FOOD AND DRUG ADMINISTRATION

+ + + + +

CENTER FOR DRUG EVALUATION AND RESEARCH

+ + + + +

ANTIVIRAL DRUGS ADVISORY COMMITTEE

+ + + + +

MEETING

+ + + + +

MONDAY

OCTOBER 16, 2000

+ + + + +

The meeting was held at the Gaithersburg Marriott Washington Center, 9751 Washingtonian Boulevard, Gaithersburg, Maryland 20878, at 8:30 a.m., Dr. Henry Masur, Chair, presiding.

PRESENT:

HENRY MASUR, M.D.	Chair
COURTNEY V. FLETCHER, Pharm.D.	
ROY M. GULICK, M.D., M.P.H.	
JOHN D. HAMILTON, M.D.	
WILLIAM CHRISTOPHER MATHEWS, M.D., M.S.PH.	
SHARILYN K. STANLEY, M.D.	
BRIAN WONG, M.D.	
RAM YOGEV, M.D.	
NANCY CHAMBERLIN, Pharm.D.	

CONSULTANTS AND GUESTS:

DOUGLAS G. FISH, M.D.	Guest
THOMAS R. FLEMING, Ph.D. (voting)	CDER Consultant
LAWRENCE FOX, M.D., Ph.D.	Guest Speaker
JON KAGAN, M.D.	Guest Speaker
DANIEL R. KURITZKES, M.D.	Guest Speaker
ALAN LANDAY, Ph.D.	
CLIFFORD LANE, M.D.	CBER Consultant (non-voting)

CONSULTANTS AND GUESTS: (cont.)

BRENDA LEIN	Patient Representative
MICHAEL LEDERMAN, M.D.	Guest Speaker
DAVID M. PARENTI, M.D.	Guest
ROBERT REDFIELD, M.D.	Guest
MICHAEL S. SAAG, M.D.	CBER Consultant (voting)
ROBERT T. SCHOOLEY, M.D.	CBER Consultant (voting)
FRED T. VALENTINE, M.D.	CDER Consultant (voting)

FDA PARTICIPANTS

JAY P. SIEGEL, M.D.	CBER
KAREN D. WEISS, M.D.	CBER
WILLIAM D. SCHWIETERMAN, M.D.	CBER

A-G-E-N-D-A

P-R-O-C-E-E-D-I-N-G-S

8:45 a.m.

CHAIRMAN MASUR: Good morning. Welcome to this session of the Antiviral Drug Advisory Committee. I am Henry Masur, the Chairperson.

We are going to have a series of statements and announcements by Nancy Chamberlin, the Executive Secretary, in just a moment. But I would like to begin the meeting by introducing all the members. So if we could start maybe with Bob Redfield. If each person could speak into the microphone and identify himself or herself and their institution. I see one of the problems is that Dr. Redfield doesn't have a microphone. But maybe he could speak loudly.

DR. REDFIELD: Bob Redfield.

DR. FISH: Doug Fish.

DR. PARENTI: David Parenti.

DR. SIEGEL: Jay Siegel, Office of Therapeutics, Research and Review, Center for Biologics, FDA.

DR. WEISS: Karen Weiss, Division of Clinical Trial Design and Analysis, Center for Biologics, FDA.

DR. SCHWIETERMAN: Bill Schwieterman, Center for Biologics, FDA.

DR. SCHOOLEY: Chip Schooley, guest, University of Colorado.

DR. WONG: Brian Wong, Yale University.

DR. MATHEWS: Chris Mathews, UC, San Diego.

DR. YOGEV: Ram Yogev, Children's Memorial Hospital in Chicago.

DR. CHAMBERLIN: Nancy Chamberlin, FDA, Executive Secretary.

DR. FLETCHER: Courtney Fletcher, University of Minnesota.

DR. HAMILTON: John Hamilton, Durham VA Hospital, Duke University.

DR. FLEMING: Thomas Fleming, University of Washington, Seattle.

DR. GULICK: Trip Gulick from Cornell.

DR. STANLEY: Sharilyn Stanley, Texas Department of Health.

DR. VALENTINE: Fred Valentine, NYU and Bellview Hospital.

MS. LEIN: Brenda Lein, Project Inform.

CHAIRMAN MASUR: And Princy Kumar will be joining us shortly. And we are expecting Michael Saag, so that will round out the members of the Advisory Committee and the consultants and guests today. We now have some statements and

announcements by Nancy Chamberlin, the Executive Secretary of the Committee.

DR. CHAMBERLIN: Welcome. The following announcement addresses the issue of conflict of interest with regard to this meeting and is made a part of the record to preclude even the appearance of such at this meeting.

Based on the submitted agenda for the meeting and all financial interests reported by the committee participants, it has been determined that all interests in the firms regulated by the Center for Drug Evaluation and Research present no potential for an appearance of a conflict of interest at this meeting with the following exceptions. In accordance with 18 U.S.C. 208(b), full waivers have been granted to Dr. Ram Yogev, Dr. Thomas Fleming, and Dr. Clifford Lane.

A copy of these waiver statements may be obtained by submitting a written request to the Agency's Freedom of Information Office, Room 12A-30 of the Parklawn Building.

In addition, we would like to disclose for the record that Dr. Michael Saag has interests which do not constitute financial interests within the meaning of 18 U.S.C. 208(a), but which could create the appearance of a conflict. The Agency

has determined, notwithstanding these interests, that it is in the Agency's best interest to have Dr. Saag participate in the committee discussions concerning the use of surrogate markers in the early development of immunomodulatory agents for the treatment of patients with HIV.

With respect to FDA's invited guests and guest speakers, Dr. Daniel Kuritzkes, Dr. Alan Landay, Dr. Michael Lederman, Dr. Donna Mildvan, Dr. David Parenti, Dr. Robert Redfield and Ms. Brenda Lein have reported interests which we believe should be made public to allow the participants to objectively evaluate their comments.

Dr. Kuritzkes would like to disclose that he has contracts with Agouron, Roche, Visible Genetics, and Triangle. He also receives consulting fees from Amgen, Bristol Myers Squibb, Glaxo, Roche, Trimeres, Triangle and Viologic. Further, Dr. Kuritzkes receives speaker fees from Bristol Myers Squibb, Dupont, Glaxo, Merck, Roche, Visible Genetics and Las Corps.

Dr. Alan Landay would like to disclose that he has grants with Chiron and Agouron and receives consulting fees from Chiron.

Dr. Michael Lederman would like to

disclose that he has research contracts with Schering Plough and Chiron, is a co-investigator on studies for Schering Plough and Chiron, and is a consultant to Schering Plough.

Dr. Donna Mildvan would like to disclose that she is the principal investigator on grants from Schering Plough, Hoffmann-LaRoche and Chiron dealing with antivirals and immunomodulators.

Dr. David Parenti is involved in investigational trials from Glaxo Wellcome, Serono, Merck, Chiron, Gilead Sciences, Pharmacia and Upjohn, OXO Chemie, Dupont, Triangle, Agouron and Bristol Myers Squibb. He is also on Glaxo Wellcome and Merck speaker bureaus, and is a consultant to Glaxo Wellcome, Merck and Agouron. Further, Dr. Parenti's minor child owns stock in Bristol Myers Squibb.

Dr. Redfield would like to disclose that he is conducting clinical trials with interferon for Schering Plough and Hoffmann-LaRoche.

Ms. Brenda Lein would like to disclose that her employer has unrestricted educational grants from Amgen, Schering Plough, Immunex, Hoffmann-LaRoche and Chiron.

In the event that the discussions involve any other products or firms not already on the agenda for which an FDA participant has a financial interest, the participants are aware of the need to exclude themselves from such involvement and their exclusion will be noted for the record.

With respect to all other participants, we ask in the interest of fairness that they address any current or previous financial involvement with any firm whose products they may wish to comment upon.

CHAIRMAN MASUR: All right. Thanks very much. We are going to begin our agenda with Jay Siegel, who will talk on the need for well-characterized biomarkers and surrogate markers. Okay, we are going to have Bill Schwieterman make some comments and then we are going to deal with Jay.

DR. SCHWIETERMAN: We actually are beginning the meeting with Siegel. I am just going to provide a few brief opening comments here. Good morning and welcome, everyone.

We at the Center, and I think we can speak for the rest of the committee, are anticipating an exciting and hopefully productive

meeting of this particular advisory committee to answer some important questions about the development of biomarkers and surrogate markers for the field of immuno-based therapies for the treatment of HIV.

We at the Center for Biologics have for a number of years been offering guidance to sponsors who are developing these therapies and have worked closely with the community and with the activists as well as with academicians on the many challenges and hurdles that investigators face when developing their products in Phase I through Phase III.

There is no question that there is a need for new therapies given the toxicities and some of the failures associated with the currently existing regimens. And for these and other reasons, we decided that a meeting of this sort, a gathering of the experts, would be not only timely but possibly a beginning of a, I think, fruitful process by which these particular surrogate and biomarkers could be developed for this field.

So we have assembled this meeting for the following purpose. I divided this purpose into three different parts, and I will go through. The purpose of this meeting is to facilitate the

development of immune-based therapies for the treatment of HIV, and to do this by beginning a productive interaction between investigators, academicians, industry sponsors and patients and their advocates on how to optimally develop and use biomarkers for these therapies. It is a long purpose, but I think it speaks to some of the complications and confounders and challenges involved with this. We very much believe that by identifying and clarifying the issues regarding the use of surrogates and biomarkers that we can facilitate the development of this field. And that we also very much believe that this can only be a beginning given that there are many issues and many nuances to be discussed. And that finally it is only through the development of these biomarkers and surrogate markers that this field can be optimally developed.

And so I very much want to thank the committee members and the speakers for assembling here, because I think we have an expert panel that is going to be able to help us with this particular purpose.

There are many objectives of this meeting. I have listed four central ones here. I believe that the principle objectives of this

meeting are to identify and clarify the following four areas. The usefulness and the limitations of biomarkers; special challenges for biomarkers for immune-based therapies; candidate biomarkers of promise for immune-based therapies; and finally, to identify and clarify mechanisms, and this is both scientific and organizational, by which this field can be fostered and developed.

I should say just one quick word on the use of the terms biomarkers and surrogate markers. Because this is not an unimportant point. Biomarkers, as others will undoubtedly get into, are measures of product bioactivity. Parameters that can be used to characterize a particular product with respect to any particular outcome.

Surrogates, by their definition, are substitutes. Substitutes for another outcome measure. In this particular case, almost always we are using surrogate markers for clinical efficacy. And Dr. Fleming and others will get into this particular area and how it is very important to distinguish these two concepts when discussing any of this in a forum such as this.

Finally, my last overhead is just a brief overview of the agenda. We have assembled this morning's session into really three different

sections with the afternoon then being devoted to the discussion and questions. Dr. Siegel and Dr. Fox, if he gets out of traffic, will discuss the need for new therapies and biomarkers as an introduction.

And then there will be a series of discussions by Dr. Landay and by Dr. Lane and by Dr. Mildvan if she is feeling better, and perhaps by Dr. Kagan if she is not, on the review of candidate markers, disease pathophysiology and clinical data. Dr. Lederman and Kuritzkes and Fleming will discuss respectively the perspectives they have on virologic immunologic biomarkers. And then finally the usefulness and the limitations of surrogate markers and biomarkers in the development of therapeutics. And then finally, as I mentioned, there will be an open public hearing and questions to the committee focusing on ways that we can identify and clarify the issues, and very importantly, ways that we can go forward from here.

So with that, I will turn the podium back to you, Dr. Masur.

CHAIRMAN MASUR: Thanks very much, Bill. Now, with that introduction, we will move to Jay Siegel, who will talk -- do, I guess, the first

of two presentations on the need for well-characterized biomarkers and surrogate markers.

Can everybody hear in the back? Because I get the sense that maybe the microphones are not turned on. Could the audio people -- all right. Can you hear now? I think you can probably turn it up even a little bit more. All right, can you hear in the back now? Okay. Again, just raise your hands if you can't hear. So, Jay?

DR. SIEGEL: Thank you very much. Good morning. I want to thank the committee for coming here to work with us on this important topic and thank all the speakers as well. It is a pleasure to attend this meeting as well as to address it.

Well, the need for biomarkers for immune-based therapies is apparent to everyone who is involved in addressing this field. And while there were some remarks about beginning to work on this, I would like to acknowledge that many of you and many of us, of course, have been working in this area for five or ten years. But I think we are, in fact, at a critical junction where we understand much more many therapies under development. I think this is an excellent time to take stock of where we are and try to move forward as Dr. Schwieterman mentioned.

One critical need for biomarkers is to optimize therapies in Phase I and II particularly, but also in Phase III. It is clear in the development of all therapies that there are large numbers of questions that need to be answered. And in therapies for HIV infection, it is simply impossible to answer all of them with measures of clinical benefit. What is the optimal dose, regimen and route to administer a therapy.

For immunomodulators in particular, this could be quite critical. In some cases, minor differences in dose or route can make the difference between an immunizing effect and a tolerogenic effect like an opposite effect. Also, unlike drugs, pharmacokinetics is often not useful or not highly useful. For drugs, if you know an active level, you can adjust the dosing to pharmacokinetics to get that level. In effects for immune therapies, there is often not a simple relationship between serum levels and effects. Effects often persist well beyond the disappearance of levels from the serum.

One also in many cases needs to optimize the target population. Will a therapy work best in patients who have active circulating -- high levels of circulating HIV, for example, or low

levels. Patients whose immune function is still relatively intact or patients whose immune function is more impaired and so forth and will they work best in combination with other medications. Which ones may impair their ability to activate the immune system and which ones will protect that ability perhaps by controlling viral infection. Very many important questions.

Other needs, important needs for biomarkers for immune-based therapies, as for all therapies, are to select among the many candidate therapies for further testing at all phases of development. One needs to make guesses as to where is the best place to put one's resources, particularly when one is speaking of clinical trials, which can be lengthy and costly. And of course to develop biomarkers as potential surrogates for clinical measures of efficacy.

Here I want to draw somewhat of a contrast, but I think an important one, between two of these uses of biomarkers and what characteristics are desirable for their use. The use to guide early drug development and the use to develop these biomarkers as surrogates. I have the same characteristics listed here, although in a somewhat different order.

To guide early drug development -- dose, route, regimen, patient population, concomitant medications and the like. Critically important is a sensitive and rapid measure. One needs a measure that one can deal with. There are so many questions and one can get answers from dozens of patients. One needs a measure that one can -- again, because there are so many patients -- that one can get answers rapidly, typically a time frame of days to weeks is highly desirable if not necessary. Of course, one needs a reproducible measure. It is desirable for those measures to predict clinical benefit, but not perhaps critical. One can optimize a therapy against its ability to elicit antibodies or its ability to elicit CTL responses and then determine whether that predicts clinical benefit.

When looking to develop a biomarker for a surrogate, which is to use in place of a measure of clinical benefit, the ability to predict clinical benefit rises well to the top of the list. Sensitivity and repetivity are important, but repetivity -- but a surrogate marker can be quite useful if it is sensitive enough to measure effects in hundreds of patients as opposed to dozens, and it only needs to really work in a small number of

critical trials to answer one -- or a small number of critical questions. And similarly, it can be useful if it is measuring on the order of months to a year or two. Again, it needs to be reproducible.

This is a reminder. I am sure all the members of the committee are familiar with this. But determinations of efficacy, at least as made by the FDA for the purposes of product approval maybe based on clinical endpoints. They may be based on validated surrogate endpoints, endpoints that have been shown to be predictive of clinical benefit for a given disease and drug or drug class. Or they may be best on surrogate endpoints which are determined to be reasonably likely to predict clinical benefit, in which case approval is possible under our accelerated approval regulations, which require confirmation of clinical benefit in the post-approval period.

Now in developing a surrogate for an immune-based therapy, some of the issues to consider is of course at the present time there is not any definitive clinical efficacy data against which to correlate surrogates for immune-based therapy. So at this point we could not validate a surrogate with such data. One can and should use the available data to assess likely candidates and

that will be one of the focuses of this meeting.

And this is a point -- the next point is an important point that is sometime overlooked and I do want to focus on for a couple of minutes. The data and conclusion from one class of therapies, and notably antiviral drugs, for which we have substantial efficacy data, may not apply to therapies with different mechanisms, for example various immune-based therapies.

And that is largely because the mechanism of action on a surrogate is critical to the type of inference one can draw, and we will be hearing more about this, I am sure, from other speakers. I will just quickly note, for example, an antiviral therapy has a rather direct impact on viral load measures. That impact may be beneficial. In fact, it has been proven for many drugs to correlate with or predict clinical benefit.

Conceivably, it could also be a measurement artifact. It could indicate decreases in virus in the circulating department, but not in other more critical departments or other potential interpretations that might be less predictive of benefit.

But when you see if an antiviral effect therapy substantially affects an important immune

parameter, that might suggest that the antiviral effect is beneficial. That in some way -- for example, if it is an CD4 count, it may in some way suggest that the antiviral effect is impairing the viral mediated destruction or inhibition of CD4 cells. Conversely, an immune-based therapy may affect some immunologic parameters directly. Depending on the nature of the therapy, it may have a direct effect on some parameters and others may be affected more directly.

And possibly a decreased viral load certainly would be suggestive that an immunological effect -- that the immunological effects are beneficial. If you have a drug that affects the immune system and secondarily you see a change in viral load, that at least would suggest that the immunological effects may be pertinent to the control of HIV virus.

So the types of implications are different, and I am sure we will hear a lot of discussion of the data regarding those points.

This is a -- there is a bunch of questions on this slide, which I think of as sort of a thought experiment. I am not going to answer any of these questions, just put them out to consider. Just to make the point of how drugs of

different classes with different mechanisms may have very different implications regarding the implication of a surrogate. The top of the slide, which may not be legible, says what would an increased CD4 cell count imply regarding effective immune responses against HIV or opportunistic infections and regarding effective antiviral mechanisms if the therapy had been an antiviral drug?

