evaluated was estimated with a prespecified exact 90% confidence interval. Details of the historical control group, participant monitoring, and laboratory and statistical methods are outlined in the Supplementary Methods section in the Supplementary Appendix.

RESULTS

STUDY PARTICIPANTS

The A5340 trial enrolled 14 participants, all of whom were male, with a median CD4 T-cell count at enrollment of 896 cells per cubic millimeter (interquartile range, 579 to 1053) and a median duration from the initiation of ART to study entry of 4.7 years (interquartile range,

3.8 to 6.0). One participant was excluded from the analyses of time to viral rebound because he discontinued ART before the administration of VRC01.

The NIH trial enrolled 10 participants (8 men and 2 women) with a median CD4 T-cell count of 724 cells per cubic millimeter (interquartile range, 630 to 926) (Fig. S1 in the Supplementary Appendix) and a median duration from the initiation of ART to study entry of 10.0 years (interquartile range, 7.7 to 13.3). In the NIH participants, the median frequency of CD4 T cells carrying HIV proviral DNA was 881 copies per 10^6 cells (range, 154 to 2079) (Fig. S2 in the Supplementary Appendix). Table 1 lists the baseline characteristics of the participants in both trials and the historical controls.

SAFETY

All participants completed VRC01 infusions per protocol. One serious adverse event occurred in a participant who required a brief hospital admission to recover from conscious sedation following upper gastrointestinal endoscopy to evaluate a history of possible hematemesis after alcohol ingestion. In the A5340 trial, 14 participants received from 1 to 3 infusions of VRC01, and none had a grade 3 or higher adverse event or a grade 2 VRC01-related adverse event (0%; 95% confidence interval [CI], 0 to 23). In the NIH trial, participants received 2 to 6 infusions (median, 3.5) of VRC01, and no adverse events occurred during the infusion or immediate postinfusion period. Complete data on adverse events are provided in the Supplementary Appendix. These safety results are consistent with those of much larger and ongoing studies of the same product.

In 24 participants, ART was reinitiated on confirmation of viral rebound and their plasma viremia was suppressed again. In the A5340 trial, participants had not received ART for a median of 6 weeks (range, 3 to 13). The median time from the first detectable HIV RNA level of 200 copies per milliliter or more to the first suppressed HIV level of less than 200 copies per milliliter after ART reinitiation was 6 weeks (range, 3 to 14). In the NIH trial, participants did not receive ART for a median of 8 weeks (range, 3 to 17). The median time from the first detectable HIV RNA level of 200 copies or more per milliliter to the first suppressed HIV level of less than 200 copies per milliliter after ART reinitiation was 11 weeks (range, 4 to 20). No participant had a confirmed decrease in the CD4 T-cell count below 350 cells per milliliter; 1 participant had a decrease of more than 30% from the baseline CD4 T-cell count; this led to the reinitiation of ART per protocol.

TIME TO VIRAL REBOUND

In both trials, the administration of VRC01 did not produce durable suppression of plasma viremia. In the A5340 trial, 12 of 13 participants with data that could be evaluated had viral rebound of more than 200 copies per milliliter at or before week 8 (92%; 90% CI, 68 to 100), with a median time to rebound of 4 weeks (interquartile range, 3 to 5) (Fig. 2A, and Fig. S3 in the Supplementary Appendix). This is a small delay in viral rebound as compared with the delay in historical controls from previous ACTG studies; 38% of the participants versus 13% of the controls had viral suppression at week 4 (P = 0.04 by a two-sided Fisher's exact test) and 8% and 3%, respectively, had viral suppression at week 8 (P = 0.44 by a two-sided Fisher's exact test) (Fig. 2C). One participant (Participant A07) in the A5340 trial had

prolonged viral suppression, and detectable plasma viremia developed at week 11 of the analytic treatment interruption when plasma VRC01 levels had waned significantly (plasma VRC01 concentration, 25 μg per milliliter) (Fig. 2A, and Fig. S3 in the Supplementary Appendix). One participant (Participant A11) was excluded from the evaluation of time to viral rebound, since he had detectable plasma viremia at the time of VRC01 infusion (Fig. S3 in the Supplementary Appendix).

In the NIH trial, viral rebound to more than 40 copies per milliliter occurred during VRC01 treatment in all 10 participants, with a median time to rebound of 39 days (interquartile range, 29 to 39) or 5.6 weeks (interquartile range, 4.1 to 5.6) (Fig. 2B, and Fig. S5 in the Supplementary Appendix). However, as compared with the time to plasma viral rebound (HIV RNA level, ≥200 copies per milliliter) in historical controls, VRC01 infusion led to a longer time to rebound (≥200 copies per milliliter), and 80% of the participants versus 13% of the controls had viral suppression at week 4 ($P<0.001$ by a two-sided Fisher's exact test) and 10% and 3%, respectively, had viral suppression at week 8 ($P = 0.37$ by a two-sided Fisher's exact test) (Fig. 2C). Nine of 10 study participants resumed ART because of virologic failure; ART was reinitiated in the remaining participant (Participant N03) because of a significant decrease in the CD4 T-cell count (>30%) (Figs. S1 and S5 in the Supplementary Appendix) associated with low-level plasma viremia (HIV RNA level, 471 copies per milliliter). One participant (Participant N04) self-administered antiretroviral drugs for 3 days off protocol after the first ART interruption; self-administration of antiretroviral drugs may have contributed to a brief period of aviremia (Fig. S5 in the Supplementary Appendix). However, his plasma viremia rebounded shortly after the second ART interruption.

VRC01 PHARMACOKINETIC CHARACTERISTICS

Plasma levels of VRC01 that were achieved by passive infusion were similar to levels reported in previous trials.[23,24] In the A5340 trial, participants received 40 mg of VRC01 per kilogram every 3 weeks for three doses and maintained measured plasma VRC01 levels well above 50 μg per milliliter for 8 weeks (median, 175 μg per milliliter; range, 68 to 1494) (Fig. S6 in the Supplementary Appendix).

In the NIH trial, participants received 40 mg of VRC01 per kilogram every 2 weeks for the first three doses, then monthly for up to 6 months. NIH trial participants maintained levels of VRC01 in plasma above 100 μg per milliliter at almost all time points throughout the trial (Fig. S5 in the Supplementary Appendix).

Measured values of plasma VRC01 at the time of rebound were greater than 50 μg per milliliter in all trial participants, except for Participant A07 in the A5340 trial, who had delayed rebound at week 11 of analytic treatment interruption with VRC01 levels of approximately 25 μg per milliliter (Fig. 2D). No anti-VRC01 antibodies were identified in any trial participants.

SEQUENCE EVIDENCE OF VRC01-MEDIATED VIRUS RESTRICTION

Different strategies were applied in each trial to elucidate the mechanisms leading to early viral rebound. To characterize rebounding viral populations in the A5340 trial, single-

genome sequencing[25] of plasma HIV *env* genes from pre-ART plasma samples (available in 8 participants) and rebound plasma samples from the first and second weeks of detectable viremia (available in 13 participants) was performed. When analyzed together in a maximum likelihood phylogenetic tree, the pre-ART and rebound sequences of the 13 participants with data that could be evaluated clustered independently, indicating the relatedness of pre-ART and rebound viruses (Fig. S7 in the Supplementary Appendix).

Three independent studies have recently shown that without any additional intervention besides ART, viral rebound after analytic treatment interruption is consistently polyclonal because of the reactivation of multiple latent viruses.[26–28] Genetic evidence of VRC01-mediated restriction of viral rebound was assessed by analyzing the clonality of rebound virus or by enumerating genetically distinct virus populations that composed rebound viremia. In 3 of 12 participants (25%) in the A5340 trial who had viral rebound in the presence of high concentrations of VRC01, sequence evidence suggested VRC01-mediated restriction of viral rebound. As shown in Participant A04 in Figure 3A and Participants A02 and A12 in Figure S8 in the Supplementary Appendix, pre-ART plasma virus from persons with chronic infection formed characteristic diverse trees,[29] whereas rebound virus clustered into single low-diversity lineages of nearly identical sequences (Figs. S9 and S10 in the Supplementary Appendix).

The remaining 9 of 12 participants (75%) had polyclonal rebound akin to what is reported in historical analytic treatment interruption without intervention,[26,27] suggesting possible preexisting resistance. As shown in Figure 3B, rebound virus in Participant A05 clustered into multiple genetically distinct rebound lineages that align throughout the pre-ART virus phylogeny, whereas multiple rebound lineages in Participant A03 clustered unevenly within the pre-ART virus population (Fig. 3C). The rebound sequences in Participant A07 in the A5340 trial (Fig. 3D), who had virus suppression maintained until week 11 of analytic treatment interruption and had rebound with lower VRC01 concentrations, formed two closely related lineages.

Evidence of selective pressure exerted by VRC01 was also explored at the molecular level in the NIH trial by amplifying *env* genes by means of single-genome sequencing from pre-ART (a median of 1.8 months before the initiation of ART) and rebound plasma samples. As in the A5340 trial, the majority of NIH trial participants had phylogenetic evidence of multiple viral lineages in rebound plasma virus (Fig. S11 in the Supplementary Appendix). Specific amino acid changes within the VRC01 antibody-binding site were examined with the use of a neutralization-based epitope prediction algorithm.[30] In four of the six NIH trial participants, changes were identified in or near the VRC01 epitope, mainly in the V5 loop and the CD4-binding loop (Fig. S12 in the Supplementary Appendix); this outcome suggested VRC01-mediated selective pressure on rebounding virus. Similar patterns were seen in the VRC01-binding site in participants in the A5340 trial (Fig. S10 in the Supplementary Appendix).

SELECTION FOR VRC01-RESISTANT REBOUNDING VIRUSES

The role of resistance to VRC01 at viral rebound in the presence of high VRC01 concentrations in the A5340 trial was assessed by cloning selected *env* genes from 46 quasi-

species and lineages collected throughout the pre-ART and rebound periods of the trial (median, 3 pre-ART and 3 rebound *env* genes per participant). These *env* genes were cloned, expressed as pseudoviruses, and tested for sensitivity to neutralization by VRC01. Notably, nearly all participants who had viral rebound early with high concentrations of plasma VRC01 had rebound Env pseudoviruses with IC_{50} neutralization titers higher than 1 μg per milliliter (median, 4.1 μg per milliliter; range, 1.9 to >50.0), conferring what is generally perceived to be at least moderate resistance to VRC01.[5,31,32] Only Participants A02 and A07, who had rebound at week 8 and 11 after analytic treatment interruption, respectively, had rebound viruses with IC_{50} neutralization titers below 1 μg per milliliter (Fig. 3E).

Similarly, all participants who had viral rebound early had preexisting resistant virus as either dominant or minor populations in the pre-ART virus, as shown in the phylogenetic trees of the four participants in Figure 3A through 3D. The prevalence of preexisting resistance predicted the pattern of rebound. In participants with VRC01-resistant virus in multiple pre-ART variants (e.g., Participants A05, A06, and A09) (Fig. 3, and Fig. S8 in the Supplementary Appendix), VRC01 therapy was followed by rapid, polyclonal rebound with highly resistant virus. In participants in whom there was a range of neutralization sensitivities in the baseline virus (e.g., Participants A03 and A04 [Fig. 3] and Participant A12 [Fig. S8 in the Supplementary Appendix]), VRC01 therapy led to monoclonal or compartmentalized rebound after variable durations of suppression (range, 2 to 6 weeks). Finally, Participants A02 and A07, who had highly sensitive virus throughout their tested pre-ART populations, had suppression maintained for 7 and 10 weeks, respectively, and had rebound with relatively sensitive monoclonal or oligoclonal virus.

Regardless of the time to rebound, resistance to VRC01 increased almost universally in participants in the A5340 trial during treatment with VRC01. In an exploratory analysis, pre-ART and rebound Env pseudoviruses were compared in the eight participants in whom both samples were available. This analysis showed significantly increased VRC01 resistance at rebound by IC_{50} (mean increase by a factor of 3.44, $P = 0.006$ by a two-sided random-effects model) and IC_{80} (mean increase by a factor of 3.79, $P = 0.004$ by a two-sided random-effects model) (Fig. 3E and 3F, and Fig. S13 in the Supplementary Appendix).

NEUTRALIZATION CAPACITY OF VRC01 AND OTHER BNABS AGAINST AUTOLOGOUS HIV BEFORE AND AFTER ANTIBODY INFUSIONS

In the NIH trial, the role of resistance to VRC01 and other bNAbs was explored by testing fully replication-competent autologous HIV isolates recovered from the CD4 T cells of trial participants before and after antibody infusions. Multiple viral isolates (182 total) were generated from peripheral-blood mononuclear cells obtained from trial participants before infusions of VRC01 (eight participants, 75 isolates) and after infusions of VRC01 (nine participants, 107 isolates) and the discontinuation of ART. The susceptibility of the infectious isolates to neutralization by VRC01 and other bNAbs (3BNC117, 10–1074, and PGT121), and anti-CD4 antibody (UB-421) was then measured with the latter antibodies serving as controls, both for comparison with antibodies currently being tested in monotherapy trials as well as for their possible inclusion in future combination antibody trials. As shown in Figure 4A, the capacity of VRC01 to neutralize the preinfusion viral

isolates was significantly lower than that of 3BNC117 (P = 0.008), 10–1074 (P = 0.03), and UB-421 (P = 0.008); this finding strongly suggests that the preexisting viral reservoir of the majority of NIH trial participants harbored VRC01-resistant HIV.

Next, the capacity of VRC01 to suppress the preinfusion and postinfusion autologous virus was evaluated in the seven participants from whom infectious isolates could be recovered at both time points (Fig. 4B, and Figs. S14 and S15 in the Supplementary Appendix). Notably, virus sensitivity to VRC01 diminished significantly after multiple infusions of VRC01 during analytic treatment interruption in several participants. Participant-to-participant variability prevented us from concluding that the observed decrease in sensitivity was universal (P = 0.08). Nonetheless, in Participants N03, N04, N08, and N10, there was strong evidence of the emergence of HIV isolates that were less sensitive to VRC01, highly resistant to VRC01, or both (Fig. 4B). It is noteworthy that the preinfusion isolates obtained from Participant N09 were already highly resistant to VRC01 and remained resistant after infusion. In contrast, there were no detected changes in susceptibility of pre-ART versus rebound viral isolates to neutralization by 3BNC117, 10–1074, PGT121, and UB-421 (Figs. S14 and S15 in the Supplementary Appendix). The rebound VRC01-resistant isolates from two participants (Participants N04 and N08) were also resistant to the CD4-binding site bNAb 3BNC117 (Figs. S14 and S15 in the Supplementary Appendix). Collectively, the analyses of viral isolates in the NIH trial corroborate the sequence-based analysis observed in the A5340 trial and show selection for preexisting VRC01-resistant virus and the capacity for VRC01 to further drive resistance during analytic treatment interruption.

DISCUSSION

In two similarly designed clinical trials, we found that the passive administration of multiple doses of VRC01 monotherapy generated high plasma VRC01 concentrations, and no safety concerns were identified. In persons with chronic HIV infection who were undergoing analytic treatment interruption, as compared with historical controls, VRC01 therapy slightly delayed plasma viral rebound; however, viral suppression beyond 8 weeks was not achieved. Sequence-based and neutralization analyses suggest that VRC01 can restrict the clonality of rebounding virus in some participants, select for preexisting resistance, and drive the emergence of VRC01-resistant virus. Baseline resistance to VRC01 was common in both trials, suggesting that persons with chronic infection may frequently harbor archived resistant virus to this antibody.

Our results suggest that the prevalence of clinically significant archived resistance to VRC01 may present a considerable challenge in the use of bNAbs as therapeutic agents for HIV infection. Preexisting resistance to bNAbs is biologically plausible, since before the initiation of ART, persons with chronic HIV infection have extensive exposure to a polyclonal autologous B-cell response that results in archived escape variants to many antibody specificities, including those of bNAb target epitopes.[33–36] Indeed, a previous study tested replication-competent viral isolates derived from the latent viral reservoir and showed resistance to VRC01 in a substantial proportion of persons in an autologous culture system.[17] In participants with only sensitive pre-ART virus (tested as infectious isolates or envelope-pseudo-typed virus) who had rebound with VRC01-resistant virus, it is unclear

whether this rebound indicates selection for low-frequency resistant viruses that were not sampled or the emergence of new VRC01 resistance. The development of methods to characterize the phenotypic characteristics (e.g., neutralization sensitivity) of the persistent replication-competent viral reservoir will be needed to distinguish between these different mechanisms of failure of HIV-specific bNAbs.

Although extensive preexisting resistance limited the efficacy of VRC01 in both trials, it is notable that in a previous study of viral isolates[17] and the present NIH clinical trial, other tested bNAbs appeared to have less prevalent archived resistance (Fig. 4A). The efficacy of any given bNAbs in persons with chronic HIV infection will be dependent in part on whether these persons have resistant virus to that bNAb, even at very low frequencies, in persistent viral reservoirs. Future clinical trials may consider prescreening for resistance, although this is a complex task.

The emergence of VRC01-resistant HIV after infusions of VRC01 and discontinuation of ART was observed in both trials. However, a fraction of infectious HIV isolates in some trial participants remained sensitive to VRC01 despite viral rebound in the presence of high levels of VRC01 in plasma. This could be explained by a possible artifact of culture whereby isolates from the persistent viral reservoir[37–39] were induced by the ex vivo conditions needed to stimulate cells into producing replication-competent viral isolates, but they may not have been actively replicating after discontinuation of ART. In the A5340 trial, virus that rebounded early in the presence of high concentrations of VRC01 was almost universally resistant to VRC01. Only two participants (including Participant A07, who had viral rebound after plasma VRC01 concentrations had waned substantially) had VRC01-sensitive envelope glycoproteins.

Our findings highlight an important consideration for the design of future clinical trials of passive immunotherapy in HIV-infected persons. During the early years of development of antiretroviral drugs for HIV infection, the nucleoside reverse transcriptase inhibitor zidovudine used as a single agent resulted in a decrease of approximately 0.5 log copies per milliliter in plasma viremia that almost invariably rebounded[40] with zidovudine-resistant mutants.[41] The advent of additional antiretroviral drugs directed at different viral targets and used in combinations led to more potent viral suppression for longer durations of time.[42,43] Analogous to current regimens of highly successful combination ART that targets multiple HIV gene products,[44] our data suggest that immunotherapy will probably require multiple bNAbs that target different sites on the HIV envelope glycoprotein.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

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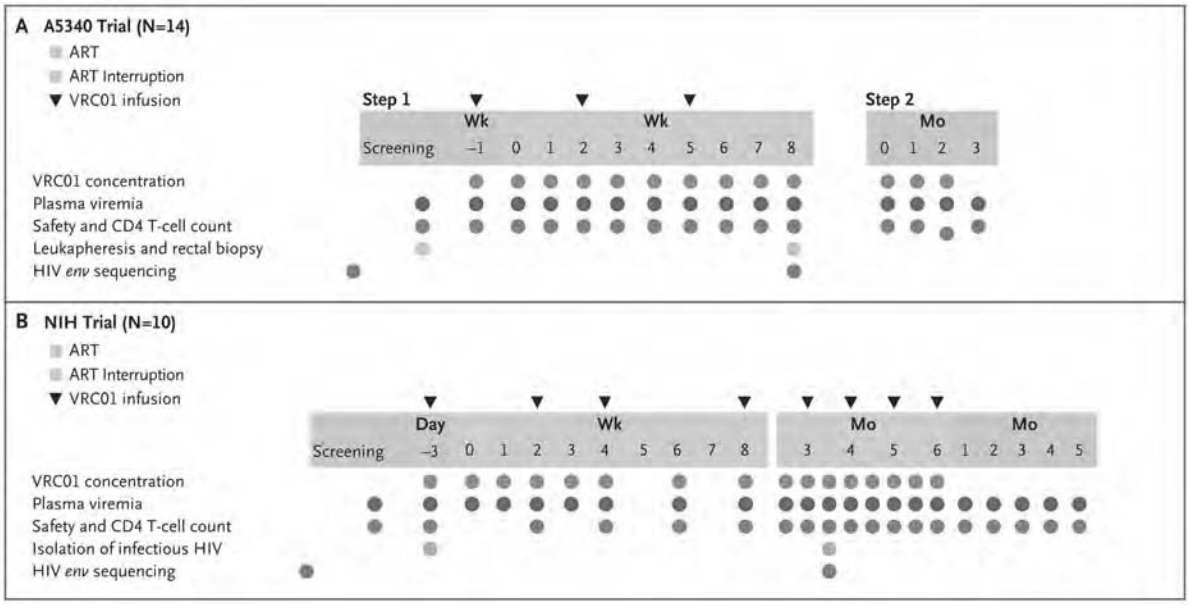

Figure 1. Trial Designs

As shown in Panel A, the A5340 trial had two steps. In step 1, participants received an intravenous infusion of VRC01 (at a dose of 40 mg per kilogram of body weight) (triangles) 1 week before and 2 and 5 weeks after discontinuation of antiretroviral therapy (ART). ART was discontinued 1 week after the first VRC01 infusion. The treatment interruption was open-ended, and participants were monitored weekly until viral rebound. On confirmed plasma viral rebound, participants entered step 2 and ART was reinitiated. Participants were then followed until plasma viremia decreased below 50 copies per milliliter. HIV *env* sequencing (gray circles) was performed with the use of plasma samples obtained before the initiation of ART and after viral rebound. As shown in Panel B, in the National Institutes of Health (NIH) trial, participants received infusions of VRC01 (at a dose of 40 kg per kilogram) (triangles) 3 days before and 14 to 28 days after discontinuation of ART, and monthly thereafter. Infectious viral isolates (orange circles) were generated from samples obtained before VRC01 infusion and after plasma viral rebound. HIV *env* sequencing (gray circles) was performed with the use of plasma samples obtained before the initiation of ART and after viral rebound.

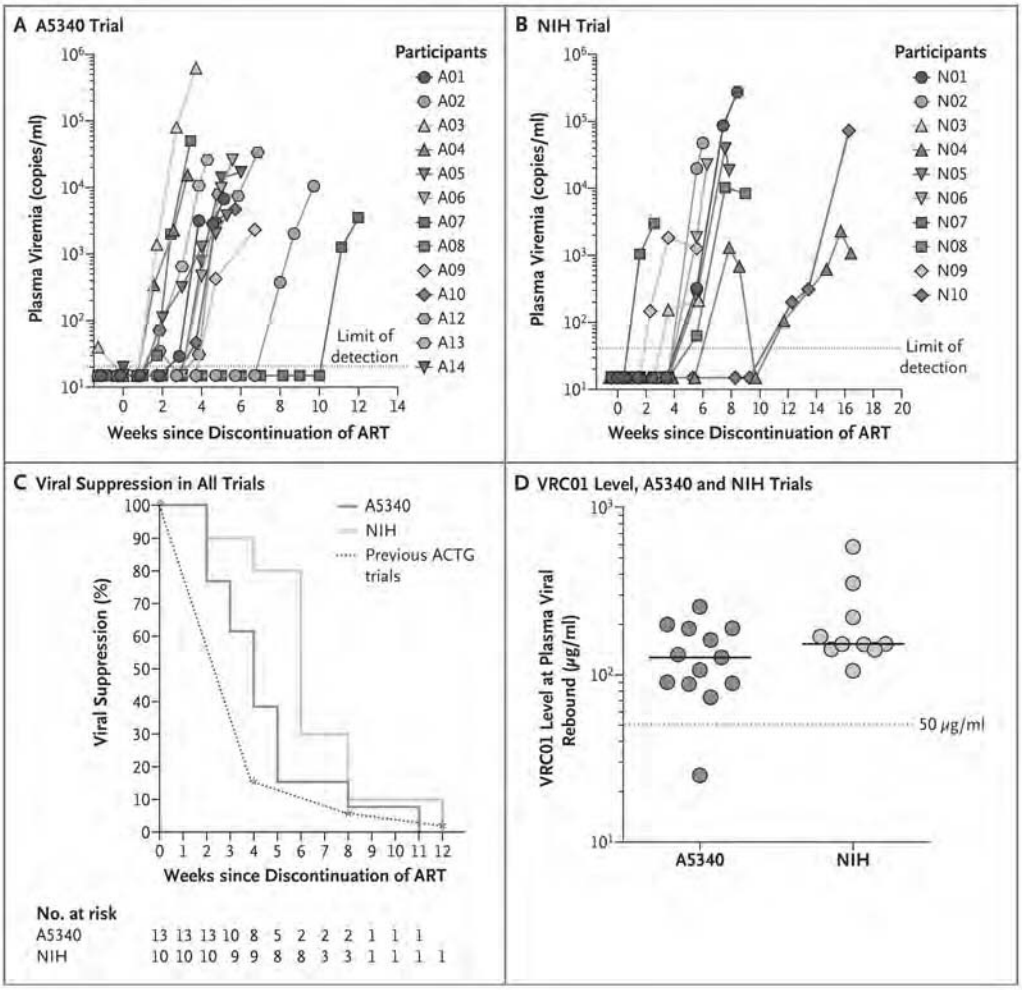

Figure 2. Plasma Viremia and Levels of VRC01 in Trial Participants after Discontinuation of ART

Panel A shows the plasma viremia of participants in the A5340 trial after the interruption of therapy. The gray dotted line indicates the limit of detection of the assay (HIV RNA level, 20 copies per milliliter). Panel B shows the plasma viremia of NIH trial participants after interruption of therapy. The gray dotted line indicates the limit of detection of the assay (HIV RNA level, 40 copies per milliliter). Panel C shows the Kaplan–Meier curve of plasma viral suppression (<200 copies per milliliter) after VRC01 administration and analytic treatment interruption in A5340 and NIH trial participants as compared with historical participants in AIDS Clinical Trials Group (ACTG) trials who underwent interruption of therapy without other immunotherapeutic interventions. Panel D shows in vivo plasma levels of VRC01 at the first detectable plasma viremia. The limit of detection of VRC01 was less than 0.98 μg per milliliter.

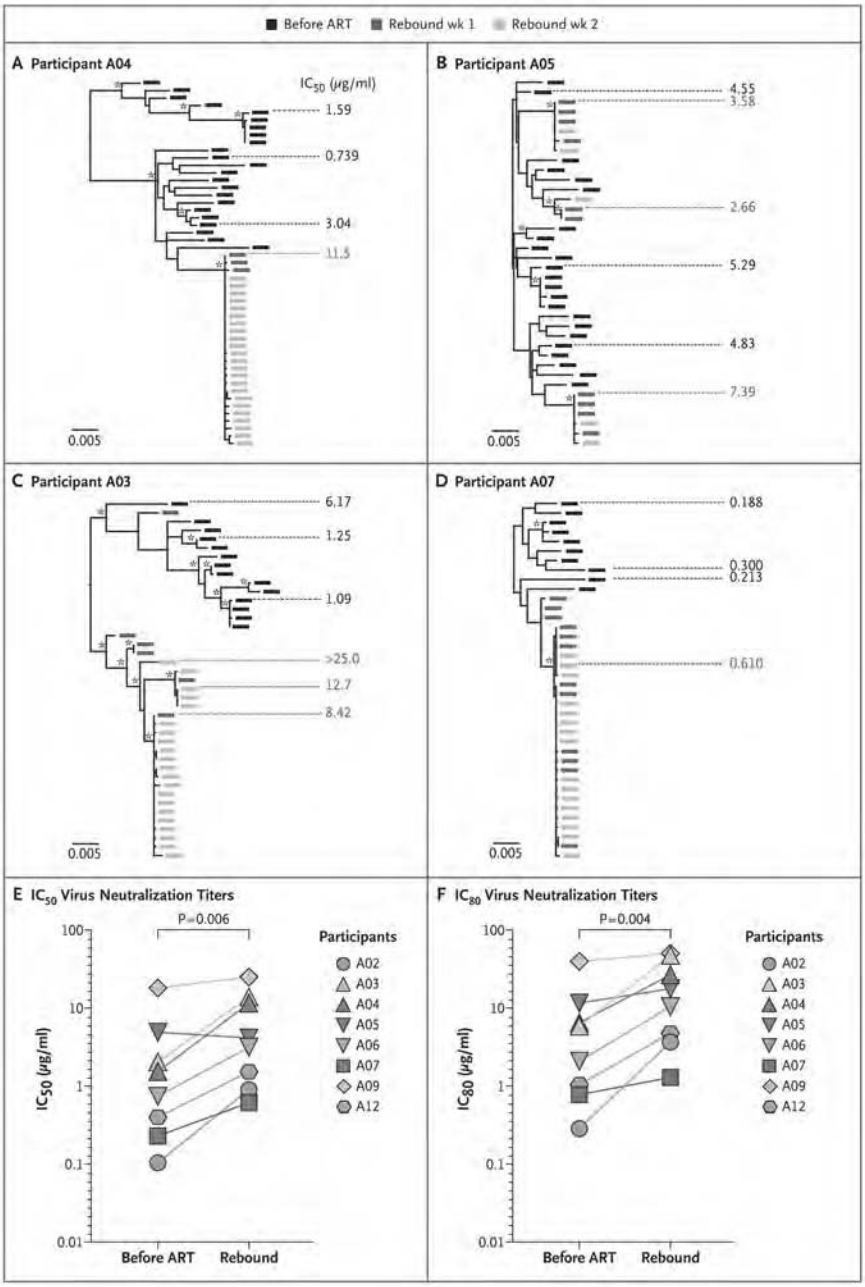

Figure 3. Rebound Virus Clonality and Resistance to VRC01

Panels A through D show maximum likelihood phylogenetic trees of single-genome sequencing–derived *env* sequences from pre-ART and rebound plasma virus and neutralization titers to VRC01 from four participants. Participants A04, A05, and A03 had early viral rebound despite high levels of VRC01; Participant A07 had delayed rebound with lower plasma VRC01 levels. Black rectangles indicate pre-ART plasma *env* sequences, and red and orange rectangles indicate the *env* sequences from the first and second weeks of detectable viremia. The scale bar indicates genetic distance. Fifty percent inhibitory concentration (IC_{50}) neutralization titers are shown to the side of each tree aligned to the

specific envelope glycoprotein that was cloned and tested for phenotypic features. Asterisks indicate bootstrap support of greater than 80%. As shown in Panel A, Participant A04 had monoclonal rebound with VRC01-resistant virus. As shown in Panel B, Participant A05 had polyclonal rebound with VRC01-resistant virus. As shown in Panel C, Participant A03 had polyclonal rebound with VRC01-resistant virus. Multiple rebound lineages arise clustered within one area of the phylogeny. Sequences from Participant A03 were tested for clustering; Slatkin–Maddison and Hudson's "nearest neighbor" tests support sequence compartmentalization ($P<0.001$ and $P = 0.004$, respectively). As shown in Panel D, Participant A07 had polyclonal rebound of VRC01-sensitive virus. As shown in Panels E and F, pre-ART and rebound Env pseudotyped virus from the eight participants with available samples were compared for changes in neutralization sensitivity by IC_{50} (truncated at 25 μg per milliliter) (Panel E) and 80% inhibitory concentration (IC_{80}) (truncated at 50 μg per milliliter) (Panel F) with the use of multilevel random-effects models (random intercept and slope) to account for multiple clones per participant at each time point. A two-sided P value for the estimated difference in pre-ART and rebound resistance was calculated. Mean titers are shown for pre-ART virus on the left and rebound virus on the right.