Well, we discussed that. I mentioned that in the last slide. A CD4 cell growth factor. Well, that would perhaps not surprisingly expand CD4 cells. One might have some questions about whether the cells that are there have appropriate functionality and durability. What if the therapy were expanded autologous CD4 cells? Again, those cells, depending on how they were treated and activated, may have very different functions and very different effects.

In durability, they may increase the CD4 count if they are there during measurements, but their implications raise significant questions. What if the treatment were beads coated with CD4 that simply registered in the CD4 assay? Well, that would probably be artifact, although such beads theoretically could impact the disease.

What if the treatment were expanded autologous CD8 cells? You know, you gave somebody CD8 cells and it may be antigen specific and the CD4 cell count went up. Well, that might have a different implication. What if the treatment were a drug which inhibits CD4 cell margination and increases circulatory time so that the CD4 cells spend more time in the circulation and the counts go up? Or what if the treatment were an HIV vaccine? Well, again, these are not simple questions to answer, but they highlight the fact that the mechanism and the type of therapy, not simply whether it is an antiviral or an immune-based therapy, but even the immune mechanism is going to have a significant impact upon the implications of a biomarker.

This is my final slide. So it is clear the need for these markers and for useful biomarkers is great. And also that the challenge is great. The available data are limited. There is a great deal of useful data. I don't mean to imply that. And we are going to be hearing about some of that and considering it. But there is not yet, for the immune-based therapies, the clinical data we would like to correlate with and also even in other areas there are still many data needs that we hope

to help identify here.

There are many immune-based therapies of varied classes and mechanisms. We will be hearing more about some of those. And there are many immune effector mechanisms and functions, many of which are known to be relevant to HIV. And there are many ways, I might add, to measure each of those. Each function has many potential markers.

So in conclusion, as with all scientific questions, insight and data will be critical to the answer. And as with perhaps all but certainly this one, cooperation amongst all of us, the need for standardized tests to focus amongst these many mechanisms and these many potential measures to focus on important ones, to standardize the methodology and to develop the data necessary to assess their usefulness will be critical for our success. Thank you.

CHAIRMAN MASUR: Okay. Thanks very much, Jay. I guess in the absence of a trial that to date has demonstrated efficacy for an immune-based therapy, this is going to be a challenge. But hopefully over the course of the day, we will make some headway in terms of adding some clarity to this.

We are now going to go to the need for

new therapies with Larry Fox from the Division of AIDS. We appreciate Larry coming here from the Million Family March.

DR. FOX: Thank you. Well, the point that I wanted to make is that we do need new therapies. I recall about three years ago at a review of an immune-based therapy protocol, someone saying why are they bothering with this. Don't they know about HAART? That is all you need. This was a very scholarly scientist who made this point and it occurred to me, well I guess he hasn't been in the clinic for a while.

So let me make the point that HAART, while wonderful, is not adequate for control of disease given the spectrum of antiretrovirals that we have available now. I will go through the benefits of HAART, the limitations of HAART, the virologic failures that we see, the immunologic failures that we see, the toxicity and even the end-organ disease that we are encountering now.

The benefits of HAART are absolutely unchallengeable. We have people that had been at death's door that are now continuing to have healthy and productive lives. We see suppression of viremia. We see reduction in virus shedding, both in semen and vaginal secretions, which in turn

is likely to reduce the risk of transmission.

So this alone is a benefit. We certainly see increased CD4 count in most cases, not all. We see reduced immune activation, which has been responsible for sequestering CD4 cells and contributing to a variety of diseases associated with HIV infection. We even see restoration of lymph node architecture in cases of advanced HIV disease. We certainly see clinical improvement. There is ample statistics to demonstrate prolonged survival, fewer opportunistic infections and HIV-associated malignancies, although not all malignancies have been reduced. Certainly Kaposi sarcoma has been reduced. But we are seeing plenty of lymphoma still. And we have certainly demonstrated that people can discontinue opportunistic infection prophylaxis and maintenance therapy.

This is a slide that I borrowed from Mike Lederman, along with a few others that I will be showing you. This is date from Case Western looking at the annual deaths that they have seen at that clinic. And there is a very clear decrease in deaths up until 1998. This is associated with HAART as well as the passing of the crest of the epidemic. And then suddenly we see a slight rise

here. So what is happening at the end are many factors contributing to people no longer deriving the maximum benefit of HAART.

So HAART does not work in all cases. There are plenty of people who have had, unfortunately, sequential monotherapy. Those of us that were in the clinic at the time that we began using triple therapy know that what we did at first was add one nucleoside to another nucleoside and then add a protease inhibitor to failing nucleoside regimens, until we caught on to the idea that we needed to suddenly change all three at once.

So there are plenty of people out there that have had one after another after another medication added instead of all changed at once and developed multi-resistance to all the antiretrovirals available, and this even occurs in people who have had three added at once. ACTG 315, we found one-third of our patients had developed multi-antiretroviral resistance despite the fact that they were extremely compliant.

HAART does not clear the latently infected cell pool. We had a dream once that we were going to purge the body of all HIV. We had calculations of how many years it would take and now those calculations have continued on to about

60 years or more. The latently infected cell pool remains latently infected.

HAART does not restore HIV-specific immunity. We have seen dramatic restoration of OI-specific immunity in many cases -- not all. But HIV-specific immunity, unless HAART is started very shortly after infection, is not preserved. It does not prevent relapse, and I will show you some statistics on how often that happens. And for most of the world, it doesn't offer anything affordable at all.

This illustrates most of the world. We are over here comfortably taking HAART and having problems with it. And over there is the rest of Western Europe. And HIV is not only concentrated in Sub-Saharan Africa, although that is where most of it is. It is now spreading into India, which has more HIV-infected patients than any country in the world, although it is not much more percentage-wise than the infections in the United States.

It will be soon if it doesn't -- if something is not done dramatically there. Infections are spreading in China. Infections are spreading in Southeast Asia. We have infections everywhere. And most people can't begin to afford what we use here and it is still not enough.

We experience virologic failure. This is statistics from clinical trials. So this is the best case scenario. These are people who have enrolled in clinical trials with determination to be compliant. And yet we still have 20 or 30 percent experiencing virologic failure at the end of the year. For those that manage to get through that year without failure, the rates are a little bit better, 8 to 15 percent failure.

But in the clinic -- and this is again data that Mike Lederman provided me with -- we see a much higher rate of failure. This is what it is like out in the real world, not in clinical trials. And in many places, people reporting that half their patients are failing. And it is associated with many things. They are all listed here. The CD4 nadir, peak plasma viremia, gender, the time that they started protease inhibitors, the number of missed clinic visits per year and resistance mutations.

There is a very clear connection between adherence and virologic suppression. More adherence, more suppression, less adherence, less suppression. Why are people having such a hard time adhering? Well, you've got to take 24 pills a day sometimes and you've got to take them with the

right food and you've got to take them at the right time of day and you've got to take them on a empty stomach or a full stomach. You've got to keep them refrigerated or not. And if you don't have a home to live in, you certainly can't keep them refrigerated.

I have never gotten through a 10-day course of antibiotics without missing a dose. You've got to be 95 percent adherent to HIV regimens because protease inhibitors are non-polar and they slip right through the cell membrane the minute you stop taking them. And that is what you need to have constantly intracellularly in order to avoid having the virus break through.

And even if you are perfectly compliant and you manage to suppress the virus, there are people who still do not experience immunologic success. Somewhere around 10 or 15 percent of patients, despite the fact that they have suppressed their viremia, do not experience an increase in CD4 count. And that is what you need to have increase in order to reduce your risk of opportunistic infections. That is what kills you.

We have got a number of problems with HAART. So in South Africa, we experience people screaming, I want therapy, I want therapy, I want

therapy. In the United States, we hear people saying, get me off this stuff, get me off this stuff, get me off this stuff.

Because we experience many things lumped together as lipodystrophy. We have lipoatrophy, abnormal fat accumulation, and hyperlipidemia, and that is enough to put people at risk for heart disease. We have insulin resistance, bone loss reported in many of the new publications -- dramatic bone loss, and marrow suppression of course. Pancreatitis and hepatitis -- those are old problems that we are seeing even more of recently with death associated with it.

Those of us that are in the clinic know that we have seen patients die with undetectable viral loads and end-organ failure. And of course we are seeing nephropathy and neuropathy and many other problems. So we would like something that would spare our patients these complications of the same wonderful drugs that are prolonging their lives, but now as they live longer are contributing to the morbidity of the disease.

This is an example of lipoatrophy. The face now does not look normal, and fat accumulation in abnormal portions of the body. Now remembering that as the epidemic has changed, as it has shifted

-- it is moving into younger and younger and younger people. So that now fully 50 percent of the new cases of HIV disease reported last year were in people under the age of 25. Imagine having in your adolescence to have to adhere to a complicated drug regimen, and if you adhere, this is what you will look like. People don't want to do that.

And on top of all that -- not due to the drugs alone, absolutely not. But due to many complicating factors -- due to HIV itself, due to Hepatitis C virus and Hepatitis, immune restoration syndrome, which is what happens when your immune system starts kicking in again after it has been tolerating opportunistic infections or tolerating Hepatitis C and you suddenly restore the immune system and now inflammation begins to damage your organs and you develop retinitis or hepatitis or lymphadenitis and the toxicities associated with drugs.

We have had an increased incidence of liver and renal failure. A couple of years ago, people didn't live long enough so that we worried about this. You were going to die of your HIV infection before you died of anything else. Now we have people clamoring for organ transplants. We have a couple of requests per week at Pittsburgh

for liver transplants. And we are beginning to develop protocols to explore whether or not this is something you can do in HIV positive organ recipients without shortening their lives. If we can improve the quality of life, we will do this.

So in summary, HAART isn't enough once again. It is wonderful. It has prolonged lives. It has taken people at death's door and brought them back to productive lives. And people are not willing to tolerate HAART for life. We have found that the best way to preserve the immune response to HIV is to start therapy as soon as possible after initial infection.

But then imagine how many years you would have to remain on a toxic drug regimen. People are now looking at structured treatment interruptions, STI. Cycles of HAART are being explored as ways of reducing toxicity and allowing people a better quality of life. Instead of wanting to start therapy as soon as possible, when their CD4 count falls to 500 or 350, people are now looking at can we wait until it is 200. How long can we wait before starting? And remembering that the epidemic is moving increasingly into people of younger and younger age, people are very reluctant to accept HAART and the toxicities associated with

it. And that is why we are looking for immune-based therapies. Thank you.

CHAIRMAN MASUR: Thanks very much, Larry. We are now going to move into the section on biomarkers and surrogate markers that are currently being considered. And the first of three presentations will be given by Alan Landay from Rush Presbyterian. Al, thanks very much for joining us. Are the microphones on?

DR. LANDAY: Thanks, Henry. And I would like to thank the committee for inviting me to talk. As I have been working in this area for a long time, I think there is clearly an opportunity to develop, and as we will see throughout the day, to look at some of these newer markers and try to put them in the context of the therapeutic options for patients. What I would like to do is to give an introductory talk on some of the basic immunology to get the committee sort of up to speed on a number of the newer markers and assays we will hear more about today in the context of basic immunology and immune development so that we can see how the various assays fit in.

In terms of T-lymphocytes, which we believe are probably in the HIV-infected host the most important of the immune responses, although I

will talk a bit about some of the other humoral and innate immunity as well, that we are interested in looking at T-lymphocyte development.

And clearly in looking at the paradigm of how this works, we start with the progenitor cells in the bone marrow, and as I'll go through, have approaches that we can use to measure those progenitor cells. We have approached this certainly for some of the stem cell therapies and mobilization certainly in the gene therapy approaches of looking for those particular progenitors. Once they leave the bone marrow, then they go to the thymus, under which they undergo thymic maturation. What we have learned over the last three to four years is that the thymus can be functional in adults.

Much of what we learned even when I was a graduate student in immunology that we thought during adult that the thymus was really no longer functional in adults and that after birth you have this sort of immunological decline from birth on when you are born with your thymus being completely mature. But now we are understanding that there still is thymic function and have the ability to measure that and look at that as an important, again potential, biomarker.

Once cells leave the thymus, they represent the naive T-cells, which are the important pool from which one generates new immune responses. And when these come in contact with antigen, one has the expansion of that pool to memory T cells and then those memory T cells will expand and be effector cells, both for CD4 and CD8 T cells.

In order to control that expansion of the memory pool, one has the mechanisms of program cell death or apoptosis that allows one to then control this expansion so that you don't get overrun with lymphocytes during a normal host immune response.

This just represents the various cellular elements showing you that we have been in search for this and clearly a lot of work goes on looking at the pluripotent hematopoietic stem cells that could be used to derive both the lymphoid progenitors, again B and T cells, or myeloid and then the other megakaryocyte and red cell lineages. And clearly we are interested predominantly in the lymphoid populations and progenitors and studying what happens in the context of HIV, as we will hear from Dr. Lane in terms of the T and B cell arms of the immune system.

We can use techniques such as flow cytometry to look at the stem cells here identified by the marker CD34 as one of the markers for hematopoietic stem cell looking at a peripheral blood sample where we gate and then look at the cells that here express the CD34 antigen on their surface. So we have certainly techniques that are available in most clinical settings for us to evaluate the stem cells and quantitate them clearly in the peripheral blood using various mobilization strategies.

As I said, the thymus is a critical organ that we know can function in adults and clearly is impacted by HIV infection. We know that there is a disruption of the thymus and its architecture, and the question is does sufficient thymic function remain to allow reconstitution of immune function with HAART or other immune-based therapies.

And we have a variety of approaches that we have used to do this. First, use of thymic scans and naive markers. The repertoire -- as we know, the T-cell repertoire is critical for the host immune responses. We also can use telomere length, which is another marker of cell division and cell age. And then finally the T-cell receptor

excision circle or TREC as another biomarker for evaluating the function of the thymus in peripheral blood T cells.

One of the things we have to realize is most of the immune system is present not in the peripheral blood, where only 2 percent of the immune system exists, but in tissue. And so how do we now use what is probably the most readily available source of material for us, clinically the blood, to evaluate that. We really, I think, through the uses you'll see for many of these markers can use peripheral blood samples at least as a correlate to tissue. But we are also trying to adapt techniques to look at the tissue as well.

This just shows you from a publication this year from some of our work in ACTG looking at a thymic scan just to point out the thymic mass in this individual here being graded at a thymic index score of 4 and the grading scale here goes from 0 to 5, with 0 being undetectable thymic mass and 5 being a thymoma. And clearly one can see in this CT scan the evidence of thymic tissue, and this can be graded and we have shown correlates of this in the context of HIV.

One can also use T cell receptor diversity, in this case using spectrotyping or

molecular techniques, and some data provided me from Crystal Mackall from the NCI, just showing you a technique can be used using peripheral blood looking at the diversity of the T cell repertoire with molecular PCR techniques, and looking here at the V-beta repertoire based on the number of peaks one has.

This shows you some data indicating an age-associated reduction in TCR repertoire diversity. Looking in blue here representing the cord blood sample, in red a 22-year-old, and then in blue the 44-year-old. And you can see then in a dilutional input here of CD4 cell number that you dilute out more rapidly in the older age population compared to cord blood samples here where you get a more robust repertoire of diversity present. And one can use again these techniques to study what happens following infection and/or therapy to see does one have some alterations in this important repertoire which represents the host response against HIV and other pathogens.

We can also apply, as I said, the technique of the T cell receptor excision circle. The original work done by Danny Douek and Rick Koup really have brought to bear the importance of using these. Again, another molecular technique to

measure what happens when T cells undergo their normal rearrangement and expression. And this just shows you how that occurs for the V-beta rearrangement. What you do is you get excised pieces of DNA during that period of T cell rearrangement.

One can then measure these excised pieces of DNA in cells from the periphery by PCR techniques as these either signal or coding joints. And what I have shown you below is some data from our own laboratory just looking at the age-related decline in adults from 20 on through 60, showing a significant correlation in the reduction of the TREC numbers in both the CD4 and CD8 compartments. So that one sees normally in the aging process the decline in the TREC value, so that one has to be cognizant when studying an HIV infected host. Clearly one has to match for the appropriate age-related changes that one can see.

We also know that there is an importance of the telomeres. Telomeres are the ends of chromosomes that are essentially repeats. These TTAGGG repeats at the end of chromosomes essential for chromosome stability. And what happens is you get a progressive shortening of the telomeres over time with the aging. And what

happens is approximately 50 to 100 base pairs per year are lost in vivo. And you can also note that telomere lengths are maintained by a ribonuclear protein called telomerase, an enzyme that maintains the telomere lengths.

nd what we have noted in HIV at least is that there is a replicative senescence of particular cells, especially the CD8 cells. There is an increase in the cell -- the CD8+/CD28- cells. These cells have shortening of telomeres and that there seems to be a result of extensive replicative history as a result of the clonal expansions. And what happens essentially in an HIV infected adult is they have telomere lengths similar to that of a 100-year-old individual compared to an age-matched healthy control individual in their 20's and 30's. So clearly there is a significant impact and one can measure this.

On the next slide, it just shows you some data that we have generated and published last year measuring then the telomere lengths in two patients showing you -- in this case looking at some evidence post-antiretroviral therapy that one can actually induce an increase in telomere length in both the CD4 and the CD8, so that there is some impact of the therapy in blocking viral replication

and actually restoring now the telomere length. And we are beginning to look at this certainly further in many other approaches. But it is another measure of cell age and cell function. In our own laboratory, we are beginning to compare the telomere in the TREC assays in terms of their role in measuring new T cell development.

We also know, as I mentioned, the importance of apoptosis as a normal process of cell death, and this slide really just shows you the comparison of the necrotic cell processes of death versus those of apoptosis and then the apoptotic bodies that are created are uptaken by macrophages which basically can phagocytize that. We can measure the process of apoptosis by a variety of techniques. I have just shown one of them here, which is a common technique we use in the laboratory using flow cytometry and measurements by DNA content.

This is the normal DNA content of a cell, showing you the cell cycle components of the G-zero, G-one-S and G-two-M. And what happens in a cell undergoing apoptosis is you increase this peak to the left of the G-zero/G-one, indicating a lower DNA content in that cell as a measure of apoptosis when you get DNA fragmentation. And this can be

used and applied in a routine setting. As I will also highlight in a moment, a number of other assays can also be used to measure apoptosis, and whether therapy impacts that is, again, another critical marker in the pathogenesis.

The next slide will highlight for you the importance of apoptosis in HIV infection itself. It is, as I said, a morphologic finding resulting from the process of programmed activation death characterized by the condensation of nucleus and giving this distinctive pattern of DNA fragmentation. And it is really very much of interest in HIV infections because it appears to be one of the most important mechanisms of CD4 cell depletion. One in which when we are looking at therapeutic interventions, we want to reverse the process. And clearly one can show increased numbers of apoptotic cells in HIV. And as I mentioned, we do have laboratory techniques that can be used to quantitate this, especially those with flow cytometry and some of the simple PI methods which I mentioned.

So to summarize the first part in terms of lymphocyte development and function, one can see we have a variety of markers looking from the progenitor cell, which would be represented here,

to the thymus looking at both the TREC and telomere approach. This is another marker reported on by Louis Picker's group, CD103, as a potential marker that could identify recent thymic immigrants specifically on the CD8 cell population, which was published in the last year. That is another marker which we really haven't explored very much in the context of HIV infection.

In terms of naive cells, we have a variety of ways of defining naive cells by a phenotypic criterion. Flow cytometry, as I have listed here, and we are looking at again many of these to correlate these in my own laboratories, the phenotypic correlates here of these various naive phenotypes and the TREC and telomere assays. We also note that measuring memory cells, one can define these both phenotypically here and looking at cell death, we can use phenotypic markers such as CD95, which is the Fas antigen marker of apoptosis. We can also use Annexin V, which is a measure by flow cytometry of early stage of the apoptotic pathway, where you have the beginnings of the membrane being turned inside out and one can measure that. Also a Tunel assay, another flow method for measuring the DNA fragmentation. And then finally the propidium iodide method.

And then finally as I will talk, the effector activity that one can measure of memory cell responses which can easily be adapted in the clinical setting, those of the delay type hypersensitivity or DTH skin test responses, which have been used extensively in clinical practice for a number of years and clearly are being applied now in the setting of HIV in patients as well.

Once we move beyond the maturational stages of the immune system, we move to the really important functional host components of the immune response. And these represent the critical elements of the immune system. I have talked about the CD4 and CD8 T cells and their derivation. We also know that there is an important role for antigen presenting cell activity, both dendritic cells and macrophages that were important host components of the antigen presenting cell activity, dendritic cells being found at much lower levels, 10 to 100-fold lower than the monocytes in the blood, but also given what they lack in numbers, they make up in function in terms of their potency.

We also know the critical role for B cells in antibody production and potentially their role in making neutralizing antibody. And then finally NK cells, another part of the innate immune

system, which we really haven't studied extensively in the context of either therapy or responses in immune-based therapy. I think again another host component that clearly can respond to a number of the factors that Dr. Siegel had originally talked about in terms of T cell growth factors that can also respond.

How do we assay then and perform immunologic function, which I will go through these I think to provide the sort of basic assays that we can use to approach and understand the function of these important components in the context of markers, biomarkers or potentially surrogate markers. Firstly, as I will go through, the lymphoproliferative assay, which has been a standard almost now for 25 years in the field for measuring T cell responses. That is an in vitro measure. One can also use the in vivo measures of delayed type hypersensitivity. And then some more recently developed approaches, ICC, intracellular cytokine detection by flow cytometry, which I think is really going to revolutionize the ability to standardize and apply these techniques clearly in the laboratory.

We also know CD8 cells are critical as effector functions in the context of HIV. Using the

CTL assay, which has been the classic approach, cytotoxic T lymphocyte response. We can also use intracellular cytokine measurements. ELISPOTs, which can be used as an in vitro measure to look at particular cytokine production and specificity of CD8 cells to respond to HIV antigens or peptides. And then finally the newest of the CD8 approaches, the MHC class I tetrameres, which I think again are going to really allow us now in a very well defined way to look at the immune response against HIV and other pathogens in a highly quantitative approach as I will come to at the end.

I also bring up NK cells as an important cell that we should be looking at as another potential marker for responses to therapy by both their abilities to kill directly or through antibody-dependent killing. And then finally B cell responses, which one can measure by a variety of immunization with things like diphtheria, pertussis or tetanus and measuring antibodies against these through immunization strategies.

This just shows you then the basic elements of the immune response from a cellular immunologic perspective, indicating the role of the dendritic cell and macrophages producing cytokines that in turn activate the helper cells. These make

then the important regulatory cytokines of interferon-gamma and IL-2 that are critical for the CD8 effector function. This is what would be a standard approach to any viral or chronic viral illness in terms of the role of the cellular immune system.

We also note that we can use a variety of cell surface markers and multiparameter flow cytometry to define these cells in the context of their potential functional role, and one can look at a variety of activation markers -- maturation markers of either mammary or naive cells, and then functional markers here, CD95, a marker for the apoptotic pathways, and CD28, a marker as a co-receptor critical for the interactions of either CD4 or CD8 T cells with the antigen presenting cell.

So this our cartoon just showing you the interaction. You will have to just page through this on the next -- just showing in the docking. This is my first PowerPoint. I actually wanted to show a little bit how you could actually use this to do docking of T helper cell in TH-zero cell, showing you now the TCR interaction here with the antigen and MHC class II. Also, the recognition of CD4 and then the CD3 molecule, and then the co-

receptor, CD28, and then CD40.

Next we will show the IL-2 receptor. We then induce the CD40 ligand on the T cell, making induction then through that of B7. So you have the important co-receptor interactions here -- I mentioned the CD28 on the T cell interacting with the CD80 molecule. And then also the CD40 and CD40 ligand. These are critical elements in the immune response. If you don't have the expression of those markers, the immune system will not respond appropriately. Instead of the proliferative responses one normally would get with antigen 1 then undergoes the cell death or apoptotic pathways. You can see IL-2 binding to its receptor. As it dies undergoing cell division producing then interferon-gamma to activate the macrophage. Next coming around and making IL-1, IL-6 and TNF-alpha, which can again activate the T helper cells.

So that is the basic cellular interaction pathway between the T cell and APC. And how we measure this -- again, the in vitro approach is our lymphoproliferative assays; in vitro, coreless cellular immunity, important in control of viral and intercellular pathogens. It quantitates T cells to a variety of stimuli -- again, mitogen,

alloantigens. And one can show that there is strong LPA responses to antigens that are associated in HIV with controlled replication.

This just lists for you some examples of what one can use in a lymphoproliferative response. Again, looking at HIV-specific responses with P24 antigen. Pathogen-specific responses, CMV or MAC. Your recall antigens, mitogen, or neoantigen. So all of these can be applied in a routine setting in an in vitro system.

We can also look not only at the proliferative responses overall to the interaction of the cellular components I spoke about, but individually we can now measure cytokine production intracellularly and define cells based on their type I, which makes cytokines driving cellular immunity -- and this lists the various cytokines -- IL-2, 12, gamma-interferon and IL-15, critical for type I health. Or type II health, IL-4, 5, 6, 10 and 13 and driving cellular immunity. And again, there is a cross-regulation between these two arms of the immune system. And the important cellular elements that make these.

One can apply then the intracellular cytokine approach. It is a relatively new assay and may have better precision with in-between labs,

which we are now looking at. Again, it is a faster turnaround time and no radioisotope use. We can study numerous cell types directly and give more information and can be quantitative both for CD4 and CD8 rich frequencies.

This shows you some representative data from Louis Picker, who really I think put this technology out into the field in the last couple of years. This is showing quantification of viral specific CD4 memory cells in a normal subject. Just showing you the breadth of a normal response to things like adenovirus, flu, measles, mumps or CMV, measuring in this case intercellular gamma-interferon and looking at the activated population here and the control and then showing you the various stimulated populations. Just as an example, one can do this very rapidly within a matter of six hours as an assay.

We could also apply it with the appropriate stimulus as well, here using overlapping peptides for interferon-gamma production, again in CD8 cells. This is looking at HIV-specific responses looking to the various gag, pol, nef, rev and bpr and tat. So you can look at all of the various both structural and regulatory gene products of HIV having the appropriate

peptides available and can quantitate now in a very rapid fashion immune responses to CD8. As well these same peptides could be used to stimulate CD4 cells.

We also know that cytotoxic mechanisms are critical. This just shows you the basic killing mechanisms which involve perforin, granzymes, cytokines and finally Fas ligands. All of these are involved in the killing mechanisms, as you can see on the right. And one can measure these as well. There have now been techniques developed to measure intercellular perforin, granzymes, cytokines and also surface Fas-fas ligand production as a way of functionally evaluating the CD8 cytotoxic function.

These are the classic assays for measuring cytotoxicity, looking at the target cells which are labeled, the chromium 51, and then effector cells added, and then measuring the release of chromium here and determining the percent specific lysis. So one has this as a sort of standard method for measuring and classic method of looking at the target effector cell for CD8 interaction.

But we have now -- and again, John Altman, who pioneered this work, developed the

tetrameres technology that allows us using flow-based techniques to look at specific CD8 cells. Again, one can make these tetrameres techniques. These are looking at class I tetrameres, single peptide ligand. And you can alter the specificity by the particular peptide here represented in blue with the HLA molecule. Depending on the right HLA haplotype and the specific peptide recognized by that HLA. So one can adapt this technology clearly to HIV or other pathogens or other antigens as well.

Just to give an example of what the data looks like, this is actually in the primate model looking at the Mamu-A1 response here, which is the HLA equivalent in monkeys, and looking at that particular response on the CD62L positive cells. And gating that again on the CD3/CD8 population, one can rapidly quantitate in this case HIV-specific responses.

So in summary, what we think we have really developed is a paradigm that we are trying to adapt in the context of new approaches to measuring biomarkers. We have had, as I have talked about, the very classic assays of lymphoproliferation and cytotoxicity, which really are being replaced by many of the newer assays like

intercellular flow cytometry to look at APC function, both for APC CD4 and CD8. We have the development of the class I tetrameres technology that I just showed you examples of for looking at the specific responses to HIV or pathogens, and also the more recent development now of class II tetrameres, the same approach being taken to look at HIV-specific CD8 cells can now actually be applied to HIV-specific CD4 cells, and one can do that in a very quantitative way. And one can combine with the class I tetrameres, the ability to look at perforin or granzyme as the lytic effector molecules and identifying then the HIV-specific cell and whether or not they are functionally competent.

This is my last slide just to say that in the future I think we are really going to be looking at this and we will hear more about this today, standardized assays for immune assessment. We have adapted DTH responses. One could do this again for classic recall responses. And then one could also look at those following immunization for both recall or neoresponses as well. And then in vitro, looking at some of the intracellular cytokine combined with the tetrameres technology to look at the axis of the APC-CD4-CD8. And clearly in

vivo we can measure both the cellular and humoral arm to the immune system by measuring antibody as well as in vivo cellular responses.

So I think where we have come is really an interesting and new paradigm to really evaluate and have a whole host of new markers. We are really not at the point, as we will hear, for these being validated as markers, but I think we have the ability to look at these markers and see how they correlate in the context of the clinical trials and approaches that we will take in the future. Thank you very much.

CHAIRMAN MASUR: Alan, thanks very much for both a good talk and a new standard for the committee in terms of technology in a presentation. So we appreciate the docking.

With that introduction to candidate biomarkers and surrogate markers, Cliff Lane from the National Institute of Allergy and Infectious Disease is now going to talk about how these markers relate to disease pathophysiology.

DR. LANE: So I'm going to go back to low tech probably in more ways one. So I've got slides there in the back. I don't know if there is anyone back there to keep a handle on those slides for focus or things. But if there could be, that

would be great.

What I have been asked to do is talk about how some of these laboratory markers play a role in helping us understand HIV pathophysiology. Clearly knowing that we are here at the FDA, I have subtitled this, "Why is it so easy to license antiretrovirals and so difficult to license immune-based therapies." And I think what you will see -- or I hope you will see as we go through this is that as Alan has pointed out, there are a lot of assays that the immunologist has at their disposal to be able to measure different parameters of the immune system. But unfortunately, we don't really know which of these markers for the most part are relevant to host offense in a common environment. Which of these markers translate to increased survival and which of these translate to increased quality of life. So we have a bit of a problem.

So the theme that I will try to stay with is that the measurements we make directly in the patient really seem to be the ones that are most relevant to where that patient is headed. And while we can do a lot of things in the laboratory, I think we still have some difficulty predicting with these things what will happen to the patient.

There will be a fair amount of

discussion, I know this morning, about the two laboratory markers that have been clearly demonstrated to have clinical relevance in patients with HIV infection. These are the levels of helper inducer or CD4+ T-lymphocytes and the levels of HIV RNA. This is just an old cartoon showing that as the CD4 count declines, one begins to develop different clinical consequences of HIV infection such that as long as the count remains above 500, one rarely has much in the way of difficulty. That once the count drops below 500, yet is still above 200, one may see a variety of more minor AIDS defining illnesses such as Kaposi sarcoma, orally hairy leukoplakia, thrush zoster.

And then once the count drops below 200, one begins seeing some of the more serious life-threatening AIDS-defining illnesses such as pneumocystis carnii pneumonia, disseminated microbacterial infections, toxo and CMV retinitis. This just looks at it as a cartoon. There are an enormous number of data in the literature showing this relationship between CD4 count and opportunistic infection. Similarly the data in the literature correlating levels of HIV RNA in plasma and disease progression -- these are the MACS data from John Mellors -- again, showing very clearly

that these two direct in vivo measurements have correlations with what happened to the patients clinically.

There is a lot of discussion and I think now there are fortunately a lot of data that bear on the relative importance of these two markers. Again, I think some of the confusion in this area has been the fact that when one looks at different studies, one marker may appear to be more important than another. Part of that, I think, is due to the range of values for the cohort.

In other words, if you have a cohort where everyone has a CD4 count between 200 and 300, the CD4 count won't be as predictive as the viral load. Similarly, if you are looking at a time interval of one week, the viral load may not be as relevant as the CD4 count. So, again, these are parameters that will reflect the relative importance of these two markers. But I think suffice it to say that when one looks at these overall, they do each have a degree of independent predictive value.

What I am going to do then is focus my comments for the remainder of the talk on some of these other laboratory markers which Alan has mentioned in his area. I am going to talk about it

really in four different areas, because I think they are related but they may reflect different things. HIV-specific immunity, lymphocyte subsets, activation markers, and T cell receptor rearrangement excision circles or TRECs.

When one looks at some of these potential markers of HIV-specific immunity, one can look at the two main T cell pools that may be conferring that immunity, the CD4 T lymphocyte pool, where the most prominent assay has been that of in vitro blast transformation of P24 antigen, and then the CD8 cytotoxic T lymphocytes, where the assays have focused on cytolytic activity or cytokine production in response to HIV antigens.

I think there is two general points I would like to make before discussing data. The first one I think -- and again, this is my perspective on this. I think for something to be considered an important element of HIV specific immunity, a laboratory marker should show some direct correlation with plasma HIV RNA levels. In other words, just because I have a measurement of something that is stimulated by HIV antigen, if it doesn't correlate with what is happening inside the patient, I am not really sure what to make of it. In other words, I think it is very important to

distinguish between elements of the immune system that bind to and are able to be stimulated by HIV antigens from those elements of the immune system that appear to be important in the control of production or clearance of virus. I think this is really an area where we get into some difficulties and have gotten into difficulties.

So just to talk a little bit about the P24 lymphocyte blast transformation response. This P24 antigen is the major structural protein in the HIV virion. It is a major component of the inactivated HIV present in remune. It has been shown as an antigen to elicit in vitro blast transformation and peripheral blood mononuclear cells of early seroconverters treated with combination antiretroviral therapy, a subset of long-term nonprogressors, and patients immunized with remune.

The question is, though, what does this assay give us overall. I am just wondering if someone can focus really the bottom two parts of that slide, the most important part. What this is looking is from a large cohort that we follow at the NIH, and it is looking at on the bottom viral load going from lower to higher in the log scale -- you can get an idea of the log scale looking here

at the middle one. And looking at in vitro blast transformation to first pokeweed mitogen, P24 antigen, and then tetanus toxoid at the bottom.

And I think if you look at the extremes for P24, just focusing your attention on the middle for a moment, that patients with low viral loads have a range of responsiveness, some who have quite high responses. If you look at the other extreme, patients with very high viral loads, there is very little evidence of responsiveness. But in-between, there is very little correlation. This is an R value of .15. This correlation between in vitro blast transformation to P24 antigen and viral load is actually no better, in fact even a tiny big worse, than a similar correlation for tetanus toxoid. In other words, these types of responsiveness may reflect a state of activation of the immune system, precursor frequencies of different antigen specific cells, but it is not really clear that it is a direct measurement of how well the host is able to control HIV.

On the CD8 side, we have again a variety of responses that have been measured -- CTL, antigen-specific CD8 cells, making cytokines by ELISPOT in response to tetramere stimulation or antigen-induced cytokine production. Here again, it

is to me confusing to -- or difficult to pull out an element that is important.

But here I think there is some interesting correlations with in vivo phenomenon, but it is not clear that they are in the direction one would necessarily have predicted. So these are data from a study at the NIH, again where patients with persistent levels of HIV RNA less than 50 copies had their antiretroviral stopped. As you can see, within a couple of weeks, all these patients showed an increase in levels of plasma HIV RNA.