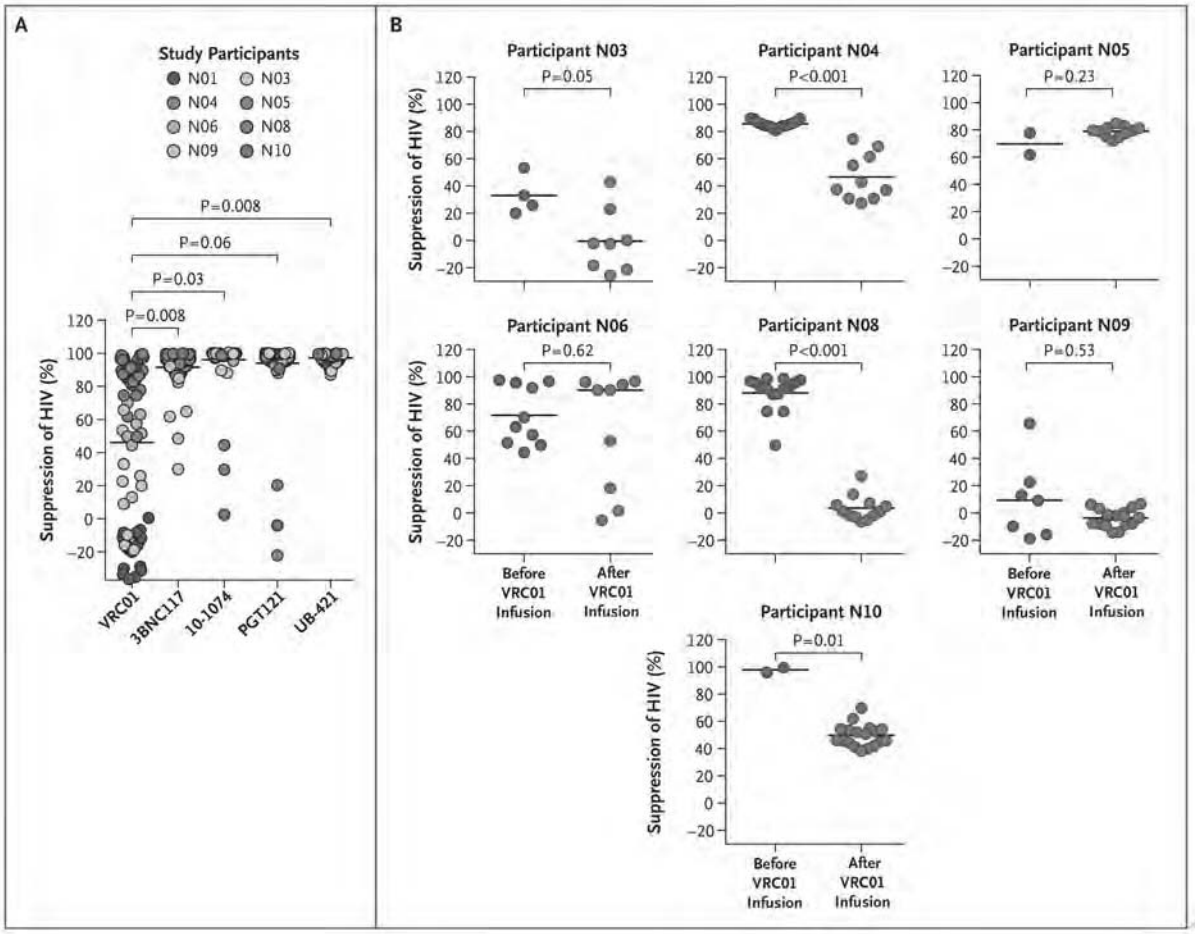

Figure 4. Characterization of Autologous, Replication-Competent HIV Isolates before and after Infusions of VRC01 and Discontinuation of ART in the NIH Trial

Panel A shows neutralization of preinfusion autologous viral isolates by VRC01 and other monoclonal antibodies. Susceptibility of preinfusion infectious isolates obtained from eight trial participants to neutralization by VRC01 and other broadly neutralizing antibodies (3BNC117, 10–1074, and PGT121) and anti-CD4 antibody (UB-421) is shown. The percent suppression of HIV was calculated with the use of the following formula: (1 − [luciferase activity in the presence of test antibody ÷ luciferase activity in the presence of control antibody IgG]) × 100. Luciferase activity was expressed in relative light units. Gray horizontal bars indicate mean values. P values were computed with the use of a paired permutation test. Panel B shows neutralization of preinfusion and postinfusion viral isolates by VRC01 in seven trial participants from whom infectious isolates could be recovered at both time points. Gray horizontal bars indicate mean values. The P value for each participant was computed with the use of the Wilcoxon–Mann–Whitney test.

Table 1

Characteristics of the Participants at Baseline.*

Characteristic	A5340 Trial (N = 14)	NIH Trial (N = 10)	Historical Controls from Previous ACTG Studies (N = 61)
Sex — no. (%)			
Male	14 (100)	8 (80)	53 (87)
Female	0	2 (20)	8 (13)
Age — yr			
Median (IQR)	38 (34–44)	51 (44–56)	44 (40–50)
Range	27–52	33–59	27–73
Race or ethnic group — no. (%)†			
White non-Hispanic	6 (43)	6 (60)	41 (67)
Black non-Hispanic	6 (43)	3 (30)	13 (21)
Hispanic, regardless of race	2 (14)	1 (10)	7 (11)
Weight — kg			
Median (IQR)	86 (77–102)	83 (78–89)	NA
Range	60–115	75–100	NA
HIV RNA — copies/no. (%)			
<50 copies/ml	13 (93)	10 (100)	61 (100)
≥50 copies/ml	1 (7)	0	0
CD4 T-cell count — cells/mm^3			
Median (IQR)	896 (579–1053)	724 (630–926)	852 (686–1048)
Range	470–1586	577–1616	350–1667
Nadir CD4 T-cell count — no. (%)			
<201 cells/mm^3	0	2 (20)	3 (5)
201–500 cells/mm^3	12 (86)	3 (30)	39 (64)
>500 cells/mm^3	2 (14)	4 (40)	16 (26)
Unknown	0	1 (10)	3 (5)
Duration from initiation of ART to study entry — yr			
Median (IQR)	4.7 (3.8–6.0)	10.0 (7.7–13.3)	5.6 (4.1–6.7)
Range	2.7–14.5	7.0–17.2	0.7–16.8
Duration of suppression — yr			
Median (IQR)	NA	8.3 (6.8–12.9)	NA
Range	NA	3.0–16.8	NA
ART regimen — no. (%)			
Abacavir–lamivudine–dolutegravir	4 (29)	3 (30)	0
Abacavir–lamivudine–atazanavir	0	1 (10)	0
Emtricitabine–tenofovir–ritonavir-boosted atazanavir	1 (7)	1 (10)	0
Emtricitabine–tenofovir–ritonavir-boosted darunavir	3 (21)	0	0
Emtricitabine–tenofovir–dolutegravir	2 (14)	0	0

Characteristic	A5340 Trial (N = 14)	NIH Trial (N = 10)	Historical Controls from Previous ACTG Studies (N = 61)
Emtricitabine–tenofovir–efavirenz	0	1 (10)	0
Emtricitabine–tenofovir–elvitegravir–cobicistat	3 (21)	2 (20)	0
Emtricitabine–tenofovir–raltegravir	1 (7)	0	0
Emtricitabine–tenofovir–rilpivirine	0	2 (20)	0
Zidovudine–lamivudine–nelfinavir	0	0	15 (25)
Zidovudine–lamivudine–indinavir	0	0	10 (16)
Zidovudine–lamivudine–ritonavir-boosted indinavir	0	0	4 (7)
Stavudine–lamivudine–indinavir	0	0	6 (10)
Stavudine–lamivudine–nelfinavir	0	0	5 (8)
Stavudine–didanosine–nelfinavir	0	0	2 (3)
Other protease inhibitor–based regimen	0	0	19 (31)

*ACTG denotes AIDS Clinical Trials Group, ART antiretroviral therapy, HIV human immunodeficiency virus, IQR interquartile range, NA not available, and NIH National Institutes of Health.

†Race or ethnic group was self-reported.

EXHIBIT 37

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Rebound of plasma viremia following cessation of antiretroviral therapy despite profoundly low levels of HIV reservoir: implications for eradication

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Abstract

Objectives—Sustained suppression of plasma viremia in HIV-infected individuals is attainable with antiretroviral therapy (ART); however, eradication of virus that would allow discontinuation of ART has been hampered by the persistence of HIV reservoirs. It is of great interest to identify individuals who had received ART for prolonged periods of time with extremely low or undetectable HIV reservoirs and monitor plasma viremia following discontinuation of therapy.

Methods—We measured the size of HIV reservoirs in $CD4^+$ T cells of individuals on long-term ART and monitored plasma viremia following cessation of ART in one individual with an exceptionally low viral burden after a decade of therapy.

Results—We demonstrated undetectable levels of HIV DNA in the blood of eight of 45 infected individuals on long-term ART. Among those eight individuals, the frequency of cells carrying infectious virus was significantly lower in those who initiated ART during the early versus the chronic phase of infection. One individual with undetectable HIV DNA in both blood and tissue and a profoundly low level of infectious virus experienced plasma viral rebound 50 days following discontinuation of ART.

Conclusions—Our data suggest that a significant reduction in the size of viral reservoirs may be achievable in selected individuals who initiate standard ART early in infection. However, given re-emergence of plasma viremia in an individual with an extraordinarily low viral burden, therapeutic strategies aimed at specifically targeting these extremely rare HIV-infected cells with novel interventions may be necessary in order to achieve eradication of virus.

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Keywords

antiretroviral therapy; discontinuation of therapy; eradication; human immunodeficiency virus; viral reservoirs

Introduction

Despite the development of successful therapeutic strategies [1], it has thus far been impossible to eradicate HIV in infected individuals receiving effective antiretroviral therapy (ART), mainly due to the persistence of viral reservoirs [2–4]. Previous studies have demonstrated the persistence of HIV in $CD4^+$ T cells in blood as well as in gut-associated lymphoid tissue (GALT) of infected individuals receiving ART who had maintained undetectable levels of plasma viremia for significant periods of time [5], an observation that may help explain the relatively rapid rebound of plasma viremia upon treatment interruption [6,7]. In this regard, it has been demonstrated that detectable levels of plasma viremia (>50 copies of HIV RNA per ml) re-emerge on average 9 days following discontinuation of ART in individuals who experienced ART-induced suppression of plasma viremia [7]. However, the vast majority, if not all, of such infected individuals carried readily detectable HIV reservoirs prior to cessation of ART and had received antiretroviral drugs for less than 3–5 years [6,7]. Although viral reservoirs, which persist in spite of clinically effective ART, pose a major impediment to complete eradication of virus, evidence for continual decay of a pool of infected $CD4^+$ T cells has also been demonstrated in a subset of HIV-infected individuals, especially those who initiated ART during the early phase of infection [8,9]. In this regard, it would be of interest to determine if treatment with ART for extended periods of time (up to and longer than a decade) could bring the viral reservoirs to extremely low and possibly undetectable levels, and whether under such conditions the occurrence, timing, and level of viral rebound would be influenced upon discontinuation of therapy. We conducted the present study to address this issue.

Materials and methods

Patient population

Forty-four individuals with documented HIV infection who had received ART for a median of 7.7 years (range 3.0–10.5) and who had achieved prolonged suppression of plasma viremia were studied. One of 44 patients had been reported in a previously published paper [9]. Nine of the 44 individuals initiated ART during the early phase of infection (<6 months after acquisition of HIV). All participants included in this study maintained undetectable levels of plasma viremia (<50 copies/ml) without viral 'blips' after initiation of ART as determined by frequent blood sampling (at least three times per year). Any individual with detectable plasma viremia (>50 copies/ml) during the course of ART was excluded from this study. Blood and tissue specimens were collected from the study participants in accordance with protocols approved by the Institutional Review Boards of the University of Toronto, Toronto, Canada, University of Washington, Seattle, and by the Office of Human Subjects Research at the National Institutes of Health.

Isolation of $CD4^+$ T cells in blood and preparation of cells in gut-associated lymphoid tissue

Peripheral blood mononuclear cells (PBMCs) were obtained from blood draw and leukapheresis by Ficoll-Hypaque density gradient centrifugation. $CD4^+$ T cells were isolated from PBMCs of HIV-infected individuals using either a column-based cell or an automated separation technique (StemCell Technologies) as previously described [10]. The purity of

enriched $CD4^+$ T cells was generally greater than 95% assessed by flow cytometry. In order to examine cells in GALT, sigmoidoscopy was conducted in two study participants. Tissue samples (>10 biopsies) were incubated with 0.5 mg/ml collagenase (Type II-S; Sigma) in RPMI containing 5% fetal bovine serum, HEPES, and Pen/Strep at 37°C for 30 min. After frequent pipetting and vortexing, cells were washed and stored on ice and the remaining undigested tissue was treated with 1.0 mg/ml collagenase for an additional 30 min. The cells obtained above were subjected to $CD8^+$ cell depletion (Invitrogen-Dynal).

Quantitative real-time PCR for measurements of HIV DNA

In order to determine the frequency of $CD4^+$ T cells carrying HIV proviral DNA in infected individuals, realtime PCR was carried out on genomic DNA isolated from 1–2 × 10^6 purified $CD4^+$ T cells using the Puregene DNA isolation kit according to the manufacturer's specifications (Gentra). 1 μg of DNA was then used as a template for real-time PCR in an iCycler (Bio-Rad). The amplification reaction was carried out in triplicate using 0.5 μmol/l primers, 0.2 μmol/l fluorescent probe, 0.8 mmol/l dNTPs, 5 mmol/l $MgCl_2$, and 2.5 U Platinum Taq Polymerase (Invitrogen) in 50 μl total volume. The following primers were used: 5′-GGTCT CTCTGGTTAGACCAGAT-3′ (5′ primer) and 5′-CTG CTAGAGATTTTCCACACTG-3′ (3′ primer) along with the fluorescent probe 5′-6FAM-AGTAGTGTGTGCCCGTCTGTT-TAMRA-3′. PCR conditions consisted of a denaturation step at 95°C for 3 min followed by 45 cycles of 15 s at 95°C and 1 min at 59°C. Serially diluted ACH-2 DNA (40 000, 8000, 1600, 320, 64, 12.8, 2.56, and 0.56 cell equivalents per well in triplicates) was also subjected to the above PCR to obtain standard curves. The detection limit of the assay was 2.6 copies of HIV DNA. In order to determine the frequency of HIV infection in GALT, approximately 200 000 CD8-depleted cells were lyzed in 10 mmol/l Tris–HCl pH8 containing 100 μg/ml proteinase K (Roche Applied Science) for 1 h at 56°C followed by heat inactivation of the enzyme. Real-time PCR specific for human β-actin DNA (Applied Biosystems) was carried out on the above cell lysates in order to determine the exact copy number of cells per μl of cell lysate. Serially diluted ACH-2 DNA was also subjected to the above PCR to obtain standard curves. Finally, real-time PCR specific for HIV DNA was carried out as described above and the copy number of HIV DNA per 1 × 10^6 $CD4^+$ T cells was calculated based on the results obtained from the PCR experiments.

High-input quantitative coculture assays

In order to determine the frequency of $CD4^+$ T cells carrying replication-competent HIV, high-input quantitative coculture assays were carried out in which multiple wells containing 1 × 10^7 $CD4^+$ T cells were subjected to activation in 12-well tissue culture plates as described previously [11]. Briefly, highly enriched $CD4^+$ T cells were enumerated using an automated cell counter (Guava PCA, Guava Technologies) and precisely 10 × 10^6 $CD4^+$ T cells were seeded to each well in 12-well plates. Subsequently, 8 × 10^6 irradiated PBMCs from HIV-negative healthy donors were added to each well along with anti-CD3 antibody and incubated overnight in the presence of culture medium including recombinant IL-2 (20 units/ml). 1 × 10^6 CD8-depleted and anti-CD3 stimulated PBMC blasts from HIV-negative donors were added to each well the following day and again on day 7. The cultures were subjected to removal of 33% of the cell suspension every three days and replenished with fresh media. The culture supernatants were subjected to HIV p24 ELISA between days 14 and 21. The viability of cultures was periodically measured using dyes that stain cell membrane and DNA (Guava PCA, Guava Technologies). The infectious units per million cells (IUPM) values from the high-input coculture (HIC) assays were determined as described [2] except that the Newton-Raphson algorithm with a convergence criteria of relative change of the estimated IUPM value less than 1 × 10^{-6} was used. When high-HICs

were negative, the IUPM value was estimated to be lower than a number that assumes that one well containing 10 million cells was indeed culture-positive.

Determination of plasma viremia following discontinuation of antiretroviral therapy

Plasma viral loads were monitored longitudinally using a branched DNA assay (the limit of detection of 50 copies of HIV RNA per ml) after one study participant voluntarily discontinued all antiretroviral drugs.

Results

In order to determine the frequency of $CD4^+$ T cells carrying HIV DNA from the study participants, genomic DNA was prepared from highly purified $CD4^+$ T cells and subjected to real-time PCR specific for HIV proviral DNA (limit of detection 2.6 copies of HIV DNA per μg of genomic DNA or 150 000 cell equivalent) [10]. The median copy number of HIV proviral DNA for all study participants examined was 417.1 (range <2.6–8804.4) per 10^6 $CD4^+$ T cells (Fig. 1a). The median copy number of HIV proviral DNA in the study participants who had initiated ART within 6 months of infection was significantly lower (4.6 copies per 10^6 $CD4^+$ T cells) compared to those who had initiated ART during the chronic phase of infection (949.4 copies per 10^6 $CD4^+$ T cells) ($P = 0.003$). Of note, no measurable HIV proviral DNA was detected in four of nine early treated (44.4%) and four of 35 chronic treated (11.4%) individuals.

In order to examine the frequency of $CD4^+$ T cells carrying infectious virus, a HIC assay [11], which allows examination of large numbers of cells, was conducted using highly enriched $CD4^+$ T cells from the eight infected individuals in whose cells no measurable HIV proviral DNA had been detected. As shown in Fig. 1b, infectious virus was recovered in all infected individuals using the HIC assay on $CD4^+$ T cells. In one particular individual, the first HIC assay involving 3×10^8 purified $CD4^+$ T cells failed to produce replication-competent virus (7.6 years after initiation of ART). The infectious viral burden was estimated to be below 0.0012 per 10^6 $CD4^+$ T cells, using the assumption that one additional well would have resulted in HIV p24-positive outcome. However, a subsequent HIC assay conducted 10.5 years after initiation of ART using 1.56×10^9 $CD4^+$ T cells resulted in one out of 156 wells being positive for infectious virus. This translated into an infectious viral burden of 0.00064 per 10^6 $CD4^+$ T cells or one infected cell per 1.7×10^9 $CD4^+$ T cells. This is the lowest infectious HIV burden recorded to date in our laboratory and is considerably lower than the previously reported average frequency of 0.059 infectious units per 10^6 $CD4^+$ T cells in HIV-infected individuals having received ART for more than 7.6 years [10]. When the coculture data were stratified by time of initiation of ART, the frequency of cells carrying infectious virus in infected individuals who initiated therapy within 6 months of infection was significantly lower (median: 0.0074 infectious units per 10^6 $CD4^+$ T cells, range 0.00064–0.0173) than that of infected individuals who initiated therapy during the chronic phase of infection (median 0.0666 infectious units per 10^6 $CD4^+$ T cells, range 0.0345–0.0847) ($P = 0.03$).

Colonoscopy was performed on two infected individuals in order to measure levels of HIV in GALT. As shown in Table 1, real-time PCR conducted on CD8-depleted cells from sigmoid colon biopsies from one infected individual (who initiated ART during the chronic phase of infection) in whom HIV proviral DNA was undetectable in peripheral blood $CD4^+$ T cells, but in whom infectious virus was recovered, revealed readily detectable HIV DNA (89 copies of DNA per million cells). However, HIV DNA was undetectable in CD8-depleted cells isolated from the sigmoid colon biopsies of the infected individual (who initiated ART during the early phase of infection) in whom HIV proviral DNA was undetectable and the level of infectious virus was extraordinarily low (one infected cell per

1.7×10^9 $CD4^+$ T cells) in peripheral blood $CD4^+$ T cells (Table 1), suggesting that a profound reduction in the size of viral reservoir was achieved in this study participant.

In order to investigate whether discontinuation of ART in the individual who was started on ART early in the course of infection and who had an extraordinarily low HIV reservoir would result in rebound of plasma viremia, and what the kinetics of such a rebound would be if it occurred, we discontinued ART in the patient upon his consent and monitored plasma viremia. As shown in Fig. 2, plasma viremia was not detected for the first 50 days following discontinuation of therapy. Subsequently, plasma viremia rebounded to 1593 copies of HIV RNA per ml followed by spontaneous suppression to an undetectable level. However, plasma viremia then rebounded again to 8684 copies of HIV RNA per ml on day 143 at which point ART was re-initiated.

Discussion

The persistence of HIV proviral DNA and/or infectious virus in $CD4^+$ T cells of infected individuals receiving ART and in whom plasma viremia was suppressed below the level of detection for prolonged periods of time has thus far made the prospect of eradicating virus extremely problematic [2–4,10]. In the present study, we demonstrated undetectable levels of HIV DNA in the blood of eight infected individuals on long-term ART, including one individual in whom HIV proviral DNA could not be detected in the GALT and infectious HIV burden was extraordinarily low. Among the eight individuals whose peripheral blood $CD4^+$ T cells had undetectable HIV DNA, the frequency of cells carrying infectious virus was significantly lower in those in whom ART was initiated during the acute/early phase of infection compared to those who began therapy during the chronic phase of infection. It is not clear whether differential decay rates of virus in subsets of memory $CD4^+$ T cells [12] and/or efficacy of different drug regimens contributed to rapid clearance of HIV in some infected individuals receiving ART. Although we cannot rule out the existence of low levels of HIV replication [13,14] or the persistence of virus in other lymphoid tissue [5,8], our data clearly suggest that, at least in a subset of infected individuals, the profound suppression of viral replication by long-term effective ART that had been initiated early in the course of infection may lead to substantially greater reduction of residual HIV compared to those in whom ART was initiated after HIV infection had already been established as a chronic process. Of note, it is likely that a standard quantitative coculture assay would not have detected any replication competent virus in one study participant, given the unusually large number of cells (over one billion $CD4^+$ T cells) used to detect one well containing infectious HIV in the $CD4^+$ T cells. Nonetheless, the present study clearly demonstrated that the combination of early initiation of ART, an extended duration of therapy, and a profoundly low HIV burden in $CD4^+$ T cells did not eradicate HIV, nor did it indefinitely suppress the re-emergence of plasma viremia; however, it did lead to a longer period (50 days) of aviremia compared to previous studies (average 9 days) after cessation of antiretroviral drugs [7]. It is possible that profoundly low HIV burden and/or HIV-specific immune responses may have contributed to the long delay of plasma viral rebound in this infected individual [15]. The secondary plasma viral rebound may have been due to emergence of escape mutants [16]. Our data also suggest that the vast majority of, if not all, infected individuals receiving ART will experience plasma viral rebound regardless of the level of HIV in their $CD4^+$ T cell compartment at the time of discontinuation of ART.

Several attempts have been made in the past to ‘flush’ out HIV from $CD4^+$ T cells in infected individuals receiving ART without providing definitive evidence for eradication of virus [6,17,18]. Of note, we have previously demonstrated that co-administration of IL-2 and ART could lead to a dramatic diminution in the size of the $CD4^+$ T-cell viral reservoir [11]. Yet, despite the diminution in the size of the viral reservoir, HIV proviral DNA was

still readily detectable in their $CD4^+$ T cells prior to cessation of antiviral drugs. Given the presence of detectable proviral DNA, it should not have been surprising that these patients experienced rebound of plasma viremia upon discontinuation of therapy [6]. However, the present study demonstrates that despite the fact that prolonged treatment with ART initiated early in the course of HIV infection resulted in undetectable levels of proviral DNA and profoundly low levels of infectious HIV in peripheral blood $CD4^+$ T cells, virus still rebounded upon discontinuation of therapy. It appears that currently available antiretroviral drugs, even when initiated early in the course of infection and continued for prolonged periods of time resulting in 'undetectable' HIV DNA, do not eradicate HIV [19]. In order to achieve a condition under which HIV does not rebound for extended periods of time in the absence of ART, novel therapeutic strategies aimed at more specifically targeting these extremely rare infected cells may be necessary with or without the use of therapeutic vaccination to boost immune system control of viral rebound. In addition, prior to interrupting antiretroviral therapy in HIV-infected individuals, exhaustive laboratory assays, especially HIC assays that allow detection of infectious virus in large numbers of $CD4^+$ T cells, should be conducted given rebound of plasma viremia following cessation of therapy is all but certain as long as infectious viral reservoirs are present.

Acknowledgments

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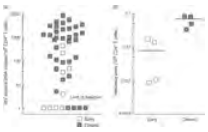

Fig. 1. Frequencies of HIV proviral DNA (a) and infectious virus (b) in CD4$^+$ T cells of HIV-infected individuals receiving effective ART for prolonged periods of time

(a) Levels of HIV proviral DNA in highly enriched CD4$^+$ T cells was determined by real-time PCR as previously described [10]. The open and closed squares represent data obtained from the 'early treated' and 'chronic treated' individuals, respectively. (b) Levels of replication-competent HIV in CD4$^+$ T cells from infected individuals were determined by high input coculture assay as previously described [11]. The median is shown as gray bars.

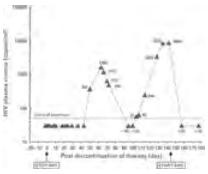

Fig. 2. Levels of plasma viremia following discontinuation and re-initiation of ART

Plasma viremia was determined by a branched DNA assay with the detection limit of 50 copies of HIV RNA per ml of plasma. ARV, antiretroviral.

Table 1

Immunologic and virologic profiles of selected HIV-infected individuals in whom sigmoid colon biopsies were preformed.

Participant	Sex	Age	Time of initiation of antiretroviral therapy	Duration of antiretroviral therapy at time of study (year)	Antiretroviral therapy at time of study[a]	CD4 cell count at time of study (cells/μl)	CD8 cell count at time of study (cells/μl)	CD4/CD8 ratio	Plasma HIV RNA at time of study (copies/ml)[b]	Level of HIV DNA in blood (copies/10^6 cells)	Level of infectious HIV in blood ($/10^6$ cells)	Level of HIV DNA in sigmoid colon (copies/10^6 cells)
1	Male	44	Chronic	8.6	ABC/3TC/EFV	410	580	0.7	<50	<2.56	0.05750	89.0
2	Male	46	Early	10.5	ABC/3TC/EFV	1060	840	1.3	<50	<2.56[c]	0.00064	<2 56

[a] 3TC, lamivudine; ABC, abacavir; EFV, efavirenz.

[b] Measured by a branched DNA assay with a detection limit of 50 copies per ml of plasma.

[c] 80 wells containing 1 μg of genomic DNA per well resulted in undetectable levels of HIV proviral DNA.

A-00449

EXHIBIT 38

IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF VIRGINIA
ALEXANDRIA DIVISION
~~~~~~~~~~~~~~~~~~~~

RICHARD ROE, et al.,

Plaintiffs,

vs. Civil Action No.
1:18-cv-01565

PATRICK M. SHANAHAN, et al.,

Defendants.
~~~~~~~~~~~~~~~~~~~~

Deposition of
CAPTAIN DEVIN KELLY, DO

March 13, 2019
10:10 a.m.
Taken at:
Dinsmore & Shohl, LLP
1 South Main Street, Suite 1300
Dayton, Ohio

Kimberly A. Kaz, RPR, Notary Public

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TRANSCRIPT INDEX

EXHIBIT CUSTODY

EXHIBITS RETAINED BY COURT REPORTER

INDEX OF EXHIBITS

misstate this acronym, JTS, which is, I believe, joint trauma system; CPG, clinical practice guideline on fresh blood transfusion.

Q. That's a written document, is it?

A. Yes.

Q. And did you read that document after she referred you to it?

A. Yes.

Q. And what information did you get from that document that's relevant to your testimony today?

MS. ZEIDNER MARCUS: Objection. Form.

Q. What was the reason that you were looking at the article on fresh blood transfusions?

A. I was looking at that article to review what the infectious risk might be with fresh whole blood transfusion. Within that article, it stated that there is an increased risk for transmission of infectious diseases such as HIV, hepatitis C with fresh whole blood transfusion.

Q. Is there anything else that Lieutenant Colonel Hughes pointed you to?

A. No. That is the -- the only resource.

Q. Okay. Were there any other personnel within the Air Force that you spoke to in preparation for your deposition today?

A. I spoke with personnel within the optometry clinic.

Q. Optometry?

A. Optometry.

Q. Okay. And why did you speak with persons in the optometry clinic?

A. I was -- I was asking or I was looking into if a member requires corrective lenses such as glasses who are deploying, what are they supplied with, and if they could be -- if they need to be replaced, is there a mechanism in place for that.

Q. Okay. And what did you learn with respect to deployed members who use glasses?

A. They stated that prior to deployment a member should have two sets of glasses and one set of gas mask inserts.

Q. And what did you learn with respect to how replacements would be provided to them, if necessary?

A. The person I talked to felt that glasses may potentially be shipped through a logistic chain to get it there, however, not clear how quick those could get to a member who needs a replacement. And there are some situations where someone may actually have to return from a forward-operating area to get a replacement at a more fixed facility.

Q. Okay. Do you know whether a situation's ever occurred that a deployed Air Force member needs to obtain a replacement pair of corrective lenses?

A. Yes, I do know of one situation.

Q. Okay. And when did that occur?

MS. ZEIDNER MARCUS: Objection. May call for privileged information with respect to doctor/patient confidentiality. If we could take a break. Is that --

THE WITNESS: Yeah.

MS. ZEIDNER MARCUS: We have a consent to have privileged information.

MS. BAUER: Okay. Then I'll have a break. But, obviously, he is the 30(b)(6) designee on this and I think he has to answer the questions, but we can go off the record.

MS. ZEIDNER MARCUS: Okay. Thank you.

(Recess taken.)

THE WITNESS: On a personal level, I know of one situation because my wife is an active duty Air Force physician who is deployed to a location, I'm not sure I can say, was in the Middle East region, and an active duty member had to be removed from that location and taken back to Kuwait to replace corrective lenses.

Q. Okay. And can you tell me approximately when this occurred?

A. It occurred approximately in the year 2017.

Q. Okay. And did the optometry clinic provide you with any information in preparation for the deposition today about any other instances where that had occurred?

A. No.

Q. Okay. And you also told me that you reviewed some HIV medical literature in preparation for your deposition today; is that right?

A. Yes.

medications?

A. Those medications are considered some of the first line treatment options for HIV, and I was interested in seeing if there were any particular storage requirements that the manufacturer has listed for those medications.

Q. And did the manufacturers list any particular storage requirements for any of those four medications?

A. Manufacturers give a range of temperatures in which the medications should be stored at.

Q. And do you recall what the range of temperatures was?

A. I do not know all the ranges. Some of them varied, but in general, most, if not all the medications should not be in temperatures greater than 30 degrees Celsius.

Q. Okay. And did they indicate a minimum temperature that they should not be exposed to?

A. Yes, they did indicate a minimum temperature, which I cannot recall at this time.

Q. Okay. Did they indicate if there were any other special storage or handling requirements for these four medications?

A. That is all the special storage or handling requirements that I can recall.

Q. Okay. Temperature restrictions?

A. Yes.

Q. Okay. Do you agree that persons who are diagnosed with HIV in a timely manner and provided with appropriate care and treatment with the antiretroviral medications experience no noticeable effects on their physical health?

MS. ZEIDNER MARCUS: Objection. Compound.

THE WITNESS: Can you repeat the question?

Q. Sure. Would you agree that persons who are HIV positive who are diagnosed in a timely manner and provided with appropriate care and treatment with antiretroviral medications experience no noticeable effects on their physical health?

MS. ZEIDNER MARCUS: Same objection. Form.

THE WITNESS: In general, persons who are living with HIV who are diagnosed and treated and have virologic suppression may have a expectancy similar to someone who's living without HIV, however, there are some documented impacts on that person's health who is living with HIV. Some of that may be HIV associated neurocognitive disorder, increased risk for cardiovascular disease, and potentially affecting other organ systems as well.

Q. And do you know, have you read anything -- strike that. I'll come back to that.

In your role as chief of infectious diseases at Wright-Patterson Air Force Medical Center, have you seen any HIV positive individuals who are suffering from HIV related neurocognitive disorder?

A. While serving as chief of infectious disease at Wright-Patterson Air Force Medical Center, I cannot definitively say I've seen my patient who have been diagnosed with an HIV associated neurocognitive disorder, however, some of those findings or diagnoses may be very subtle and may require specialized

neurocognitive testing. And as my role in training as an infectious disease fellow, I have seen at least one patient who may have had an HIV associated neurocognitive disorder.

Q. And why do you say that? Why do you believe that a patient may have had HIV associated neurocognitive disorder?

A. HIV neurocognitive disorder can be a complex diagnosis. There are multiple factors that go into it. The patient did not have an alternative diagnosis, and based on the prevalence of HIV neurocognitive disorder, which ranges from asymptomatic nerve cognitive impairment to moderate cognitive disorder and the most severe, but less common form, HIV associated dementia. If you take all those combined, some studies around the prevalence may be around 50 percent. And in a patient who is displaying impaired neurocognition with longstanding diagnosis of HIV is -- is -- as a diagnosis, we strongly consider in that patient.

Q. But it was not a diagnosis that was made in the patient that you were talking about?

A. From my knowledge, I don't know if that was ever a firm diagnosis placed in the chart.

Q. Okay. And what is your understanding as to how long a person -- what the time period is between an HIV diagnosis and the showing some victims of some neurocognitive impairment related to HIV?