Interestingly, you see one patient here who seems to maintain relatively low levels. There is a second patient from this cohort not plotted here with similar data. That patient is interesting in that he and the one shown here both had their antiretroviral therapy started very soon after the initiation of therapy, and that is very analogous to the patients that Eric Rosenberg and Bruce Walker published on a couple of weeks ago.

Well, if you look at this cohort of patients, and now looking at CD8+ T cell responses, you see that as levels of virus went up in these patients, the levels of HIV-specific CD8+ T cells by flow cytokine again went up as well. If you went to correlate the levels of these CD8+ T cells

with the viral load, you would see the higher the viral load went, the higher the number of these CD8+ T cells went.

So what does that mean? I am not entirely sure. The one thing I can say is I think that having more virus expressed caused an expansion of CD8+ T cells that could respond to viral antigens. Does that mean then that these patients with the highest levels of CD8+ T cells who happen to have the highest levels of virus have the best HIV-specific immunity? I wouldn't come to that conclusion. These patients down here who had very little increase in virus and then very little increase in CD8+ T cells seem to me to have much better HIV-specific immunity.

Well, perhaps this is adding something. And I don't really know a good way to assess that right now. One of the ways we thought we might assess it was looking at the rate of decline of the viral load when these patients all went back on antiviral. So if indeed these are important elements of host immune response, maybe those who have higher levels would drop more rapidly.

In fact, what we found was that the rate of drop of virus was not influenced at all by the percent of CD8+ T cells present in the

peripheral blood expressing cytokines in response to HIV antigen. So, again, while there is certainly a balance and these play some role in host immune response, merely having them in your blood doesn't mean that you have a better immune response to the virus.

So I would like to move now and talk about lymphocyte subsets. As you heard from Alan, the pool of lymphocytes is a quite complicated mix of cells. It is generated as undifferentiated stem cells that migrate into a thymic environment under the influence of the thymus. The T cell receptor genes undergo rearrangement.

There is positive and negative selection such that the cells that eventually enter the pool of CD4 T cells for humans recognize self plus antigen, do not recognize self alone. They have a predefined specificity and are considered naive until they encounter antigen. As we come back and we talk about the TRECs a little bit later on, they also will have some of these fragments of the T cell receptor rearrangement present in these cells.

Well, as the T cell pool evolves during life as naive T cells encounter antigen, those cells that are stimulated by antigen will expand in

numbers just like we use the more common letters of the Roman alphabet more commonly if we are speaking English. The letters of the Greek alphabet, we see less and less of it. They are not used.

If you were trying to analogize the T cell pool to the alphabet using two different examples. You see as you age, as Alan mentioned, the diversity of the pool declines and the size of the pool declines. But what is happening is at the same time it is becoming a more appropriate pool for you. So if you are constantly stimulated by CMV and toxo antigens, you will have more T cells out there with specificities for CMV and toxo antigens. That is probably one reason why the number of CD4 T cells can drop quite dramatically before one begins to see clinical problems.

We can measure some of these differences of lymphocytes by flow cytometry using markers for naive or memory subsets and through analysis of the T cell receptor repertoire. If we look at what happens to just naive and memory T cells now within the CD4 T cell pool, what you find shown here as a set of laboratory data are similar to what I showed you in the cartoon.

Namely, as the CD4 T cell count declines -- and this is for a cohort of 16 patients

with HIV infection whose counts went from 650 to 50 -- as the number of CD4 T cells decline, the fraction of cells in that naive pool goes down, thus the fraction of cells in that memory pools goes up. In other words, again, as the pool shrinks in size, you hang on to those cells that are more relevant to you given your antigenic environment. And when we come in and treat with here protease inhibitor therapy -- these are data from Indinavir -- you see that both naive and memory T cell numbers come up.

And again, this is going to get back to this issue of what is the role of the thymus. How much of a role does the thymus play in immune reconstitution? Where are the T cells coming from that come up in number with HAART. Are they redistribution of cells? Are they thymic immigrants of cells? Are they expansions of the peripheral pool as the three main sources?

This top set of patients are patients who had a relatively high number of naive cells prior to therapy, the bottom group a relatively low number. I think you can see focusing first at the bottom that this cohort of patients with very few naive cells had a nice increase in total CD4 T cell count, but all of that was pretty much within that

memory pool and very few within the naive pool over this period of three months.

In contrast, for the patient with the higher number of naive cells, both naive and memory cells increase with the initiation of therapy. So in other words, what you see with the immediate initiation of therapy are changes in the pools of cells reflective of the pools that are there.

There are two important elements of the pathophysiology of HIV infection. I think one is immune system activation and the other is immuno-deficiency. What you see with the rapid increase is probably a reflection of quieting of immune system activation, which is very tightly correlated with viral load. The longer term increase is probably more related to immune system reconstitution.

This just shows in a cartoon fashion the relationship between these different sources of entry or exit of CD4 T cells from the CD4 pool. Again, stem cells differentiating through a thymic environment add genuine new diversity to the pool. Cells within the pool expanding peripherally just add to the size of the pool without increasing diversity. Cells leave the pool all the time through natural death and through HIV-induced cell

death.

Now we can measure the size of the pool pretty well. And we can actually measure naive versus memory T cell pools relatively well also. And we can measure TRECs quite well, and that gives us an idea of cells coming out of the thymus. But what we can't measure very well is actually the diversity of the pool. As Alan mentioned, we can use the immunoscope technique to look at CDR3 size diversity within the beta chain of the T cell receptor. The trouble there is you are breaking up to -- you are grouping up to 10^{15} different specificities into around 192 boxes. So it doesn't really give you the type of specificity you need to know whether or not you can respond to one antigen or another.

Another way of trying to look at diversity is to look at the ability to produce antibody in response to immunization or the ability to monitor DTH in response to immunization. These are some data that were generated by Alannah Fogelman when she was at CBER showing that if you immunize a group of healthy volunteers with a neo-antigen bacteriophage Fiex 174, you get a pretty good increase in antibody production and you can boost that with subsequent immunization.

Again, this is on a logged scale. The shaded gray area is the normal control values for this assay in Hans Ochs's lab. Hans was the one who actually made these particular measurements. Well, if you take a group of patients with HIV infection who have done well with respect to their antiretroviral who have HIV RNA levels less than 500 copies and you perform the same type of immunizations, you see that some of the patients look quite normal and some of them look quite abnormal. And again, there are some very important qualitative aspects of the immune system that we really have ways of measuring, but we don't know how this correlates with overall survival.

I mean, who needs immunity to Fiex 174 bacteriophage. I mean, the problem is you are not going to come into this. It is not a pathogen. What we don't know is how to capture the range of pathogens. What we don't know is whether or not this type of assay will correlate to clinical outcome. In other words, will these patients who fall within the shaded area do better than these patients who don't.

I mean, I think these patients probably have a better immune system, but it is not clear that it isn't good enough. And again, if you look

at studies of the immune system, you can say, yes, this looks more diverse than that or this response looks higher than that. But what you really want to know is is this patient healthier than that one. And that is really the trick and that is the challenge, I think, that faces us.

Activation markers -- again, activation is a component of HIV disease. These are some data -- the activation marker we like to look at is bromodioxyuridine incorporation. It is a measurement of cell cycle progression and a measure of T cell turnover. You can see that you can make this measurement. It is quite simple to do. It correlates very nicely with viral load as shown on the left. It does not correlate with CD4 count as shown on the right. So this is the measurement on the Y axis of a cell turnover, log viral load on the X axis. Again, the measurement on the left is corrected for CD4 count and on the right for viral load. You see a very striking correlation between these two parameters. It is even more striking if you look at what happens with therapy.

So take patients -- this is a cohort of 11 patients who are protease inhibitor naive. Their cell turnover rate was monitored in the weeks following initiation of HAART as indicated by the

yellow symbols. The viral load was monitored as indicated by the purple symbols, and you see a very, very tight correlation between these two measurements over time. So in other words, what you have with HIV infection is a state of immune system activation. That activation does things to make the immune system not function as well. It will not give you as good an in vitro blast transformation assay when you have an activated immune system. You quiet that immune system and in vitro blast transformation will get better.

I think the rapid recovery of a variety of different opportunistic illnesses that we see following the initiation of HAART reflect not immune system recovery in the sense of immune reconstitution or repopulation of the CD4 pool, but really I think a fact that you quieted down this immune system activation so the T cells that are there can work better.

The last area I will comment on is the T cell receptor rearrangement excision circles or TREC. As Alan mentioned, these are a by-product of T cell receptor rearrangement in stem cells. Now the thing that is important, I think, in trying to look at TRECs is that there are two things that determine the level of TREC. How many are entering

the circulation -- in other words, how many new T cells are leaving the thymus, and how rapidly those in the peripheral circulation are being diluted out. In other words, this TREC -- there is one. It does not replicate with cell division. So it will be divided out or dilute out as the cells divide out. So levels of TRECs will be dependent upon the rate of thymic output and the rate of T cell turnover.

So here are some data again from the study that was mentioned by Alan by Danny Douek and Rick Koup looking at levels of TRECs in the lymph nodes of four patients with HIV infection on the right and four healthy controls on the left. You see less TRECs per cell on the right than you do on the left. The question is is that due to decreased thymic output or is that due to more rapid dilution of the cells that are leaving the thymus in these patients.

So we have done some work looking at the correlation between changes in TREC and changes in rates of now used T cell turnover again. It is the now used T cell pool in which these TRECs will be enriched. And we find a very striking correlation with an R^2 of .96 between change in the number of TRECs per million cells and full change

in T cell turnover within the now used T cell pool. In other words, it would seem to us that the driving factor between changes in TRECs isn't number of new T cells leaving the thymus, but really rather the rate at which those T cells are being diluted out.

So unfortunately, I come back to a final slide that is not too different from one of my earlier slides, that there are two laboratory markers of clear clinical relevance in patients with HIV infection. I think these are levels of CD4 T cells and levels of HIV RNA. Hopefully as we learn more about the disease and as we see more about how we perturb different levels of these markers with immune-based therapies, we may have some better correlates than what we currently have.

CHAIRMAN MASUR: Okay. Thanks very much, Cliff, for that perspective. We are not going to move on to the next talk which is on clinical studies, where are we and where do we go from here. And Jon Kagan will be pinch-hitting for this talk. So, Jon, we appreciate your ability to quickly transform yourself.

DR. KAGAN: I don't usually have such a great excuse to give a lousy talk.

CHAIRMAN MASUR: All right. We will

move on to the next speaker.

DR. KAGAN: So thank you for letting me talk to you today. Hearing the way the talks have gone this morning, I really did want to keep this right after Cliff's talk and I thank you for setting up Cliff. Even though this was not staged (off the microphone).

CHAIRMAN MASUR: I think the microphones need to be turned on.

DR. KAGAN: That's okay. I don't have a tie to hook it onto. That is only because they didn't call me early enough this morning.

CHAIRMAN MASUR: Dr. Fox will lend you his uniform.

DR. KAGAN: Okay. Does this work? Can you hear? Okay. What I hope to do in the remarks in the next couple of minutes is to give you some words that might be of use in taking the comments, and particularly some of the discussion about the markers and what they theoretically can teach us about the immune system and HIV disease and how those markers might be used in the context of evaluating immune-based therapies. And then I think some of the sanguine remarks of Cliff Lane regarding the potential for delusion between things that we like to see and things that mean something

to patients with HIV infection.

So I want to put up on here the slide comes from a Prentice paper years ago that really sets the standard for a rigorous definition of a surrogate marker. I even see that even on the agenda for the meeting. I think there is a lot of room for potential misinterpretation about this word and I think we should be rigorous in sticking to a definition of what this word means. And I am going to go to the extent of reading it to you because I think that what often falls by the wayside is that we, in talking about surrogates, are talking about markers that so strongly relate to patients' clinical outcomes in the context of treatment that they may substitute themselves for clinical endpoints in the context of therapeutic efficacy trials.

We are going to hear a lot today, and we have already heard a lot, about things that change in the context of HIV disease, treated or untreated. But it is a long road from things that change to things that can be used to predict the clinical outcome and that can be used to assess the potential therapeutic benefits of interventions that have not been validated with clinical endpoints.

These are some tired examples that probably many of you have seen, but for those who haven't are worth repeating. They give examples of when surrogates work, as in the case of HIV RNA, to very strongly predict clinical outcome in the presence of antiretroviral therapy to cases, both positive and negative, where surrogates either overestimate clinical benefit and then could, if relied upon inappropriately, lead to the approval of agents that actually do more harm than good or prematurely discard agents that look like they are no good when in fact they do confer clinical benefit, it just doesn't happen to be reflected in the surrogate or surrogates that we chose to investigate in the study.

So this is the question I asked to Alan Landay and to others. So many observations -- so many immunologic observations made over all these years, 20 years of reading the Journal of Immunology, and LPA, CTL, cytokines, you name it, up and down, over the course of the disease. Why is it that Cliff Lane gets up here, and I agree with him and can only say that CD4 and viral load are the only useful markers. Okay? And I put it on all of us that we just haven't done the right studies. We have not done the rigorous studies to

validate these markers. Granted the technology is going to open up new doors. But I am here to tell you that we need to work better -- we need to work a lot better than we have so far. We have failed to validate markers in large studies. We have pursued markers that have obscure relationships to disease pathology. We have pursued markers that have lack of specificity for disease. It doesn't mean that they can't be useful, but it makes it hard for people to grab onto them. Yes, there are technical barriers which I think will always fall along the wayside. And as we know, there are the potential for misestimates, examples of which I show you, which lead to diminished enthusiasm on the part of the clinical community to embrace these things. And it is not easy. It is not easy to validate a marker as a surrogate.

So I want to run through with you very briefly a paradigm that has been put out there to try to help clarify what markers can do and in what context. Probably some of you have seen this before and the proposal from Donna Mildvan has been to use a terminology that helps delineate between different applications of markers. Type 0 being a natural history -- I will give you examples of these. Type I a marker of biological activity of a

compound in vivo. And Type II the true surrogate for clinical efficacy of which we have been speaking just for a moment here.

This is the kind of data, data that John Mellors showed from the MACS study in which a single baseline RNA measurement could predict proportion to AIDS-free survival at three years down the road from the MACS. Okay? That is natural history data, Type 0 marker, very useful in the overall context of telling us what the relationship is between a marker and overall outcome. It has nothing to do with therapeutics -- with therapeutic interventions. It has no ability to jump from Type 0 to surrogate marker of clinical efficacy, Type II. And the startling example of that, everybody should know, is P24, which I was interested by Cliff's example. But everybody in this room should remember that although P24 -- people with high P24 have worse prognosis and P24 drops dramatically upon the introduction of effective antiviral therapy. Those people whose P24 plummets versus those people whose P24 either doesn't change or increases or goes from negative to positive, there is no difference in the ability of P24 to predict therapeutic outcome. So the lesson here is look and learn and remember that this is great for

prognosis, very useful for telling us something about the pathogenesis of the disease, and completely, until proven, useless for leaping to the point of surrogate.

Type I we put up there was the drug or therapeutic activity marker in vivo. And this is what we mean as a marker that will reflect the activity of an intervention in a person, this being a stylized ideal, and this being data from real trials from multiple -- actually, the protocol 35 from Merck with Indinavir, showing particularly in the earliest phase that antiviral effect is better with three drugs than two than one. That is the kind of data we need to validate that a compound has activity in vivo as an antiretroviral. Still doesn't mean that a compound -- that a marker, in this case viral and RNA, did not prove that the marker would have any surrogacy for clinical outcome.

This is the data. And without this data, viral RNA is not a surrogate. This is the data that relates change in viral RNA at both a million and a hundred thousand copies at baseline to the dramatic decreases in the risk of death only can be confirmed by clinical endpoint studies and looking at the correlation of the relationship

between those changes. This is a crappy slide from a meta-analysis that Michael Hughes did some years ago. And don't even bother looking. I can just tell you, though, it proves the same thing across a whole variety of antiretroviral studies, and it was -- I think this data was highly influential before this committee some years ago in the advocating for the use of HIV RNA as a surrogate in the manner that we use it today.

So going to the immunologic markers, this is the sorry history. And you can add to this list from what has been presented today. But the point is that we have been pretty good at doing some studies in the context of cohort studies to gather some of the Type 0 information. You can see what I mean by here is that if there is a plus, that means there is some evidence that apoptosis has been -- there is some data relating apoptosis to prognosis. And the more pluses obviously the strong the relationship.

But as you move to the right across the chart, here proof of the relationship between the change in the marker and drug effect in vivo. You can start to see the fall-out. And you get over to this pretty sad looking column where we just have not done the studies. The question mark says we

don't know. Okay? And you can see that the only thing that really stands out here very well, CD4 1+, HIV RNA 2+, and there has actually been a little bit of data to support Beta-2 and neopterin. But unfortunately those markers are probably good examples of the point I mentioned earlier in terms of lack of specificity for the disease and probably not offering anywhere near the predictive outcome of plasma RNA, especially as it becomes cheaper and easier to do that test.

So here is a slide that was prepared specifically for this meeting that hones in a little bit more on the immunologic measures and comes to the same conclusion. You can start on the right and go to the left here. Again, you can start with all the question marks, but my point is that we are still only in this gray zone here for proving relationship to activity detection of drugs in vivo. I mean, we are off the ground in terms of proving a relationship between these markers and prognosis. But the point is that it takes very highly coordinated studies pulled off in conjunction with study groups and organization and planning and money to do this stuff.

So we have been working off the existing paradigm for the last several years about

the need to conduct marker validation studies in the context of interventions that show clinical benefits. The point being right that you can't prove the utility of a surrogate unless you can test it in the context of clinical benefit.

So I think that where we are now is that we need to move a little bit. And we need to move into an area where we don't simply use clinical benefit as the outcome against looking at surrogates, but look at benefit in terms of relevant outcomes in today's treatment environment. And these are some that I would pose to you as perhaps year 2000/2001 potential outcomes to be looking at for the validation of markers. All still in the context, I think, that Dr. Lane was trying to give you, and that is real world stuff that means something to patient's health. I think maybe some of us in the room might be willing to say that looking at immunologic restoration immunologic failure on therapy and viral rebound off therapy. These are the kinds of things that get closer than picking your favorite marker out of the Journal of Immunology and saying, well, this looks really good because this goes up or this goes down. I think this is the kind of thing we have to start looking at. Rebound on therapy, suppression of

reservoirs and of course maintain the opportunity to seize on all the opportunities that we have with the decreasing number of clinical endpoint trials to validate these markers.

And why does it seem so obvious to do this but that this really doesn't happen? Well, in terms of -- I am going to jump ahead of myself here -- in terms of planning these studies prospectively, we could build into a lot of studies these immunologic markers of interest. You can see that it would allow us to do a lot of tests that we currently can't do or can only do in real time. We can target specific interventions, et cetera. But the tests are often costly. They often have huge variability problems between one center and another. And so we balk at building these kinds of exploratory tests into prospective studies because we have no crystal ball. On the other hand, when we go into the retrospective studies where we know the outcome and there is an opportunity to look at a marker and its relationship to the outcome that is known, lots of times we are limited by what it was that was collected in terms of samples for those trials, whether or not there were enough of them given the variability of the assays that we want to do to be able to come to any meaningful

conclusions, whether populations were excluded, et cetera.

So there are pitfalls and advantages of both the prospective and retrospective approaches. This is just a snapshot of what is going on in the AIDS clinical trials group. The first kind of two-thirds of the diagram just giving you an idea about retrospective studies. There is something in there about what we are doing. Let me just kind of walk you through it a little bit. These are some of the older studies, because there are still older marker studies that are going on in the ACTG, where we are looking at the relationships between these markers and these traditional endpoints, AIDS and death in this case, the OI-specific lymphoproliferative as relating to the clinical opportunistic pathogen endpoints. But there is movement now to move to the areas of trials where the outcomes -- or studies where the outcomes are looking at discordance between RNA and CD4, radioimmunologic restoration and virologic suppression. And probably some of the most exciting here looking at these immunologic markers in the context of an STI readout that is viral rebound upon withdrawal of an antiretroviral therapy.

So I think that where we are now is the

tough job of not using sight of what it is we are trying to do. Keeping a rigorous eye on what a surrogate really is. And on not being led astray by our theoretical interests about roles of different cells or soluble factors in the immune system. And lastly, to kind of bolster the point that I was making about this work being hard is that I think to really tackle this problem of the validation of markers for the use and the proving of immuno-based therapies is going to take some Herculean efforts between academia, industry and government to do studies the likes of which I don't think we have seen so far. Those are my comments to you.

CHAIRMAN MASUR: Okay, Jon. Thanks very much for helping to define these issues of biomarkers and surrogate markers. Again, we appreciate your pinch-hitting for Donna.

I think it is actually impressive that not only did we have three excellent talks, but also that we stayed on time. So we will take a 15 minute break and reconvene at 10:45 for Dan Kuritzkes.

(Whereupon, at 10:30 a.m., off the record until 10:53 a.m.)

CHAIRMAN MASUR: We are going to get

started again in about two minutes. So if the committee could take their seats. Okay, we will wait for one more minute while we assemble the tardy committee members. Okay, I guess we have reached a quorum and we won't wait for your boss. The next talk is on perspective on viral load and CD4 counts by Dan Kuritzkes from the University of Colorado. So, Dan, welcome.

DR. KURITZKES: Thanks very much. It is a pleasure to be here. I would like to especially thank Sherry Lord and Bill Schwieterman for inviting me and for the several conference calls that helped to focus my talk.

I am going to be picking up really directly from where Jon Kagan left off and also picking up from the talk that Cliff Lane gave to discuss the use of viral markers to assess the activity of immune-based therapies.

By way of introduction, I think I should emphasize a couple of things. First of all, I was really asked to look forward in terms of how we might think of creatively using both available markers and markers in development. And secondly to emphasize that much of what I will be talking about is about the use of viral markers as measures of activity of immune-based therapy. So really the

kind of Type I markers that Jon Kagan was talking about. And acknowledge at the outset that there is a very long road to go between providing evidence of activity and evidence of surrogacy.

It is useful in thinking about how we might use viral markers to evaluate immune-based therapies to ask what the goals of immune-based therapy are. And I think clearly the goals are to -- that immune-based therapy is directed at modulating the immune system in order to control HIV replication and enhance overall immune function. Now I will be focusing really on the first of these goals of immune-based therapy in terms of my talk and the use of viral markers.

There are several potential mechanisms of action of immune-based therapy with regard to viral markers. IBT's may enhance HIV-specific immunity, as we have already heard from the earlier talks this morning. They may do this through the direct effects of some of these therapies such as HIV vaccines or the presumed effects of strategic treatment interruptions which can be thought of as endogenous vaccines. Or indirectly through the action of cytokines that might lead to enhanced HIV-specific immunity.

There can also be general increases in

immune competence, either directly through the action of cytokines or indirectly through immune reconstitution that follows from effective control of virus replication.

An area of immune-based therapy that gets less attention these days but is still a possible use for such therapies is to decrease cellular activation directly through immune suppressive agents like Cyclosporin or Cyclophosphamide or corticosteroids, or indirectly again through controlling virus replication which in turn leads to diminished activation.

And then there is a category of agents that block virus entry which are sometimes thought of as immune-based therapies. Although with apologies to my immunologic colleagues, I would argue that although these may make use of the immune system or take advantage of the immune system to generate these agents, in fact they really are the antiviral agents and I think should be thought of from the point of view of the whole process of demonstrating activity of these agents and moving them forward as antivirals of a unique class, and these would include virus-specific antibodies and agents that block cellular receptors for virus entry.

Well, what are the possible virologic effects of enhanced HIV-specific immunity? There could be decreased virus replication, acceleration in the clearance of infectious virions as Cliff already eluded to. One might eliminate productively infected cells, presumably by agents that are directly targeted or cellular effector mechanisms that are directly targeted at these cells. Eliminate latently infected cells, those that continue to express HIV antigens even if they are not actively producing infectious virus. Decrease the size or accelerate the clearance of the latently infected pool of cells. Diminish the pool of available or productively infected target cells. Again, this speaks to the decrease of cellular activation.

But there is a paradox as relates to the immune-based therapies and the approach of IBT's, and that is that these therapies seem to work best in patients who already have the most intact immune systems. That effective control of virus replication in general tends to provide the optimum substrate for the use of immune-based therapies. And therefore the efficacy of antiretroviral therapies makes it difficult to demonstrate and incremental benefit of immune-based

therapy, particularly when focused on viral markers.

Now the traditional viral markers can still be used in the evaluation of immune-based therapies in several ways. One can think of them in their current use, again as stressed by Jon Kagan, as measures of activity and in certain contexts as measures of efficacy, looking at a decrease in plasma HIV RNA from baseline, looking at the proportion of patients or subjects with plasma HIV RNA levels that are suppressed to below the limits of detection or at that time to virologic failure. But in the context where you already start with patients who are maximally suppressed or where immune-based therapies need to be given with maximally effective antiviral therapy, these markers are -- it is going to be very difficult to demonstrate any increase in activity of the regimen based on these markers.

But these markers may also have an important role to play as safety measures for immune-based therapies, particularly in patients who start off with suppressed virus replication. Because you would like to exclude evidence of an increase in plasma virus load as evidence of activation of viral replication as a consequence of

immune-based therapy and exclude an increased rate of virologic failure.

Now the problems with using these traditional markers in evaluating IBT's I already eluded to in part when I talked about the paradox of IBT's. But obviously current antiviral regimens suppress plasma RNA below the limit of detection in a large majority of patients, particularly in the context of clinical trials. Virologic failure rates are low, especially the true virologic failures -- not the dropouts, but the actual virologic failures. In the most recent studies of the last two or three agents to be approved and reviewed by this committee, I think you are well aware that we are looking at true virologic failure rates on the order of 5 percent or so. And this has the consequence of requiring studies of extremely long duration or very large sample size in order to show some incremental benefit of an immune-based therapy added to antiviral therapy. And i think we all recognize that it is impractical for the purpose of selecting agents that should move forward into further development to be relying on 800-patient studies simply to decide which agents would truly deserve large efficacy trial.

And of course the potency of antiviral

therapy continues to improve as witnessed by some of the more recent combination agents or pharmacologically enhanced protease inhibitor agents that have now come into clinical practice.

So in thinking about how viral markers might be useful, especially how we might look at them from a novel point of view, I wanted to review just very briefly the dynamics of HIV infection and then contrast what we see in the pre-treatment steady state to the treated patient, and of course these are data that are well familiar to the members of the committee and the audience. But we start with plasma virus, which has an infectious half-life of one or two hours or less according to the most recent data from the group with Aaron Diamond and Alan Perelson. If the virus encounters a susceptible cell, generally a CD4+ T cell, those cells go on to become productively infected, releasing new infectious virions and die with a half-life of about a day and a half, leading to the completion of this cycle in a period that seems to occur over a two to two and a half day period. Some of the time, though, these cells, either at the time of initial infection or subsequent to infection and the completion of reverse transcription and integration, become resting cells

and therefore contribute to the pool of latently infected resting CD4 T cells and presumably also monocytes, and these cells have a half-life that is much longer than the productively infected cells and the real half-life of these cells I think we don't have an accurate estimate of yet. Although these cells can obviously be activated at any time to enter into the pool of productively infected cells, after which they die quite quickly.

Now another factor to be considered, especially when we begin to think about some of the markers that might be used, is that some of the time

-- and the proportion of this pathway is really not at all clear. Virus that is capable of completing the entry, reverse transcription and integration steps may nevertheless lead to dead-end infection because there is some subsequent block to production of infectious virus. And these defective pro-viruses then accumulate in cells and the actual turnover of cells that are infected with dead-end virus is also not known, but these cells can confound some measurements of the pool of latently infected cells depending on the marker being used.

Now in the patient who is on antiviral

therapy and is fully suppressed, the relative importance of these pools may shift, at least from the point of view of attempting to quantify what is going on. The plasma virus is either unmeasurable or barely measurable with concentration techniques and highly sensitive assays. There may be persistent virus replication occurring, but it is occurring at very low levels and may require access to tissue compartments in order to detect. And so what we are left with is a pool of circulating latently infected cells and this pool of defectively infected cells or cells infected with defective pro-virus which contribute to the quantification.

So what markers might be available to use in this setting then to see whether we can identify activity of immune-based therapies in this context. We could attempt to quantify pro-viral DNA. To quantify by quantitative culture latently infected resting CD4+ cells. To make some quantitative assessment of residual virus replication through the use, for example, of 2-LTR circle assays, in situ hybridization for spliced and unspliced HIV RNA, or in a more cumbersome approach by looking at viral sequence evolution, and I will show some illustration of each of these

approaches.

There are also indirect measures of the effects of HIV-specific immunity on virus replication. These would include looking at alterations in the rate of viral rebound after treatment interruption, and Cliff already introduced this concept. Looking at the magnitude of virus rebound after treatment interruption. Or looking at the proportion of patients who have spontaneous control of viremia to below some threshold value, and here it is obviously not spontaneous but the hypothesis is that this control is the consequence of the immune-based therapeutic in question. But whether this threshold should be below the level of detection as currently we hold antiviral agents to below some higher threshold such as 500 copies per ml or 10,000 copies per ml, I think these are important questions which have important implications.

It is also important to keep asking how these different changes correlate with more traditional immunologic measures, in particular the CD4 cell count, and what the durability of either the slowly rebounding virus replication rates would be or the new threshold is.

Well, what about proviral DNA

quantification? The advantage to this approach is there are a number of prototypic assays that are currently in development. These are easily amenable to standardization using existing PCR technologies and can be used with stored specimens, which is a really very important advantage and gets over some of the hurdles as far as looking at stored specimens from previous cohorts where we already have clinical endpoints and outcome data, at least for looking at its utility retrospectively.

Unfortunately, though, there are these two compartments or two pools, the defectively infected cells and the cells that are latently infected, and these pools turn over quite slowly. So we would expect to see changes in the quantitative nature of this marker that would occur very slowly. And we really don't know what the relevance of the cells that harbor defective provirus is to overall infection and to what extent they will confound this measurement.

Now looking at the decay of latently infected resting CD4 cells -- these are the data from Finzi, et al., from Bob Siliciano's lab showing the very slow decay in a group of patients, many of whom may have been nonadherent to therapy.

And again when they looked at the aggregate slope, they couldn't find a difference that was statistically meaningful from zero. So it is conceivable that the decay could be enhanced by altering the host virus specific immune response and that demonstrating acceleration in the decay of this pool might be taken as activity that the agent was at least doing something. Whether that something is of clinical importance or not would require further study. But it would be some reason for hope and moving forward and further evaluation of that agent.

And then, of course, as Bharat Ramratnam and the group at Aaron Diamond have shown, these slopes are actually quite variable and may depend very much on the extent to which the patient is adhering to the baseline antiviral therapy, since those patients who had no blips above 50 copies had already negative slopes in the decay of this pool, whereas patients who had intermittent viremia had apparently shallower slopes, and those who had frequent blips either had no decay or in fact even an increase in the size of this pool.

The other problem, of course, is that these are extremely labor intensive assays. The

inter-assay variation is quite great and to do these assays properly requires a fair volume of blood, which would make their routine application in large clinical trials quite cumbersome.

Looking at evidence for persistent virus replication, perhaps one of the most promising assays, although one that is a long way from validation, is the use of the so-called 2-LTR circle to provide evidence of recent virus replication. Recall that the viral genomic material has two long terminal repeats, one at the five prime end and one at the three prime end. And when the virus undergoes reverse transcription to generate linear double-stranded DNA that the linear DNA molecule is the molecule that integrates. But in cells where there is some blocked integration, one of two circular forms can be generated -- either the circle which has a single LTR or a circle that has two LTRs. And for the point of view of this assay, the only significance to the 2-LTR circle is that one can design PCR primers that uniquely detected 2-LCR circles and cannot be confused with either integrated linear proviral DNA or with genomic RNA. And since the only time you have two LTRs together in this kind of apposition is as a result of this process. Now it is believed

largely from the result of in vitro work, although there is still some in vivo validation to be done, that 2-LTR circles are relatively unstable and decay quite rapidly after unsuccessful infection of a cell as shown on the left panel here using HIV 1-LAI, and these are data from Sharkey, et all, published in Nature Medicine earlier this year. And that if you completely block further rounds of replication, you see the decay of two LTR circles over the course of three days.

This was then taken as evidence -- one can then -- if it is true that these circles decay quite quickly after the cessation of virus replication, then the persistence of circles might be taken as evidence of ongoing replication and evidence particularly of recent replication. And so they looked at a group of patients who had undetectable plasma HIV RNA for many months up to a year and a half and could see that even in patients out at 15 months, those who had plasma viremia that fell below the limits of the current plasma HIV RNA assays, they were nevertheless able to detect in circulating cells evidence for recent virus infection. And so one could imagine that if this assay were standardized, and certainly because it is a PCR-based assay and could even be improved by

the use of TAC man technologies for example, that these assays could be made quite precise and reproducible. And that an immune-based therapeutic that led to the disappearance of 2-LTR circles from the circulating cells of patients who were already virologically suppressed again might be taken as evidence that the agent was doing something.

More cumbersome measures of ongoing virus replication include in situ hybridization for spliced and unspliced messenger RNA. And here usually both species are measured because unspliced HIV RNA may be genomic RNA that is being produced by the cell or packaged RNA inside virion particles attached to the surface of the cell, whereas spliced RNA is evidence that there is some active transcription occurring and processing of viral RNA intercellularly. Here you see from a recent Nature Medicine paper by Reinhart, et all, the evidence looking at -- these are cells that have been transfected with plasmins that produce rev and gag messenger RNA and looking at unspliced and LTR spliced message and then looking at tissues This is with an SIV specific probe, but the same kinds of probes can be generated to look for HIV, here looking at spliced message, and you see here in the germinal center evidence of transcriptional

activity, and then an unspliced message shown here in the surrounding region.

Or looking at gut tissue in this paper by Lingi Zhang and again the group from Aaron Diamond showing evidence of these rare cells, which on higher power show evidence of persisting virus production. But if we needed to get tissue samples in order to do this, again the ability to sample frequently and to sample in large numbers would be a major challenge. And also the inter-assay reproducibility or inter-patient variation in these measures is completely undefined.

Another hypothetical approach, one that has been used to argue for the persistence of virus replication as well as to look at the source of virus from different pools following stimulation is to examine sequence evolution. Again, from the same Zhang paper, there was evidence here of continuing virus evolution that argued for persistent virus replication in the apparent setting of controlled replication because of undetectable plasma viremia. This would be a very cumbersome and time consuming process, although one that could be done using stored samples.

What about the issue of looking at some of the less direct evidence using viral rebound

following treatment interruption. There are several studies now that have looked at viral rebound in different context. These are the data from ACTG 343 that were published by John Ioannidis and the 343 team for patients who were failing therapy after a switch to a simpler maintenance regimen. And these are data from the Spanish treatment interruption study from Garcia, et al., and then also data from Avidon Neumann using a data set from the Dutch group with the two cycles of initiation of therapy and then interruption. I think enough data have been generated now that we can actually begin to get some sense of what the interpatient variation is in the rates of viral rebound, so that at least for the purposes of sample size calculation, we begin to have something to go on as far as what the expected rebound rates are, how many patients would be needed to accurately determine the rebounding rates and what sort of sample size you would need to be able to detect the difference in the rate of rebound between a treated and untreated group.