A. In general, that is a variable period and more severe neurocognitive impairment that may go to HIV associated dementia was more common in the past when treatment was not as available, however, I can't say a particular time frame. The -- it is variable and may be present early or late in the disease process.

Q. And is the HIV associated neurocognitive impairment thought to stem from the underlying HIV or the treatment, or is it unclear, or both, I suppose?

A. It is unclear the exact mechanism for the cause. There is literature that shows even persons living with HIV who are virologically suppressed may have some form of HIV associated neurocognitive disorder, and

that it may be related to increased inflammation within the central nervous system which may be related to increased glutamate levels. The exact mechanism, I believe, is still unclear, but some progression of neurocognitive disorders can be seen even on -- even in patients living with HIV who are virologically suppressed.

Q. Okay. What is the current treatment regiment within the Air Force for a person after they have been diagnosed as HIV positive?

A. Can you clarify treatment regimen?

Q. Sure. What I want to know -- not a very good question, but after they have been tested as HIV positive, how often are they seen? I take it they go quickly on an antiretroviral therapy; is that right?

A. Yes. After initial diagnosis, they -- members diagnosed with HIV are started on HIV therapy quickly.

Q. Okay. And how frequently after that initial diagnosis, then, are they seen by a doctor?

A. Can you clarify what type of

A. Yes.

Q. Okay. And how is Section E2 to Enclosure 3 being applied in the -- by the Air Force?

A. For any active duty member who is seropositive for HIV prior to deployment, the cognizant combatant command must approve that deployment.

Q. So the way the Air Force is applying the section, even those persons who are -- who are -- have asymptomatic HIV cannot deploy without the permission of the cognizant combatant command surgeon. Is that your understanding?

MS. ZEIDNER MARCUS: Objection. Form.

THE WITNESS: To my knowledge, yes. The Air Force is implementing this Department of Defense Instruction that requires the cognizant combatant command to approve medical clearance for deployment.

Q. Okay. And the cognizant combatant command surgeon is not consulted only in cases of HIV antibody positive with the presence of progressive clinical illness or immunological

deficiencies; is that correct?

A. Can you repeat?

Q. Sure. Let me ask a different question.

Is the medical condition that usually precludes contingency deployment that's referred to in Section E2, a diagnosis of HIV positive with the presence of progressive clinical illness or immunological deficiency, as opposed to all persons who are HIV positive?

A. The cognizant combatant command must review all cases of HIV seropositively prior to clearance.

Q. Regardless of whether that person is showing signs of progressive clinical illness or immunological deficiency?

A. Yes. That's correct.

Q. Okay. And when you say the cognizant combatant command surgeon shall be a consultant, do you mean that the cognizant combatant command surgeon has to approve the deployment?

A. In general, yes.

Q. As an infectious disease specialist, would you say that there's any

reason that a person with asymptomatic HIV who does not have clinical progressive illness or immunological deficiency cannot deploy?

MS. ZEIDNER MARCUS: Can we just pause for one second? I just want to look at the question 'cause I wasn't --

Will you just read back the question, please?

(Question read back as requested.)

MS. ZEIDNER MARCUS: Objection to scope.

THE WITNESS: There are certain considerations when you're talking about someone who is seropositive for HIV in a deployed setting. In a -- for example, in a contingency deployment or other situation, there may be a situation in which there is a mass casualty event that would require fresh whole blood transfusion which may have an increased risk for transmission of bloodborne pathogens, for which HIV is considered a bloodborne pathogen. The safety of the blood supply is an important factor when considering who is able to deploy.

Other considerations may include

access to medical care, if needed. The Department of Health and Human Service guidelines do recommend every three- to six-month visits with an HIV provider, which may include laboratory testing depending on the length of deployment. Someone living with HIV may not reach those intervals for recommended guideline follow-up with their provider.

Medication supply potentially could be impacted. Active duty Air Force members can deploy to austere environments, which may be remote and have limited supply chains to reach them. If they do not have their HIV medications, there are potential health risks to someone living with HIV. Going a period of time without HIV medications can lead potentially to resistance, which then may require further testing, different types of HIV treatment regimens, who may have more side effects that are less tolerated than their current regimen. There are several published studies looking at interrupted therapy in persons who are HIV seropositive. Interruptions in therapy can lead to increased for progression to AIDS, opportunistic

infections, and the presence of noninfectious complications from HIV, which may involve the central nervous system, liver, kidneys.

Q. In general, how long does the treatment need to be interrupted for an HIV positive person to develop resistance to their treatment regimen?

A. It's difficult for me to say the exact time that is required for that to occur. There is one published study that's showing interruption of three months can lead to adverse outcomes in a person living with HIV. Certain medications that we use to treat HIV include NNRTIs, non-nucleoside reverse transcriptase inhibitor, and that may be in combination -- it's usually in combination with two NRTIs, nucleoside reverse transcriptase inhibiters. True NRTIs are a typical backbone in combination with another class of medication, which may be an NNRTI, it may be an integrase inhibiter, potential a protease inhibitor. So it's fairly standard to give a two NRTI backbone plus another class of medication and NRTIs have a longer half-life, meaning that they stay in the body longer than

Q. Did you finish your answer?
A. Yes.
Q. Okay. And just so I'm clear, in the first part of that answer where you're saying that there's literature that suggests in a stressful environment, HIV positive people might be less compliant with their treatment regimen?
A. CDC, on their website for patient information, does state that.
Q. For HIV positive persons in particular?
A. To my knowledge, yes, that is located under information for persons living with HIV.
Q. Okay. And is that true also for people who take medication for other chronic medical conditions that -- that stressful situations can make them less compliant with their treatment regimens?
MS. ZEIDNER MARCUS: Objection. Exceeds the scope of designated testimony.
THE WITNESS: In general, as a medical provider, that is -- that is -- yes, that may apply to other medical conditions.

Q. Tell me with respect to Paragraph 3, which provides that any required ongoing health care medications anticipated to be needed for the duration of the deployment are available in theater within the military health system, and then it goes on to talk about medication. Tell me how that condition might not be met by HIV positive Air Force men.

A. To my knowledge, HIV medications are not available -- are not readily available within theater. Depending on location, temperature requirements as laid out by the manufacturer may be outside of the range of what is recommended.

Q. Okay. And we discussed those temperature ranges earlier this morning, right?

A. Yes.

Q. Okay. Do you know if preexposure prophylaxis for HIV is available in theater?

A. Can you repeat?

Q. Sure. You're familiar with pre-exposure prophylaxis for HIV?

A. Yes.

Q. Okay. Is that medication available in theater?

A. In general, no, pre-exposure prophylaxis is not available.

Q. How about post-exposure prevention treatments, are those available in theater?

MS. ZEIDNER MARCUS: Objection. Exceeds the scope of designated testimony.

THE WITNESS: Post-exposure prophylaxis may be available at higher levels of care, but may not be available in different -- in different areas.

Q. Okay. Tell me what you mean by "higher levels of care."

A. There's a tiered system with health care in a deployed setting, level one, level two, level three, level four, level five. Level one may be the most basic of care such as buddy-care, self-care, tourniquets, very minimal. Level two may have some surgical capabilities for stabilization. Level three will have higher surgical capabilities, more reliable labs, imaging, ICU care. Level four, the facilities would include locations in Germany and Hawaii, which those hospitals operate on a level of hospitals here in the states. Level five are larger medical centers

hepatitis C, that you're unaware of any order that a service member would hepatitis C not give blood?

A. In my practice, I do treat hepatitis C in a similar situation of my hepatitis B patients. All the patients I have treated for hepatitis C have been beneficiaries, not active duty to date. I would advise them not to donate blood, however, I do not know of an order such as Attachment 13.

Q. Okay. You don't know of an order?

A. No, I do not know of an order.

Q. Okay. I just wanted to make sure I heard you correctly.

A. Yeah.

Q. If I could direct your attention to Topic 14 in Kelly Deposition Exhibit No. 1. Topic No. 14 provides the process by which the Air Force provides airmen requiring daily medication with that medication while they're deployed in the cent com area of responsibility. Do you see that?

A. Yes.

Q. And are you prepared to address

that topic on behalf of the Air Force today?

A. Yes.

Q. Okay. What can you tell me about the process by which the Air Force provides airmen requiring daily medication with that medication while they're deployed in the cent com area of responsibility?

MS. ZEIDNER MARCUS: Objection. Calls for a narrative.

THE WITNESS: In general, when deploying to the cent com area take with them an adequate supply of medication with them, however, there may be some situations in which a refill may be needed or medication could be lost, destroyed. If that were the case, refilling the medication or resupplying would depend on the area where the service member is. Are they in a remote austere area in which it may be difficult to send medications? Certain areas may have a mission in which different supplies. For example, ammunition may be the highest priorities in the logistic chain to get to that area depending where it is, what location that may be through air, may be through convoy. There is a -- it's a priority,

depending on the mission, to get certain supplies there, and there is a possibility that medications may not be able to be restocked in certain time frames. It depends on the situation and the location.

Q. Okay. And, again, just to make sure I understand, you're saying even within cent com, the answer to that question can vary depending on the specific location within cent com?

A. Correct.

Q. Okay. And when you say in general, the service members take with them an adequate supply of medicine with them, they take with them -- tell me if I'm wrong, but more pills than they think they're going to need for the length of their anticipated deployment. Is that the idea?

A. Service members should take supply for approximately 180 days.

Q. Okay.

A. Approximately, and there's some situations in which deployments can get extended or return can be delayed because of logistical reasons.

Force service members with those chronic infectious diseases have deployed?

A. I am not aware of those situations.

MS. ZEIDNER MARCUS: Can I just clarify? Were you talking about is he personally aware as opposed to the Air Force?

MS. BAUER: Well, I'll ask -- I guess I can ask it both ways, right?

Q. So you're not personally aware of anyone with a chronic infectious disease who's been deployed?

A. I'm not personally aware.

Q. Okay. But, presumably, the Air Force has information on whether or not people with chronic infectious diseases have been deployed or not?

MS. ZEIDNER MARCUS: Objection. Exceeds the scope of the designated testimony, but you can answer.

THE WITNESS: I am not aware, but a chronic infectious disease that is well controlled and poses no risk of health to the service member or others, someone may have deployed, but I'm not aware of that situation.

Q. Okay. We were talking a minute ago

about how medications were provided to Air Force service members while deployed to cent com, and you -- you said in certain circumstances, a refill might be needed or their medication might be lost or destroyed; is that right?

A. Yes.

Q. And do I understand your answer to mean that in some circumstances, a refill of that medication could just not be gotten to the deployed service member?

A. There is a possibility that a medication may not be able to get to that service member or it may be delayed in that time frame. I cannot state specifically. It'll depend on each situation.

Q. Okay. What happens if an Air Force service member develops some type of an acute health problem while they're deployed, how does the Air Force treat that person?

A. So it would depend on location, services available. Can that acute medical problem be handled sufficiently by the medical personnel and resources available and location? If there's an acute problem that is beyond the

Exceeds the scope of designated testimony.

THE WITNESS: The screening for infectious diseases of fresh whole blood may depend upon the location and situation and resources. Fresh whole blood can be screened with rapid tests, which may include tests for HIV. Rapid tests are not the standard for screening blood as far as FDA approved. Those samples will also be sent back for testing in states for those FDA approved rapid tests. The rapid tests may not have the same performance characteristics at FDA approved tests, meaning they may not be as sensitive and they may miss some cases, but that is what is feasible in certain locations. I speak in general terms. In reviewing the fresh whole blood clinical practice guideline, however, I'm not the expert on blood blank safety. That would probably be best be answered by members who are involved in the blood blank and publishing such guidelines.

Q. Okay. And we've seen in earlier documents today that the Air Force orders HIV positive service members not to donate blood; is that right?

A. Yes. That is correct.

Q. Okay. And do you know in the case of a transfusion, is the person who is donating the blood also asked at that time whether they have a condition that would preclude them from donating blood?

MS. ZEIDNER MARCUS: Objection. Exceeds the scope of designated testimony.

THE WITNESS: In a controlled situation, someone donating blood will often be asked if they have a condition that would preclude them from donating blood. It may be possible in certain situations that there may be limited members who are available to donate blood, and having that -- having a safe blood supply is important to reduce risks of transmission to members requiring blood transfusions.

Q. Okay. I'm not sure that I understood that answer.

A. In certain situations, there may be a mass casualty event that would require activation of a walking blood bank and donations of fresh whole blood. And depending on the situation, there may be very limited amount of individuals who would be able to

donate fresh whole blood. Having members available who can donate blood would increase the chances of -- having members who do not have an infectious disease that can be transmitted through blood will increase the chance of having available members who can donate blood, if needed.

Q. Okay. But you're not suggesting that in any incidents when it was determined that the person was not eligible to donate blood because they had an infectious disease, they wouldn't use that blood, would they, or do they, in some circumstances, use it --

MS. ZEIDNER MARCUS: Objection.

Q. -- despite the risk?

MS. ZEIDNER MARCUS: Exceeds the scope of designated testimony.

THE WITNESS: Can you repeat the question?

Q. Sure. Let me phrase it differently.

What I'm trying to understand is if there's a situation where they need to activate a walking blood bank and someone is asked can you donate blood and they say no, I'm not

facility or duty station that has -- does not have an infectious disease provider, the treating providers from San Antonio Military Medical Center can coordinate labs remotely that they need out of that particular lab.

Q. Okay. But do I understand they could not coordinate those labs if the person was -- if the service member was deployed to cent com?

A. It depends on their location and resource available.

Q. Okay.

A. There will be locations where there are no blood tests available.

Q. And are there other locations within cent com where there would be blood testing facilities available?

A. I do not know of a location in cent com that does an HIV viral load.

Q. Okay.

A. How about -- it may be present, but I do not know of them.

Q. Okay. Do you know of other combatant commands where those blood testing facilities are available?

MS. ZEIDNER MARCUS: Objection. Exceeds the scope of designated testimony.

THE WITNESS: I'm unsure of the exact location where all testing is available as in general, Germany has capabilities to function similar to a hospital in the states. If they cannot get testing within their own lab, there may be local labs that they can get testing.

Q. In Germany specifically?

A. Correct.

Q. Okay.

A. There may be other locations that I'm not knowledgeable of.

Q. Okay. The second concern that you listed was that medications could be lost or destroyed and there might be logistical difficulties in getting a refill to the service member; is that right?

A. Yes.

Q. Are there any -- does that risk present any different risk than for any other deployed service member who needs medication on a daily basis?

A. That depends on location and

availability of certain medications. If -- just in general, if a medication is lost or destroyed and that is not in stock in a remote area, it may be difficult to replace certain medications, and that's -- that could be all medications depending on how that can get resupplied. Part of consideration with HIV is going without medication for a period of time can lead to significant long-term health complications. As a physician who takes care of persons living with HIV, we advise to take medications every day and not to miss a dose. A longer -- a break in taking medications, although difficult to find to say exactly how long that is, we know that three months risk involve -- risk goes up for long-term health complications. Where does that start? I can't say exactly, but we advise not to miss any days of therapy.

Q. Okay. And that's true for any number of medications as you're taking them to manage chronic medications; is that right?

MS. ZEIDNER MARCUS: Objection. Exceeds the scope of designated testimony.

THE WITNESS: It depends on the

THE WITNESS: I am uncertain that that the HIV medications are available. There are many different preferred regimens for HIV and, oftentimes, members living with HIV could be on different preferred regimens, or even further down the list, other regimens that are not on the preferred list. There are many different medication treatment regimens for HIV, and I'm unsure if all that may be needed.

Q. Is there any reason that you're aware of that the HIV treatment medications could not be provided in cent com?

MS. ZEIDNER MARCUS: Objection. Exceeds the scope of designated testimony.

THE WITNESS: Can you repeat the question?

Q. Sure. Is there any reason that you're aware of that the HIV treatment medications could not be made available in cent com?

MS. ZEIDNER MARCUS: Same objection.

THE WITNESS: That area falls outside my scope as a physician treating HIV and depends more upon logistics, combatant

commands.

Q. Okay.

MS. BAUER: Okay. Why don't we take another break and let me look through my notes.

(Recess taken.)

Q. Several hours ago, you told me that in preparation for your deposition, you talked to a gentleman named Richard Davis, who I understand was an endocrinologist. And why did you talk to Dr. Davis?

A. I was interested in gathering information if a service member has diabetes or hypothyroidism, if they would require a waiver for deployment.

Q. And what did you learn from Dr. Davis on that topic?

A. That once stable on therapy, that they may require a waiver. It would depend on the combatant command potentially, individual combatant commands.

Q. Okay. And was that true for both diabetes and for hyperthyroidism?

A. For those diagnoses, those may require a waiver.

Q. Okay. For both of them?
A. Correct.
Q. Okay. And did he indicate to you whether those waivers are being granted for either of those conditions?
A. No. He was -- he said for further information, you could contact PEBLO, which I did not have a contact at PEBLO to get further information on that.
Q. Let me ask you: A few minutes ago when we were talking about Topic 15, the concerns about the Air Force's ability to provide necessary health care to airmen deployed to cent com, and you talked about the concern about the ability to provide HIV positive service members with their routine medical visits and testing. Do you remember that?
A. Can you repeat that?
Q. Sure. Let me rephrase it.
Just a few minutes ago, we were talking about the Air Force's concerns with its ability to provide necessary health care to HIV positive service members if they were deployed to cent com, and you enumerated four separate

occur?

A. If testing is needed and not available at a particular location, that member may be evacuated to a location that had those capabilities.

Q. Are there situations in which evacuation would be a response because of a lack of treatment as well as a lack of testing?

A. Yes, potentially.

Q. What means might be used to evacuate a service member on a deployment in cent com?

MS. BAUER: Objection.

THE WITNESS: It depends on the location, how remote the person is. Is it safe to evacuate? It may involve helicopter evacuation, airplane evacuation, potentially land vehicle. It really depends on location, what kind of vehicle resources are available to get to that area and return to an area where the capabilities are located.

Q. And being evacuated -- the possibility of being evacuated a concern related to airmen living with HIV who may be deployed to cent com?

A. Can you restate the question?

Q. Yeah. The possibility of -- is the possible need to evacuate a service member who is living with HIV part of the concern of providing necessary health care to individuals who are deployed in cent com?

A. The need to evacuate a patient would be a concern and the ability to provide medical care in that area because evacuating any service member for any reason may impact the mission of where they're located. That individual may be required for their skills they have for the mission.

Q. Are there other problems or concerns with -- related to evacuations? For example, are there safety concerns?

MS. BAUER: Objection.

THE WITNESS: There can be safety concerns with evacuation depending on location. It may be certain times, situations where it may be unsafe to have the appropriate mechanism for evacuation to get to that area and also relieve that area. There may be dynamic situations in that area or threats that may make it unsafe to evacuate at certain points in

time.

Q. Is evacuation resource intensive?

MS. BAUER: Objection.

THE WITNESS: Evacuation can be resource intensive. What resources are required depend on location, distance, geography, factors that all play a role.

Q. Okay. When you testified earlier that you did not know whether cent com includes any areas where HIV blood testing was possible, were you talking about your personal knowledge as opposed to the Air Force's knowledge?

A. That's my personal knowledge.

Q. Would the combatant command know what HIV blood testing was available in an area of deployment?

A. My understanding is that a combatant command will -- will know what resources are available.

Q. Is that a reason for the waiver process?

A. Yes. The reason we have a waiver process is to ensure that the appropriate resources to take care of a member with any chronic condition has those -- has those

A. Can you clarify?
Q. How is a rapid test of blood conducted?
A. Rapid tests can mean many different things. There are different techniques that may depend on which type of testing you're doing or what you're testing for.
Q. So for a transfusion purposes, what -- how are rapid tests of blood to be used in a full body transfusion conducted?
A. In general, I'm not the expert on rapid diagnostics or screening blood supply, and I'm unsure of the exact techniques the rapid tests that are used or utilized, what type of mechanism, how much of the exact mechanism of the rapid test that are utilized.
Q. Could be like a dipstick?
MS. BAUER: Objection.
Q. Just trying to get -- do you have any sense of what the rapid test consists of?
A. A rapid test may detect antigen or antibody or a combination of both, and that may be something that reacts with the antibody or an antigen and has potential colorimetric change, change in distance that it's on, say,

capillary paper. There's -- when you add, say, blood for a rapid test from a fingerstick or other specimen, the rapid test needs to be able to recognize something and indicate that to the person interpreting the test.

Q. How quickly can rapid tests provide a result?

A. It depends on individual rapid tests. It may be within hours. I'm not sure what the quickest turnaround is.

Q. What is the -- are rapid tests always available in deployment locations within cent com?

A. There may be situations or locations where rapid tests are not available.

Q. Does it depend on the location of the deployment within cent com?

A. It may depend on location, resources available, resources that members may be able to carry with them. There's many factors that go into that.

Q. Would the combatant command know the availability of rapid tests within a particular deployment location?

A. To my understanding, I believe that

medication to service members deployed overseas?

A. It can be variable. I mean, there's a large range. I cannot speak to exactly what that time frame is, but depending on location and safety of transport, it's possible it could be weeks. I'm not sure of the exact time frame. Perhaps a logistician may be the best person to answer those exact time frames.

MS. ZEIDNER MARCUS: I have no further questions. Thank you.

EXAMINATION OF CAPTAIN DEVIN KELLY, DO

BY MS. BAUER:

Q. Captain Kelly, do the antiretroviral medications that you identified earlier in your deposition require refrigeration?

A. From my review of the manufacturer information, refrigeration was not a requirement.

MS. BAUER: Thank you very much. Nothing further.

(The deposition was concluded at 5:40 p.m.)

Whereupon, counsel was requested to give instruction regarding the witness's review of the transcript pursuant to the Civil Rules.

SIGNATURE:

Transcript review was requested pursuant to the applicable Rules of Civil Procedure.

TRANSCRIPT DELIVERY:

Counsel was requested to give instruction regarding delivery date of transcript.

Ms. Bauer original regular.

Ms. Zeidner Marcus copy regular.

REPORTER'S CERTIFICATE

The State of Ohio,)
SS:
County of Fairfield.)

I, Kimberly A. Kaz, RPR, a Notary Public within and for the State of Ohio, duly commissioned and qualified, do hereby certify that the within named witness, CAPTAIN DEVIN KELLY, DO, was by me first duly sworn to testify the truth, the whole truth and nothing but the truth in the cause aforesaid; that the testimony then given by the above-referenced witness was by me reduced to stenotypy in the presence of said witness; afterwards transcribed, and that the foregoing is a true and correct transcription of the testimony so given by the above-referenced witness.

I do further certify that this deposition was taken at the time and place in the foregoing caption specified and was completed without adjournment.

I do further certify that I am not a relative, counsel or attorney for either party, or otherwise interested in the event of this action.

IN WITNESS WHEREOF, I have hereunto set my hand and affixed my seal of office at Cleveland, Ohio, on this 5th day of April, 2019.

Kimberly A. Kaz, RPR, Notary Public within and for the State of Ohio

My commission expires March 31, 2023.

Veritext Legal Solutions
1100 Superior Ave
Suite 1820
Cleveland, Ohio 44114
Phone: 216-523-1313

April 5, 2019

To: Lisa Zeidner Marcus, Esq.

Case Name: Roe, Richard, et al. v. Shanahan, Patrick M., et al.

Veritext Reference Number: 3255391

Witness: Captain Devin Kelly, DO Deposition Date: 3/13/2019

Dear Sir/Madam:

Enclosed please find a deposition transcript. Please have the witness review the transcript and note any changes or corrections on the included errata sheet, indicating the page, line number, change, and the reason for the change. Have the witness' signature notarized and forward the completed page(s) back to us at the Production address shown above, or email to production-midwest@veritext.com.

If the errata is not returned within thirty days of your receipt of this letter, the reading and signing will be deemed waived.

Sincerely,
Production Department

NO NOTARY REQUIRED IN CA

DEPOSITION REVIEW
CERTIFICATION OF WITNESS

ASSIGNMENT REFERENCE NO: 3255391
CASE NAME: Roe, Richard, et al. v. Shanahan, Patrick M.
DATE OF DEPOSITION: 3/13/2019
WITNESS' NAME: Captain Devin Kelly, DO

In accordance with the Rules of Civil Procedure, I have read the entire transcript of my testimony or it has been read to me.

I have made no changes to the testimony as transcribed by the court reporter.

_____ _____

Date Captain Devin Kelly, DO

Sworn to and subscribed before me, a Notary Public in and for the State and County, the referenced witness did personally appear and acknowledge that:

They have read the transcript;
They signed the foregoing Sworn Statement; and
Their execution of this Statement is of their free act and deed.

I have affixed my name and official seal

this _____ day of_____, 20____.

_____
Notary Public

_____
Commission Expiration Date

DEPOSITION REVIEW
CERTIFICATION OF WITNESS

ASSIGNMENT REFERENCE NO: 3255391
CASE NAME: Roe, Richard, et al. v. Shanahan, Patrick M.
DATE OF DEPOSITION: 3/13/2019
WITNESS' NAME: Captain Devin Kelly, DO

In accordance with the Rules of Civil Procedure, I have read the entire transcript of my testimony or it has been read to me.

I have listed my changes on the attached Errata Sheet, listing page and line numbers as well as the reason(s) for the change(s).

I request that these changes be entered as part of the record of my testimony.

I have executed the Errata Sheet, as well as this Certificate, and request and authorize that both be appended to the transcript of my testimony and be incorporated therein.

30 Apr 2019
Date

Captain Devin Kelly, DO

Sworn to and subscribed before me, a Notary Public in and for the State and County, the referenced witness did personally appear and acknowledge that:

They have read the transcript;
They have listed all of their corrections in the appended Errata Sheet;
They signed the foregoing Sworn Statement; and
Their execution of this Statement is of their free act and deed.

I have affixed my name and official seal this 30th day of April, 2019.

Kathleen O. Biser
Notary Public

KATHLEEN O BISER
Notary Public
In and For the State of Ohio
Recorded in Montgomery County
My Commission Expires
March 9, 2024

Commission Expiration Date

ERRATA SHEET

VERITEXT LEGAL SOLUTIONS MIDWEST

ASSIGNMENT NO: 3/13/2019

PAGE/LINE(S)	CHANGE	REASON
17/17	"periods" to "areas"	believe to be written/transcribed incorrectly
23/3	"offers" to "authors"	
35/13	"nerve cognitive" to "neurocognitive"	
47/10	"continuous sea" to "contingency"	
75/18	"true" to "two"	
110/10	"pathonomic" to "pathognomonic"	
115/15	"duly" to "duty"	
128/9	"route" to "throughout"	

30-Apr-2019
Date

Captain Devin Kelly, DO

SUBSCRIBED AND SWORN TO BEFORE ME THIS 30th

DAY OF April, 2019.

Kathleen O. Biser
Notary Public

KATHLEEN O BISER
Notary Public
In and For the State of Ohio
Recorded in Montgomery County
My Commission Expires
March 9, 2024

Commission Expiration Date

EXHIBIT 39

Low prevalence of neurocognitive impairment in early diagnosed and managed HIV-infected persons

Nancy F. Crum-Cianflone, MD, MPH
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Scott Letendre, MD
Mollie Poehlman Roediger, MS
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Amy Weintrob, MD
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ABSTRACT

Objective: To describe the prevalence of neurocognitive impairment (NCI) among early diagnosed and managed HIV-infected persons (HIV+) compared to HIV-negative controls.

Methods: We performed a cross-sectional study among 200 HIV+ and 50 matched HIV-uninfected (HIV−) military beneficiaries. HIV+ patients were categorized as earlier (<6 years of HIV, no AIDS-defining conditions, and CD4 nadir >200 cells/mm^3) or later stage patients (n = 100 in each group); both groups were diagnosed early and had access to care. NCI was diagnosed using a comprehensive battery of standardized neuropsychological tests.

Results: HIV+ patients had a median age of 36 years, 91% were seroconverters (median window of 1.2 years), had a median duration of HIV of 5 years, had a CD4 nadir of 319, had current CD4 of 546 cells/mm^3, and 64% were on highly active antiretroviral therapy (initiated 1.3 years after diagnosis at a median CD4 of 333 cells/mm^3). NCI was diagnosed among 38 (19%, 95% confidence interval 14%–25%) HIV+ patients, with a similar prevalence of NCI among earlier and later stage patients (18% vs 20%, $p = 0.72$). The prevalence of NCI among HIV+ patients was similar to HIV− patients.

Conclusions: HIV+ patients diagnosed and managed early during the course of HIV infection had a low prevalence of NCI, comparable to matched HIV-uninfected persons. Early recognition and management of HIV infection may be important in limiting neurocognitive impairment.
Neurology® 2013;80:371–379

GLOSSARY

BDI = Beck Depression Inventory; **CI** = confidence interval; **GDS** = Global Deficit Score; **HAART** = highly active antiretroviral therapy; **HCV** = hepatitis C virus; **IQR** = interquartile range; **NCI** = neurocognitive impairment; **OR** = odds ratio.

Despite the availability of highly active antiretroviral therapy (HAART), HIV-infected persons remain at risk for neurocognitive impairment (NCI).[1] Although severe forms of neurologic disease (e.g., HIV-associated dementia) have declined, the risk of other forms of NCI remains elevated compared to the general population.[1–4] The burden of NCI among HIV-infected persons remains substantial, occurring in approximately half (range 18%–73%) of patients.[1,5–7]

Since most studies have evaluated HIV-positive patients with unknown dates of HIV seroconversion and 35%–45% of newly diagnosed HIV-positive patients in the United States meet AIDS-defining criteria within 1 year of diagnosis,[8] elevated rates of NCI may result from late diagnosis and uninhibited viral replication in the CNS causing irreversible brain injury prior to diagnosis or initiation of therapy. A history of AIDS and low nadir CD4 counts has been associated with NCI.[1,2,9] However, few studies have determined the rate of NCI among HIV-positive patients managed in an optimized setting of early diagnosis, free access to care, and few concurrent comorbidities.

From the Infectious Disease Clinical Research Program (N.F.C.-C., M.P.R., L.E., A.W., A.G., E.J., R.D., B.K.A., B.R.H.), Uniformed Services University of the Health Sciences, Bethesda, MD; HIV Clinic (N.F.C.-C., R.D., B.R.H.), Naval Medical Center San Diego, San Diego; Naval Health Research Center (N.F.C.-C., B.R.H.), San Diego; Health Neurobehavioral Research Program (D.J.M., S.L.), University of California, San Diego; Biostatistics Division (M.P.R., L.E.), University of Minnesota, Minneapolis; Infectious Disease Clinic (A.W.), Walter Reed Army Medical Center, Washington, DC; Infectious Disease Clinic (A.G.), National Naval Medical Center, Bethesda, MD; and Infectious Disease Service (E.J.), San Antonio Military Medical Center, San Antonio, TX.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

We determined the prevalence of NCI among US military HIV-infected persons who have routine HIV testing, mandatory follow-up evaluations after diagnosis, open access to early antiretroviral treatment, and low rates of comorbidities including active substance use. We assessed the prevalence of neurocognitive impairment evaluating HIV-positive patients both earlier (i.e., <6 years of HIV infection since diagnosis, no AIDS-defining condition, and CD4 nadir >200 cells/mm^3) and later in the course of HIV infection, and compared these participants with a matched HIV-uninfected group of military beneficiaries.

METHODS Study design and participants. We performed a cross-sectional study among 200 HIV-infected and 50 HIV-uninfected military beneficiaries (active duty members, retirees, or dependents). All active duty members, including those in this analysis, are HIV seronegative upon entry into military service and undergo repeated mandatory HIV testing. Active duty members who become HIV-positive are evaluated by an HIV specialist at least semiannually, and all military beneficiaries have open access to early antiretroviral treatment and low rates of comorbidities including active substance use.

Of the 200 HIV-infected participants, 100 were classified as earlier stage (<6 years of HIV infection since diagnosis, no prior AIDS-defining condition, and CD4 nadir >200 cells/mm^3), and 100 as later stage (not meeting all 3 criteria). A control group of military beneficiaries (n = 50) were matched to the HIV-infected subjects by age (<35 vs 35–50 years), gender, race/ethnicity (Caucasian vs other), and rank (officer vs enlisted vs other including retirees and spouses). Inclusion criteria for both HIV-infected and HIV-uninfected groups included military beneficiaries who were 18–50 years of age. Exclusion criteria were current/recent suicidal ideation, inability or unwillingness to complete the neuropsychological battery, and presence of an acute medical condition that could impact the participant's ability to complete the tests (e.g., febrile illness). The control group had an HIV-negative ELISA test within 1 year of study enrollment.

Standard protocol approvals, registrations, and patient consents. All study participants provided written informed consent and the study was approved by a central military institutional review board. The trial was registered at ClinicalTrials.gov (registration #NCT00893815).