It is also possible, as was done in this paper by Richard Harrigan and the group at University of British Columbia in Vancouver to extrapolate backwards from the rate of rebound to

what the likely pre-treatment interruption level of plasma -- total plasma body virus must have been, assuming that there are constant rates, although that is a huge assumption and one doesn't know that there wasn't some plateau here and then a take-off, because you are really extrapolating well below the limits of detection, and those are indicated by these dotted lines that don't project very well.

Well, what does all of this mean? Is the rate of rebound related to the eventual steady state, or do you simply take a longer or shorter time to get to the same steady state. How does the viral load plateau, following post-immune-based therapy rebound relate to the risk of disease progression? What about the T cell count in these patients, and what is the clinical significance of decreasing the pool of latently infected cells, and is there an incremental benefit to extinguishing residual viral replication? We would like to think that there is, but it is not -- since we can't find good evidence for the emergence of drug resistance in some of these patients, it is uncertain exactly what the meaning of this residual pool is.

And to illustrate some of the difficulty in relating the effects of an immune-based therapeutic to an antiviral effect, the very

recent paper in Nature Medicine by Hel and colleagues I think makes the point very nicely. This is actually the cartoon from the accompanying news piece by Eric Rosenberg and Bruce Walker. But in essence, they took 24 macaques who were acutely infected with SIV and then the macaques were either treated with a potent antiviral regimen and given a Sham vaccine, treated with therapy and given an SIV vaccine that expressed several SIV antigens or given vaccine alone. Although even I am having trouble from this point reading the numbers, in essence what they found after a period of vaccination and/or treatment that four of the seven animals who received treatment alone had spontaneous control of viremia. Six of eight who received therapy and vaccine had spontaneous control of viremia, and only one of eight who received the vaccine alone had spontaneous control of viremia. And this was after the therapy was then stopped.

Now just to summarize this for you, because again the slides won't be readable, but the dilemma here was that there were -- the group that got vaccination and treatment had the best evidence of immune response. But having better immune responses in this case didn't really translate into

having anything different in terms of control of viremia. Because the same numbers of animals had spontaneous control or similar proportions of animals had spontaneous control of viremia in when they therapy in these animals was stopped. And this led the authors to conclude in a statement that seemed somewhat rueful, "The effect of antiviral therapy alone has interfered with our ability to reach unequivocal conclusions on the contribution of vaccination to the containment of viremia following treatment suspension. And I think this captures really very nicely the dilemma that we are all living with.

And similarly with the data that Bruce Walker has recently published, where there were five patients who were able to maintain viremia below 5000 copies per ml. The 5000 copy threshold is an arbitrary threshold here, in part because most of the patients fell below it one suspects, and it is really not certain how this relates to our more traditional ways of thinking about the risk of disease progression given a particular viral setpoint.

You've seen this slide a couple of times already this morning, but remember that these data from Mellors look at patients following

unmanipulated natural infection. And whether an HIV RNA level or setpoint of less than 5000 copies in the absence of any intervention has the same risk of disease progression as a plasma HIV RNA level of less than 5000 copies following such manipulation is something that remains to be reestablished, I think.

It is also important to remember from the meta-analyses that have been done -- and this is more recent data from the same meta-analysis that Michael Hughes led, that it is not just the control of virus replication, but also the improvement in CD4 cell count as a marker of immune reconstitution that confer clinical benefit. So here looking at the proportion of patients progressing to AIDS or death, those patients who had the best response or the best prognosis were those who had control of both virus replication and an increase in CD4 count. But those who had an increase in CD4 count, even without complete control of virus replication, had the next best outcome, and control of virus replication without evidence of CD4 cell reconstitution had an outcome that was not quite as good. And this has been shown more recently with more potent therapies by the European group. Actually, I believe this is

from the French Aquitaine cohort. The data are just plotted in the inverse direction. So this is proportion remaining AIDS free. And again, those who had no virologic or immunologic response had the worst prognosis, but having either a complete response, meaning both an immunologic and virologic response, or having a partial virologic suppression with a good immune response, that is a significant rise in CD4 count, led to an outcome that was not substantially different. So even if we focus on viral markers as evidence for the activity of immune-based therapies, we will still need to be asking what are they doing to CD4 cells and to the immune function of the host overall.

So in terms of selecting viral markers for immune-based therapies or for trials of immune-based therapies, I think there ought to be going into the study a hypothesis regarding the mechanism by which the immune-based therapeutic is expected to produce a virologic benefit. And the choice of the virologic marker then should be based on the proposed mechanism of action of the agent in question.

To conclude, I would say that treatment-associated change in some of the novel viral markers might be useful for establishing

proof of concept and that such a change might be used to justify a larger, randomized trial, but that these novel markers are going to require validation of surrogate markers before they can be used in Phase III studies for the further study of -- or development of immune-based therapeutics. And I will stop there and turn this back over to the chair.

CHAIRMAN MASUR: All right. Thanks, Dan. We will have, I am sure, considerable discussion about this this afternoon. We are now going to move on to perspective on other markers of immune function by Mike Lederman from Case Western Reserve.

DR. LEDERMAN: Thanks, Henry. Thanks, Dan, for your introduction. And I will be talking a little bit about the use of immune-based -- how do we use markers to validate the potential utility of immune-based therapies. And in contrast to Dan, who has been talking about agents that could be useful in terms of limiting viral replication, I am going to talk primarily about agents that may have some utility in terms of enhancing immune responses in a more general way.

I think this is particularly important now because it is clear that we are going to have -

- there are an increasing number of interesting-looking molecules. And in time it is likely that we are going to have even more interesting molecules. And unless we -- the timing is very good now to try to reconfigure and rethink how we develop these molecules for their potential clinical utility.

So if I could have the next slide, please? I know we will get through this. There we go. The first thing that I want to say is to amplify on some of the discussions that were made earlier, presentations by Larry Fox, are that immune restoration, even in people with excellent suppression of viral replication, is incomplete at best. And these are simply some data taken from one ACTG study that demonstrate the CD4 cell rise after a year of therapy. And although you can't see the Y axis very well here, the total CD4 count at the end of a year - this is the median CD4 cell count in this population -- was about 400. Actually, I think it was 350. And at the end of three years of therapy on these same patients, among those who had excellent suppression of viral replication, the median CD4 cell count was just around 400, indicating that more than half of the patients in this study had circulating CD4 cell numbers that were below the 95 percent confidence limits among

normal, healthy HIV-uninfected persons.

Next slide please. When one looks at functional ability, and this is an in vivo measure of immune competence, which has some utility in terms of cross-sectional and longitudinal studies in terms of predicting outcome in HIV disease, we can see that even at the end of 48 weeks, only about a third of patients -- a little more than a third of patients -- have any delay-type hypersensitivity reactivity at all, whereas 60 percent of patients remain anergic, and in a healthy population, 90 percent of persons should have some DTH response to any one of a panel of these DTH antigens. So both in terms of phenotype and in terms of function, the immunologic restoration that we see with suppressive antiviral therapies is incomplete at best.

So how do we develop agents that may improve immune responses in HIV disease. Dan talked a little bit about how one might be able to monitor the activity of agents that may enhance HIV-specific offenses. But we also need to look at agents that may enhance immune function that may prevent against opportunistic infections and other related complications, and that is what I will focus on in the remaining moments that I have.

So can we use laboratory indices to evaluate the potential utility of these agents that could improve immune competence? To date, as has been emphasized by a couple of the speakers, there are only two laboratory markers that have been shown to predict clinical benefit in the context of antiretroviral treatment trials, and those are levels of HIV replication and the circulating number of CD4+ T lymphocytes.

Circulating CD4+ T cells are a very useful marker that predicts the outcome in HIV disease. They predict the outcome in natural history studies. They increase with antiretroviral therapies and predict the clinical course, and they can be used as a guide for the administration of prophylaxis against opportunistic infections. So in a general way of looking at these numbers, it is a fairly good reflector of immune competence.

So what happens when we give an immune-based therapy? Well, here is some data from an ANRS study that show a nice CD4 cell rise - a nice rise in the circulating numbers of CD4+ T cells among persons receiving Interleuki-2.

So the key question that I know everyone

-- many groups here are wrestling with is whether

or not a CD4 cell increase after IL-2 therapy confers clinical benefit. The increases are polyclonal. The cells are clearly functional ex-vivo. And in terms of the relative significance of this, one can draw an analogy to what we see in terms of the CD4 cell increase after HAART, which is the first phase CD4 cell increases, which are largely redistributive in nature, are temporally associated with a clinical benefit. Now the caveat here is that these increases are also associated with diminished viral replication and also diminished consequences of viral replication on immune activation that could also play a role. But we have reason to think that these numbers -- just increasing the numbers of these cells may turn out to be useful.

So what I am going to do is move ahead a couple of years and a couple of tens and perhaps even hundreds of millions of dollars now. The results of ESPRIT and SILCAAT are out and they confirm the clinical benefit of Interleukin-2 administration in HIV infection. And not only will this make many people very happy, but it may be very useful for us in terms of validating a clinical marker or a laboratory marker of immune competence.

Well, the news is even better. The news is even better because ESPRIT and SILCAAT show that the benefit of IL-2 administration is completely explained, as statisticians like to put in parenthesis, by an increase in the circulating CD4+ T cells counts. And so this really validates the concept that increasing CD4+ T cells is enough to enhance immune function.

So as my grandmother used to say, how will this be good for us Jews. And the answer is that it is not clear. It is not clear. Because demonstrating this and showing that an increase in CD4+ T cells may not help us at all in terms of developing other interesting immunologic molecules. For example, it is not clear that showing that an increase in CD4+ T cells will help us in the development of agents like Interleukin-12, Interleukin-15, Interleukin-16, flt-3 ligand, CD40 ligand, CPG motifs, B cell stimulators like BlyS, Interleukin-7, GM-CSF. So there are all these things that are floating out there that may be of some utility in human disease. But showing -- just demonstrating and validating a clinical marker in one immune-based therapeutic trial may not help us in terms of developing another immune-based therapy.

So what about in vitro laboratory markers? Well, we have got lots of them. And the advantage of these markers is that we can examine them both prospectively and in retrospective studies, and they are particularly useful in terms of asking questions about disease pathogenesis. But they may have limited utility for the development of immune-based therapeutics, because the promising reagents that we have available to us exploit multiple different mechanisms and multiple different pathways for regulation of immunologic responses.

So I think what I would like to suggest, and what I am going to propose to this group is that we try to develop some final common pathway readout for immune competence.

So let's go back again. In this regard, what -- maybe I would like to look at a clinical endpoint trial maybe from an overview and different perspective. So when you generate a clinical endpoint trial, when you put one together, you have individuals who agree to participate in the study, and they may or may not be randomized to a treatment regimen. And then they are observed for their ability to mount or maintain a protective, adaptive immune response to a microbial challenge.

And that is really what we are trying to do in terms of a clinical endpoint study in HIV disease.

Now one of the limitations of these studies is that the investigators have limited or essentially no control over the challenge, and it places persons at risk for morbidity and death, which are in fact the endpoints of these studies.

So this raises the question as to what really are we talking about when we talk about adaptive immunity. In contrast to what Jon Kagan always says about the adaptive immune system, he maintains that this is a means to keep clinical immunologists employed. In fact, there is another role for adaptive immunity. And in a nutshell, it is largely a mechanism that permits the survival of large, bulky organisms that have limited reproductive potential and great love for their few offspring, meaning that they have a faithful DNA polymerase, by promoting the ability of these organisms to evolve in the absence of germ line mutation. And I think really -- I mean, that is really what an adaptive immune response is. That is, I think, why we have it. And so an adaptive immune response pretty much mediates the evolution of an immune response to a microbial challenge.

So can we develop a model to test in vivo? And I think that Cliff is exactly right when he says in vivo veritas. The ability to mount an adaptive immune response to a microbial challenge.

Well, one thing that we can think about in terms of an in vivo measure of immune response is delayed type hypersensitivity responses to skin testing. One, DTH responses are predictors of outcome in natural history studies. Two, they improve with suppression of HIV replication. Three, they are relatively simple and certainly safe. Four, they measure primarily CD4+ T cell responses, but they can be manipulated using peptides to measure CD8+ T cell responses. On the downside, these assays are not standardized. They are not terribly reproducible between individuals or even in the same individual over time. And they measure the efferent limb of the adaptive immune response to microbial antigens.

What about immunization? Well, in fact immunization really is a form of microbial challenge, and one can utilize complex or simple antigens to measure a CD4+ T cell response or a B cell response to a microbial challenge. An immunization strategy or an immunization test can

test both the afferent limb and the efferent limb of the immune response, and one can even utilize methods for intracellular gene expression to induce a CD8+ T cell response either using a DNA or an RNA vector or perhaps even a virus or a viral vector or an attenuated viral vector to get a Class I restricted T cell response.

So you can use antibody levels, measurement of antibody levels, to measure a B cell response. One can use DTH to measure a CD4+ T cell response and possibly a CD8+ T cell response in vivo. And you can also use in vitro assays, any one of a number of the assays that Alan reviewed earlier this morning, to provide a detailed, cell-specific fine characterization of responses.

So has there been a lot of experience with looking at the response to immunization after HAART? Well, there has been a limited study by the ACTG 375 group that demonstrated that the magnitude of responses as measured either in terms of lymphocyte proliferation or delay type hypersensitivity or antibody levels was related to the degree of HIV inhibition to decreases in immune activation and also to expression of a co-receptor for T cell activation, CD28. What is more, in these particular studies, the appropriate

representation of naive cells determine the ability to respond to neo-antigen immunization and the appropriate representation of memory CD4 cells predicted the ability to have a recall response.

So another advantage of trying to develop this strategy as a means to evaluate the activity of immune-based therapies is that you can time your opportunistic infection. You can time the OI type challenge by the trial design. That is, if you make a determination that you are going to apply an immune-based therapy, you don't have to wait for something to happen. But you can actually say on week four or on week eight or on week twelve or whatever the appropriate timing is, you can challenge a person with a microbial antigen or antigens.

This approach, at least in developmental studies, avoids the morbidity of clinical endpoint trials. And I am not saying that we don't need to validate the utility of this approach in the context of clinical endpoints. But just in terms of early development, one can attempt to do this without a clinical endpoint study. You can define the study. You can power your study and end up with a more rapid trial completion and obviously fewer subjects will be needed to

contribute to the OI challenge. That is fewer subjects are needed because all individuals in the study will be part of the -- will be candidates for the challenge.

So in order to take this any farther, I think we need to have systems for immunization and we must standardize them. We need consensus methods and reagents for immunization, whether these be complex antigens or mechanisms to deliver intercellular -- sequences for intercellular gene expression. We need to have vectors to test B cell, CD4+ T cell and CD8+ T cell responses. And of course we need consensus methods for measuring these responses to microbial challenge.

So finally, how do we validate the immunization responses as a predictor of OI protection? And I think there are a couple of things that we have to start. Once we have identified what sorts of standards and what sorts of assays we are going to use, we can perhaps look at some cross-sectional studies to see if there is a reasonable relationship between the ability to respond to immunization and the stage of disease. One can look at this in the context of response to antiviral therapies and as well ultimately perhaps in the context of response to immune-based

therapies. I will now turn this over to Dr. Masur. Thank you.

CHAIRMAN MASUR: Thanks very much, Mike. We are now going to go to the last of our presentations before lunch, which will be limitations and complexities of biomarkers, and we are delighted to have Tom Fleming from the University of Washington here.

DR. FLEMING: Thank you, Henry. Can you hear me? Jon had pointed out that there are several levels of types of measures of biologic activity, and specifically a key interest is looking at these measures of biologic activity as replacement endpoints, or I think he referred to it as tie-2. And what I would like to do in particular then over the next 20 minutes or so is discuss the limitations and complexities that we encounter in using these biologic markers as surrogate or replacement endpoints for true measures of clinical benefit.

So in essence just to quickly review. If we are looking at identifying endpoints in a pivotal study, there are a couple of major criteria that we would focus on. One is we want those measures to be sensitive to the effects of treatment. And just as a simple example, if we were

looking at an analgesic in a terminally ill patient, certainly survival is very relevant, but pain relief is going to be particularly sensitive.

Our interest in biologic markers is partly based on the fact that they certainly are anticipated to be sensitive to the intended mechanisms of the intervention. But it is also critically important that they be clinically relevant and the considerations of clinical relevance depends on whether we are looking at a Phase II screening evaluation or a Phase III definitive evaluation. Certainly in a screening evaluation, it is key to assess biologic activity. And as we have seen, measures of viral load or immune status are going to be particularly sensitive and allow us to establish plausibility that will be able to achieve clinical benefit.

If we have established that plausibility, typically then we want to move into a Phase III or definitive evaluation to define the role of the intervention in the clinical practice. And the measures really in particular of interest are clinical efficacy. And when I refer to that, I am thinking of measures that unequivocally reflect tangible benefit to patients. Duration of survival and overall quality of life measures, symptomatic

AIDS defining events, functional status. As we well know, the challenge is to be able to asses these clinical efficacy measures often takes large trials and long-term studies. So there is this great interest in looking at replacement endpoints. And frequently measures of biologic activity are of primary interest, partly because these measures can be assessed in a much shorter period of time, and generally they, by their selection, are measures that we understand are correlated with the clinical endpoints. So the typical approach, then, has been to surrogate endpoints to identify measures of biologic activity that are correlated with clinical endpoints, show the effects on these measures, and then hopefully be able to conclude that we achieved clinical efficacy benefit.