Data collection. Clinical data were abstracted from medical records including medical conditions, medications, body mass index, and fasting lipid levels. Hepatitis C virus (HCV) was defined as a positive antibody or RNA viral load. Among HIV-infected subjects, data on last HIV seronegative and first HIV seropositive dates, AIDS-defining conditions,[10] CD4 cell counts (including current, nadir, and recovery [current CD4 − CD4 nadir]), HIV RNA levels, and antiretroviral therapy (type, CNS penetration effectiveness,[11] duration, and percentage of time since diagnosis on medications) were collected. HIV infection in our cohort was primarily acquired by sexual routes[12]; data on sexual orientation were not available.

Questionnaire data included demographics, military rank and duty status, education, substance use, history of loss of consciousness or traumatic brain injury, and self-reported assessment of cognitive impairment. Illicit drug use was ascertained by a confidential questionnaire regarding drug use (past and current) and prior failure of military mandatory drug screening. Questionnaires assessed lipodystrophy, neuropathy (AIDS Clinical Trial Group Peripheral Neuropathy Screening Tool),[13] current and lifetime psychiatric diagnoses (Composite International Diagnostic Interview modules A, E, F, J, K, O, and X), and current mood (Beck Depression Inventory [BDI]–II).

All participants underwent a comprehensive battery of standardized neuropsychological tests and questionnaires (administration time 3.5–4 hours) shown to be sensitive to HIV-associated neurocognitive disorders.[1] The neuropsychological measures included an estimate of premorbid functioning (Wechsler Test of Adult Reading), verbal fluency (letter fluency [FAS], category fluency [animals], and action fluency [verbs]), attention/working memory (Paced Auditory Serial Addition Task, Wechsler Adult Intelligence Scale III [WAIS-III] Digit Span), visuospatial functioning (Judgment of Line Orientation Tests, form H; Hooper Visual Organization Test), speeded information processing (WAIS-III Symbol Search, WAIS-III Digit Symbol, Trail Making Test [TMT] A, Stroop Word and Color Tests), learning and recall (Hopkins Verbal Learning Test–R, Brief Visuospatial Memory Test–R), abstraction/executive functioning (Wisconsin Card Sorting Tests, 64-card version; TMT B; Stroop Word and Color Tests), motor speed and dexterity (Grooved Pegboard Test [both hands]), and effort (Hiscock Digit Memory Test). Neuropsychological tests were scored by trained psychometrists and raw scores were converted to demographically adjusted *t* scores corrected for effects of age, education, gender, and ethnicity. Scores were then converted to deficit scores that give differential weight to impaired rather than normal scores as previously described.[14,15] The Global Deficit Score (GDS) was used to summarize neuropsychological test results by quantifying the number and degree of impaired performances. A score ≥0.5 has been shown to be a sensitive and specific indicator of global NCI,[14] and a deficit score >0.5 was used within each domain. Importantly, impairment on the GDS has been found to be associated with biomarkers of HIV disease progression (e.g., CD4 count)[16] as well as aspects of everyday functioning declines (e.g., medication adherence).[17,18]

Statistical analyses. Descriptive statistics are presented as medians with interquartile ranges (IQRs) or as counts with percents, as appropriate. The Kruskal-Wallis rank-sum test was used to compare medians, and x^2 tests to compare percentages. For each analysis, there were 2 comparisons: earlier vs later stage HIV-infected participants, and HIV-infected vs HIV-uninfected participants. The relationships between self-reported cognitive problems and GDS and depression were explored with linear and logistic regression. Univariate and multivariate associations of factors with NCI were determined by logistic regression. Odds ratios (OR) for the prevalence of NCI were estimated with 95% confidence intervals (CI). Prespecified factors of interest (age, gender, race/ethnicity, years since HIV seropositivity, and cumulative years on antiretroviral therapy since diagnosis) along with factors with a *p* value ≤0.15 in univariate models were included in the multivariate model. All *p* values are 2-sided. Analyses were conducted using SAS software (version 9.1; SAS).

RESULTS Study population. Two hundred HIV-infected persons were studied (table 1); 91% were documented HIV seroconverters with a median seroconversion window of 1.2 years. The study population consisted of a population with low prevalence of

Table 1 Baseline characteristics of study population

	HIV+ stage, n (%) or median (IQR)					
	HIV+	Earlier stage	Later stage	*p* Value[a,b]	HIV−	*p* Value[a,c]
No.	200	100	100		50	
Demographics						
Age, y	36.4 (28.1–43.6)	28.8 (25.6–35.9)	42.1 (36.9–46.5)	<0.001	36.0 (27.0–44.0)	0.56
Male	191 (95.5)	97 (97.0)	94 (94.0)	0.50	48 (96.0)	
Race				0.15		0.001
White	97 (48.5)	44 (44.0)	53 (53.0)		25 (50.0)	
Black	58 (29.0)	27 (27.0)	31 (31.0)		4 (8.0)	
Hispanic	28 (14.0)	17 (17.0)	11 (11.0)		9 (18.0)	
Other	17 (8.5)	12 (12.0)	5 (5.0)		12 (24.0)	
Education						
Total years	14.0 (12.0–16.0)	13.0 (12.0–15.0)	14.0 (13.0–16.0)	<0.001	13.0 (12.0–14.0)	0.06
Highest completed level				0.001		0.02
Less than high school	1 (0.5)	0 (0.0)	1 (1.0)		1 (2.0)	
High school/diploma	130 (65.0)	77 (77.0)	53 (53.0)		39 (78.0)	
Bachelor degree	37 (18.5)	15 (15.0)	22 (22.0)		9 (18.0)	
Higher degree (e.g., master, PhD)	31 (15.5)	8 (8.0)	23 (23.0)		1 (2.0)	
Medical history						
Depression (BDI ≥ 20)	15 (7.5)	6 (6.0)	9 (9.0)	0.42	0 (0.0)	0.05
Significant medical conditions[d]	75 (37.5)	20 (20.0)	55 (55.0)	<0.001		
Hypertension	56 (28.0)	18 (18.0)	38 (38.0)	0.002	2 (4.0)	<0.001
Diabetes	6 (3.0)	0 (0.0)	6 (6.0)	0.03	1 (2.0)	>0.99
Ever symptoms of peripheral neuropathy	63 (31.5)	21 (21.0)	42 (42.0)	0.001		
HIV history						
Years HIV seropositive	5.2 (2.1–11.1)	2.3 (1.1–3.5)	11.1 (8.0–16.0)	<0.001		
Seroconverter	181 (90.5)	94 (94.0)	87 (87.0)	0.09		
Seroconversion window[s] (in years)[e]	1.2 (0.8–1.9)	1.3 (0.8–2.0)	1.2 (0.8–1.9)	0.89		
CD4+ (cells/mm^3)	546 (417–706)	542 (434–688)	555 (397–737)	0.79		
Nadir CD4+ (cells/mm^3)	319 (239–425)	366 (283–512)	278 (173–342)	<0.001		
Nadir CD4+ < 200 cells/mm^3	30 (15.0)	0 (0.0)	30 (30.0)			
CD4 recovery[f] (cells/mm^3)	195 (75–351)	119 (22–236)	291 (181–431)	<0.001		
HIV RNA ($\log_{10}$ copies/mL)	1.7 (1.7–3.5)	2.8 (1.7–4.1)	1.7 (1.7–1.7)	<0.001		
HIV RNA, undetectable (<50 copies/mL)	108 (55.1)	33 (34.4)	75 (75.0)	<0.001		
Antiretroviral use				<0.001		
On HAART	128 (64.0)	40 (40.0)	88 (88.0)			
Prior HAART use	13 (6.5)	4 (4.0)	9 (9.0)			
ART naive	59 (29.5)	56 (56.0)	3 (3.0)			
CPE rank of current regimen[g]	7.0 (7.0–8.0)	7.0 (7.0–7.0)	7.0 (7.0–9.0)	0.007		
Current regimen contains EFV[f]	74 (57.8)	33 (82.5)	41 (46.6)	<0.001		
Cumulative years on HAART[g,h]	4.3 (1.8–8.4)	1.4 (0.4–2.7)	7.1 (4.0–10.3)	<0.001		
Percentage of time on HAART[g,h]	62.3 (37.5–86.1)	58.2 (32.3–83.4)	63.0 (43.6–86.7)	<0.001		

Abbreviations: ART = antiretroviral therapy; BDI = Beck Depression Inventory; CPE = CNS penetration effectiveness; EFV = efavirenz; HAART = highly active antiretroviral therapy; IQR = interquartile range.
[a] *p* Values are calculated with χ^2 test or Fisher exact test, as appropriate, for proportions and Kruskal-Wallis rank sum tests for medians.
[b] Earlier stage vs later stage.
[c] HIV+ vs HIV−.
[d] Hepatitis C virus, clinical AIDS, cardiac disease, cerebrovascular disease, hypertension, diabetes, cirrhosis, renal failure, CNS infection, seizures.
[e] Time between last documented HIV-negative date and first documented HIV-positive date, limited to the 181 participants with both.
[f] Current CD4 count − nadir CD4 count.
[g] Limited to participants who have received ART (excludes ART-naive participants).
[h] Taking into account starts and stops of regimens, calculated from documented HIV+ date to enrollment date.

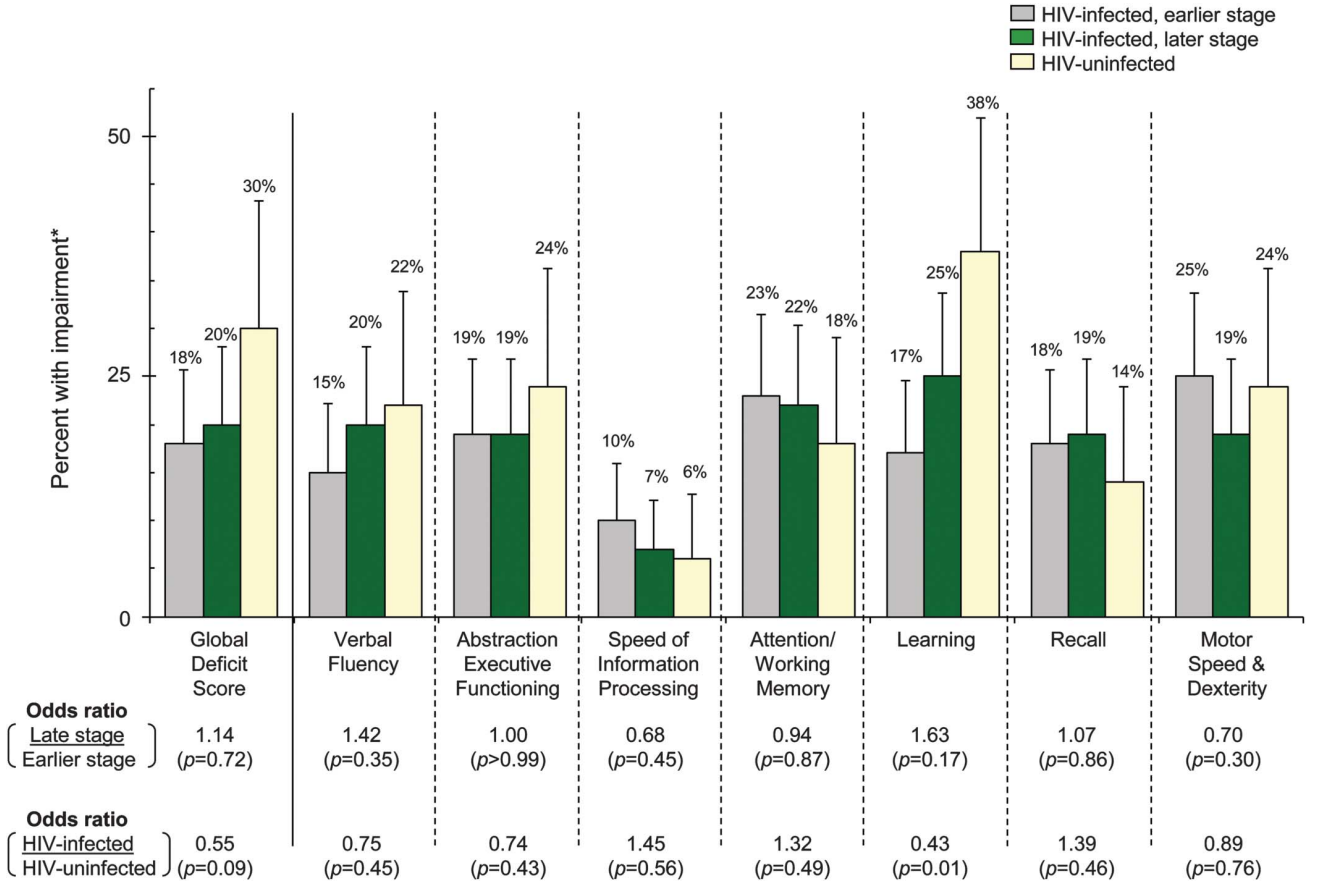
substance use—18% used tobacco, 5.5% consumed ≥6 alcoholic drinks per week, and 3.5% used illicit drugs. Sixty-four percent were receiving HAART, which was initiated a median of 1.3 years after HIV diagnosis at a median CD4 count of 333 (IQR 248–423) cells/mm^3; 81% had a HIV RNA <50 copies/mL at the time of enrollment. Among those off HAART, their median CD4 count was 523 (IQR 417–685) cells/mm^3. Characteristics by earlier vs later stage HIV-infected persons are shown in table 1. The HIV-negative control group (n = 50) was similar to the HIV-infected group except they were less likely to be African American and more likely to be "other" races, less likely to have a higher education degree, and less likely to have hypertension (table 1).

Prevalence of NCI among HIV-infected persons. NCI (GDS ≥ 0.5) was diagnosed among 38 (19%, 95% CI 14%–25%) HIV-infected participants. The prevalence of NCI was similar among earlier and later stage patients (18% vs 20%, $p = 0.72$). Later stage patients were more likely to have impairments in verbal fluency, learning, and recall, although none were statistically different from earlier stage patients (figure). The number of domains in which participants were impaired was also similar between earlier and later stage HIV participants (earlier 1.27 and later 1.31, $p = 0.84$).

We evaluated the association between self-reported cognitive problems ("Do you feel that you have a problem with memory loss or cognitive functioning?") and battery-identified NCI and found no relationship of self-reported cognitive complaints with NCI (OR 1.3, $p = 0.53$) or with the mean GDS ($p = 0.44$). Among the 55 participants who complained of cognitive issues, 12 (22%) had NCI, whereas 26 (18%) of those who did not complain of cognitive issues had NCI ($p = 0.53$). Those with symptoms of depression (BDI ≥ 20) were more likely to self-report cognitive dysfunction compared to those without depression (73% vs 24%, $p < 0.001$); however, there was no association of depression with NCI or with GDS.

Prevalence of NCI among HIV-uninfected persons. Fifteen of the HIV-uninfected participants (30%, 95% CI 17%–43%) had NCI (figure). The percentage with NCI among HIV-infected vs HIV-uninfected persons was not statistically significantly different

Figure Impairment by ability area



*Domain deficit score >0.50, global deficit score ≥0.50.

($p = 0.09$). HIV-infected persons had elevated rates of impairment in speed of information processing, attention/working memory, and recall, but none were significantly different from HIV-uninfected persons (all p values > 0.05). HIV-uninfected participants were more likely to have impairment in learning than HIV-infected persons ($p = 0.01$) (figure). Additional models adjusting for years of education and ethnicity compared HIV-infected to HIV-uninfected participants for the outcomes of NCI and each domain, separately, showed similar nonsignificant findings.

Factors associated with neurocognitive impairment among HIV-infected persons. Factors associated with NCI among HIV-infected participants in the univariate models are shown in table 2. No demographic, behavioral, or medical history variable was significantly associated with NCI. A higher number of years of education was associated with NCI (OR 1.2 per year, $p = 0.02$). Regarding HIV-specific factors, a higher current CD4 count (OR 1.07 per 50 cells/mm^3, $p = 0.08$) and higher CD4 recovery (OR 1.07 per 50 cells/mm^3, $p = 0.10$) were marginally associated with NCI (table 2).

In a multivariate model including factors of interest (age, gender, race, years HIV seropositive, and cumulative years on antiretroviral therapy) and univariate factors with a p value ≤ 0.15, only more years of education was associated with NCI (OR 1.24 per year, $p = 0.02$). Current CD4 count continued to have a marginal association with NCI (OR 1.07, $p = 0.07$); no association was found with either CD4 nadir or CD4 recovery. Analyses were also performed stratified by earlier vs later stage and for each neurocognitive domain with similar findings (data not shown).

We repeated our analyses restricted to HIV-infected participants receiving HAART who had an HIV RNA level <50 copies/mL. Similar associations were noted with additional findings that higher CD4 cell counts (OR 1.10 per 50 cells/mm^3, $p = 0.04$) and greater CD4 recovery (OR 1.15 per 50 cells/mm^3, $p = 0.01$) were significantly associated with NCI. The nadir CD4 count, HIV RNA level, and HAART information were not associated with NCI (data not shown).

DISCUSSION We found a low prevalence of NCI among HIV-infected persons diagnosed and managed early during the course of HIV infection, and nearly identical NCI rates among earlier and later HIV-infected patients in this cohort. Of note, most patients classified as later in their course of HIV infection met criteria by a longer duration of HIV infection, but the majority had preserved CD4 counts and few had prior AIDS-defining conditions. Further, the prevalence of NCI among our HIV-infected participants was not significantly greater than a matched HIV-uninfected group. These data provide important and novel information suggesting that the early recognition and management of HIV infection may be important in limiting NCI.

The importance of preventing NCI is severalfold as it impacts both the quantity and quality of life. Regarding survival, HIV-infected patients with NCI are at increased risk of death, even after controlling for other medical factors.[3,19] Further, NCI can have a substantial impact on patients' daily functioning and their ability to pursue career endeavors.[20] Finally, NCI may reduce antiretroviral adherence, hence adversely affecting the outcome of HIV-infected persons.[17] Hence, preserving cognitive function among HIV-infected persons may be an important step in further improving quality and life expectancies of patients.

During the pre-HAART era, 16% of patients with AIDS had HIV dementia, with an annual incidence of 7%.[21] After the introduction of HAART in 1996, there was a sharp decrease in HIV dementia; however, milder forms of neurocognitive disease continued to be diagnosed.[4,22] Studies demonstrated that patients with symptomatic seroconverting illness as well as high HIV RNA levels and low CD4 counts early after infection were at highest risk.[23,24]

The prevalence of NCI in the HAART era has varied in prior studies, likely related to clinical disease stage, comorbid diseases, and other factors.[6,7] A recent study found a NCI prevalence of 52% among HIV-infected patients seen at academic US HIV clinics regardless of comorbidity level.[1] Our study found a much lower NCI rate (19%), but was similar to a recent study evaluating HIV-infected persons with suppressed HIV RNA levels.[25] The relatively low prevalence in our study may be due to a combination of factors. Early diagnosis and active disease management, few comorbid conditions (low prevalence of concurrent medical conditions including HCV, illicit drug use, and alcohol), young age, frequent monitoring of vocational functioning, and the lack of AIDS events or low CD4 counts likely contributed to our low impairment rate. Further, despite the later group having HIV for a median of 11 years, their risk of NCI remained similar to the earlier group (median 2 years). This suggests that length of HIV duration itself may not be a risk factor if patients maintain good HIV control, avoiding AIDS-defining events and low nadir CD4 counts.[6]

In our study, HIV-infected persons had a similar prevalence of NCI compared to matched HIV-uninfected persons. Further, HIV-infected patients were not more likely to be impaired in any of the 7 cognitive domains. A recent Danish study found the overall risk of severe neurocognitive disorders is now similar among HIV-positive and -negative persons.[3] It should be noted that the HIV-negative controls in this study are likely to be an accurate control population relative to prior studies, as the military is relatively homogeneous in

Table 2 Factors associated with neurocognitive impairment (GDS ≥0.5) among HIV-infected persons

	Neurocognitive impairment, n (%) or mean (SD)		Univariate models	
	Yes, n = 38	No, n = 162	OR (95% CI)	p Value
Demographics				
Age, y	36.7 (8.7)	35.7 (8.5)	1.01 (0.97–1.06)	0.51
Race				
White	18 (47.4)	79 (48.8)	1.0	—
Black	9 (23.7)	49 (30.2)	0.81 (0.34–1.94)	0.63
Hispanic	8 (21.1)	20 (12.3)	1.76 (0.67–4.62)	0.25
Other	3 (7.9)	14 (8.6)	0.94 (0.24–3.62)	0.93
Education				
Total years	15.2 (2.3)	14.2 (2.3)	1.20 (1.03–1.39)	0.02
Highest completed level				
Less than high school	0 (0.0)	1 (0.6)	-	-
High school/diploma	19 (50.0)	111 (68.5)	1.0	-
Bachelor degree	11 (28.9)	26 (16.0)	0.53 (0.21–1.36)	0.19
Higher degree (e.g., master, PhD)	8 (21.1)	23 (14.2)	1.32 (0.46–3.83)	0.61
Medical history				
Depression (BDI ≥20)	4 (10.5)	11 (6.8)	1.61 (0.48–5.38)	0.43
Significant medical conditions[a]	14 (36.8)	61 (37.7)	0.97 (0.46–2.01)	0.93
Hypertension	10 (26.3)	46 (28.4)	0.90 (0.41–2.00)	0.80
Diabetes	0 (0.0)	6 (3.7)	-	-
Ever symptoms of peripheral neuropathy	11 (28.9)	52 (32.1)	0.86 (0.40–1.87)	0.71
HIV history				
Years HIV seropositive	8.1 (6.9)	7.1 (6.2)	1.03 (0.97–1.08)	0.35
Seroconverter, yes	34 (89.5)	147 (90.7)	0.87 (0.27–2.78)	0.81
Seroconversion window[b] (in years)	1.4 (0.9)	1.5 (1.0)	0.97 (0.66–1.44)	0.90
HIV stage (earlier vs later)	18 (47.4)	82 (50.6)	0.88 (0.43–1.78)	0.72
Current CD4+ (cells/mm^3)	640.7 (282.2)	566.5 (214.2)	1.07 (0.99–1.15)	0.08
Nadir CD4+ (cells/mm^3)	344.0 (197.3)	339.1 (158.7)	1.01 (0.91–1.12)	0.87
Nadir CD4+ <200 cells/mm^3	7 (18.4)	23 (14.2)	1.36 (0.54–3.46)	0.51
CD4 recovery[c] (cells/mm^3)	296.7 (298.8)	231.6 (192.6)	1.07 (0.99–1.15)	0.10
HIV RNA ($\log_{10}$ copies/mL)	2.6 (1.3)	2.5 (1.1)	1.09 (0.81–1.47)	0.55
HIV RNA, undetectable (<50 copies/mL)	22 (57.9)	86 (54.4)	1.15 (0.56–2.36)	0.70
Antiretroviral use				
On HAART	23 (60.5)	105 (64.8)	0.86 (0.39–1.87)	0.70
Prior HAART use	3 (7.9)	10 (6.2)	1.18 (0.28–4.95)	0.83
ART naive	12 (31.6)	47 (29.0)		
CPE rank of current regimen[d]	7.9 (2.1)	7.8 (2.0)	1.02 (0.83–1.27)	0.82
Current regimen contains EFV[d]	15 (65.2)	59 (56.2)	1.46 (0.57–3.74)	0.43
Cumulative years on HAART[d,e]	6.5 (4.6)	5.1 (3.9)	1.05 (0.97–1.14)	0.27
Percentage of time on HAART[d,e]	61.7 (27.5)	59.0 (28.6)	1.02 (0.38–2.73)	0.96

Abbreviations: ART = antiretroviral therapy; BDI = Beck Depression Inventory; CI = confidence interval; CPE = CNS penetration effectiveness; EFV = efavirenz; GDS = Global Deficit Score; HAART = highly active antiretroviral therapy; OR = odds ratio.

[a] Hepatitis C virus, clinical AIDS, cardiac disease, cerebrovascular disease, hypertension, diabetes, cirrhosis, renal failure, CNS infection, seizures.

[b] Time between last documented HIV-negative date and first documented HIV-positive date, limited to the 181 participants with both.

[c] Current CD4 count − nadir CD4 count.

[d] Limited to participants who have received ART (excludes ART-naive participants).

[e] Taking into account starts and stops of regimens, calculated from documented HIV+ date to enrollment date.

regards to socioeconomic status, lifestyle, and other factors such as substance abuse.

Early events in HIV infection such as loss of vital CD4 reserves and uncontrolled HIV replication may trigger irreversible CNS damage and may cause the "residual" NCI seen in long-term survivors. Prospective studies are needed to determine if early diagnosis and initiation of antiretroviral therapy would reduce the burden of NCI among HIV-infected persons.[26] A recent study showed that patients with early HIV infection had similar neurocognitive functioning compared to HIV-uninfected persons, suggesting that detrimental effects of HIV on the brain may not occur immediately, potentially providing an opportunity for early intervention.[27] Clinical trials are underway, including a substudy of the Strategic Timing of Antiretroviral Treatment (START) trial, examining neurocognitive functioning among those treated immediately compared to later in their disease course.

We did not detect strong associations between immunologic or virologic control and the presence of NCI. A low CD4 nadir was not associated with NCI as seen in other studies[1,2,7,9] including a recent study which suggested that these factors may lead to structural brain damage.[28] Our lack of association may reflect that few of our patients experienced very low CD4 nadirs, or that CD4 nadirs are not predictive in persons who are managed early in infection and who avoid reaching very low counts ($<$200 cells/mm^3). Regarding current HIV counts, we noted a marginal association between higher current CD4 counts and CD4 recovery with NCI. Interestingly, when restricting our analyses to participants receiving HAART with a HIV RNA $<$50 copies/mL, these associations became stronger. We examined the association of PI use (which may result in higher CD4 counts, but has limited CNS penetration) and found no associations between specific antiretroviral class and NCI. These data suggest a possible immunologic component, such as immune reconstitution inflammatory syndrome–like reaction, in the pathogenesis of NCI; further studies are needed. Finally, we found no associations between HAART use and NCI. Although prior studies have shown that cognition improves shortly after HAART initiation,[5,29] our data suggest that NCI may persist despite ongoing HAART use, signifying that chronic neuronal inflammation and injury may continue. Since the benefit of HAART is incomplete,[30,31] strategies to prevent the initial development of NCI are paramount.

The prevalence of NCI among our HIV-uninfected persons was higher than expected with the estimated rate in the general population of 16%.[32] Although the reasons for this are unknown, it may have been due to self-selection bias as this group was enrolled from different settings (military bases) than the HIV-positive group (within HIV clinics). There were also differences in education and ethnicity, which may have contributed to the observed differences. The HIV-infected group had a reasonable proportion of individuals with higher degrees (e.g., Master, PhD, MD), whereas only one individual in our HIV-uninfected group had a higher degree. It may be that the more highly educated subjects in our HIV-infected group have greater levels of cognitive reserve, making declines due to HIV infection less likely. Moreover, our normative data adjust for African American and Caucasian ethnicities; the higher proportion of Hispanic and "other" ethnicities in our HIV-uninfected group may not have been appropriately corrected for among the HIV-uninfected group, leading to higher rates of NCI.

Our study had some limitations. We conducted a cross-sectional study, hence could not assess temporality or causation between factors of interest and the development of NCI. Furthermore, the low prevalence of NCI may have limited our ability to identify associated factors. We also evaluated a distinct population consisting of military members who may differ from other HIV-infected populations; however, our data provide important information about NCI in an optimized setting of early diagnosis, comprehensive medical care, stable socioeconomic factors, and few comorbidities (e.g., illicit drug use, HCV). Further, our data provide important information about the cognitive functioning of HIV-positive military personnel, and suggest that rather than disqualifying all seropositive members from performing certain occupations (e.g., aviators) due to concerns of NCI, it may be more prudent to perform neurocognitive testing in these groups.[33] Additionally, our study advocates for formal neurocognitive testing as self-reports of neurocognitive complications were more strongly related to depressed mood than cognitive functioning. Since we evaluated a US military population consisting of mostly men, our study cannot be generalized to women. Finally, the main objective of the study was to determine the prevalence of NCI among HIV-infected persons, and it was not specifically powered for comparisons to the HIV-uninfected arm.

HIV-infected persons diagnosed and managed early in infection have low rates of NCI, which are comparable to those of HIV-uninfected persons. Patients with longstanding HIV infection (median $>$10 years) had similar NCI rates compared to those with more recent infection, suggesting that early management and avoidance of comorbid conditions may be important in preserving cognitive function.

AUTHOR CONTRIBUTIONS

Nancy F. Crum-Cianflone, MD, MPH: study concept and design, acquisition of data, analysis and interpretation, critical revision of the manuscript for important intellectual content, and study supervision. David

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DISCLOSURE

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EXHIBIT 40

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Changing Clinical Phenotypes of HIV-Associated Neurocognitive Disorders

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Abstract

HIV-associated neurocognitive disorders (HAND) remains a common cause of cognitive impairment and persists in 15–55% of HIV+ individuals in the CART era. Combination antiretroviral therapy (CART) is now the primary treatment for HAND, but it is effective in only a subset of patients. In the pre-CART era, HIV-associated dementia was the most common form of HAND. However, in CART-treated patients the prevalence of HIV-associated dementia has declined substantially, and milder stages of HAND, *i.e.*, ANI and MND predominate. HIV+ patients with mild neurocognitive disorder (MND) can still have significant functional impairment in some activities of daily living. There have been several other significant changes in the clinical features of HAND in the CART era. The mean survival for an individual diagnosed with HIV dementia has increased dramatically. In HIV+ individuals on CART with a suppressed systemic viral load, the majority of individuals with HAND remain stable, with a small proportion showing deterioration. Extrapyramidal signs are now less common in patients with HAND on CART. In the CART era, HAND may have a mixed pattern of both cortical and subcortical features with greater deficits in executive functioning and working memory. Despite the milder clinical phenotype, in the CART era, patients with HAND still have persistent laboratory and neuroimaging abnormalities in the central nervous system even with systemic viral suppression. As the HIV+ patient population ages, cerebrovascular disease risk factors such as hypertension, diabetes, and hypercholesterolemia are increasingly recognized as risk factors for cognitive impairment in HIV+ patients on CART. HAND remains a common neurological condition globally in the CART era, necessitating the need for new animal models to examine pathogenesis and potential treatments for HAND.

Keywords

HIV; dementia; cognitive disorder

In the past 20 years, there have been several advancements in the treatment of human immunodeficiency virus (HIV) infection. Combination antiretroviral therapy (CART), introduced in 1996, can provide effective systemic suppression of HIV replication (1). The

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introduction of CART has resulted in a 50% decline in mortality rates associated with HIV infection, and CART has reduced the incidence of opportunistic infections associated with acquired immunodeficiency syndrome (AIDS). Another advancement in the management of HIV-seropositive (HIV+) patients is the ability to monitor the efficacy of CART with CD4+ helper T cells, ultrasensitive plasma HIV RNA levels, and antiretroviral resistance profiles which are now used commonly in clinic settings.

In addition, results from the Strategic Timing of Antiretroviral Therapy (START) trial (2) have proven the benefits and safety of early CART initiation on AIDS and non-AIDS related events in this international study of >4600 CART-naïve HIV+ individuals with CD4+ T cells counts > 500 cells/μL and HIV+ individuals with CD4+ T cells counts <350 cells/μL. CART is now begun as early as possible in an HIV+ patient, regardless of the CD4+ T cell counts.

The syndrome of HIV-associated neurocognitive disorders (HAND) was defined in 2007 with 3 stages of cognitive impairment: (1) asymptomatic neurocognitive impairment (ANI), (2) mild neurocognitive disorder (MND), and (3) HIV-associated dementia (3). ANI is defined by neuropsychological test performance that is at least 1 SD below the mean of demographically adjusted normative scores in at least two cognitive areas, but without impairment in everyday functioning. MND is defined by mild-to-moderate cognitive impairment with neuropsychological test performance that is at least 1 SD below the mean of demographically adjusted normative scores in at least two cognitive areas, and is associated with mild interference in daily functioning. HAD is defined by a moderate-severe cognitive impairment with neuropsychological test performance that is at least 2 SD below the mean of demographically adjusted normative scores in at least two cognitive areas, and is associated with marked interference with day-to-day functioning. The ANI category was added to this modified staging criteria by emphasize this mildest stage of cognitive impairment in CART-treated HIV+ patients (4).

CART is now the only option to prevent or delay progression of HAND, but it is effective in only a subset of patients. Indeed, in the START trial, early CART initiation did not have an effect on HAND (2). However, a recent study suggests that CART initiation very shortly after HIV acquisition results in greater improvement in HIV+ individuals' neurocognitive performance over time compared to deferred CART treatment 24 weeks later (5). HAND remains a common cause of cognitive impairment and persists in 15–55% of HIV+ individuals in the CART era (1). Over the past 20 years there has been little change in the overall prevalence of HAND (6). In the CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) cohort, HAND was present in 47% of the cohort, with ANI present in 33%, MND in 12%, and HIV-associated dementia in only 2% of the cohort. In the pre-CART era, HIV-associated dementia was the most common form of HAND. However, in CART-treated HIV+ patients the prevalence of HIV-associated dementia has declined substantially, and milder stages of HAND, *i.e.*, ANI and MND predominate (7).

Although HIV+ patients with the stage of ANI are asymptomatic, the stage may still be clinically relevant. In a CHARTER cohort study, HIV+ individuals who had a diagnosis of ANI at baseline were two to six times more likely to develop symptomatic HAND [(MND) and HIV-associated dementia] during several years of follow-up than those individuals who

were neurocognitively normal at baseline (8). However, confounding causes of cognitive impairment can contribute to the neuropsychological test impairment seen with ANI, so a diagnosis of ANI should be reserved for research studies, and not used in clinical settings.

In the past decade increasing attention is being given to HAND in resource limited settings. Almost 70% of the global HIV+ population resides in Sub-Saharan Africa, and multiple studies suggest that HIV+ individuals in this region have worse neurocognitive performance than HIV− individuals (1, 9–15). In a study among CART naïve HIV+ participants in Uganda 49% had HAND, with 20% of the cohort diagnosed with MND and 18% diagnosed with HIV-associated dementia (16). After two years on CART in this study from Uganda, 15% had HAND, with 10% of the cohort diagnosed with MND and only 2% with HIV-associated dementia. In another study of CART-naïve HIV+ individuals from South Africa, 42% of the cohort had MND and 25% had HIV-associated dementia. If these proportions are seen throughout resource-limited countries in the world, than HAND would likely be the most common form of neurocognitive impairment in young adults worldwide (1).

In the CART era, there have been significant changes in the clinical features of HAND. With respect to survival, the mean survival for an individual diagnosed with HIV-associated dementia in 1993–1995 was five months, but the mean survival for an individual diagnosed with HIV-associated dementia in 1996–2000 was 38.5 months (17). HIV+ patients diagnosed with HAND on current CART regimens have a near normal lifespan. However, it is not a full lifespan, as a population based study of 1602 HIV+ individuals demonstrated an approximately 3 fold increased mortality risk among individuals with HAND compared to individuals without HAND (18).

The temporal progression of HAND has also changed in the CART era. Prior to the use of CART, HIV-associated dementia was a rapidly progressive dementia. In HIV+ individuals on CART with a suppressed systemic viral load, the majority of individuals with HAND remain stable. In a four year study of 197 HIV+ participants receiving CART, 77% remained neurocognitively stable, 13% deteriorated, and 10% improved their neurocognitive performance (4).

Clinical features of HAND have also changed in the CART era. Extrapyramidal signs such as bradykinesia, rigidity, and tremor which were common in the pre-CART era in patients with HIV-associated dementia, are now less common in patients with HAND on CART.

The neuropsychological profile of HAND may also be different in CART-treated patients. The overall severity of cognitive impairment is milder, in HIV+ patients on CART. Also, the overall neuropsychological test profile may be different as well. In the pre-CART era, HAND was characterized as a subcortical disorder characterized by psychomotor slowing, motor dysfunction, and memory impairment. In the CART era, HAND may have a mixed pattern of both cortical and subcortical features with greater deficits in executive functioning and working memory (7).

Risk factors for HAND have also changed in the pre-CART era. Low CD4+ T cell count, high plasma and cerebrospinal fluid HIV RNA levels prior to antiretroviral therapy, HIV-related medical symptoms (*e.g.* anemia, low weight, and fatigue), and the presence of

extrapyramidal signs on neurological examination, depression, and psychomotor slowing on neuropsychological testing were all associated with an increased risk for dementia in HIV+ patients (19–21). In CART-treated individuals, many of these risk factors are no longer associated with HAND, as patients on CART have less immunosuppression and commonly have suppressed viral replication in both plasma and cerebrospinal fluid. Risk factors for HAND in the CART era include advanced age, a low CD4 nadir, cardiovascular risk factors, use of illicit drugs (*e.g.*, methamphetamine), sleep disorders as well as psychiatric comorbidly including depression and anxiety (1).

Despite the milder clinical phenotype, in the CART era, patients with HAND still have persistent abnormalities in the central nervous system even with systemic viral suppression. Biomarkers for HAND which remain elevated in CART-treated patients include cerebrospinal fluid (CSF) markers of inflammation such as neopterin (22), and markers of active axonal injury such as neurofilament protein light chain (23). Additional laboratory biomarkers of HAND currently under evaluation include markers of neuronal injury (*e.g.*, CSF quinolinic acid), immune activation (*e.g.*, plasma sCD14 and sCD163, CSF fractalkine, osteopontin and MCP-1), oxidative stress (*e.g.*, CSF ceramide, sphingomyelin, protein carbonyls), and energy metabolism (*e.g.*, CSF Krebs cycle substrates) (1, 24–29).

In addition, neuroimaging markers demonstrate both caudate/putamen atrophy and cortical atrophy in HIV+ individuals with suppressed viral load (30, 31). These structural changes in the brain indicate ongoing CNS injury in HIV+ patients despite systemic virological suppression of the HIV virus.

Autopsy studies of HAND in the CART era suggest that HIV encephalitis and neuronal loss do not explain the cognitive impairment seen in HIV+ patients on CART. In one study, the frequency of HIV encephalitis at autopsy was reduced from 54% in the pre-CART era to 15% in the CART era. The pathogenesis of HAND is associated with macrophage and microglial activation, increased cytokines, chemokines, glutamate, and neurotoxic viral proteins, and bioenergetics disturbance causing functional alterations in neurons rather than neuronal death (1). Additional discussion of the neuropathology of HAND can be found in the accompanying article by Dr. Benjamin Gelman.

An important demographic change in the CART era is the aging of the HIV+ population (32). As of 2015, more than one-half of all HIV-infected individuals in the United States are greater than 50 years old (33). In a study of 202 HIV+ adults in the Hawaii Aging with HIV-1 Cohort, older HIV+ individuals greater than age 50 years are twice as likely to have HIV-associated dementia compared to younger HIV+ individuals in an age range of 20 to 39 years (25). In the CHARTER study, age and HIV infection may have synergistic effects leading to worse performance on a summary measure of neuropsychological testing than either factor alone (33, 34).

Cerebrovascular disease risk factors such as hypertension, diabetes, and hypercholesterolemia are increasingly recognized as risk factors for cognitive impairment in HIV+ patients on CART. In the Multicenter AIDS Cohort Study (MACS) cohort, hyperglycemia and increased carotid intima media thickness as measured by carotid

ultrasound, a measure of large vessel atherosclerotic disease were both predictors of poor psychomotor speed performance among older HIV+ individuals (35). In another study, HIV + patients with pre-existing cardiovascular disease risk factors had a 6.2 fold higher odds of cognitive impairment compared to HIV+ patients without cardiovascular risk factors (36).

Neurodegenerative diseases such as Alzheimer's disease is another potential cause of cognitive impairment in patients with HIV infection. One autopsy study suggests that amyloid plaques are more frequently seen in HIV+ cases compared to age-matched HIV− control brains among subjects between age 30 and 59 years (37). Amyloid deposition also can be detected using position emission tomography (PET) radioligands, and studies of increased cortical uptake using the [^{18}F] AV-45 ligand in HIV+ individuals have been described (38). However, additional studies are needed to evaluate the association of amyloid deposition and cognitive impairment among older HIV+ individuals.

In summary, HAND remains a common neurological condition globally in the CART era. HIV dementia is rare in HIV+ patients with suppressed viral replication, and less severe forms of HAND predominate. The risk of HAND increases with age and when an HIV+ individual has cardiovascular risk factors. Latent HIV persists in the brain even when systemic virological control is achieved with CART, thereby hampering efforts to eradicate HIV, with the brain serving as a potential reservoir for the virus. Although many biomarkers have been evaluated, an easily obtainable, validated surrogate marker for HAND has not entered clinical practice in the CART era. The primary treatment for HAND remains CART, though trials for adjunctive therapies are ongoing.

Because of the changing phenotype of HAND, new animal models are needed to examine the pathogenesis and potential treatment for HAND. The remainder of this issue will describe some of these novel animal models for HAND.

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EXHIBIT 41

HIV-associated neurocognitive disorders: Epidemiology, clinical manifestations, and diagnosis

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All topics are updated as new evidence becomes available and our peer review process is complete.

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INTRODUCTION

Changes in memory, concentration, attention, and motor skills are common in HIV-infected patients and present a diagnostic challenge to the clinician [1]. Since these symptoms can be caused by a variety of disorders, accurate diagnosis is critical for patient treatment [2]. When not clearly attributable to an alternate cause other than HIV infection, such impairments have been collectively classified as HIV-associated neurocognitive disorders (HAND).

The epidemiology, clinical manifestations, and diagnosis of HIV-associated neurocognitive disorders will be discussed here. Management of HIV-associated neurocognitive disorders are discussed elsewhere. (See "HIV-associated neurocognitive disorders: Management".)

An overview of the range of neuropsychiatric conditions associated with HIV infection and more detailed reviews of other specific conditions are discussed separately. (See "Overview of the neuropsychiatric aspects of HIV infection and AIDS" and "Depression, mania, and schizophrenia in HIV-infected patients" and "Substance abuse and addiction in HIV-infected patients".)

TERMINOLOGY

The presence of neurocognitive deficits in certain HIV-infected individuals without alternative explanation other than HIV infection has long been recognized. However, the terminology to refer to this phenomenon has undergone substantial evolution since its initial characterization. In order to assist in diagnosis and categorization for research and clinical purposes, a working group supported by the United States National Institutes of Health published a classification scheme in 2007 that was initially proposed by the HIV Neurobehavioral Research Center at the University of California, San Diego [3]. This classification, often referred to as the "Frascati criteria," has been widely, but not universally, adopted [4]. It includes three levels of impaired neuropsychological test performance and functional impairment within an umbrella term, HIV-associated neurocognitive disorders (HAND):

- Asymptomatic neurocognitive impairment (ANI) – defined by a score of one standard deviation or more below the mean in at least two cognitive domains on standardized neuropsychological testing without a symptomatic or observable functional impairment.
- Mild neurocognitive disorder (MND) – defined by a score of one standard deviation or more below the mean in at least two cognitive domains on standardized neuropsychological testing with at least mild symptomatic or functional impairment.
- HIV-associated dementia (HAD) – defined by a score of two standard deviations or more below the mean in at least two cognitive domains on standardized neuropsychological testing with concomitant impairment in activities of daily living.

The definitions are applied only when the observed impairment cannot be explained by other conditions, either alternative neurological diagnoses (such as opportunistic infection, stroke, or metabolic or toxic encephalopathy) or underlying "confounding" comorbidities that might alter neuropsychological test performance (such as severe substance abuse, prior head trauma, or severe psychiatric disease).

These diagnostic categories were developed to apply a common set of criteria for research studies and, in the strict sense, they should not be used in the clinical setting without formal neuropsychological testing. However, because of the functional impairment associated with HAD, it is usually possible to apply this term to the severe form. The two milder forms of

HAND require this testing for characterization, and, in particular, ANI cannot be diagnosed without formal testing.

HAD has also been previously referred to as AIDS dementia complex, HIV encephalitis, or HIV encephalopathy, all terms that referred to a circumscribed, often subacute syndrome of progressive cognitive and motor dysfunction. (See 'HIV-associated dementia' below.)

PATHOPHYSIOLOGY

HIV disseminates to the central nervous system (CNS) during the initial days of systemic infection and can be detected in the cerebrospinal fluid (CSF) of most untreated patients thereafter [5-7]. However, the character of CSF infection changes over the course of infection and disease evolution. Initially, CSF viruses are genetically identical to those in blood and likely originate from trafficking CD4 cells. Later, CNS infection can become "compartmentalized," with the virus evolving independently from the virus found in blood. Additionally, the cell tropism of the CNS virus may change to become largely macrophage-tropic (M-tropic), in contrast to blood virus which characteristically maintains tropism for T-lymphocytes.

Whether viruses with M-tropism originate within the CNS or are introduced by infected monocytes is uncertain. However, macrophages and microglia are clearly critical to the switch from seemingly benign meningeal infection involving mainly T-lymphocytes to a more invasive encephalitis. Perivascular macrophages and related cells sustain compartmentalized brain infection and, whether infected or not, serve as important sources of the toxic signaling pathways involved in brain dysfunction that underlie HIV-associated dementia (HAD) and perhaps milder forms of HIV-neurocognitive disorders [8].

While HIV also appears to infect astrocytes [9], this is usually nonproductive (ie, does not propagate infection), and its pathogenic significance remains uncertain. There is little evidence that HIV infects neurons or oligodendrocytes. Thus, alteration of the metabolism or death of such cells occurs by "indirect" mechanisms, through the aforementioned toxic signaling pathways, which likely involve both viral and cellular molecules [10].

Autopsy studies of AIDS patients with HAD show characteristic white matter pallor, microglial nodules, multinucleated giant cells, and perivascular infiltrates [11-13]. Basal ganglia and nigrostriatal structures can be affected early in the course of the dementia, with

subsequent diffuse neuronal loss resulting in up to 40 percent reduction in frontal and temporal neurons. More subtle structural brain changes (eg, decreased cortical grey matter volume) may be detected even within the first year of HIV infection in the absence of overt encephalitis more clearly associated with HAD [14].

Although antiretroviral therapy (ART) reduces HIV RNA in CSF, a substantial proportion of patients continue to show biomarker evidence of mild immune activation within the CNS even after years of durable viral suppression [15]. The pathophysiologic mechanisms that drive this persistent inflammatory response are unknown, although persistent CNS infection below levels detected in CSF is one possible explanation [16-20].

Other comorbidities that are common in HIV-infected patients may also play a pathogenic role in the development of neurocognitive impairment. (See 'Comorbidities' below.)

EPIDEMIOLOGY

Prevalence in the ART era — The widespread use of suppressive combination antiretroviral therapy (ART) has been associated with a marked decrease in the incidence of more severe neurocognitive deficits (ie, HIV-associated dementia [HAD]) [21]. Data from 15,380 HIV-infected patients followed in the CASCADE cohort (Concerted Action on Seroconversion to AIDS and Death in Europe) demonstrated a decrease in the incidence of HAD from 6.49 per 1000 person-years in the pre-ART era to 0.66 per 1000 person-years by 2003 to 2006 [22]. Similarly, a Danish population study reported that the incidence of severe neurological deficits in those with HIV infection was approaching that of the uninfected population [23].

In contrast to the major impact of ART on the incidence of HAD, a number of reports document a continued, substantial prevalence of milder impairment on testing in the setting of HIV infection (ranging from 20 to 69 percent in various series), even among patients with viral suppression [24-31]. In a study of 1521 HIV-infected patients from the eras before and after combination ART, neurocognitive impairment of any type was seen slightly more frequently in the post-ART compared with pre-ART cohorts (40 versus 33 percent, respectively) [32]. In a separate analysis of the same post-ART cohort, the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) study, asymptomatic neurocognitive impairment (ANI) was most common, occurring in 33 percent, with mild neurocognitive

disorder in 12 percent and HAD in only 2 percent of 1316 HIV-infected patients (the majority were on ART) [28].

The pattern of neuropsychological impairment seen in HIV-infected patients may also be affected by the use of ART. Whereas early reports of HAD emphasized the "subcortical" character of the dementia associated with HIV, with prominent slowing of processing and difficulty with attention and concentration, more recent observations have suggested a more "cortical" pattern of deficits [33]. In a smaller study of HIV-infected patients, the prevalence of neurocognitive deficits was similar among 51 patients treated with single-drug ART and 90 patients treated with combination ART [34]. However, the patterns of deficits differed: the use of combination ART specifically was associated with improvement in attention, verbal fluency, and visuoconstruction defects, but deterioration in learning efficiency and complex attention.

A critical issue is whether milder cognitive impairment in virally suppressed patients is a residual effect of earlier, possibly subclinical brain injury that developed before treatment was started or whether brain injury can continue despite effective viral suppression. This issue is not yet fully resolved. Additionally, the frequency or degree to which the cognitive impairments in treated patients can be attributed to HIV infection itself is unclear. Other unrecognized co-morbidities that may contribute include substance use, medical morbidities, and the effects of aging that may advance more rapidly in those with HIV infection than those without. Finally, there is concern that ART itself may have chronic toxic effects on the brain and contribute to the impairments in test performance [35]. A study that followed a subset of patients from the CHARTER cohort with repeated neuropsychological testing over a mean of three years noted a stable neurocognitive status in 61 percent, improvement in 17 percent, and worsening in 23 percent [30]. The factors associated with neurocognitive deterioration were complex and related to HIV disease and treatment status, but also involved other comorbid conditions.

Compared with the uninfected — Neurocognitive deficits are reported to be more common in HIV-infected individuals, including those using or not using antiretroviral therapy (ART), than in those without HIV infection in many [32,36,37], but not all [24,38], studies. One large study compared the prevalence of neurocognitive impairment as measured by comprehensive neuropsychological testing among several cohorts of HIV-infected (n = 1521) and uninfected patients (n = 273) from the eras before and after the introduction of combination ART (1988 to 1995 and 1999 to 2004, respectively) [32]. The prevalence of any

impairment was 16 to 19 percent among HIV-uninfected patients, but ranged from 25 to 52 percent among HIV-infected individuals, depending on the era (pre- or post-ART) and disease state. In a prospective cohort study from China, 53 of 192 HIV-infected patients (over half of whom had clinical AIDS and were on ART) developed cognitive decline after 12 months of follow-up based upon serial neuropsychological testing, compared with only 5 of 101 HIV-uninfected controls [36]. In contrast, in a subsequent study of 200 HIV-infected patients, among whom the median CD4 cell count was 546 cells/microL and HIV viral suppression was achieved in 55 percent, the rate of cognitive impairment was comparable to that observed in 50 HIV-uninfected controls [24]. Similarly, in a study of over 1000 women, the effect of HIV status on cognition was less than that of years of education, age, race, income, and reading level [39].

Risk factors — Risk factors for the development of HIV-associated neurocognitive disorders (HAND) include HIV disease factors, other comorbidities, and possibly host genetic factors.

HIV disease factors — In several studies, lower nadir CD4 cell count has been associated with an increased risk of neurocognitive impairment among HIV-infected patients on ART [26,28,40]. In the CASCADE cohort of 15,380 HIV-infected patients followed longitudinally, risk factors for the development of HAD specifically included lower CD4 cell counts, older age at seroconversion, duration of HIV infection, and the presence of a prior AIDS-defining diagnosis [22]. A period of severe immunosuppression appears to confer a long-lasting impact on neuropsychiatric performance regardless of subsequent viral suppression and immune reconstitution.

The underlying reasons for this are not known. There is possibly a higher incidence of HIV-associated brain injury in those with more advanced immunosuppression, which leaves residual impairment and a legacy of diminished "brain reserve" [7]. Alternatively, advanced infection and immune injury may initiate a neuropathic process that continues even after treatment and CD4 cell restoration, regardless of ongoing viral replication or gene expression within the CNS. Evolution of compartmentalized infection with macrophage tropism, which is more common in advanced infection, may predispose to persistent CNS infection [41].

Comorbidities — HIV-infected adults older than 50 years have an increased prevalence of neurocognitive deficits and dementia compared with patients younger than 40 years [42-

44]. However, whether increased age affects cognitive function in HIV-infected patients to a greater extent than in HIV-uninfected patients is unclear. In one study, age greater than 50 years was associated with similarly decreased performance on neuropsychological testing among 115 HIV-infected and 30 uninfected individuals [45] (see "HIV infection in older adults"). Furthermore, while it has been speculated that brain aging accelerates in those with HIV infection, the pathological basis of this is not clear. HIV-infected patients have not yet been shown to have a higher incidence of Alzheimer's disease, and the CSF and imaging findings characteristic of Alzheimer's disease are not found in HAD patients [46-48].

Other general comorbidities that have been associated with HAND include anemia, vascular disease, and metabolic abnormalities (including increased waist circumference and insulin resistance), particularly in older adults [49-55]. Coinfection with hepatitis C virus (HCV) has also been identified as a possible, but not universally identified [56], risk factor for greater neurocognitive deficits in the setting of HIV infection, possibly because of independent neurotoxic effects of HCV [57-59]. As an example, HCV RNA as well as nonstructural and core proteins have been detected in cerebrospinal fluid and brain tissue; HCV may traffic into the CNS via macrophages and infect microglia and astrocytes, and HCV core protein may cause neuronal damage through the production of proinflammatory cytokines [60-65]. Prior infection with toxoplasma has also been associated with neurocognitive deficits [66].

Host genetic factors — Polymorphisms in several genes, including those encoding apolipoprotein E4, the chemokine receptor CCR2, and monocyte chemoattractant protein-1, have been associated with the presence or development of HAD [67-69]. However, a genome-wide association study that involved 1287 patients from the Multicenter AIDS Cohort Study did not detect an association between those or any other polymorphisms and HAND or HAD [70].

CLINICAL FEATURES

Spectrum of findings by ART status — Antiretroviral treatment (ART) status impacts the presentation of HIV-associated neurocognitive disorders (HAND). The most severe form, HIV-associated dementia (HAD), typically occurs in patients with advanced, untreated HIV infection, with CD4 cell counts <200 cells/microL and high plasma viral levels. This is much less likely to occur in patients on ART, among whom it is more common to find milder deficits that are residual or develop more indolently. Rarely, successfully treated patients

can present with a "CNS escape syndrome," in which deficits as severe and subacutely progressive as those seen in HAD develop with detectable HIV in the CSF despite plasma viral suppression on antiretroviral therapy (ART).

The following sections discuss the clinical findings of these syndromes in more detail.

HIV-associated dementia — HIV-associated dementia (HAD), in its classic form, is primarily characterized by subcortical dysfunction, with attention-concentration impairment, depressive symptoms, and impaired psychomotor speed and precision; this is consistent with pathology suggesting that HIV affects predominantly subcortical and deep grey matter structures [71,72]. It occurs predominantly in untreated patients with advanced HIV infection. The onset of the impairments is typically subacute. Some emphasize that the deficits associated with HAND may wax and wane over time [73], unlike the progressive neurological decline seen in other neurodegenerative diseases, such as Alzheimer's disease.

Cognitive deficits — Prominent features of HAD include substantial memory deficits, impaired executive functioning, poor attention and concentration, mental slowing, and apathy [74,75]. Typically, cognitive deficits are evident and clearly impair functional status. Patients with HAD are often too slow and forgetful to work or prepare meals and can get lost while walking or driving [76]. Judgement, however, frequently remains relatively intact.

The absence of higher cortical dysfunction including aphasia, agnosia, and apraxia help distinguish HAD from classical "cortical" dementia, such as Alzheimer's disease. However, the distinction between cortical and subcortical dementias can be blurred as a patient with late and severe HAD may have dysfunction in both language and praxis.

Behavioral and mood changes — Behavioral changes in HAD are commonly characterized by apathy and lack of motivation [76]. Patients with HAD may also display irritable mood, sleeplessness, weight loss, restlessness, and anxiety. Although these changes may be attributed to depression, patients with HAD are not typically dysphoric, and lack crying spells or reported sadness.

However, mood-changes associated with HAD may progress to psychosis with paranoid ideas and hallucinations. Furthermore, a small proportion of patients with HAD may develop mania. (See "Depression, mania, and schizophrenia in HIV-infected patients".)

Motor symptoms and signs — Most patients with frank HAD exhibit slowness of movement (tested by rapid finger opposition or toe tapping). In addition, patients can experience impaired saccadic eye movements, marked difficulty with smooth limb movement (especially in the lower extremities), dysdiadochokinesia, hyperreflexia, and frontal release signs such as grasp, root, snout, and glabellar reflexes.

Imaging findings — In patients with HAD, cerebral atrophy is typically evident on brain imaging [77,78]. It affects mainly the basal ganglia (particularly the caudate) and white matter, but also cortical regions. On magnetic resonance imaging (MRI), T2-weighted images also demonstrate diffuse or patchy white matter hyperintensity (image 1), which may correlate neuropathologically with high levels of HIV in those regions of the brain [79].

More advanced neuroimaging techniques, such as magnetic resonance spectroscopy, functional MRI, and positron emission tomography (PET), also demonstrate abnormalities in the subcortical regions, in some cases even in patients with more mild neurocognitive deficits [46,77,80,81].

CSF findings — In observational studies, patients with HAD who are not on antiretroviral therapy often have elevated CSF protein and elevated HIV levels in the CSF. Lymphocytic pleocytosis in the CSF is variable. However, these findings are not specific to patients with HAD and are observed in many untreated HIV-infected patients without neurocognitive impairment. As an example, in a cross-sectional study that included 46 untreated HIV-infected patients, the mean CSF viral level in this population was 3.6 log copies/mL, and the CSF WBC count ranged from 0 to 11 [82]. There was no association between the presence of neurological deficits and the CSF HIV level.

Additionally, in untreated patients with HAD, the drug resistance profile of the CSF virus may differ from that of the plasma virus, although there has been no direct evidence that such differences are clinically important [83].

Of note, testing of HIV viral levels and genotype in the CSF is not widely available, is not diagnostically informative in most cases of HAD, and is not routinely performed. (See 'Severe deficits, not on ART' below.)

Milder neurocognitive deficits — In the full spectrum of HAND, the cognitive deficits reported are more diverse and variable than those initially reported in HAD [84]. Successfully treated patients typically present with milder cognitive impairments.

The main cognitive deficits reported in milder presentations of HAND include difficulty with attention and working memory, executive functioning (eg, complex problem solving), and speed of informational processing [3,77]. The majority of HIV-infected patients who have evidence of such neurocognitive defects on testing actually have no evident symptoms or impairment in functioning (ie, asymptomatic neurocognitive impairment [ANI]). For those with mild symptomatic disease (ie, mild neurocognitive disorder [MND]), these deficits may manifest as difficulty with reading, performing complex tasks, or maintaining concentration in conversations or activities. Such symptoms may be subtle and overlooked or attributed to fatigue or other illness. Early language deficits are uncommon [85].

The onset and time course of cognitive deficits in milder forms of HAND is generally more indolent than the typically subacute presentation of HAD, and deficits may remain stable or seemingly unchanged for years [76].

Motor problems are not common in milder cases of HAND. Early motor symptoms can include unsteady gait, slowed or clumsy fine hand coordination (eg, a change in handwriting), or tremor [85]. Individuals may notice difficulties in performing activities that depend on fine coordination or rapid movements. On exam, rapid movement and gait may be slowed.

Affective disturbance is frequently associated with HAND [86]. Early manifestations may include apathy, lethargy, loss of sexual drive, and diminished emotional responsiveness. HAND-associated mood disturbances are frequently distinguished by lack of crying spells or reported sadness, in contrast to patients with major depressive disorder.

Some patients with mild neurocognitive deficits have brain imaging findings similar to those of HAD, with cerebral atrophy or white matter abnormalities; others have normal imaging. Thus, imaging findings cannot distinguish these patients. Likewise, routine CSF studies do not distinguish them from HIV-infected patients without neurocognitive impairment. (See 'Imaging findings' above and 'CSF findings' above.)

Although some patients with mild neurocognitive deficits may have imaging findings characteristic of HAD (see 'Imaging findings' above), many have normal neuroimaging.

CNS viral escape syndrome — Several studies have described an uncommon condition in ART-treated patients who present with severe new-onset neurological deficits and exhibit "CNS viral escape," in which there is evidence of CNS HIV replication in the CSF despite

low viral levels or viral suppression in the plasma [87-90]. In most, there is also documented drug resistance in the CSF virus.

As examples, two retrospective studies described 10 and 11 patients, respectively, who developed new neurocognitive symptoms without alternate diagnosis and had detectable CSF HIV RNA (range 558 to 12885 and 134 to 9056 copies/mL) despite stable ART and virological control in the plasma (at least <500 copies/mL, most had <50 copies/mL) [87,89]. The clinical presentations were heterogeneous, with some having a HAD-like presentation and others with more focal neurological manifestations accompanied by multifocal MRI lesions. CSF pleocytosis was present in most. A predominance of CD8 pleocytosis has also been described in a few patients with potential CNS escape syndrome [90]. Viral drug resistance mutations were identified in the majority of cases in which genotyping of the CSF virus was performed, suggesting "compartmentalization" of resistant virus within the CSF. In this isolated clinical setting, assessment of the level of CSF HIV RNA for diagnosis and drug resistance for selecting changed treatment can be valuable in clinical management. (See 'Severe deficits, on ART' below.)

This syndrome of symptomatic CNS escape in a previously asymptomatic or minimally symptomatic patient, as described in these cases, is rare. It should be clearly distinguished from asymptomatic, minor elevations of CSF HIV RNA levels despite plasma viral suppression that have been reported in several studies. As an example, in a study of 849 HIV-infected patients with viral suppression on ART who were followed for a median of 30 months and underwent serial lumbar puncture testing, detectable CSF HIV RNA was observed during 88 visits (2.7 percent) in 60 patients (7.1 percent) [91]. Detectable CSF HIV RNA on two consecutive visits was observed in only nine patients (1 percent), and there was no association with detectable CSF HIV RNA and neurocognitive performance. Previous smaller retrospective series, generally consisting of less than 100 patients with plasma virological suppression, described detectable CNS HIV RNA >50 copies/mL in 5 to 23 percent [88,89,92]. There were no clear associations with detectable CSF HIV and neurocognitive function in those studies either, although there was an association with mild elevations of CSF neopterin, a marker of macrophage activation.

SCREENING FOR DEFICITS

For patients who do not report or have evident neurocognitive deficits, screening may be able to identify mild deficits. However, the value of broadly screening HIV-infected patients for neurocognitive impairment is controversial. Arguments in its favor include the potential value of identifying deficits that might affect medication adherence. However, the speculation that patients identified by neurocognitive impairment screening might benefit from use of antiretroviral regimens that optimally penetrate the central nervous system has not been supported by clinical trial evidence [93]. Other arguments against the practice include its consumption of precious resources in the busy clinical setting. Given limited resources and the absence of clear evidence to support changing management on the basis of mild deficits, we do not routinely screen for such deficits. However, when resources allow, screening is reasonable to establish a baseline assessment of a patient's neurocognitive function in case there is subsequent deterioration.

Screening for deficits can be done by inquiring about symptoms and/or performing brief neurocognitive tests in the clinic. Some experts have suggested the following series of questions [25,94]:

- Do you experience frequent memory loss (eg, do you forget the occurrence of special events, even the more recent ones, appointments, etc.)?
- Do you feel that you are slower when reasoning, planning activities, or solving problems?
- Do you have difficulties paying attention (eg, to a conversation, a book, or a movie)?

For each question, patients can answer "never," "hardly ever," or "yes, definitely." A "yes, definitely" answer to any of the three questions can prompt further evaluation. While a symptom-based approach would overlook the subset of patients with asymptomatic neurocognitive impairment (ANI), it may be the easiest for clinicians to implement.

Additional screening tools for neurocognitive function have been evaluated in HIV-infected patients. The Montreal Cognitive Assessment (MoCA) can be performed in the office in less than 10 minutes and may be the most practical bedside test for periodic assessment [95-99]. The CogState-based assessment has reasonably good performance but is not publicly available [100]. The HIV Dementia Scale and the International Dementia Scale may not be highly sensitive for mild cognitive impairments [101-103]. Additionally, the mini-mental status examination does not thoroughly test executive function and is an insensitive tool for detection of HAND [101,104].

Some experts have recommended a screening interval of 6 to 24 months, depending on the presence of risk factors for HAND (see 'Risk factors' above) [105,106]. As an alternative, an approach that involves regularly screening every patient presenting for HIV care (such as having them complete a self-report screen in the waiting room), if feasible, allows the clinician to focus on determining next steps for those who screen positive instead of trying to decide whom and how often to screen.

EVALUATION

The possibility of an HIV-associated neurocognitive disorder (HAND) is suspected in HIV-infected patients who present with symptomatic cognitive deficits or are found to have deficits on screening tests. The diagnosis requires evaluation for pre-existing or alternative disorders that could account for the observed cognitive impairment, including other neurologic or neurodegenerative disorders, primary psychiatric disorders (eg, major depression, schizophrenia), and severe substance use disorders. Depending on the presentation, excluding alternate conditions may involve a number of laboratory tests and potentially neuroimaging (MRI) followed by cerebrospinal fluid (CSF) analysis.