Well, the issue is given that our goal is to be able to ultimately understand the effects of the interventions on measures of clinical efficacy, showing effects on biologic markers certainly does establish biologic activity and the plausibility of achieving clinical benefit, but does not necessarily give us definitive evidence of that clinical benefit. And to give a few illustrations here of how this paradox in fact can specifically arise. A given disease process may

well causally induce an effect on a surrogate marker as well as on a true clinical endpoint. And yet if this surrogate does not lie in the pathophysiological pathway by which the disease process induces the clinical outcome, even though these two are correlated, having an effect on the surrogate does not necessarily reliably predict an effect on the clinical endpoint.

As an illustration of this, if we look at a setting where I have spent a lot of my own personal time in research, which is maternal to child transmission of HIV in developing countries, in this setting the disease is infection in the mother. The true clinical endpoint is transmission of the infection to the infant. A goal in this setting in developing countries in particular is to find interventions that can be delivered at the initiation of labor and delivery. We know in this setting that CD4 count is correlated with risk of transmission, but it is highly implausible that an immune-based therapy delivered at the initiation of labor and delivery that would affect CD4 count, for example, would have an impact on transmission of HIV.

A second major explanation of this disconnect between an effect on a marker and an

effect on a clinical endpoint is explained by the realization that a disease process can actually have several causal pathways through which the clinical endpoint is induced. And the surrogate may, in fact, lie in only one of these pathways. So if we, for example, continue to consider the setting of an HIV-infected woman but now look at transmission of -- heterosexual transmission of HIV as the clinical endpoint, if the surrogate endpoint is of plasma viral load and we look at an effective intervention on plasma viral load, that in fact may represent part of the overall risk, ultimately though it may be viral load in the vaginal mucosa, which is much more indicative of risk of heterosexual transmission. And if the intervention's effect is predominantly on plasma viral load, we may be significantly overestimating the effect of the intervention on risk of heterosexual transmission. Or conversely, if the intervention's effect is predominantly on vaginal mucosa viral load, we may be underestimating the effect by looking at viral load in the plasma.

And certainly if we were looking at immune-based therapies, these same issues arise. We have heard a lot of informative discussions today about the myriad of different immune-based

immunologic measures or effects that can be induced. So, for example, if we are looking at one pathway that is related to CD4-based effects and another on CD8 or cytologic T lymphocyte based effects, if we have targeted as our surrogate the specific pathway that actually is the lesser important in terms of the overall progression of HIV, then in this setting we would be overestimating the ultimate effect and in this setting we could be underestimating the ultimate effect.

And I think we often don't give proper attention to the fact that over-reliance on surrogate measures can actually lead to an underestimate or a missing of potentially effective interventions. And I think Jon referred to one example of an immune-based therapy in an immuno-compromised patient population where reliance on a surrogate led to an underestimate. I will just briefly elude to that again. It is the setting of chronic granulomatous disease, which is a setting in which microorganisms -- basically the intervention is gamma-interferon, and it was of interest in this setting because microorganisms engulf the overall infectious cells but are ineffective through a lack of a generation of an

oxygen burst to kill those and they ultimately lead to a risk of recurrent serious infections. Gamma was of interest because of its ability to increase bacterial killing and super-oxide production, and there was an interest that in designing a short-term trial to show that gamma-interferon was effective in generating this specific intended immune response. Ultimately, though, because of a fear that this could lead to an overestimate of the treatment effect, there was a longer term clinical trial conducted, and that trial, in fact, did show a striking effect on the clinical endpoint of recurrent serious infections. Interestingly, with this larger amount of data, it was of interest that when we looked at whether or not gamma actually had the intended effect on bacterial killing and super-oxide production, there was essentially no effect on these biologic markers. And so an immune-based therapy that in fact did have the intended clinical effect would have had that effect underestimated because of the lack of proper targeting of what the actual mechanism of action was.

In addition, even if in fact an intervention has the intended effects on the multiple causal pathways of the disease process, one other explanation for a potentially misleading

result is that the intervention itself may in fact have unintended effects that also influence the clinical outcome. And so it may be with immune-based therapies that we are, in fact, able to induce the effects that are intended on surrogates on CD4, CD8 or other immune-based measures. But it may be that the intervention has unintended effects on viral bursts, long-term viral load, or other specific processes that influence outcome or in fact have other toxic effects. And there are a myriad of examples in the literature to show that even though you achieve the intended effect on the marker, the ultimate effect on the clinical endpoint may be very different. We heard of one of the classic examples being with ecanide/fleconide in suppression of erythema.

This is a setting that in a sense ought to represent the ideal. This is a setting in which the surrogate marker lies in the pathophysiological pathway by which the disease process influences the clinical endpoint and the intervention's effect is solely on the intended pathway. But even in this setting, the surrogate may over or under-represent the true effect. If we are looking at, for example -- in early infection if we look at measures of CD4, it could be that the variability or noise in

those measures of CD4 lead to an underestimate of the actual effect in the clinical endpoint. Conversely, if we are looking in early infection at early measures of viral load, it may be that those early measures of viral load do not give us a reliable prediction of what the long-term clinical effect is on ultimately delaying progression to symptomatic AIDS-defining events or death.

And even in fact when we are looking at a clinical endpoint, short-term clinical endpoint in a long-term chronic risk setting, it may be that that short-term effect does not reliably predict the overall clinical profile. And if we go back about a decade or so and take a look at the experience from monotherapy with AZT, the HIV trial collaborative group in 1999 in Lancet presented this meta-analysis overview that reflected the fact that monotherapy AZT does in fact provide a very substantial immediate effect. But when one looks over the longer term of risk, the profile is very different.

At ICAAC about four weeks ago in Toronto, Jim Neaton's conclusions from these types of observations were that there is a great need for large randomized trials with long-term follow-up. And long-term follow-up in particular because

short-term trials cannot address the longer term risks and benefits. And of large size because smaller studies are challenged in being able to reliably assess the treatment effects on clinical outcomes.

So, for an example -- and this reflects the SILCAAT and ESPRIT type trial designs -- if one is looking at an immune-based therapy added to antiretroviral therapy and one is looking at clinical endpoints, progression to AIDS and death, if a study were of five years duration, the types of size that we are looking at depends on the disease setting. In an earlier stage disease setting, we might be looking at a study of 4,000 to 8,000. In a more advanced disease setting, two to four-fold reduced sample sizes. But substantial sample sizes with follow-up over a fairly lengthy period of time.

I would like to take a few minutes to talk about the issue of how does one go about validating a surrogate endpoint given these challenges that are apparent with using replacement endpoints. And has been referred to earlier, some famous conditions that have been put forward as sufficient conditions for validating a surrogate are two-fold.

The first is that the surrogate endpoint must be correlated with the clinical outcome. And it is an important issue here to pause and recollect that this is often the criterion that people think of as the sufficient criterion. This is all one has to show. But in fact in Jon's presentation earlier, this is really in essence just establishing the marker as a Type 0 or at best Type 1 marker, and ultimately we want a Type 2 marker, one that allows us to say that we can reliably replace the clinical endpoint with the marker when one is looking at establishing definitive evidence of benefit. So the second and much more difficult condition to establish is that the surrogate endpoint must fully capture the net effect of the treatment on the clinical outcome.

And in essence, the way this has often been addressed using data from trials that provide evidence on the clinical endpoint and on the surrogate can be represented in this slide. And just to quickly talk you through this. If one is looking -- if Z represents therapy and if one is talking about an immune-based therapy, code that as 0, and this would be the control, which would be antiretroviral therapy, and one is looking at modeling the relationship of that immune-based

therapy with the risk of the clinical endpoint, let's say progression to AIDS or death, then lambda naught of T just represents the failure rate on the immune-based therapy. And so E to the alpha times that would be what it is on the control. So if you were doubling the failure rate on the control versus the immune-based therapy, then alpha would be -- E to the alpha would be 2.

Now the key issue is suppose we want to assess whether or not a surrogate such as CD4 over time is a valid surrogate. In essence then we would model not only the effective treatment but also the surrogate on the risk of the clinical endpoint.

And if in fact the surrogate, CD4 over time, is fully capturing the effect of the intervention on the risk of the clinical endpoint, which is Prentice's second criterion, then mathematically this term beta should be relatively close to zero. So the estimation of the proportion of the treatment effect explained by the surrogate is just one minus beta over alpha. This is an approach that has been frequently used to establish whether or not we are fully capturing the net effect. There is a statistical -- immediate statistical problem that arises here, and that is

estimating beta over alpha takes much more data than estimating alpha alone. The practical consequence of that is to validate a surrogate takes much more evidence and much more data than it takes to simply directly show what the effect of the intervention is on the clinical endpoint. And actually as much of a sobering issue as that is, the issue is even much more complex than what this simple transparency shows. For example, suppose in truth the effect of an intervention on the immune system would lead to a four-fold improvement in time to the event?

If the marker that we are using, CD4, would predict only a two-fold increase and what we observe in the data is a two-fold increase, we are inclined to say, aha, we have got a marker that is fully explaining the treatment effect on the immune system and there are no unintended effects. Well, what may be happening is that may be wrong on both accounts. It may be that the intervention is influencing the immune system in a way to generate this four-fold effect, but unintended effects are nullifying some of that benefit giving you a net two-fold effect, which is what the data are showing.

So the challenge is we are looking at

data that is not nearly as multi-dimensional as what in reality is happening with an intervention that is affecting multiple pathways that are intended as well as unintended pathways.

So from a statistical perspective, we would say to begin to have statistical evidence to validate a surrogate, we need to have a myriad of studies that look at the effect on the potential markers that could be surrogates as well as on the clinical endpoints in order to be able to have the level of statistical evidence.

But that in itself is not enough, i.e., it is not going to ultimately be or it is not ultimately a statistical solution at all when one is looking at validating markers. The issue is very much clinical in the sense that to be able to truly validate a marker, one has to have a comprehensive understanding of the causal pathways of the disease process. So if we are in fact looking at a specific potential biologic marker, we have to have a clear understanding of the relationship of that specific marker to the overall HIV disease pathophysiology. And furthermore, it is critical to understand the intervention's intended and unintended mechanisms of action. These are both extraordinary requirements, and it is a continuum, obviously, in

achieving this level of understanding. But these are certainly extraordinary requirements that ultimately indicate why being able to fully validate surrogates is such an enormous challenge.

Let me just mention one other specific limitation we have to realize, and this has been alluded to two or three times already today. And that is the issue of bridging. If we go back approximately a decade again, at that point in time there was great interest with nucleoside analogues in looking at whether CD4 could be a valid surrogate for HIV AIDS death. And ultimately to address this, one had to establish that the relationship of the effects of antiretroviral therapy on CD4 was reliably predicting the effects on clinical endpoints. And of course as I have mentioned, by the time you have achieved that validation, you already know the effect of the nucleoside analogues on the clinical endpoints. But the thought is if you have now validated these surrogates, you can now use these for future interventions.

And in fact the FDA was being asked whether or not, if this validation could occur, could these immune measures be used for immune-based -- for approvals of immune-based therapy.

And back, in fact, in I think it was November 16, 1991, the FDA Vaccine and Related Biological Products Advisory Committee addressed this issue and specifically said that even if for a given class of treatments such as nucleoside analogues CD4 levels could be validated as a surrogate marker for AIDS and death, that it may not necessarily be reliable as a surrogate marker for a new class of immune-based interventions if this interventions and the nucleoside analogues had differing mechanisms of action.

So in conclusion, this slide says, "What is the use of surrogate markers?" And actually probably the title of this would better be, "What is the appropriate use of measures of biologic activity?"

And in fact, as we have heard discussed today, the usefulness is of critical importance in drug development. Most specifically, screening trials provide a critical step in establishing risk and benefit.

It is not practical to assume that we could, for every potential intervention do a large scale clinical trial that would require the amount of resources that would be required for a Phase III study. So these screening trials with surrogate

markers or biologic measures of activity as the primary endpoint provide us an efficient and effective way and a sensitive way to establish plausibility of efficacy.

In the definitive trials then, these markers also provide important supportive data on the mechanism of action. The critical and obviously controversial issue, though, is can they be used as replacement endpoints. And the issues that we have discussed elucidate the major challenges that we must face before these markers can be reliably used as replacement endpoints in clinical endpoint studies.

CHAIRMAN MASUR: Tom, thanks very much for those provocative comments. (Off the microphone.)

(Whereupon, at 12:00 noon, the meeting was adjourned for lunch to reconvene this same day at 1:08 p.m.)

A-F-T-E-R-N-O-O-N S-E-S-S-I-O-N

1:08 p.m.

CHAIRMAN MASUR: All right. I think we are ready to start the afternoon session. I appreciate the committee coming back in a timely way. This is the open public hearing, and we appreciate the fact that there are six individuals who have asked to address the open public hearing. We would like each person to confine his or her comments to no more than 7 to 10 minutes. The first presentation will be by Vernon Maino, the Scientific Director for BD Biosciences.

DR. MAINO: Thank you for allowing me to come say a few words about your technology. I am Skip Maino from BD Biosciences. And I am going to talk about this flow-based assays for measuring T cells responses which Alan Landay actually alluded to this morning and talked about. I am not going to really talk a whole lot about the technology except about some of the features that we have been working on to help validate and establish this as a reproducible, clinically viable assay.

So there are a number of markers that can be used in these types of assays, including antigen-specific -- it doesn't have to be HIV-specific. Basically any antigen can be used in

these kinds of assays to measure CD4 and CD8 T cell cytokine responses to specific antigens. Or we can measure proliferative responses by measuring incorporation of BRDU. So flow-based assays to measure proliferative responses. And then finally APC functional responses, based on the detection of intracellular cytokine expression and using a multi-parametric flow-based analysis.

The rationale for validating these kinds of assays has to do with, again, a couple of points that were raised by a number of speakers this morning, Jon Kagan especially. And the first rationale is for measuring the expected immune activity of the therapeutic intervention. For example, vaccines -- measuring a response of T cell cellular response to that vaccine is an expected response to that vaccine and can be measured with these kinds of assays.

The second measurement is a lot more difficult and a lot more difficult to establish, that is that the assay itself is related to the efficacy of the treatment. And this has to do with associating with clinical endpoints, and again speakers have talked about why this is a complex problem.

Just to show you a summary of some

clinical data from a collaboration we have with Immune Response Corporation measuring responses to -- CD4 T cell responses to introduction of three vaccines at 12 weeks, 24 and 28 weeks. And most of these patients had been on HAART therapy for longer than six months. So consistent with earlier observations that we published with Louis Picker that long-term HAART patients make very little response. Just showing you that in the setting of HAART treatment, you can observe -- and this using these kinds of assays -- significant responses in most of these individual patients that we have assessed. There were 18 patients analyzed in all.

This is the basic assay, and I am not going to go into a lot of detail. I just want to show you that we are working on some improvements that allow you to -- that are working toward automation of this assay to allow handling of clinical samples, even if they come in as late as 5:00. We now have the procedure down to where you can leave at 5:10 and get the assay done by using these automated cooling procedures.

The assay can actually be broken into two parts, so that you can add the whole blood to the tube. And now we have stabilized antigen in the Brefeldin A preparations that can be a unit dose,

so the tube themselves can come with the Brefeldin A and the antigen. It is simply adding whole blood for six hours. At the end of that period, you add a lysing solution, and that whole mixture, that fixed cell suspension, can be put into a freezer at minus 80 degrees and stored indefinitely for later batch analysis. So we are really thinking about now the sample handling problems that are associated with doing large clinical trials, and we have, of course, put this assay in multiple sites now to evaluate reproducibility.

Just the basic output of this assay is cytokine positive CD69+ cells and gated on T cells. And we now have automated kinds of analysis algorithms that are going to be able to handle that analysis a lot more reproducibly as well.

Some of the newer kinds of antigen preparations we are working on to standardize the antigen preparation really comes out of the work that Louis and Florian Kern have been working on that use an algorithm where they make multiple peptides spanning the entire length of a protein, and these are 15 amino acid peptides overlapping by 11 amino acids. So, for example, for CMV pp65, that would be 138 peptides. For HIV p55, that is 120 peptides. It turns out that you can put up to

300,000 peptides in a single -- if you could get that many in your mix, without affecting adversely the specific response to a single peptide.

So here is an example in two HIV individuals, seropositive individuals, that we're measuring response now to mixtures of peptides derived from envelope, gag, pol and nef. These are random selected. So one of the advantages of using these mixtures of peptides is that you don't have to worry about the HLA backgrounds and let the biology sort the appropriate presentation out.

So this is a CD8 T cell response to these subunit mixtures of proteins. Now these are optimized peptides that are 9 amino acids, but you get the idea that you can measure dominant responses in different subunits and that these can be different for different individuals. This is a seronegative individual.

One of the advantages of the assay is that in most cases, constituative background for the cytokine expression is very low, close to zero.

And then finally, the other advantage here is that you can measure both CD4+ and CD4- cell positive responses. This is an example of a CMV peptide mixture looking at a normal individual. Comparing the peptide mixture to the CD4 response

to a CD4 response with a whole protein. And here, you can't see the numbers, but these numbers are -- one is .52, and this is .57 percent. We see this consistently with both HIV and CMV peptide mixtures that to the CD4 response is very close to what you see with whole protein. But in this case with the peptide mixtures, you now see a CD8 response. And the ratio of these responses can vary depending on the antigen and depending on the individual that you are looking at.

So where we are headed is to develop unit dose preparations of antigen plus Brefeldin-A to allow the sample handling. We can use -- with this mixture, the other advantage of using this is that we can use either archive frozen PBMc's or whole blood and we get exactly the same kinds of answers. And the other advantage is that you can use even older blood. The peptide mixtures help you using much older blood. Because the cell that takes a beating during the sample handling is the antigen presenting cell, not the T cell as it turns out.

And we are now working on a sample prep device that will allow you to automate perm, stain and wash. And then with the software and loading kind of capability that we have with flow cytometry, we will have walk-away loading,

standardized instrument set-up, analysis and data base compatibility. This goal here is for handling large numbers of samples. Today we can handle certainly smaller trials and experimental kinds of settings. We can certainly handle enough samples for that sort of activity.