Because of the frequency with which comorbid conditions exist in those with HAND, the assessment for persistent cognitive deficits should be made when pre-existing conditions are under optimal control. The differential diagnosis of cognitive impairment in HIV-infected patients is discussed in further detail below. (See 'Differential diagnosis' below.)

Initial assessment — The goal of the initial assessment is to characterize the degree, time course, and functional impact of any cognitive deficits, establish the stage of HIV disease and treatment status, and start to evaluate for other potential causes of cognitive symptoms.

Characterization of the cognitive impairment — Characterizing cognitive deficits begins with a history from the patient and, if possible, family members or someone who knows the patient well. History should assess the domains affected by the cognitive impairment (eg, learning and memory, language, executive function), any accompanying behavioral or personality changes, the time course of symptoms, and particularly, the degree that any of these difficulties lead to functional impairment in common activities and in work. (See 'Cognitive deficits' above and 'Milder neurocognitive deficits' above.)

For patients who present with subacute or progressive symptoms typical of HIV-associated dementia (HAD), cognitive impairment that leads to functional deterioration is usually evident on such history and confirmed on bedside cognitive testing (see "Evaluation of cognitive impairment and dementia", section on 'Cognitive testing'). In such cases, formal neuropsychological testing is superfluous.

When the history is less clear and suggests a more static impairment or if impairments are only detected on screening tests, formal neuropsychological testing can be useful for establishing both the magnitude and profile of impairment. If performed, neuropsychological testing usually evaluates at least five of the following cognitive abilities [3]:

- Verbal/language
- Attention/working memory
- Abstraction/executive functioning
- Learning/recall
- Speed of informational processing

Additional history and physical exam — In addition to elucidating the extent and impact of cognitive impairment, history should evaluate for other potential causes of neurological impairment, including prior neurologic insults (eg, traumatic brain injury, vascular insults, other CNS infections) and major depressive, anxiety, and substance use disorders.

Both a general physical exam to evaluate for signs of alternate causes of cognitive impairment (eg, physical findings suggestive of thyroid or liver disease) and a dedicated neurologic exam should be performed. Findings of psychomotor slowness and hyperreflexia are suggestive of HAD. Other neurological findings could be suggestive of other causes of cognitive decline, such as rigidity and tremors in Parkinson's disease and a focal deficit in stroke or other focal brain lesions. Patients with HAND are characteristically alert; altered consciousness would be suggestive of alternative conditions. (See 'Cognitive deficits' above and 'Behavioral and mood changes' above and 'Motor symptoms and signs' above.)

Laboratory testing — Assessment of the stage of HIV infection (ie, with CD4 cell count and plasma viral load) is an important element in the evaluation of HAND. The CD4 cell count informs the likelihood that progressive cognitive impairment is due to HAD, which occurs predominantly (though not exclusively) at counts <200 cells/microL in untreated patients. It also informs the differential diagnosis, as opportunistic infections that could

cause cognitive impairment are more likely at lower CD4 cell counts. (See 'Differential diagnosis' below.)

Other laboratory testing that should be performed to evaluate for other causes of cognitive declines include a complete metabolic panel that includes liver function tests and glucose, vitamin B12 level, folate level, thyroid stimulating hormone, and serologic testing for syphilis and hepatitis C virus. (See "Evaluation of cognitive impairment and dementia", section on 'Laboratory testing' and "Neurosyphilis", section on 'Diagnosis' and "Diagnosis and evaluation of chronic hepatitis C virus infection", section on 'Diagnosis and testing approach'.)

Further evaluation — The focus of further diagnostic evaluation depends on the severity of the presentation and whether the patient is on ART, as outlined below.

Severe deficits, not on ART — HAD develops almost exclusively in untreated HIV-infected patients, particularly when the CD4 cell count is <200 cells/microL. Thus, additional diagnostic evaluation usually focuses on ruling out other opportunistic processes that such patients are at risk for and that can present with neurocognitive decline.

Neuroimaging with magnetic resonance imaging (MRI) is typically performed first to evaluate for other neurological disorders (infection, neoplasm, infarction, leukoencephalopathy) that can occur in untreated HIV-infected patients. Although these disorders are often suspected on the basis of history or exam, these disorders may sometimes present with predominantly cognitive impairment and no other localizing findings (see "Approach to HIV-infected patients with central nervous system lesions"). Diffuse cerebral atrophy and subcortical or periventricular white-matter changes (hypodense on computed tomography and bright on T2-weighted MRI) are consistent with HAD, but are neither sensitive nor specific. (See 'Imaging findings' above.)

Similarly, performance of other tests, including lumbar puncture for CSF evaluation, is done mainly to assess for other possible diagnoses. Such testing is guided by the clinical presentation and imaging findings:

- In patients with focal clinical or imaging features, toxoplasma blood serology (to assess susceptibility), CSF JC virus PCR (for diagnosis of progressive multifocal leukoencephalopathy), CSF EBV PCR (for primary CNS lymphoma), and CSF CMV

PCR may be useful. (See "Approach to HIV-infected patients with central nervous system lesions", section on 'CSF examination'.)

- In patients with a nonfocal presentation, CSF syphilis serology, CSF cryptococcal antigen, and CSF CMV PCR are usually assessed.

CSF findings in HAD can include elevated CSF protein and elevated ratio of CSF to blood albumin, but these routine assessments do not distinguish these patients from those without HAD. CSF HIV RNA levels in HAD also do not distinguish HAD patients. (See 'CSF findings' above.)

Severe deficits, on ART — In patients who are on ART, particularly if viremia is suppressed and the CD4 cell count has rebounded, neurological signs and symptoms are more likely due to non-HIV-related processes, such as cerebrovascular or neurodegenerative disease. Thus, the evaluation of such patients focuses on other non-HIV-related causes of neurocognitive decline, as would be done for uninfected individuals with cognitive decline. MRI and lumbar puncture are typically included in the evaluation. This is discussed in detail elsewhere. (See "Early-onset dementia in adults", section on 'Initial evaluation' and "Early-onset dementia in adults", section on 'Additional testing'.).

If no other causes are identified, there is the rare possibility that CNS escape syndrome underlies the cognitive decline. If this is suspected, CSF HIV RNA should also be evaluated, as CNS escape is characterized by detectable CSF HIV RNA despite plasma viral suppression. CSF pleocytosis in this setting may also be suggestive of CSF escape, since a normal CSF cell count is typically seen in patients on ART. Additionally, genotypic testing of the CSF virus can potentially help guide management in this limited setting. CSF HIV viral load testing is not readily available to all clinicians since it is not a generally approved laboratory test. If the clinical laboratory will not perform HIV viral load or genotypic testing on CSF, CSF can be frozen and sent to a research or commercial laboratory that does perform those assays. (See "HIV-associated neurocognitive disorders: Management", section on 'Evaluating CSF for HIV'.)

Even more rarely, new-onset severe neurological symptoms may represent immune reconstitution inflammatory syndrome (IRIS) related to HIV CNS infection itself. This can present with severe CD8 encephalitis, with diffuse white and gray matter abnormalities on MRI and CD8 cell pleocytosis in the CSF [90]. MRI and CSF findings should be helpful in suggesting this syndrome.

Mild deficits — Whether additional neurological evaluation is warranted for patients with mild deficits depends on the severity of symptoms and signs, the presence of alternate explanations, and whether the impairment is of recent onset or longstanding. In general, for patients with very mild symptoms, without functional impairment in work or daily life, and without recent onset or progression, it is reasonable to follow symptoms and signs without further dedicated neurological evaluation. In those with more prominent symptoms, functional impairment, or recent onset of deficits, additional evaluation is similar to that for patients with more severe deficits, with MRI and, in some instances, lumbar puncture for CSF evaluation. (See 'Severe deficits, not on ART' above and 'Severe deficits, on ART' above.)

DIAGNOSIS

The diagnosis of an HIV-associated neurocognitive disorder (HAND) is made in an HIV-infected patient who has neurocognitive impairment (particularly if it is new onset or there is suggestion of progression) by history and exam or by detection of cognitive impairment through neuropsychological testing, and whose deficits cannot be fully explained by alternate conditions or pre-existing causes after thorough evaluation. (See 'Evaluation' above.)

Strictly speaking, classification of deficits as HIV-associated dementia (HAD), minor neurocognitive disorder (MND), or asymptomatic neurocognitive impairment (ANI) requires formal neuropsychological testing. However, comprehensive neuropsychological testing is costly, requires highly skilled professionals for administration and interpretation, and may not be readily accessible. From a practical standpoint, HAD can often be diagnosed in the absence of formal neuropsychological testing on the basis of more severe cognitive and motor dysfunction that substantially impairs functioning, and MND on the basis of symptoms or signs of milder cognitive decline that impair functioning. ANI, by definition, can only be diagnosed by formal testing. (See 'Terminology' above.)

The rare CNS viral escape syndrome can be diagnosed in patients who have virologic suppression on antiretroviral therapy (ART) and yet develop subacute progressive cognitive deficits that are unexplained by other conditions and accompanied by detectable CSF HIV RNA levels.

DIFFERENTIAL DIAGNOSIS

Cognitive impairment in HIV-infected patients may be a presenting symptom of numerous other disease processes. The differential diagnosis depends on the degree of immunosuppression and whether the patient is off or on antiretroviral agents. Among these are the following:

- Central nervous system (CNS) infections — In patients with CD4 cell counts <200 cells/microL, other CNS infections that can cause cognitive deficits are toxoplasmosis and progressive multifocal leukoencephalopathy (PML, caused by JC virus infection), both of which usually present with focal deficits and can be detected by MRI. Cryptococcal meningitis is also relatively common in this setting, is usually nonfocal with headache and altered mentation, and is diagnosed by cryptococcal antigen or growth in the CSF. CMV encephalitis also can occur in these patients and may be nonfocal or occasionally include focal features; it is diagnosed by CSF CMV PCR testing. In patients with any CD4 cell count, neurosyphilis is an important consideration and can be differentiated by CSF testing for syphilis. (See "Toxoplasmosis in HIV-infected patients" and "Progressive multifocal leukoencephalopathy: Epidemiology, clinical manifestations, and diagnosis" and "Epidemiology, clinical manifestations, and diagnosis of Cryptococcus neoformans meningoencephalitis in HIV-infected patients" and "Syphilis in the HIV-infected patient", section on 'Neurosyphilis'.)

- CNS malignancies (eg, primary CNS lymphoma) — Primary CNS lymphoma associated with EBV infection presents much like toxoplasmosis and PML with focal manifestation and a mass lesion on imaging, although depending on the location of the mass, it may present only with changes in cognition and behavior. Imaging findings usually distinguish it from HIV-associated dementia (HAD). (See "AIDS-related lymphomas: Primary central nervous system lymphoma".)

- Other dementia syndromes — With longer survival, patients with well-controlled HIV infection on antiretroviral treatment may develop other dementing illnesses. Alzheimer's disease is usually distinguished by early and relatively isolated memory loss followed by other "cortical" abnormalities, such as aphasia and apraxia. In difficult cases, CSF biomarkers of Alzheimer’s disease (t- and p-tau and amyloid beta 1-42) or amyloid positron emission tomographic (PET) scanning can be used to establish the Alzheimer’s diagnosis. Vascular dementia can present much like HAD with subcortical

features, but is usually distinguished by a background of hypertension, episodes of lacunar stroke, and distinct MRI findings. (See "Evaluation of cognitive impairment and dementia".)

- Nutritional deficiencies (eg, vitamin B12 deficiency) – Cognitive impairment secondary to vitamin B12 deficiency can be accompanied by other neurologic symptoms, including paresthesias and sensory deficits. Vitamin B12 deficiency is not uncommon in the setting of HIV infection and can be easily identified through laboratory testing. (See "Treatment of vitamin B12 and folate deficiencies".)

- Endocrine disorders (eg, thyroid dysfunction and adrenal insufficiency) – Hormonal aberrations are also frequent findings in HIV infection and can lead to confusion and other cognitive deficits. These can also be easily identified through laboratory testing. While gonadal insufficiency is a common finding in the aging HIV population, the impact of that on cognition is not well established. (See "Clinical manifestations of hypothyroidism" and "Clinical manifestations of adrenal insufficiency in adults" and "Hypogonadism in HIV-infected males".)

- Severe substance use or psychiatric disorder – These are frequent comorbidities and confounders in the diagnosis of HAND and are often evident on detailed history. (See "Substance abuse and addiction in HIV-infected patients" and "Depression, mania, and schizophrenia in HIV-infected patients".)

- Delirium – Patients with delirium have a reduced ability to sustain, focus, or shift attention, which occurs over a short period of time. The presence of a disturbance in consciousness is a key factor in distinguishing delirium from HAND, in which the patient has a clear level of consciousness. (See "Overview of the neuropsychiatric aspects of HIV infection and AIDS", section on 'Delirium'.)

SOCIETY GUIDELINE LINKS

Links to society and government-sponsored guidelines from selected countries and regions around the world are provided separately. (See "Society guideline links: Primary care of the HIV-infected adult".)

INFORMATION FOR PATIENTS

UpToDate offers two types of patient education materials, "The Basics" and "Beyond the Basics." The Basics patient education pieces are written in plain language, at the 5th to 6th grade reading level, and they answer the four or five key questions a patient might have about a given condition. These articles are best for patients who want a general overview and who prefer short, easy-to-read materials. Beyond the Basics patient education pieces are longer, more sophisticated, and more detailed. These articles are written at the 10th to 12th grade reading level and are best for patients who want in-depth information and are comfortable with some medical jargon.

Here are the patient education articles that are relevant to this topic. We encourage you to print or e-mail these topics to your patients. (You can also locate patient education articles on a variety of subjects by searching on "patient info" and the keyword(s) of interest.)

- Basics topics (see "Patient education: HIV-associated dementia (The Basics)")

SUMMARY AND RECOMMENDATIONS

- Changes in memory, concentration, attention, and motor skills are common in HIV-infected patients. When these occur without an evident cause other than HIV infection, such impairments have been collectively classified as HIV-associated neurocognitive disorders (HAND). Depending on the severity and impact on daily functioning, cognitive deficits can be classified into three conditions: asymptomatic neurocognitive impairment (ANI), HIV-associated mild neurocognitive disorder (MND), and HIV-associated dementia (HAD). (See 'Introduction' above and 'Terminology' above.)

- The widespread use of combination antiretroviral therapy (ART) has been associated with a decrease in the prevalence of more severe neurocognitive deficits (ie, HAD), but milder cognitive deficits without alternative explanation remain common in the setting of HIV infection, even among patients with viral suppression. Risk factors for HAND include low nadir CD4 cell count, age, and other comorbidities, such as cardiovascular and metabolic disease. (See 'Epidemiology' above.)

- HAD occurs predominantly in untreated patients with advanced HIV infection. In its classic form, it is primarily characterized by subcortical dysfunction, with attention-

concentration impairment, depressive symptoms, and impaired psychomotor speed and precision. The onset of the impairments is typically subacute. Cerebral atrophy is typically evident on brain imaging, which can also demonstrate diffuse or patchy white matter hyperintensity. (See 'HIV-associated dementia' above.)

- The main cognitive deficits reported in milder presentations of HAND include difficulty with attention and working memory, executive functioning, and speed of informational processing. The onset and time course is generally more indolent than the typically subacute presentation of HAD, and deficits may remain stable or seemingly unchanged for years. There are no specific imaging findings. (See 'Milder neurocognitive deficits' above.)

- The value of broadly screening HIV-infected patients for neurocognitive impairment is controversial. Given limited resources and the absence of clear evidence to support changing management on the basis of mild deficits, we do not routinely screen for such deficits. However, when resources allow, screening is reasonable to establish a baseline assessment of a patient's neurocognitive function in case there is subsequent deterioration. (See 'Screening for deficits' above.)

- The possibility of HAND is suspected in HIV-infected patients who present with symptomatic cognitive deficits or are found to have deficits on screening tests. The goal of the initial assessment is to characterize the degree, time course, and functional impact of any cognitive deficits, establish the stage of HIV disease and treatment status, and start to evaluate for other potential causes of cognitive symptoms, such as other neurologic or neurodegenerative disorders, primary psychiatric disorders, and severe substance use disorders. (See 'Initial assessment' above and 'Differential diagnosis' above.)

- The focus of further diagnostic evaluation depends on the severity of the presentation and whether the patient is on ART. For patients with very mild symptoms, without functional impairment in work or daily life, and without recent onset or progression, it is reasonable to follow symptoms and signs without further dedicated neurological evaluation. For patients with severe deficits, magnetic resonance imaging (MRI) and lumbar puncture for cerebrospinal fluid evaluation are generally performed to evaluated for other potential etiologies. In patients not on ART with CD4 cell counts <200 cells/microL, these include mainly opportunistic processes, and in patients with viral

suppression on ART, these include non-HIV-related processes, such as cerebrovascular or neurodegenerative disease, or the rare CNS escape syndrome. (See 'Further evaluation' above and 'Differential diagnosis' above.)

- The diagnosis of HAND is made in an HIV-infected patient who has neurocognitive impairment (particularly if it is new onset or there is suggestion of progression) by history and exam or by detection of cognitive impairment through neuropsychological testing, and whose deficits cannot be fully explained by alternate conditions or pre-existing causes after thorough evaluation. (See 'Diagnosis' above.)

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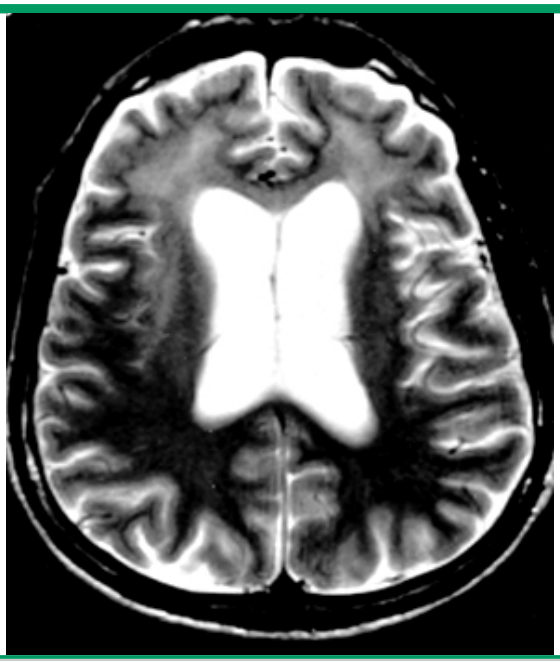
Topic 3723 Version 22.0

GRAPHICS

MRI of the brain from a patient with HIV-associated dementia



Bilateral symmetrical high T2 signals without mass effect are present in the white matter of both frontal lobes, associated with subcortical atrophy.

MRI: Magnetic resonance imaging.

Graphic 82180 Version 4.0

Contributor Disclosures

Richard W Price, MD Other Financial Interests: Gilead Sciences, Inc [Honorarium and travel expenses as speaker at a meeting (Tenofovir Alafenanamide and Fumerate, Rilpivarine, Emtriva, Elvitegravir and various combinations of these in single pill doses)], Merck & Co [Honorarium and travel expenses as speaker at a meeting (raltegravir)]. **John G Bartlett, MD** Nothing to disclose **Allyson Bloom, MD** Nothing to disclose

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Conflict of interest policy

EXHIBIT 42

Dement Neuropsychol 2016 March;10(1):42-46

Original Article

Risk factors for neurocognitive impairment in HIV-infected patients and comparison of different screening tools

Elisa Moreira de Souza[1], Caroline Schleiffer Buoniconti[1], Frederico Cunha Valim[1], Alexandre Sampaio Moura[2]

ABSTRACT. HIV-associated neurocognitive disorder (HAND) is relatively frequent among HIV-infected patients and is often underdiagnosed. Assessment of HAND in daily clinical practice is challenging and different tools have been proposed. **Objective:** To evaluate risk factors and compare different screening tools for neurocognitive impairment in HIV-infected patients. **Methods:** HIV-infected patients were evaluated using the International HIV-Dementia Scale (IHDS), Mini-Mental State Examination (MMSE) and a neurocognitive self-perception questionnaire recommended by the European AIDS Clinical Society. Sociodemographic, clinical and laboratory data were obtained through chart review and patient interview. **Results:** Among the 63 patients included, low performance on the IHDS was observed in 54.0% and IHDS score was inversely associated with age (OR 0.13; 95%CI [0.02–0.67]). Regarding cognitive self-perception, 63.5% of patients reported no impairment on the three domains covered by the questionnaire. Among those patients self-reporting no problems, 42.1% had low performance on the IHDS. None of the patients scored below the education-adjusted cut-off on the MMSE. **Conclusion:** IHDS scores suggestive of HAND were observed in more than half of the patients and lower scores were found among older patients. There was low agreement between the different tools, suggesting that the MMSE may be inadequate for assessing HAND. The self-assessment questionnaire had low sensitivity and might not be useful as a screening tool.

Key words: HIV, dementia, Mini-Mental State Examination, International HIV Dementia Scale.

FATORES DE RISCO PARA ALTERAÇÕES NEUROCOGNITVAS EM PACIENTES INFECTADOS PELO HIV E COMPARAÇÃO DE DIFERENTES FERRAMENTAS DE TRIAGEM

RESUMO. As alterações neurocognitivas associadas ao HIV (HAND) são relativamente frequentes entre pacientes infectados pelo HIV, porém são subdiagnosticadas. Avaliação de HAND na prática clínica diária é desafiador e diferentes ferramentas têm sido propostas. **Objetivo:** Avaliar fatores de risco e comparar diferentes ferramentas de rastreamento de alterações neurocognitivas em pacientes infectados pelo HIV. **Métodos:** Pacientes infectados pelo HIV foram avaliados usando a Escala Internacional de Demência pelo HIV (IHDS), Mini Exame do Estado Mental (MEEM) e um questionário de autopercepção neurocognitiva recomendado pela Sociedade Clínica Europeia de AIDS. Dados sociodemográficos, clínicos e laboratoriais foram obtidos por revisão de prontuário e entrevista com o paciente. **Resultados:** Entre os 63 pacientes incluídos no estudo, um baixo desempenho no IHDS foi observado em 54,0% e o escore no IHDS esteve inversamente associado à idade (OR 0,13; IC95% [0,02–0,67]). Em relação à autopercepção cognitiva, 63,5% dos pacientes não relataram nenhum prejuízo nos três domínios avaliados pelo instrumento. Nenhum paciente apresentou escore no MEEM abaixo do ponto de corte ajustado para escolaridade. **Conclusão:** Escores no IHDS sugestivos de HAND foram observados em mais da metade dos pacientes e valores mais baixos foram encontrados entre pacientes mais velhos. Houve pouca concordância entre os diferentes métodos de avaliação, sugerindo que o MEEM é inadequado para avaliação de HAND e o questionário de auto-avaliação tem uma baixa sensibilidade, não parecendo ser útil como ferramenta de triagem.

Palavras-chave: HIV, demência, Mini Exame do Estado Mental, Escala Internacional de Demência pelo HIV.

This study was conducted at the Universidade José do Rosário Vellano, Belo Horizonte MG, Brazil.

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INTRODUCTION

The central nervous system (CNS) is a major target for HIV infection with high viral loads often observed in cerebrospinal fluid and in different anatomical sites such as the caudate nucleus and the hippocampus of HIV-infected patients.[1] In the CNS, HIV infects and replicates on macrophages, microglia and multicellular glia, resulting in the release of neurotoxic factors and subsequent cell damage.[2]

In 1991, the American Academy of Neurology divided HIV-associated neurocognitive disease (HAND) into two different categories: HIV-associated dementia (HAD) and mild neurocognitive disorder. HAD is characterized by impairment in multiple domains, particularly learning of new information, information processing, and attention or concentration, that impact at least two activities of daily living and result in at least one functional or psychosocial change. In mild neurocognitive disorder, there is a reduction in mental accuracy with loss of efficiency at work and reduced performance on domestic tasks,[1] but with a much lower impact on activities of daily living.

A decade later, Antinori et al.,[3] introduced a new category of asymptomatic neurocognitive disorders based on the finding that some individuals have subclinical impairment on neurocognitive evaluation without any impact on activities of daily living. Prevalences of asymptomatic neurocognitive disorder (ANA), mild neurocognitive disorder and HAD are 30–35%, 20–25% and 2–3%, respectively.[4] CD4+ cell counts lower than 200 cells/mm^3, age greater than 50 years and low educational status were found to be risk factors for HIV-associated neurocognitive disorders (HAND).[4]

The gold standard for diagnosing neurocognitive impairment is a battery of neuropsychological tests applied by a trained neuropsychologist. However, such comprehensive assessment is not feasible in daily clinical practice and simpler screening tools are needed.[4,5]

The IHDS is a rapid assessment tool that evaluates memory-recall and both motor and psychomotor speed.[5,6] It consists of three subtests: [1] timed finger tapping which measures motor speed; [2] timed alternating hand sequence test which assesses psychomotor speed; and [3] recall of 4 words (blue, dog, hat and apple) at 2 minutes which assesses memory registration and recall. Each of these tests is rated on a scale of 0–4 and the maximum possible score on the IHDS is 12. Validation of the IHDS in Brazil was conducted by Rodrigues et al.,[5] and showed sensitivity and specificity for detecting HAND of 55% and 80%, respectively. A moderate-to-high interobserver agreement was observed and a there was reasonable agreement between the IHDS and other neuropsychological tests.[5]

Another neurocognitive assessment tool is the Mini-Mental State Examination (MMSE) that evaluates orientation, attention and calculation, registration, recall, language and the ability to follow simple commands.[7] However, the MMSE was originally developed to screen for cortical dementia such as Alzheimer's disease and there might be limitations on its use to assess subcortical disorders, such as those observed among HIV-infected patients.[7]

A self-perception questionnaire was recently proposed by the European AIDS Clinical Society as a first step in neurocognitive evaluation of HIV-infected patients.[9] The questionnaire has three items related to memory, attention and information processing, based on a previous study conducted by Simioni et al.[8] However, the performance of the questionnaire in clinical setting has not been systematically evaluated.

The aim of this study was to evaluate the factors associated with performance on the IHDS and MMSE and level of agreement between scores on these screening tools and patients' self-perception of neurocognitive status.

METHODS

Between October 2013 and February 2014, consecutive HIV-infected patients were recruited for the study at CEASC-UNIFENAS, a public university-based ambulatory-care unit for infectious diseases located in Belo Horizonte, Brazil. All patients included were adults and had confirmed HIV diagnosis. Patients were excluded if they were illiterate, had severe psychiatric conditions or current CNS opportunistic infection. Sociodemographic, clinical and laboratory data were obtained through chart review and patient interview.

Neurocognitive evaluation using validated versions of the IHDS[5] and MMSE[7] was conducted by trained researchers. For the IHDS, a score less than or equal to 10 was considered to be altered, based on the study of Rodrigues et al. that showed a sensitivity and specificity for detection of HAND of 55% and 80%, respectively.[5] For the MMSE, a cut-off score based on years of education was used as following: 18 points for patients with four years of education or less and 26 points for those with more than four years of education.[7]

Cognitive self-perception was assessed by a questionnaire recommended by the European AIDS Clinical Society guideline.[8,9] The questionnaire includes three items: [1] "Do you experience frequent memory loss?"; [2] "Do you feel that you are slower when reasoning, planning

activities or solving problems?"; [3] "Do you have difficulties paying attention?". For each of the questions, patients must choose one of the following answers: [a] never, [b] hardly ever or [c] yes, definitely. The EACS guideline recommends that patients be submitted to a more thorough neurocognitive evaluation if the response on at least one of the items is "yes, definitely".

Patients' depressive symptoms were assessed using the Beck Depression Inventory (BDI),[10] a self-rated 21-item questionnaire validated in Brazil by Gorenstein and Andrade.[11] A score of < 14 suggests the presence of no or minimal depressive symptoms while scores from 14–19, 20–28 and 29–63 are suggestive of the presence of mild, moderate or severe depressive symptoms, respectively.

Demographic information, CD4+ cell count, HIV viral load, antiretroviral regimen, smoking history, use of illicit drugs and alcohol abuse were obtained from patients' medical records.

Descriptive analysis of frequency and proportions were used for categorical variables. Comparison of proportions was conducted using Pearson's Chi-square test. Means and standard deviation were used for normally distributed continuous variables. Statistical significance was set up at 0.05. The Epi info statistical package (Version 3.5.4, July 30 (2012)) was used to conduct all analyses.

This study was conducted in accordance with the Helsinki declaration and approved by the local Research Ethics Committee. All subjects gave their written informed consent.

RESULTS

Sixty-four patients were assessed throughout the study period and one was excluded due to illiteracy. Among the 63 patients included, 45 (71.4%) were male, with a mean age of 42.9 years (range 19.0–73.0), 39 (61.9%) individuals were non-white and 38 (60.3%) had eight or less years of education. Only two patients (3.2%) had a CD4+ count less than 200cells/mm^3. Fifty-eight patients were on antiretroviral therapy (ART) and 30 (51.7%) of these were using efavirenz. Among the forty-four patients that had been on ART for more than 24 weeks, 37 (84.1%) had undetectable viral load.

Twenty-four (33.9%) patients showed symptoms of depression, where most of these were suggestive of mild depression. Only nine patients had a BDI score suggestive of moderate or severe depression (Table 1).

Neurocognitive assessment showed that 34 (54.0%) patients had low performance (<11) on the IHDS (Table 2) and scores were inversely associated with age (OR 0.13; 95%CI 0.02-0.67). Performance on the IHDS was not significantly associated with efavirenz use, gender, CD4+ cell count; viral load or depressive symptoms (Table 3).

None of the patients included had an MMSE score below the cut-off level.

Regarding the self-assessment questionnaire, 25.7% of the patients answered positively for at least one of the

Table 1. Baseline demographic and clinical characteristics of all patients included.

	N=63
Age (years)	42.9 ± 10.9*
Years of education (%)	
1-8	60.3%
9-12	30.1%
>12	9.5%
Male (%)	71.4%
Non-white (%)	61.9%
CD4+ T-cell count < 200 /mm^3 (%)	3.2%
CD4+ T-cell count (cells/mm^3)	556.6 ± 189.1*
Viral load <50 copies/ml (%)	58.7%
Current ARV use (%)	92.0%
Efavirenz use	51.7%
Beck Depression Inventory score (%)	
0-13 points	66.1%
14-19 points	25.4%
20-28 points	8.5%
>29 points	6.3%

*mean ± standard deviation.

Table 2. Performance of HIV-infected patients on different neurocognitive assessment tools.

	N=63
IHDS	
≥ 11	36.0%
< 11	54.0%
MMSE	
≥ cut-off point*	100%
< cut-off point*	0%
EACS self-perception questionnaire	
Reported no problems	74.3%
Reported at least one problem	25.7%

EACS: European AIDS Clinical Society; IHDS: International HIV Dementia Scale; MMSE: Mini-Mental State Examination; *Adjusted by educational level

Table 3. Univariate analysis of factors associated with performance on the International HIV Dementia Scale (IHDS).

	IHDS≤10 (n=34)	IHDS>10 (n=29)	OR	95% CI	P value
Age ≤ 50 (years)	22 (64.7%)	27 (93.0%)	0.13	0.02-0.67	< 0.01
Efavirenz use	18 (54.9%)	12 (41.3%)	1.73	0.61-4.90	0.30
CD4 count < 200 cell/mm^3	1 (2.9%)	1 (3.4%)	0.84	0.05-14.19	0.90
Viral load ≥ 50 copies/ml	14 (41.1%)	12 (41.3%)	1.0	0.36-2.75	0.49
BDI score < 13	20 (58.8%)	19 (65.5%)	0.75	0.26-2.09	0.66

BDI: Beck Depression Inventory.

questions (Table 2). Among those patients self-reporting no problems, 42.1% had low performance on the IHDS.

DISCUSSION

A high proportion of HIV-infected patients were found to have impaired performance on the IHDS. A similar high prevalence of HAND, as suggested by low scores on the IHDS, was also reported by Oshinaike et al. in Nigeria[12] and Rodrigues et al. in Brazil.[5]

With the introduction of highly-active antiretroviral therapy (HAART), a reduction in the incidence of HAND was observed but its prevalence has increased due to improved patient survival.[5,10] Currently, as the HIV population is becoming older, both incidence and prevalence of HAND appear to be increasing.[13] Indeed, we have found age to be associated with performance on the IHDS - a finding also reported by others.[14,15]

We failed to find an association between CD4+ cell count and performance on the IHDS, such as the associations found by Antinori et al.[3] However, the number of patients with CD4+ cell count less than 200 cells/mm^3 in the present study was too small to make meaningful comparisons.

Efavirenz is associated with a variety of psychiatric and neurological conditions due to its neurotoxicity.[16] However, we found no association between efavirenz use and performance on the IHDS, in agreement with the results of Lopardo et al.[15]

Depression is frequent among HIV-infected patients. Kagee & Martin conducted a study in South Africa using BDI and estimated a prevalence of moderate and severe depression of 37.4% and 20%, respectively.[17] Our prevalence of depressive symptoms was much lower and was not associated with IHDS performance.

None of the patients in the present study showed alterations on the MMSE. This finding reinforces the claim made by Sacktor et al.[6] that the MMSE is useful for cortical dementias but might lack sensitivity when evaluating subcortical neurological disorders such as those associated with HIV.

Regarding the self-assessment neurocognitive questionnaire recommended by the EACS,[8] we have found an unacceptable high rate of patients without complaints (42.1%) that had impaired performance on the IHDS, thereby limiting its utility as a screening tool. Simioni et al.,[8] (also reported a high prevalence of HAND among HIV-infected patients with long-standing undetectable viral load without neurocognitive complaints.

Misdiagnosis of HAND can have a significant impact on HIV care. HAND overdiagnosis might reduce patients' self-esteem, lead to inappropriate medical interventions and increases the already high cost of AIDS treatment.[18] Conversely, lack of early diagnosis might delay appropriate interventions such as antiretroviral therapy modifications.[19]

The present study has several limitations. First, patients were recruited from a single referral center and might not reflect the overall HIV-infected population. In addition, the small sample size may have limited the power for identifying some risk factors for HAND such as CD4 cell count. Finally, the study did not include a thorough neuropsychological assessment. However, the IHDS has been previously shown to have good performance compared to more comprehensive neurocognitive assessment.[5]

In summary, a high proportion of HIV-infected patients had poor performance on the IHDS, suggestive of HAND, a trait that seemed to increase with age. The self-assessment questionnaire recommended by the EACS and the MMSE might have a limited role as screening tools for neurocognitive impairment in HIV-infected patients.

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Author contributions. All authors have contributed significantly and are in agreement with the content of the manuscript. Elisa M. Souza participated in the design of the study, undertook the collection of data, performed the statistical analysis and drafted the manuscript. Caroline S. Buoniconti participated in the design of the study, collection of data and reviewed the manuscript. Frederico C. Valim performed the statistical analysis and reviewed the manuscript. Alexandre S. Moura participated in the design of the study, undertook the collection of data, performed the statistical analysis and drafted the manuscript.

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EXHIBIT 43

Asymptomatic HIV-associated neurocognitive impairment increases risk for symptomatic decline

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ABSTRACT

Objective: While HIV-associated neurocognitive disorders (HAND) remain prevalent despite combination antiretroviral therapy (CART), the clinical relevance of asymptomatic neurocognitive impairment (ANI), the most common HAND diagnosis, remains unclear. We investigated whether HIV-infected persons with ANI were more likely than those who were neurocognitively normal (NCN) to experience a decline in everyday functioning (symptomatic decline).

Methods: A total of 347 human participants from the CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) cohort were NCN (n = 226) or had ANI (n = 121) at baseline. Neurocognitive assessments occurred approximately every 6 months, with median (interquartile range) follow-up of 45.2 (28.7–63.7) months. Symptomatic decline was based on self-report (SR) or objective, performance-based (PB) problems in everyday functioning. Proportional hazards modeling was used to generate risk ratios for progression to symptomatic HAND after adjusting for baseline and time-dependent covariates, including CD4+ T-lymphocyte count (CD4), virologic suppression, CART, and mood.

Results: The ANI group had a shorter time to symptomatic HAND than the NCN after adjusting for baseline predictors: adjusted risk ratios for symptomatic HAND were 2.0 (confidence interval [CI] 1.1–3.6; $p = 0.02$) for SR, 5.8 (CI 3.2–10.7; $p < 0.0001$) for PB, and 3.2 (CI 2.0–5.0; $p < 0.0001$) for either SR or PB. Current CD4 and depression were significant time-dependent covariates, but antiretroviral regimen, virologic suppression, and substance abuse or dependence were not.

Conclusions: This longitudinal study demonstrates that ANI conveys a 2-fold to 6-fold increase in risk for earlier development of symptomatic HAND, supporting the prognostic value of the ANI diagnosis in clinical settings. Identifying those at highest risk for symptomatic decline may offer an opportunity to modify treatment to delay progression. *Neurology*® 2014;82:2055–2062

GLOSSARY

ANI = asymptomatic neurocognitive impairment; **ART** = antiretroviral therapy; **BDI** = Beck Depression Inventory–II; **CART** = combination antiretroviral therapy; **CHARTER** = CNS HIV Anti-Retroviral Therapy Effects Research; **CI** = confidence interval; **HAD** = HIV-associated dementia; **HAND** = HIV-associated neurocognitive disorders; **HCV** = hepatitis C virus; **IADL** = instrumental activities of daily living; **MMT-R** = revised version of the Medication Management Test; **MND** = mild neurocognitive disorder; **NCN** = neurocognitively normal; **NNRTI** = non-nucleoside reverse transcriptase inhibitor; **PAOFI** = Patient's Assessment of Own Functioning Inventory; **PB** = performance-based; **PI** = protease inhibitor; **SR** = self-report.

Combination antiretroviral therapy (CART) has reduced morbidity and mortality among those living with HIV (HIV+), but HIV-associated neurocognitive disorders (HAND) remain prevalent.[1–3] While the prevalence of the most severe form of HAND, HIV-associated dementia (HAD), is now uncommon (2%[2]), milder forms of HAND (termed asymptomatic neurocognitive impairment [ANI] and mild neurocognitive disorder [MND] according to the Frascati criteria[1]) have been reported in 40%–56% of HIV+ cases and may be more prevalent at earlier (less severe) disease stages in the CART era.[2,4,5]

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ANI is the most common form of HAND,[2] accounting for approximately 70% of cases, and is characterized by impairment on neurocognitive testing with no obvious accompanying interference in daily functioning.[1] Recent commentaries have called into question the clinical relevance of ANI,[6,7] suggesting that the diagnostic criteria for ANI generate an unacceptably high false-positive rate[6] and a lack of evidence that patients with ANI are at greater risk for progression to more severe impairment.[7] Although previous studies have shown that an antemortem ANI diagnosis is related to both increased dendritic injury and HIV encephalitis in individuals without significant comorbidities,[8,9] these studies involved terminally ill patients, limiting generalizability to medically stable HIV+ patients on CART. The clinical relevance of ANI would be bolstered if ANI predicted future symptomatic disease, i.e., problems with everyday functioning. To evaluate this, we performed a longitudinal study comparing risk of developing symptomatic decline in HIV+ persons who at baseline were neurocognitively normal (NCN) vs those with ANI.

METHODS

Participants. Study participants were from the CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) study, details of which were reported previously.[2] Individuals from the longitudinal CHARTER cohort were selected for this study on the basis of being classified as ANI (n = 121) or NCN (n = 226) at baseline according to the Frascati criteria based on comprehensive neuromedical, neurocognitive, psychiatric, and functional evaluations.[1] No individuals had non-HIV, severely confounding comorbidities[2] that would preclude a HAND diagnosis (table 1). A total of 2,749 visits were analyzed.

Procedures. ***Neuromedical examination.*** The standardized neuromedical examination included a medical history, structured neurologic and medical examination, as well as collection of blood, CSF, and urine samples.[2]

Laboratory assessment. Laboratory measurements included routine clinical chemistry panels, complete blood counts, rapid plasma reagin, hepatitis C virus antibody, CD4+ T lymphocytes, and HIV RNA levels measured in plasma and CSF.[2]

Neurocognitive examination. The CHARTER comprehensive neurocognitive test battery covered 7 cognitive domains: motor function (perceptual-motor speed), verbal fluency, executive function, attention/working memory, speed of information processing, learning, and memory.[2,10] ANI and NCN diagnoses were rendered according to Frascati criteria.[1] Premorbid verbal IQ estimates were obtained using the oral reading subtest from the Wide Range Achievement Test.[11]

Psychiatric examination. The computer-assisted Composite International Diagnostic Interview[12] was administered to establish current and lifetime diagnoses of mood disorders and substance use disorders. Current depressive symptoms were assessed with the Beck Depression Inventory–II (BDI).[13]

Self-report measures of daily functioning. Reports of cognitive difficulties in everyday life were assessed using the Patient's Assessment of Own Functioning Inventory (PAOFI).[14] Increased dependence in performing instrumental activities of daily living (IADL) was assessed with a modified version of the Lawton and Brody Scale.[15] We also administered an employment questionnaire that asks about any decreases in work productivity, accuracy/quality of work, increased effort required to do one's usual job, and increased fatigue in association with the usual workload.

Performance-based measures of daily functioning. Medication management was assessed via a revised version of the Medication Management Test (MMT-R[15]). Briefly, the MMT-R consists of both pill dispensing and medication inference questions. Vocational function was assessed using standardized work samples (MESA SF2)[16] and the next-generation Valpar COMPASS programs.[17] The Valpar assessment consists of multimodal, criterion-referenced instruments designed to establish participant skill level in areas of vocational functioning.[15]

Classification of symptomatic status. Data-driven formulas were used to determine symptomatic status 3 ways: (1) using only self-report (SR) measures of daily functioning; (2) using only performance-based (PB) measures of daily functioning; and (3) a dual method that classifies a person as symptomatic if he or she meets any 2 of the SR or PB criteria for symptomatic impairment of daily functioning. Employment status that was associated with cognitive decline (reported decline in work ability or inability to work due at least in part to cognitive issues on the IADL) counted as one area of functional decline in all formulas, in accordance with the Frascati criteria.[1]

To determine functional decline in the SR formula, scores on the PAOFI and IADL were examined. A PAOFI score of 3 or higher (reflecting at least 3 cognitive symptoms) was used to indicate functional impairment on that measure. To control for depression in SR, participants with elevated BDI scores (BDI $\geq$ 17) needed to exhibit a higher level of complaint on the PAOFI (PAOFI $\geq$10 complaints) to qualify for functional impairment on this measure. Scores on the IADL that showed decline from "best" to "'now" in 2 or more areas that were identified as being at least partially due to cognitive problems (vs physical impairment) also qualified as functionally impaired.[1,18,19] In order to be called symptomatic by SR, participants had to be (1) PAOFI and IADL impaired or (2) PAOFI or IADL impaired and either unemployed or employed, but with self-reported difficulty in job performance, which was at least partially due to cognitive problems.

Since published, demographically adjusted normative standards are not available for the performance-based tests, we derived cutpoints for the MMT-R and Valpar from the neurocognitively normal subset of CHARTER participants (n = 375; mean age = 43.4 years; 80% male; 42% Caucasian; mean education = 12.5 years). Based on prior studies,[15] cutpoints were determined based on a normal distribution, so that 16% of the NCN cohort would be impaired at 1 SD (cutoff scores: MMT-R $<$5 and Valpar $<$24) and 2% of the cohort would be impaired at 2 SDs (cutoff scores: MMT-R $<$2 and Valpar $<$17). The MMT-R and Valpar were administered at the first longitudinal visit (6 months after the CHARTER baseline) and every 6 months thereafter, but were not performed at the baseline (entry) visit.

In the PB formula, functional impairment was defined as scores 1 or 2 SDs below the mean on the MMT-R and Valpar, also consistent with the Frascati criteria.[1] Participants were coded as symptomatic if (1) both MMT-R and Valpar scores were at least 1 SD below the mean or (2) one task was 2 or more SDs

Table 1 Comparison of participants who were neurocognitively normal and asymptomatically neurocognitively impaired at baseline

	NCN (n = 226), mean (SD), %, or median (IQR)	ANI (n = 121), mean (SD), %, or median (IQR)	*p* Value[a]
Age, y[b]	43.0 (8.6)	44.8 (8.0)	
Education, y[b]	12.9 (2.4)	13.5 (2.2)	0.04
Estimated verbal IQ[b]	97.4 (13.2)	92.6 (14.5)	0.002
% Male[c]	81.9	81.8	
% Caucasian[c]	45.6	46.3	
% Lifetime substance use diagnosis[c]	71.2	69.4	
% Lifetime major depression[c]	52.6	45.4	
% With contributing comorbidity[c,d]	22.6	44.6	<0.0001
% AIDS[c]	56.2	62.8	
Current CD4, cells/mm^3[e]	459 (290–669)	425 (286–578)	
Nadir CD4, cells/mm^3[e]	201 (61–370)	162 (38–273)	0.03
% On ART[c]	66.2	72.7	
% Undetectable in plasma[c]	38.6	45.8	
% Undetectable in CSF[c]	59.6	75.9	0.006
Estimated duration HIV+, mo[b]	117.7 (75.0)	120.7 (81.6)	
% HCV+[c]	20.4	27.3	

Abbreviations: ANI = asymptomatic neurocognitive impairment; ART = antiretroviral therapy; CHARTER = CNS HIV Anti-Retroviral Therapy Effects Research; HCV = hepatitis C virus; IQR = interquartile range; NCN = neurocognitively normal.
[a] Only *p* values <0.05 are reported.
[b] *t* test.
[c] χ^2 test.
[d] Comorbidity status was based on CHARTER classification.[2] Contributing comorbidities are non-HIV factors that can influence neurocognitive impairment but are not considered the primary cause of the impairment.
[e] Wilcoxon rank test.

below the mean and the subject was unemployed due to cognitive issues.

All the diagnostic criteria described above for functional decline were also used to define functional impairment in the SR/PB method. Measures included in each formula criterion are summarized in table e-1 on the *Neurology*® Web site at Neurology.org (at least 2 criteria were necessary for functional impairment).

Standard protocol approvals, registrations, and patient consents. These procedures were approved by the Human Subjects Protection Committees of each participating institution. Written informed consent was obtained from all study participants.

Statistical methods. Kaplan-Meier estimates were generated comparing NCN and ANI participants on time to symptomatic threshold defined by the 3 criteria: using only SR symptoms, only PB symptoms, and having SR, PB, or both (SR/PB). Proportional hazards modeling was used to generate risk ratios and their 95% confidence interval (CI) estimates for symptomatic HAND, after adjusting for baseline or time-dependent variables (table 1). Separate models were developed based on each of the 3 criteria: SR, PB, and SR/PB. Multivariable models initially included all univariably significant ($p < 0.10$) predictors. Time-varying predictors of earlier decline to symptomatic status in univariable survival analyses that were screened for inclusion in multivariate models included current CD4 count, current major depressive disorder, antiretroviral therapy (ART) use status, ART regimen type (protease inhibitor [PI]-based vs non-nucleoside reverse transcriptase inhibitor [NNRTI]-based/PI-NNRTI-based/other), CNS penetration effectiveness score of ART regimen,[20] viral load in plasma and CSF, and current substance use disorder. Nonsignificant ($p \geq 0.05$) predictors were subsequently removed from the final multivariable models. Interactions of the remaining variables were tested and retained if significant at a $p < 0.05$ level. Baseline demographic characteristics, HIV disease–related laboratory measures, AIDS status, treatment-related variables, substance use variables, and psychiatric variables were compared using *t* tests, Wilcoxon rank tests, or χ^2 tests, as appropriate, between ANI vs NCN cases (table 1) and between participants who became symptomatic vs those who remained asymptomatic (table 2).

RESULTS

Survival analysis of ANI as a predictor of symptomatic HAND. Kaplan-Meier estimates comparing ANI and NCN on time to symptomatic HAND showed that ANI was a predictor of earlier time to symptomatic status using any of the 3 measures: SR ($p = 0.003$), PB ($p < 0.0001$), or SR/PB ($p < 0.0001$) (figure). The survival analyses were repeated considering only those cases that were virally suppressed in plasma at baseline (NCN = 85, ANI = 55). ANI remained a strong predictor of earlier time to symptomatic status using PB and SR/PB ($p < 0.0001$). For SR only, the relationship did not attain significance, but was suggestive of an association ($p = 0.08$). At baseline, the ANI group had higher education than the NCN

Table 2 Adjusted relative risk for symptomatic progression: Asymptomatic neurocognitive impairment vs neurocognitively normal

Criteria for symptomatic status	Relative risk[a]	95% CI	p Value
Self-report	2.00	1.09–3.62	0.02
Performance-based	5.81	3.24–10.75	<0.0001
Self-report or performance-based	3.18	2.04–4.99	<0.0001

Abbreviations: ANI = asymptomatic neurocognitive impairment; CI = confidence interval; NCN = neurocognitively normal.
[a] Relative risk for ANI vs NCN; all risk ratios adjusted for baseline education, estimated verbal IQ, nadir CD4, $\log_{10}$ CSF viral load, and comorbidity classification[2] in proportional hazards models.

group (13.5 [2.2] vs 12.9 [2.4], $p = 0.04$), but had lower verbal IQ estimates (92.5 [14.5] vs 97.4 [13.2], $p = 0.002$), lower nadir CD4 counts (162 [38–273] vs 201 [61–370], $p = 0.03$), were more likely to have an undetectable viral load in CSF (75.9% vs 59.6%; $p = 0.004$), and a greater percent of people with moderate "contributing" vs "incidental" (minimal) comorbidities (table 1). Using proportional hazards modeling, we generated risk ratios for earlier decline to symptomatic HAND that adjusted for these variables. After adjustment, ANI remained a predictor of earlier decline to symptomatic HAND using all 3 methods of measurement (SR, PB, and SR/PB, all p values <0.05, table 3).

Baseline predictors of decline to symptomatic HAND (other than ANI status). Overall, 110 (31.7%) of the entire group (50.4% for ANI and 21.7% for NCN, $p < 0.0001$) experienced a decline to symptomatic HAND measured by either SR or PB. When comparing baseline characteristics between those who declined to symptomatic HAND and those who did not, those who declined were older, had less education, were more often female, were more likely to have a lifetime substance use disorder, had greater than incidental (minimal) comorbidity, were more likely to have an AIDS diagnosis, had a lower nadir CD4, and were more likely to be hepatitis C virus (HCV)+. Race/ethnicity, CART status, current CD4 count, plasma and CSF viral load, and estimated duration of HIV infection did not predict decline (table 3). Of the 49 patients with NCN who ultimately became symptomatic, 13 developed neurocognitive impairment at a visit before the SR or PB decline, and 36 experienced the NC decline simultaneously with the SR/PB decline. The patients with NCN who declined had worse initial global deficit scores than nondecliners and scored worse in the ability areas of learning, recall, working memory, and motor coordination (all $p < 0.05$; table e-2).

Time-dependent predictors of earlier symptomatic status. Together, baseline ANI vs NCN status and a time-dependent current diagnosis of major depressive disorder were significant predictors of time to decline using SR-based measures to define symptomatic status. Baseline ANI vs NCN status and current CD4 count, in combination, were significant predictors of time to decline using PB only and SR/PB measures to define symptomatic status (table 4).

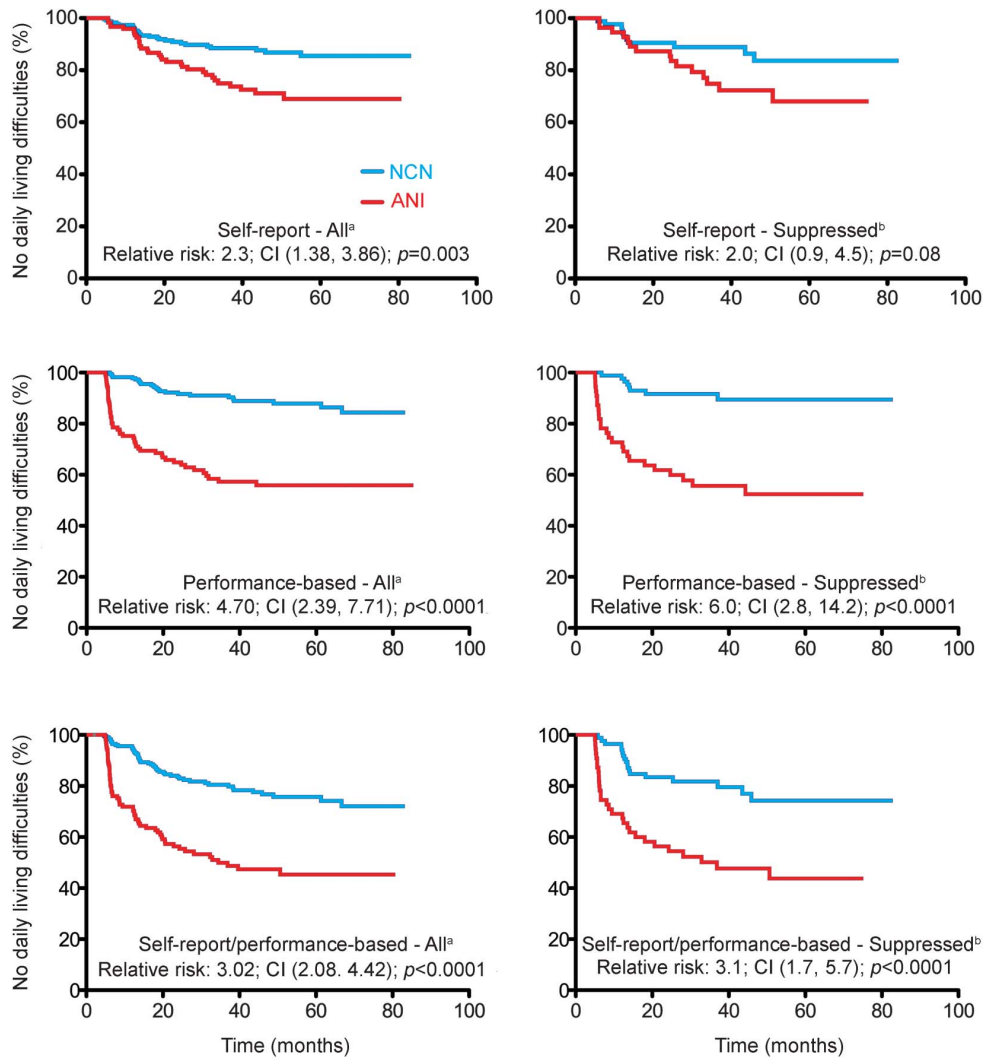
DISCUSSION While the introduction of CART has markedly reduced the rate of the most severe form of HAND—HAD—from an estimated 15% in the 1980s[5] to perhaps 2% currently,[2] this has not been accompanied by a similar reduction in the milder forms of HAND (ANI and MND),[1] with at least 40% of HIV+ patients manifesting some level of neurocognitive impairment.[4] In the cross-sectional CHARTER study of 1,316 patients without major neurologic comorbidities, 617 (47%) had some level of NCI; of these, 70% were classified as ANI, 25% as MND, and 5% as HAD.

Given that ANI is the most prevalent form of HAND, the clinical relevance of this diagnosis is clearly important. It has been argued that since ANI is "asymptomatic," it may have little clinical significance. Indeed, some have contended that establishing the diagnosis may be wasteful of resources and needlessly worrying to patients. For instance, ANI could represent brain injury that occurred in the early stages of HIV infection, is not progressive, and has no future consequences. However, our findings of increased progression in ANI argue against that interpretation. ANI patients progress to symptomatic status faster than those without ANI regardless of whether functional worsening was measured by self-report, performance, or either of these indices. Therefore, a major criticism of ANI—that it does not predict anything clinically important—is inaccurate according to this study's findings.

The magnitude of ANI as a predictor of decline to symptomatic status was substantial, ranging from relative risk of 2.3 CI (1.4–3.9) for decline based on self-report, arguably the weakest measure, to 4.7 CI (2.4–7.7) on performance-based criteria, which likely has greater reliability. Considering both criteria (the most likely scenario in many settings), the relative risk is 3.0 CI (2.1–4.4) (figure, all participants analyses). The size of this effect, therefore, is clinically meaningful.

Second, it has been suggested that ANI may simply be a statistical artifact of the particular testing procedures and algorithms proposed in the Frascati criteria.[6] While a detailed treatment of the statistical argument is beyond the scope of this article, the following 2 comments may be warranted: (1) statistical arguments rooted primarily in assumptions of Gaussian distributions of test scores may not be the most appropriate[7]; and (2) it seems unlikely that the growing number of studies that report differences in rates of impairment between HIV+ and HIV− participants can be dismissed as artifactual. In

Figure **Asymptomatic neurocognitive impairment increases risk for earlier decline to symptomatic HIV-associated neurocognitive disorders, even with viral suppression on combination antiretroviral therapy**



Relative risk for asymptomatic neurocognitive impairment (ANI) vs neurocognitively normal (NCN) as a predictor of earlier decline to symptomatic HIV-associated neurocognitive disorders (HAND) using self-report only, performance-based only, and self-report or performance-based criteria for symptomatic HAND. Viral suppression = plasma viral load ≤50 copies per mL at baseline. CI = confidence interval. [a] Total sample: ANI = 121; NCN = 22. [b] Sample with viral suppression at baseline: ANI = 55; NCN = 85.

addition, neuroimaging studies find detectable structural, functional, and spectroscopic evidence of brain abnormalities even in the acute and early phases of HIV infection.[21–25]

Of interest, those with ANI at baseline had evidence of more advanced prior HIV disease, e.g., lower nadir CD4 and greater likelihood of an AIDS diagnosis. This finding supports the concept that ANI is an HIV-driven process that, like more severe forms of HAND, is more likely with greater levels of prior immunosuppression.

We noted that women were overrepresented among those who experience symptomatic decline (table 3). Possible explanations may include that they had less education (13.4 years men vs 11.9 women; $p < 0.0001$) and had higher lifetime rates of major depression (47.5% men vs 61.9% women), both of which track with worse everyday functioning. They were also less likely to be on ART at baseline (ART = 71% men vs 51% women; $p = 0.0.03$) and had more visits during which HIV was detectable in plasma (23.0% men vs 31.8% women).

Several other cofactors such as substance use disorders and HCV coinfection were independently associated with symptomatic progression of ANI. This

Table 3 Baseline characteristics of nondecliners and decliners to symptomatic HAND (SR/PB)

	No decline (n = 237), mean (SD), %, or median (IQR)	Decline (n = 110), mean (SD), %, or median (IQR)	*p* Value	Cohen *d*/OR (95% CI)[a]
Background factors				
Age, y[b]	42.6 (8.7)	45.7 (7.4)	0.002	0.37
Education, y[b]	13.2 (2.3)	12.6 (2.2)	0.007	−0.26
% Male[c]	86.9	70.9	0.0003	2.7 (1.6–4.8)
% Lifetime substance use diagnosis[c]	65.8	80.9	0.004	2.2 (1.3–3.8)
% With comorbidity[c]	24.9	41.8	0.001	2.2 (1.3–3.5)
Disease factors				
% AIDS[c]	54.4	67.3	0.02	1.7 (1.1–2.8)
Nadir CD4, cells/mm^3[d]	204 (56–378)	163 (55–277)	0.03	−0.26
% HCV+[c]	18.1	32.7	0.003	2.2 (1.3–3.7)

Abbreviations: CI = confidence interval; HAND = HIV-associated neurocognitive disorders; IQR = interquartile range; OR = odds ratio; PB = performance-based; SR = self-report.
Ethnicity, on/off antiretroviral therapy, CD4, plasma viral load, CSF viral load, and estimated duration of HIV infection were nonsignificant ($p \geq 0.05$).
[a] *t* test.
[b] χ^2 test.
[c] Wilcoxon rank test.
[d] Cohen *d* = effect size.

is consistent with cross-sectional data showing that, for example, methamphetamine confers greater risk of poorer functional outcomes in HIV.[26] Together with the 3-year greater age of the decliners, these cofactors may produce greater CNS vulnerability to HIV-associated decline. Only 2 time-dependent factors, current CD4 and current major depression, predicted decline to symptomatic status using 1 or more criteria for symptomatic status. It is interesting that current CART, CART regimen, and virologic control did not contribute to the relative risk of decline.

Self-report of cognitive symptoms, at any point in time, requires not only the presence of everyday-functioning difficulties themselves but also awareness or insight on the part of the individual being assessed. We are unable to tell whether our participants' "declines" to symptomatic status by SR reflect actual increases in functional impairment or simply increased awareness of such impairment that may have existed even at baseline. Such increased awareness might occur if a person is faced with more cognitively demanding situations in everyday life or if there is reduced support

Table 4 Time-dependent correlates of decline to symptomatic HAND

	Univariable *p* Value	Multivariable		
		RR	95% CI for RR	*p* Value
Self-report				
ANI vs NCN	0.0007	2.81	1.65–4.76	0.0001
Current MDD	0.011	3.00	1.56–5.77	0.001
Performance-based				
ANI vs NCN	<0.0001	5.17	3.19–8.39	<0.0001
Current CD4	0.0014	1.21	1.08–1.35	0.0006
SR or PB				
ANI vs NCN	<0.0001	3.41	2.33–5.00	<0.0001
Current CD4	0.033	1.10	1.01–1.20	0.021

Abbreviations: ANI = asymptomatic neurocognitive impairment; CI = confidence interval; HAND = HIV-associated neurocognitive disorders; MDD = major depressive disorder; NCN = neurocognitively normal; PB = performance-based; RR = relative risk; SR = self-report.
Antiretroviral therapy treatment, regimen type, CNS penetration effectiveness score, plasma viral load, CSF viral load, and current substance use diagnoses were nonsignificant in univariable analyses.

from others in such situations. Whatever the mechanisms, SR of functional decline should be of clinical concern, requiring further evaluation.

Some limitations of this study include the selection of the sample and the lack of demographically adjusted norms for the performance-based measures. It is possible that requirements of participation in the longitudinal component of CHARTER (i.e., visits every 6 months, willingness to complete extra assessments) resulted in sample bias where the highest functioning (employed) participants would be less represented since they might not have the time to spare, whereas lower functioning or disabled participants might not be able to participate due to physical or cognitive limitations. In regard to norms, we used the best data available to set cutpoints for the performance-based measures that have shown evidence of construct validity in prior studies[27]; however, norms based on HIV− controls with similar demographics would improve the accuracy of any such cutpoints.

This study found that patients with ANI were about 3 times more likely to develop everyday life problems as those who were initially cognitively normal. This finding suggests that those in whom ANI has been detected deserve regular monitoring in terms of progression to symptomatic status. Future intervention studies may need to focus on such individuals to thwart further neurocognitive and functional decline.

AUTHOR CONTRIBUTIONS

Dr. Grant is the primary author on this manuscript and as such he was responsible for study conceptualization and design. All study data were available to him and he planned the statistical analyses and performed the interpretation of the results. Dr. Grant thereby assumes responsibility for the accuracy of the data, analysis, and interpretation. Donald R. Franklin is the CHARTER center manager and he provides integral coordination and dissemination of CHARTER data. Additionally, he contributed to this manuscript by assisting with study design, data analysis, drafting and revision of the manuscript. Dr. Deutsch contributed to all aspects of the manuscript, including study design, statistical analysis, and interpretation of results. Dr. Woods significantly contributed to all aspects of the manuscript, including study design, statistical analysis, and interpretation of results. He also strongly contributed in revising the manuscript. Dr. Vaida assisted with the interpretation of results along with drafting and revising the manuscript. Dr. Ellis made considerable contributions through management and coordination of the neuromedical data, and assisted with study design, analysis, and interpretation, as well as revisions to the manuscript. Dr. Letendre made considerable contributions through management and coordination of the laboratory data, and assisted with study design, analysis, and interpretation, as well as revisions to the manuscript. Dr. Marcotte assisted with the interpretation of results along with drafting and revising the manuscript. Dr. Collier assisted with primary data collection, drafting, and revising the manuscript. Dr. Marra assisted with primary data collection, drafting, and revising the manuscript. Dr. Clifford assisted with primary data collection, drafting, and revising the manuscript. Dr. Gelman assisted with primary data collection, drafting, and revising the manuscript. Dr. McArthur assisted with primary data collection, drafting, and revising the manuscript. Dr. Morgello assisted with primary data collection, drafting, and revising the manuscript. Dr. Simpson assisted with primary data collection, drafting, and revising the manuscript. Dr. McCutchan assisted with primary data collection, drafting, and revising the manuscript. Dr. Abramson assisted with drafting and revising the manuscript. Dr. Gamst assisted with drafting and revising the manuscript. Dr. Fennema-Notestine assisted with drafting and revising the manuscript. Dr. Smith assisted with drafting and revising the manuscript. Dr. Heaton significantly contributed to all aspects of the manuscript, including study design, statistical analysis, and interpretation of results. He also strongly contributed in revising the manuscript.

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DISCLOSURE

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EXHIBIT 44

EXHIBIT 45

IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF VIRGINIA
ALEXANDRIA DIVISION

NICHOLAS HARRISON and OUTSERVE-SLDN, INC.,

Plaintiffs,

v.

JAMES N. MATTIS, in his official capacity as Secretary of Defense; MARK ESPER, in his official capacity as Secretary of the Army; and the UNITES STATES DEPARTMENT OF DEFENSE,

Defendants.

No. 1:18-CV-00641-LMB-IDD

Tuesday, January 9, 2019

Videotape Deposition of LT. COL. LISA M. LUTE, taken at the Law Offices of Winston & Strawn LLP, located at 1700 K Street Northwest, Washington, D.C., beginning at 9:26 a.m., before Ryan K. Black, a Registered Professional Reporter, Certified Livenote Reporter and Notary Public in and for the District of Columbia.

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ALSO PRESENT:

Alexandra Hemmings, Winston & Strawn Law Clerk

Solomon Francis, Legal Videographer

I N D E X

E X H I B I T S

I N D E X (Cont'd)

I N D E X (Cont'd)

condition to know whether or not they would --

A. Their condition, their management, their --

Q. Whether or not they would pose --

A. Whether or not they -- they are compliant with medication or anything like that, okay?

Q. Let me finish my -- my question, --

A. I'm sorry.

Q. -- but I -- but I think you answered it. So you don't know the extent to which their HIV is under control, how it is being managed, et cetera?

A. Correct.

Q. And what I heard you say was that if the person's HIV was not well-managed, --

A. Mm-hmm.

Q. -- and you said something about a count, and I believe what you're referring to is the viral load --

A. That would be correct.

Q. -- for HIV, that if it was -- if it was not well-managed, that then they could potentially present a risk in the context of a -- a battlefield transmission?

A. There are other ways. I mean, they could present a risk. They could -- if an individual that is not properly managed or forthcoming regarding their diagnosis and they donated blood, it could impact the blood bank. And -- and, yes, we have screening on the blood bank that we use here, but we don't when you do a combat situation. It's -- it's a buddy donor-type system.

Q. Have you ever heard of anyone with HIV in the military who knew their status attempting to donate blood?

A. Yes, sir.

Q. And when did that occur, or tell me -- how -- was it more than once or one time?

A. I'm only aware of one time.

Q. And when was that?

A. I was in San Antonio, and that's as close as I can get you, okay? So I was there from, let me see, '14 -- 2014 to 2016.

MR. NORWAY: And I'm going to interpose an objection that she's not being offered for -- for this topic.

MR. SCHOETTES: Well, we'll come -- we'll come back to that.

THE WITNESS: Okay.

BY MR. SCHOETTES:

Q. So you've now identified battlefield transmission as -- as a concern for someone whose HIV is not well-controlled, and the possibility of blood donation.

A. Well, I -- I would like, if I may, to -- to clarify something. Because in the battlefield, it doesn't matter if they're well-controlled or not with blood, because there's no research to support whether or not a significant exposure to a person with HIV's blood would be reduced or limited if they were on medication and managed properly. So I -- I -- you can't -- it would only -- the only research that -- that has been put out that -- that demonstrates that -- if they are effectively managed is that it reduces the risk of transmission with sexual interactions.

Q. So I think what you just said contradicts what you said earlier, which is that if someone was virally suppressed and had less than the 200 copies per milliliter of HIV, that then would be a negligible risk, --

A. And --

that can definitively say I -- the Army G-1 will not approve this. It at least needs to make it there, and then they need to say, we can't approve this, because it falls under the -- the instruction and we don't have the authority.

Q. So when you say you would put the packet together and process it, you would process it within the Army --

A. Mm-hmm.

Q. -- and get that denial, if you will, because there's not the authority, and then at that point you would elevate it over to the Department of Defense?

A. No. I would -- I would -- it goes back to the service member.

Historically, if I know that what they're requesting is outside of what we can do, and I am involved in it in the beginning, I may tell them I will process this for a response, and I will ask them to familiarize themselves with the policies and the directives, the -- the DODIs that go along with the request.

Q. And then do you advise them after either you tell them that or -- or you process it, do you tell them that they need to submit

this through a different channel?

A. I do not. I -- I recommend to them that -- that they might want to review the DODI and consider using the DODI as a guideline to make their request.

Q. Okay. Going back to your declaration, you make the statement that, I am aware of multiple soldiers who have been granted COCOM waivers to deploy.

MR. NORWAY: Objection; scope.

You may answer.

THE WITNESS: Yes. I said that.

Is that the question? I'm sorry.

BY MR. SCHOETTES:

Q. Yes. No, it is. That was just a baseline.

So are you -- is it -- are you saying that you're aware of soldiers living with HIV who have been granted COCOM waivers to deploy?

MR. NORWAY: Objection; scope.

THE WITNESS: Yes.

BY MR. SCHOETTES:

Q. And how did you become aware that soldiers living with HIV have been granted waivers to deploy?

MR. NORWAY: Yeah. Let's go off the record.

MR. SCHOETTES: Okay.

THE VIDEOGRAPHER: The time is 4:09 p.m. We're going off the record.

(Recess taken.)

THE VIDEOGRAPHER: The time is 4:15 p.m. We're back on the record.

Please proceed, Counsel.

MR. SCHOETTES: I'd like to ask the court reporter to record for us how much time we spent on the record thus far.

THE VIDEOGRAPHER: 5:51:36 and counting.

MR. SCHOETTES: Thank you.

MR. NORWAY: And we have no redirect.

MR. SCHOETTES: All right. Well, then we're done.

MR. NORWAY: Great. And we're going to read and sign.

THE REPORTER: Yeah. Okay.

MR. NORWAY: Thank you very much, Scott.

THE VIDEOGRAPHER: Time is 4:15 p.m. This concludes today's testimony given by Lt.

Col. Lisa Lute. We're now off the record.

(Deposition concluded -- 4:15 p.m.)

Lt. Col. Lisa M. Lute

C E R T I F I C A T E

I do hereby certify that the aforesaid testimony was taken before me, pursuant to notice, at the time and place indicated; that said deponent was by me duly sworn to tell the truth, the whole truth, and nothing but the truth; that the testimony of said deponent was correctly recorded in machine shorthand by me and thereafter transcribed under my supervision with computer-aided transcription; that the deposition is a true and correct record of the testimony given by the witness; and that I am neither of counsel nor kin to any party in said action, nor interested in the outcome thereof.

WITNESS my hand and official seal this 24th day of January 2019.

Ryan K. Black

Veritext Legal Solutions
1100 Superior Ave
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Phone: 216-523-1313

January 24, 2019

To: Robert M. Norway, Esq.

Case Name: Harrison, Nicholas, et al. v. Mattis, James N., et al.

Veritext Reference Number: 3189088

Witness: Lt. Col. Lisa M. Lute Deposition Date: 1/9/2019

Dear Sir/Madam:

Enclosed please find a deposition transcript. Please have the witness review the transcript and note any changes or corrections on the included errata sheet, indicating the page, line number, change, and the reason for the change. Have the witness' signature notarized and forward the completed page(s) back to us at the Production address shown above, or email to production-midwest@veritext.com.

If the errata is not returned within thirty days of your receipt of this letter, the reading and signing will be deemed waived.

Sincerely,
Production Department

NO NOTARY REQUIRED IN CA

DEPOSITION REVIEW
CERTIFICATION OF WITNESS

ASSIGNMENT REFERENCE NO: 3189088
CASE NAME: Harrison, et al. v. Mattis, James N., et al.
DATE OF DEPOSITION: 1/9/2019
WITNESS' NAME: Lt. Col. Lisa M. Lute

In accordance with the Rules of Civil Procedure, I have read the entire transcript of my testimony or it has been read to me.

I have made no changes to the testimony as transcribed by the court reporter.

_____ _____
Date Lt. Col. Lisa M. Lute

Sworn to and subscribed before me, a Notary Public in and for the State and County, the referenced witness did personally appear and acknowledge that:

They have read the transcript;
They signed the foregoing Sworn Statement; and
Their execution of this Statement is of their free act and deed.

I have affixed my name and official seal

this _____ day of_____, 20____.

_____
Notary Public

_____
Commission Expiration Date

DEPOSITION REVIEW
CERTIFICATION OF WITNESS

ASSIGNMENT REFERENCE NO: 3189088
CASE NAME: Harrison, et al. v. Mattis, James N., et al.
DATE OF DEPOSITION: 1/9/2019
WITNESS' NAME: Lt. Col. Lisa M. Lute

In accordance with the Rules of Civil Procedure, I have read the entire transcript of my testimony or it has been read to me.

I have listed my changes on the attached Errata Sheet, listing page and line numbers as well as the reason(s) for the change(s).

I request that these changes be entered as part of the record of my testimony.

I have executed the Errata Sheet, as well as this Certificate, and request and authorize that both be appended to the transcript of my testimony and be incorporated therein.

_____ _____
Date Lt. Col. Lisa M. Lute

Sworn to and subscribed before me, a Notary Public in and for the State and County, the referenced witness did personally appear and acknowledge that:

They have read the transcript;
They have listed all of their corrections in the appended Errata Sheet;
They signed the foregoing Sworn Statement; and
Their execution of this Statement is of their free act and deed.

I have affixed my name and official seal this _____ day of_____, 20____.

_____
Notary Public

_____
Commission Expiration Date

ERRATA SHEET

VERITEXT LEGAL SOLUTIONS MIDWEST

ASSIGNMENT NO: 1/9/2019

PAGE/LINE(S) / CHANGE /REASON

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Date Lt. Col. Lisa M. Lute

SUBSCRIBED AND SWORN TO BEFORE ME THIS _____

DAY OF _____, 20_____ .

_____

Notary Public

_____

Commission Expiration Date

EXHIBIT 46

IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF VIRGINIA
ALEXANDRIA DIVISION

- - - - - - - - - - - - - - - - - - x
NICHOLAS HARRISON and :
OUTSERVE-SLDN, INC., :
Plaintiffs, :
vs. : No. 1:18-cv-00641
JAMES N. MATTIS, In His : LMB-IDD
Official Capacity As Secretary:
of Defense; MARK ESPER, In His:
Official Capacity As the :
Secretary of the Army; and the:
UNITED STATES DEPARTMENT OF :
DEFENSE, :
Defendants. :
- - - - - - - - - - - - - - - - - - x
RICHARD ROE, VICTOR VOE, and :
and OUTSERVE-SLDN, INC., :
Plaintiffs, :
vs. : No. 1:18-cv-01565
JAMES N. MATTIS, In His :
Official Capacity As Secretary:
of Defense; HEATHER A. WILSON,:
In Her Official Capacity as :
Secretary of the AIR FORCE; :
and the UNITED STATES :
DEPARTMENT OF DEFENSE, :
Defendants. :
- - - - - - - - - - - - - - - - - - x

VIDEOTAPED 30(b)(6) DEPOSITION OF DEFENDANTS
GIVEN BY AUDRA L. TAYLOR

DATE: Friday, March 1, 2019
TIME: 10:17 a.m.
LOCATION: Winston & Strawn
1700 K Street, N.W.
Washington, D.C.

REPORTED BY: Denise M. Brunet, RPR
Reporter/Notary

Veritext Legal Solutions

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(Appearances continued on the next page.)

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ALSO PRESENT: Solomon Francis, Videographer

C O N T E N T S

(*Exhibits attached to the transcript.)

P R O C E E D I N G S

THE VIDEOGRAPHER: Good morning. We are going on the record at 10:17 a.m. on March 1st, 2019. Please note that the microphones are sensitive and may pick up whispering, private conversations and cellular interference. Please turn off all cell phones or place them away from the microphones as they can interfere with the deposition audio. Audio and video recording will continue to take place unless all parties agree to go off the record.

This is media unit 1 of the video-recorded deposition of Colonel Audra L. Taylor taken by counsel for plaintiffs in the matter of Nicholas Harrison and Outserve-SLDN, Inc., plaintiffs, versus James N. Mattis, in his official capacity as Secretary of Defense, et al., defendants, and Richard Roe and Victor Voe and Outserve-SLDN, Inc., plaintiffs, versus James N. Mattis, in his official capacity as Secretary of Defense, et al., defendants, case numbers 1:18-CV-01565 and case number 1:18-CV-00641, filed in the United States District Court for the Eastern District of Virginia.

This deposition is being held at the law

Q Are individuals who have not been pre-screened as donors ever used as part of the walking blood bank?

A Not routinely. They are not the first priority. The first priority is a screened donor.

Q But it's possible that a non-screened donor could be used if screened donors were not available or had been exhausted?

A Yes, that is possible.

Q Are donors of fresh whole blood to the walking blood bank also sometimes called battlefield donors?

A Not officially, but I won't say that you've never seen that term. I could see someone using it, but...

Q Let me ask this a different way. Is a battlefield donor something different than a donor through the walking blood bank?

A Not to me.

Q Are transfusions of fresh whole blood sometimes called battlefield transfusions?

A Yes.

MR. ABBUHL: Counsel, could we take a short break on the soon side?

MR. SCHOETTES: On the soon? Sure. Let

guideline -- is slightly different than the form that is used in one of the donor centers for -- if you were to walk in today and donate blood.

MR. SCHOETTES: I don't think we have the form from the donor center, and I'd like to have that.

MR. ABBUHL: Okay.

MR. SCHOETTES: And I think it's responsive to one of our requests.

MR. ABBUHL: I'd have to double-check if we produced it, but I'll look into it.

MR. SCHOETTES: Okay.

BY MR. SCHOETTES:

Q So you described blood being -- tubes of blood from the unit being -- from the unit of blood collected being sent to the United States for testing. Are the tests that are performed on those tubes of fresh whole blood the same as the tests that would -- that are performed on blood collected at a blood center?

A Yes.

Q And in addition, you indicated that rapid testing was also conducted on fresh whole blood, correct?

A Yes, to the greatest extent possible.

Q And for what transmitted TTDs are rapid tests conducted?

A HIV, HCV, hep B surface antigen, malaria.

Q Is that it? Any others?

A That's it.

Q Is fresh whole blood FDA-approved?

A Not in theater.

Q And why is that?

A If you collect the donor and then transfuse it before all of the FDA-required testing is performed, it is not an FDA-licensed product.

Q And so the rapid tests that are performed, are those -- would those meet the standard required by the FDA?

A No.

Q And the rapid tests are always performed, but sometimes not completed before the blood is transfused, correct?

MR. ABBUHL: Objection. I think assumes facts not in evidence.

BY MR. SCHOETTES:

Q Let me ask this question: Are the rapid tests always conducted on the fresh whole blood collected in a deployed environment?

A I can't say that for certainty.

Q According to the standard operating procedures, are the rapid tests supposed to be performed for all fresh whole blood collected in a deployed environment?

A To the greatest extent possible.

Q So explain to me what that means.

A That means if time is an issue and the unit is needed before that testing can be completed, it's a -- it's a medical decision and the physician would assume responsibility for the unit.

If it's a logistical issue and the location doesn't have all of the rapid testing -- let's say they had one of the four kits or two of the four, for whatever reason -- then they would perform what they have. So to the greatest extent possible, just -- they'll do what they can with what they have in the amount of time they have.

However, testing would continue. So if the transfusion goes and they have the kits, they -- they should continue the testing.

Q So that's what I was trying to get at, I think. So for the tests -- the rapid tests that are available in a particular situation, they are

performed on the individual regardless of whether the results are going to be available by the time the blood is transfused?

A Correct.

MR. SCHOETTES: I could go a few more questions or I could break now. Up to you.

THE WITNESS: You can go a few more, then we can break.

MR. SCHOETTES: Actually, no. Let's break -- let's break now.

THE VIDEOGRAPHER: The time is 11:27 a.m. This completes media unit number 1. We are now off the record.

(Whereupon, a short recess was taken.)

THE VIDEOGRAPHER: The time is 11:40 a.m. This begins media unit number 2. We are now on the record. Please proceed, Counsel.

BY MR. SCHOETTES:

Q When is the walking blood bank activated, in what circumstances?

A It would be at the discretion of the -- of the physician. They would take into account what products are on hand, the potential for resupply of blood products, as well as the condition of the patient. And then they would

make that decision to activate the walking blood bank.

Q Is the decision always made on an individual basis per patient?

A I don't know if you would want to confine that per patient or per event. Like, it could be more than one patient. So that would dictate the -- the need to activate it if the number of patients in an event or mass -- mass casualty are going to go require more than what you have on hand of what you can be resupplied within the time frame.

Q And is a mass casualty event sometimes referred to as a MASCAL?

A Yes.

Q And is that -- the acronym for that M-A-S-C-A-L?

A All caps. Yes.

Q All caps. So what you've described would be a situation where the walking blood bank was activated based on injuries that had already occurred?

A Right. An assessment of what -- what they have coming in, yeah. So, yeah, they would need to assess the injuries, know what they have

on hand blood-wise, and then make a decision.

Q Are decisions ever made to activate the walking blood bank in anticipation of casualties?

A I don't know. I can't confirm or...

Q Does the supply of stored whole blood -- let me try again.

Is fresh whole blood generally used only when the supply of stored whole blood has been exhausted or is unavailable?

A Stored whole blood is fairly new into theater. It's not a practice that's been going on for years. So, yes, they -- they should use stored whole blood before activating a walking blood bank.

Q For how many years has stored whole blood been used in theater?

A Is it 2019 -- within the last two years.

Q So stored whole blood was not used during Operation Enduring Freedom?

A No, not -- it's called something else now. So, no. No, it was not.

Q Did you say it was called something else?

A No, I'm saying it's -- you know how they change the name of the campaigns? So when you said Operation Enduring Freedom, no, it has not

historically been used in Operation Enduring Freedom.

Q And was it used during Operation Iraqi Freedom?

A Stored whole blood, no, not per clinical practice guideline, no.

Q What about appropriate blood component products? Are those generally exhausted or unavailable before fresh whole blood is used?

A They should be exhausted, but again, I believe the decision -- the medical decision is made based on the number of casualties, the type of injury, the casualties, and what the physician feels is best for that casualty.

So depending on the nature of the -- the injury and how many -- I think that they -- they have to make that decision. They're not going to exhaust the entire supply of components, I don't believe, because you don't know what's coming in next. So you have to take a lot of factors into consideration when you decide to activate the walking blood bank and collect fresh units.

Q And are there some injuries for which blood component products might be better than fresh whole blood?

Q Actually, I should rephrase that. Ideally the test results are obtained before the blood is used, correct?

A Correct.

Q Under what circumstances would the blood be used before the results of the rapid screening tests are obtained?

A The patient's condition and the physician -- the physician requesting the unit immediately.

Q So presumably, it would be at a point where the need for that unit of blood exceeds the risk of a TTI at that point?

A Yes.

Q If a unit has not been used prior to obtaining a result, what happens to the units that return a positive result after rapid testing for any of these conditions?

A Say that again.

Q If a unit has not been transfused -- and we're talking about in the context of the walking blood bank -- and then a positive result occurs on the blood that was tested from that unit, what happens to the unit of blood?

A Quarantined and destroyed.

A FF -- which product is on the horizon or --

Q Yes.

A -- which one would be augmented?

Q Which one would be augmented?

A Fresh frozen plasma would be augmented with freeze-dried plasma. That's a product that's on the horizon. So I don't want to -- I wouldn't -- I wouldn't say substitute, because that's not the plan to substitute one of the current components with something, but it's a product that doesn't require the extensive cold chain management and something that could go to POI.

Q Point of injury?

A Point of injury, yes.

Q When it is used, the -- this new product for plasma, freeze-dried plasma, would it be used on its own or would it need to be combined with some liquid plasma?

A It would not be combined with liquid plasma because FFP, liquid plasma and freeze-dried plasma are all plasma products. The liquid plasma and the FFP require freezer, refrigerators, the cold chain management. Freeze-dried plasma would

not. So it would be in that plasma suite of products that would be available.

Q I guess what I'm trying to ask is, when you said augmented, it isn't that you would use freeze-dried plasma to augment a unit of fresh frozen plasma. It is that you're augmenting the supply?

A Yes. Yes.

Q What specific infections would be tracked in terms of transfusion-transmitted infections that have resulted through the walking blood bank?

A Any of the -- any of the tests that we screen the supply for would be tracked.

Q So HIV, HBV, HCV --

A Correct.

Q -- et cetera?

A Correct.

Q Have there been any documented transmissions of HIV through the Armed Services Blood Program blood supply in the past ten years?

A Not that I'm aware of.

Q In the past 20 years?

A I don't know.

Q What about HBV? Have there been any transmissions of HBV through the Armed Services

Blood Program blood in the past 20 years?

A Not that I'm aware of.

Q Have there been any transmissions of HTLV through the Armed Services Blood Program in the past 20 years?

A I believe there is one case potentially linked to the transfusion.

Q Have there been any transmissions of HCV through the Armed Services Blood Program in the past 20 years?

A Maybe one. And I don't know for certain if either one of those was definitely linked to the transfusion or suspected. I'm not sure.

Q Is testing for HBV required prior to deployment?

A I don't know.

Q I'm going to ask you some questions about the process by which diagnostic and other blood tests are handled for service members who are deployed --

A Okay.

Q -- to foreign bases, including those in combat zones. If an individual needs a blood sample tested -- let's say they needed an HIV viral load test done --

A -- products won't reach their destination in the correct --

Q At the correct temperature.

A At the correct temperature, no.

MR. SCHOETTES: One last thing. We're going to mark this as Exhibit 5.

(Taylor Deposition Exhibit Number 5 was marked for identification.)

BY MR. SCHOETTES:

Q Do you recognize Exhibit 5?

A Yes.

Q What is it?

A It's the OraQuick Advance Rapid HIV-1/2 Antibody Test customer letter.

Q Is this the insert that you were describing earlier?

A Yes.

Q If you could turn to page 2.

A Uh-huh.

Q And under biological principles of the test --

A Uh-huh.

Q -- it says, "The OraQuick Advance Rapid HIV-1/2 Antibody Test is a manually performed, visually read, 20-minute immunoassay for the

qualitative detection of antibodies to HIV-1 and HIV-2 in human oral fluid, whole blood obtained from a finger puncture or a venipuncture, and plasma," correct?

A Correct.

Q Is it the case, then, that this test is one that can be -- results can be obtained within -- at 20 minutes?

A Per the package insert, yes. It says 20 minutes.

Q If you'll turn to page 10, the paragraph underneath the chart, table 3 --

A Uh-huh.

Q -- talks about the sensitivity of the OraQuick Advance Rapid HIV-1/2 antibody test in these studies was calculated to be 536 divided by 538, which is equal to 99.6 percent.

Do you agree that 99.6 percent is the sensitivity of the HIV rapid test used in the walking blood bank?

A Yes.

MR. SCHOETTES: I am done.

MR. ABBUHL: We're going to need to take a few minutes, but before going off the record, I just want, before I forget, say that we will

reserve our right to read and sign the transcript.

But if we can go off the record for just a bit while we confer.

MR. SCHOETTES: Sure.

THE VIDEOGRAPHER: The time is 1:57 p.m. We are going off the record.

(Whereupon, a short recess was taken.)

THE VIDEOGRAPHER: The time is 2:21 p.m. We are back on the record. Please proceed, Counsel.

EXAMINATION BY COUNSEL FOR
THE U.S. DEPARTMENT OF JUSTICE

BY MR. ABBUHL:

Q Colonel, if you could please pick up the document marked Exhibit 5 in front of you and turn to page 10. This is the document about the rapid test for HIV, correct?

A Correct.

Q And if you look at the text below table 3, it says that -- it essentially says that the sensitivity of the HIV test was about 99 percent, correct?

A Correct.

Q Do you know the conditions under which that sensitivity was measured?

A No.

Q Do you know if it was measured in a battlefield environment?

A No. I don't know.

Q Okay. You can put away Exhibit 5 for now.

You've discussed various testing and precautions that are done to protect the blood supply; is that correct?

A Correct.

Q And you testified that those protections are done to the extent possible, correct?

A Correct.

Q So there are situations where tests might not happen that you would do in an ideal situation, correct?

A Correct.

Q And there's screening that you might do in an ideal situation, but sometimes, in a military situation, you might not be able to do it; is that correct?

A Correct.

Q Is that particularly true in situations involving being near a battlefield?

A Yes.

Q If you could also look at Exhibit 3, which is in front of you. This is the joint trauma system clinical practice guideline, correct?

A Correct.

Q Could you please turn to page 17? Could you remind me what page 17 is?

A It's the blood donor pre-screening SOP enclosure 1, ASBP 572, emergency whole blood (front).

Q And at the bottom of the page, there's a place for donor's signature; is that correct?

A Correct.

Q And could you please read the text above that line, please?

A "I verify"? That part?

Q Yes.

A "I verify that I have answered the questions honestly, I had an opportunity to ask questions. I consent to donating blood today and I feel my blood is safe to be transfused. If I am donating a unit of whole blood today, my blood will not be tested for viral diseases prior to transfusions due to the emergency situation. If for any reason I feel that my blood may not be

safe, I will not donate today."

Q Did you testify earlier that a donor should sign that line before donating blood?

A They should.

Q Are there instances where blood would be collected without a signature on this form?

A Yes.

Q And if you would also on that same page -- there's a footer at the very bottom. Do you see that?

A Yes.

Q Could you please read the footer?

A Are you talking about the blue?

Q Yes, the blue text.

A "Guideline only/not a substitute for clinical judgment."

Q Thank you. And you can also put that exhibit down.

Earlier in your testimony, you said if someone was permanently deferred from donating blood, they would not be recruited to donate blood; is that correct?

A Correct.

Q That is what you testified to earlier, correct?

A I believe so, yes.
Q Are there any military situations where you would transfuse blood before screening it?
A I'm not sure I --
Q Is it possible that a blood transfusion would take place in the military without it having been screened and then you could screen the transfusion afterward?
A In theater?
Q Correct.
A In a theater situation?
Q Is it possible?
A You're asking is it possible for a transfusion to take place prior to screening of the unit or the donor or --
Q Of the blood being transfused.
A Yes. A transfusion can take place prior to the screening --
MR. ABBUHL: I have no further questions.
THE WITNESS: -- being completed.
MR. SCHOETTES: I just have one or two.
FURTHER EXAMINATION BY COUNSEL FOR PLAINTIFFS
BY MR. SCHOETTES:
Q Going back to Exhibit 3, page 17, you just testified that there would be some instances

in which blood would be donated without the -- there could be instances in which the blood would be donated without the signature of the donor on this form. Can you tell me in what instances?

A I believe there are -- well, I used -- I think I used the word "should." It should be signed. There could be instances, maybe at a -- at a role two, if they urgently, you know, based on the number of personnel, the number of casualties and the scenario or the situation where they might not be able to get everything completed and signed ahead of time.

There could be even at point of injury, if they are to take like one of the pre -- pre-made kits that are available on the market and they're going out and they need to collect, I believe there's a form, a donor questionnaire in those kits should be signed, but, you know, depending on what the scenario is and how it's going, it may or may not be signed. So I just think it's situation and scenario-driven. It should be -- to the greatest extent possible, they should all be screened, they should all answer all the questions, have a chance to ask their questions, but I can't for certainty say that that

happens 100 percent of the time.

Q Have you ever been in a situation where the blood -- the walking blood bank was being used in which a form like this was not signed?

A Not that I recall. With the combat support hospital, no. As the JBPO, you're not -- I wasn't that -- I wasn't close to the situations, so I'm not certain if there were any that happened without the signature on the 572.

Q And then, just a clarifying a question. Counsel asked you if there could be a blood donation in the military without screening. In your answer, you talked about screening of the donor or screening of the blood. Were you indeed talking about without the blood being tested?

A Yes. That's the way I interpreted the question.

MR. SCHOETTES: That's all I have.

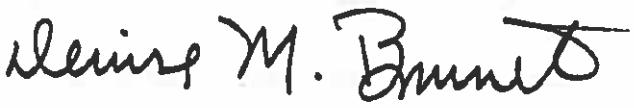
MR. ABBUHL: We will -- again, we'd like to be able to review the record and sign it.

THE VIDEOGRAPHER: The time is 2:30 p.m. This conclude today's testimony given by Colonel Audra L. Taylor. We are now off the record.

Whereupon, at 2:30 p.m., the deposition of AUDRA L. TAYLOR was concluded.)

CERTIFICATE OF NOTARY PUBLIC

I, Denise M. Brunet, the officer before whom the foregoing deposition was taken, do hereby certify that the witness whose testimony appears in the foregoing deposition was sworn by me; that the testimony of said witness was taken by me stenographically and thereafter reduced to print by means of computer-assisted transcription by me to the best of my ability; that I am neither counsel for, related to, nor employed by any of the parties to this litigation and have no interest, financial or otherwise, in the outcome of this matter.



Denise M. Brunet
Notary Public in and for
The District of Columbia

My commission expires:
December 14, 2022

Veritext Legal Solutions
1100 Superior Ave
Suite 1820
Cleveland, Ohio 44114
Phone: 216-523-1313

March 18, 2019

To: Joshua Abbuhl, Esq.

Case Name: Roe, Richard, et al. v. Shanahan, Patrick M., Et Al.

Veritext Reference Number: 3235702

Witness: Audra L. Taylor Deposition Date: 3/1/2019

Dear Sir/Madam:

Enclosed please find a deposition transcript. Please have the witness review the transcript and note any changes or corrections on the included errata sheet, indicating the page, line number, change, and the reason for the change. Have the witness' signature notarized and forward the completed page(s) back to us at the Production address shown above, or email to production-midwest@veritext.com.

If the errata is not returned within thirty days of your receipt of this letter, the reading and signing will be deemed waived.

Sincerely,
Production Department

NO NOTARY REQUIRED IN CA

DEPOSITION REVIEW
CERTIFICATION OF WITNESS

ASSIGNMENT REFERENCE NO: 3235702
CASE NAME: Roe, Richard, et al. v. Shanahan, Patrick M.
DATE OF DEPOSITION: 3/1/2019
WITNESS' NAME: Audra L. Taylor

In accordance with the Rules of Civil Procedure, I have read the entire transcript of my testimony or it has been read to me.

I have made no changes to the testimony as transcribed by the court reporter.

16 April 2019 _____
Date Audra L. Taylor

Sworn to and subscribed before me, a Notary Public in and for the State and County, the referenced witness did personally appear and acknowledge that:

They have read the transcript;
They signed the foregoing Sworn Statement; and
Their execution of this Statement is of their free act and deed.

I have affixed my name and official seal this 16th day of April, 2019.

Windy Regina Rowland
Notary Public

September 30, 2020
Commission Expiration Date

WINDY REGINA ROWLAND NOTARY PUBLIC COMMONWEALTH OF VIRGINIA MY COMMISSION NUMBER 106091

My Commission Expires September 30, 2020

DEPOSITION REVIEW
CERTIFICATION OF WITNESS

ASSIGNMENT REFERENCE NO: 3235702
CASE NAME: Roe, Richard, et al. v. Shanahan, Patrick M.
DATE OF DEPOSITION: 3/1/2019
WITNESS' NAME: Audra L. Taylor

In accordance with the Rules of Civil Procedure, I have read the entire transcript of my testimony or it has been read to me.

I have listed my changes on the attached Errata Sheet, listing page and line numbers as well as the reason(s) for the change(s).

I request that these changes be entered as part of the record of my testimony.

I have executed the Errata Sheet, as well as this Certificate, and request and authorize that both be appended to the transcript of my testimony and be incorporated therein.

16 April 2019 _____
Date Audra L. Taylor

Sworn to and subscribed before me, a Notary Public in and for the State and County, the referenced witness did personally appear and acknowledge that:

They have read the transcript;
They have listed all of their corrections in the appended Errata Sheet;
They signed the foregoing Sworn Statement; and
Their execution of this Statement is of their free act and deed.

I have affixed my name and official seal this 16th day of April, 2019.

_____
Notary Public

September 30, 2020
Commission Expiration Date

WINDY REGINA ROWLAND NOTARY PUBLIC MY COMMISSION NUMBER 106091 COMMONWEALTH OF VIRGINIA

My Commission Expires September 30, 2020

ERRATA SHEET

VERITEXT LEGAL SOLUTIONS MIDWEST

ASSIGNMENT NO: 3/1/2019

PAGE/LINE(S) /	CHANGE	/REASON
89 / 12	Alginate to Allogeneic	not spelled correctly
123 / 1	FFD to FDP	acronym
123 / 12	Freeze Dried Plasma	missing word
34 / 21	Collect	missing word
35 / 6 and 7	Units are shipped from our depot centers to those distribution centers. Note: can use "shipped or "Fed Exed"	

16th April 2019

Date

Audra L. Taylor

SUBSCRIBED AND SWORN TO BEFORE ME THIS 16th

DAY OF April 2019.

Notary Public

My Commission Expires September 30, 2020

September 30 2020

Commission Expiration Date

CINDY REGINA ROWLAND NOTARY PUBLIC MY COMMISSION NUMBER 106091 COMMONWEALTH OF VIRGINIA