So our validation strategy is to address the critical elements of the assay, which I talked to you a little bit about, address multiple levels of user experience for training and that sort of thing, validation across multiple laboratories, standardized protocols, compare this to established acceptance criteria, and then certification of completion. These are the kinds of validation concerns we have when we work with external investigators.

So we standardized both the activation, which includes the sample handling and the antigen stimulation, the acquisition and the instrument, and the analysis in terms of identifying positive populations for final data output.

So just to make a few conclusions here, we think these flow-based immune function assays can be accommodated in the clinical laboratory setting. And certainly with automation, this will become even more apparent. Validation procedures

have been developed which standardize sample handling, biological response, instrument set-up and analysis. And automation features are planned for handling large numbers of samples. We also have within BD Biosciences another group which can actually perform these assays as a service laboratory too that provide a standardized, validated approach for measuring specific T cell responses. So I think I am going to stop -- trying to stick to my 7 minutes.

CHAIRMAN MASUR: Okay. Thanks very much, Dr. Maino, for sticking to your time and thank you for your comments. The next comments will be by David Scondras, the founder of Search for a Cure.

DR. SCONDRAS: Thanks. I'm not going to be using slides. My original 15-page detailed presentation I am willing to type up and circulate to teach of you. I changed my mind halfway through this about making the original presentation because you have an extraordinary set of people in Fred Valentine and Bob Redfield and the people you invited to speak. I see Mike Saag in the audience and I see Ron Mitsayasu here. I see Fred Valentine and around the counter Brenda Lein is here, et cetera. What I am trying to say is I think you have

all of the technical expertise that is needed to talk endlessly about the development of surrogate markers.

I think what I am feeling is a sense of a lack of urgency. What I would like to convey to you is a message from the community itself rather than a scientific message. About half the folks who are taking antiretrovirals -- and that is not by any stretch of the imagination most of the people who need them -- are not doing very well on them, in fact are failing them. The other half live in a state, very often, of anxiety that at any time there may be viral breakthrough, and a constant sense of a compromise in quality of life in hundreds of different ways that are very hard to articulate to people who don't have to adhere to a regimen that is truly not designed for human beings.

It is very difficult to share with you what it is like to attend fewer funerals, but still some, and to watch friends developing lymphodystrophy syndrome, et cetera. Let me give you a sense of that frustration in context. At ICAAC a little earlier this year, one third of the clinicians at a fairly large meeting indicated that they had already gone along with a treatment

interruption for their patients.

Not because they thought it was the best medicine, but because it was necessary for the people involved. At least half of my friends have just quit. They are no longer taking their medicines. So what we really have in the real world is five percent of the world's population having access to a set of drugs, half of whom gain benefit and the other half don't like them, don't want them, can't put up with them anymore.

So I want to convey to you a sense of frustration. We have been talking about the development of immune-based therapies for at least a decade, and I don't get the impression that as much progress has been made as we really ought to have arrived at by now. Is it a money problem? Is it an organizational problem? Can somebody please clarify those things so that people like me can fix whatever it is that is broken? And don't tell me it is just a scientific problem.

I sincerely hope the message being given to the FDA and to the members of industry who are here today is not that they should not develop agents until and unless we have validated surrogate markers for clinical progression and mortality. That is not in fact what we did with the antivirals

and it should not be what we do with the immune-based therapy. So it ought to be concomitant. There is some synchronicity here. There can be concomitant development.

What are they? We need to focus on specifically what are the best guesses you have. Why am I saying that? Because we can't wait anymore. Let me give you a very specific positive spin on that fact. We have a set of trials going on, IL-2, five to seven years before we know. Why is that? Because we won't use it on people who won't take or don't take antiretrovirals.

Why is that? We have a 5057 trial that was terminated because there were inadequate resources to put enough people in it to satisfy everybody that it was properly powered. Is that really where we are at that we can't come to some resolution to determine whether or not an important immunogen may or may not be useful? We have a variety of small studies going on, for example, enhancing intracellular glutathione, which really don't have enough money poured into them. We have a set of companies not willing to engage in anything but refining existing antivirals simply because there are no indications from the FDA that something else might be important. I would suggest

to you we need a call to action, a sense of urgency communicated to the FDA that there be a broader set of markers that we look at in order to develop agents.

I am not going to pretend that I know the answer to the question, but I want to make a suggestion. Literally thousands of people are stopping therapy. There is a wealth of information to be gathered about viral rebound after therapy is stopped. There is a tool sitting there that most of us in the community are more than happy to participate in using to determine the validity of a variety of markers.

It makes a hell of a lot more sense for folks who want to stop taking their drugs to be enrolled in trials that may determine whether or not surrogate markers are meaningful and which are and also simultaneously determine which therapies might be useful. It really is a time to issue a call to action and to tell the community, look, rather than having thousands of you just stop taking drugs, we have a program. We are going to set national clinical trials up of a variety of agents which you can participate in.

At the very worst, we will gain a very good understanding of rebound in viral load and a

new tool for evaluating agents. That is the worst that could happen. At the best, we will have discovered a set of agents that may allow for a longer period without these drugs than any other.

Perhaps the best way of summarizing where the community is at is to note the fact that that shy individual, Larry Kramer, has started a new organization called Wake Up to target drug companies for issuing poison and for not dealing with the side effects that those drugs are causing. I would summarize everything I have to say in a paraphrase from T.S. Eliot. "Hurry up, please. There is no time." Thank you for taking the time.

CHAIRMAN MASUR: Thanks very much for your comments about the sense of urgency. The next presentation is by Julianna Lisziewicz from the Research Institute of Genetic and Human Therapy at Georgetown.

DR. LISZIEWICZ: Thank you very much. We are working the last five years on the immune-based therapy and actually we started to wonder about surrogate markers five years ago when our first patients stopped therapy. It was a very fortunate case. It was the famous Berlin patient who didn't rebound after stopping therapy and now he is three years. We tested him with several

different -- he was the first one who we tested several different ways and we didn't -- we was very frustrated because we didn't find a clinically relevant assay which would predict the outcome of the stopping therapy.

So, therefore, we spent a lot of time to try to develop this assay, and this is a very simple assay. It can be done basically on the same tube that the CD4 assay can be done. And what I would like to do today, I would like to just explain you the assay and give you three examples. Three examples which shows some correlation of viral control -- immune-control for HIV.

This is the assay. This is basically when we thought about the assay, we thought, okay, what do we want to measure. And what we really want to measure is what happens if the virus rebounds or tries to rebound in the patient. So we just decided to mimic exactly that in vitro. So we take the PBMCs which we isolate from peripheral blood and mix it up with the virus.

We started with replication competent virus, but now we can use replication defective virus just for safety reason in the laboratory. What we expect to happen with this virus, it goes to the antigen presenting cells and be presented to

T cells, both CD8 and CD4 T cells, and we are measuring the early signal, which is interferon gamma production, which basically measures the HIV-specific cells.

Now this assay is very relevant in our view for predict HIV rebound, because it is not only depending on the T cells, but also depending on the antigen-presenting cells which the patient has in his peripheral blood. So if you have an APC problem, this assay will not work either.

So first I just want to show this example. When you put in the normal -- check this assay in the normal donors, you do not see interferon gamma production. And if you put an unrelated antigen into the PBMCs of untreated patients who is able to control HIV, you again do not see interferon gamma production. And when you use HIV antigen, you see a very substantial, nice interferon gamma producing T cells.

Because time is short, I will concentrate in this talk on the CD8 specific interferon gamma producing cells, and I will refer that as CD8VIR. So the first example was a study which we have done in rhesus macaque. Basically we compared STI-HAART versus HAART in untreated monkeys. So we had six acutely infected monkeys

with no therapy, HAART therapy at fixed schedule, STI-HAART. We used three weeks on and three weeks off for 21 weeks. After 21 weeks of this treatment, we measured the CD8VIR activity in these monkeys. And you can see here the HAART treated monkeys had very little CD8VIR activity.

In contrast, the STI-HAART treated monkey has very high CD8VIR activity. And this is just summarizing our finding that during the treatment of STI-HAART shown here in yellow, the CD8VIR activity increased. However, the untreated monkeys or the HAART treated monkeys did not have CD8VIR activity.

So what happened when we permanently interrupted the treatment here? This is the viral load gene. Of course, the HAART treated monkeys all rebounded, shown in pink, according to the low VIR, and this correlated with the low VIR response. And none of the STI-HAART treated monkeys rebounded. So basically what we saw was a very strong, statistically significant correlation between the amount of CD8VIR and the rebound after permanent treatment interruption.

So this was the monkey trial, which was a randomized controlled monkey trial. But still, does it have any relevance in patients? So I would

like to introduce a patient which we are following up here in Washington who also got the STI-HAART treatment. The protocol here was different than in the monkeys. It was not a fixed schedule STI-HAART. It was a protocol when we reintroduced treatment when the patient was two times above 5000 copy per ml of viral load. And you can see here with pink that this is the time when the patient didn't get treated. Without treatment time, always increased after treatment interruptions. So we wondered whether the CD8VIR correlates with that. Because as you see, neither the CD4 count nor the CD8 count correlated with this increase of time to rebound.

What we see here is as is expected. At the first treatment interruption, we see CD8VIR activity, which represents HIV-specific CD8+ cells. This is 2 percent. Because this patient was an acutely infected individual. But with consecutive treatment interruption, CD8VIR increased with consecutive treatment interruption up to about 6 percent. And this CD8VIR activity that we measure with this assay basically correlates with a very vigorous CTL and perforin production in the CD8+ cells.

And what was most interesting to see is that when we correlated the days to rebound in this

patient with the CD8VIR, we could see a linear correlation. Meaning that in this patient at least, if the CD8VIR activity was low, the days to rebound was short, meaning 20 days. And when we get a higher CD8VIR activity, the days to rebound increased, suggesting that the quantity of CD8VIR correlates with the duration of immune control in patients.

So what happened in these two previous examples was an example for acute infection. The question is what happens in the chronic infection. And we have a nice model for chronic infection. We are following 12 patients since three and a half years treated with DDINT droxuria. And the reason we call this PANDA is because the patients who are treated only with two antiretroviral therapies are an endangered species despite the good results here.

I mean, nobody wants -- we cannot run clinical trials unfortunately to confirm this data because the two drugs, especially with droxuria, which is not an approved drug, would not be allowed to use. So anyway, this patient started at the baseline of around 5,000 copies and then when we look at them at week 7, all of those patients were around 2,000 copies. Here was the surprise. When

we looked at them after 122 weeks, all of these patients viral load decreased. So this was -- this is a simple double combination, and instead of seeing viral rebound, we saw all of these patients viral decreased. So our hypothesis was that maybe HIV-specific immune response acts here as an additional drug.

So we measured in this cohort CD8VIR responses, and we compared with -- we matched with 10 patients who were treated with HAART. And as is expected, after long-term HAART treatment, almost two years of HAART treatment, we see no CD8VIR response. In contrast, in the PANDA patients, we saw pretty significant amount here at this example, 1.2 percent CD8VIR response. So the question was what does it mean? Whether there is any clinical correlate. So we decided to stop these patients. So we get permission to stop these patients for 8 weeks and then restart therapy.

So what happened? As is expected, the HAART patient rebounded. However, in contrast to the HAART patients, the PANDA patients controlled HIV replication. And when we looked at the CD8VIR responses in this population, we could see that the PANDA patient had statistically significantly higher CD8VIR response compared to the HAART,

suggesting again as a third example that the quantity of CD8VIR is important for control of HIV after treatment interruption.

I would just like to sum up. We are developing this assay as a candidate predictive assay for immune-based therapies. And what we have is a quantitative assay which can determine both absolute number and the percentage of functional HIV-specific CD8+ T cells and the quantity of CD8VIR correlated with the immune control of HIV.

What are the advantages of this assay? First of all, this is so far -- I think, in my knowledge, this is the first assay which really correlated with immune control. But this is a very simple assay. It doesn't require B cell line, for example, as compared to the functional CTL assays.

his assay can analyze subtypes of these cells versus ELISPOT, which just analyzes one kind of T cells. That it is not dependent on any HLA or peptides compared to tetrameres and really requires a small amount of blood, which can be shipped overnight and analyzed the next day.

Now just to sum up. So we have a quantitative assay. We saw some correlation. My question is how can we develop -- from you, how can we develop this? Whether it is worth it to develop

it as a surrogate marker for immune-based therapy.

CHAIRMAN MASUR: Thanks very much for those comments. We are going to move on now to the next comments. They will be by Judith Britz, Ph.D., the President and CEO of the CYLEX Corporation.

DR. BRITZ: Good afternoon. Thank you for the opportunity to address this committee on such an important topic. CYLEX is a diagnostic company located in Columbia, Maryland, and we are developing diagnostic tests for measuring immunity.

Now in the next few minutes, what I would like to do is to start by referencing some very important work done by others that relates to testing functional immunity in AIDS patients. And then I would like to tell you about a test system that we have developed in collaboration with colleagues in the AIDS research community and share some of the early results.

What you are looking at right now is in 1998, Perrin and Telenti reported on the results of a cohort of HIV-infected patients that were receiving HAART therapy. Now monitoring viral load and CD4 levels, what they found is that 45 percent of the patients had the profile that you see under A. And what you can see -- the legend is probably hard to read, but this is the idealized profile of

CD4's increasing with viral load decreasing. As I mentioned, that was in only 45 percent of the patients. So the majority of patients actually had profiles that were one of these other three types. And despite the fact that many times these patients were in fact responding well to their therapy and despite what I would call discordant laboratory results monitoring CD4 and viral load.

These results are really not surprising. Because if we go back to the late 1980's, immune function was really lost in monitoring the pathogenesis of AIDS long before the decline in CD4 count. And in fact Clerici and Shear showed that up to a year before the decline in CD4 count, you could detect cellular immunity loss, and this included tests that you might consider as crude as monitoring phytohemagglutinin-induced responses measured by lymphoproliferation.

They also made the important observation that there was a progressive loss of function, first of all to HIV-specific recall antigens than general recall antigens, allo, and then mitogen was the most robust. Alan referred to these data earlier.

Now more recently, of course, Eric Rosenberg and Bruce Walker have reported that

reactivity in an LPA assay is detectable in long-term non-progressors. But in addition to that, if you look at HIV-infected individuals prior to their seroconversion and you initiate HAART therapy, you find that there is a preservation of this P24 response in an LPA assay. In addition, after going through structured treatment interruption, there actually is a strengthening of that P24-specific response. Interestingly, in patients treated with HAART therapy where the viral loads declined to undetectable, the CTL response does appear to be dependent upon a certain amount of residual virus in order to remain stimulated.

While LPA has been certainly a very useful research tool for these studies, we know that they are very time consuming. They use radioactive materials. And because they are so labor intensive, these tests are not generally available. And in September's issue of Clinical and Diagnostic Laboratory Immunology, Betensky, et al., also reported that there were shipping impacts on microbial responses in LPA. Indicating that it is kind of hard to get the sample to the lab, that the lab has to be there with the sample.

At CYLEX, we have designed a platform technology which is really based on the principle

that lymphocytes that recognize either foreign antigen or mitogen will be induced to increase their intracellular levels of ATP. And our objectives in the development of this test really were, first of all, to develop a clinical correlate of cell mediated immunity so that we would not have to skin test individuals and then bring them back a day or so later for a reading. But also that the test ideally would be performed in 24 hours or less, non-radioactively, and on whole blood.

The adaptability of the test in multiple antigen testing in a 96 well format was also desirable because I think one of the conclusions from this type of work is that there is unlikely to be a single test which will emerge as being the surrogate marker. It is going to require an algorithm.

The specifics of this test are that we start out with a sample of whole blood, stimulate the lymphocytes in the presence of CD4 or CD8. By using whole blood, we keep antigen presenting cells as well as the serum from the patient in the environment. The incubation is four hours to overnight, depending on the strength of the antigen. The test also can be made subset specific, either for CD4 or for CD8 cells, which are

magnetically separated. These are then washed and lysed to reduce their intercellular ATP, and then detected in a luminometer. They can be quantified by using ATP calibrators so that the result is actually expressed in terms of nanograms per ml of ATP.

When we compared this assay with lymphoproliferative response to mitogens or recall antigens, we got comparable dose response characteristics with the ATP assay showing slightly more sensitivity in the level of antigen used to stimulate, but also keeping in mind that the ATP test is performed in an overnight incubation compared with an LPA assay of some 96 hours.

More importantly -- well, these data, by the way, were recently published in Clinical and Diagnostic Laboratory Immunology, the March issue. More importantly, we were able to also show, as Clerici and Shear had done, that like LPA, the PHA induced response to ATP in normal individuals versus HIV-infected individuals was dramatically reduced.

In collaboration with Brett Loechelt and Maria Chan at the George Washington University School of Medicine in Washington, we also did a study in pediatric AIDS patients in monitoring

their response to HAART therapy using viral load and CD4 as traditional markers. And what you are looking at in the green is an ATP response. The two examples I am going to give you from this longitudinal study which monitored patients over six months include this first patient that was not healthy at the beginning of this study and a therapy change was recommended, which you can see indicated by the arrow. The blue and the black line indicate viral load and CD4, which did not change throughout the course of the patient's treatment. But upon a therapy change and an improvement in her clinical course, you can see that her PHA response as measured by this assay increased.

I think more importantly when we looked at a noncompliant patient, again CD4 and viral load relatively unchanged throughout a six-month period of time, but this patient who was healthy at the start of the study and quite clinically compromised at the end of the study showed again a dramatic decline in the PHA induced response by which we measured ATP.

Now I think although PHA is a marker which AIDS patients are still capable of responding to, very often specific antigens, recall antigens and P24 in particular, there is no responsiveness.

But in this particular study, Susanna Cunningham-Rundles at Cornell looked at pediatric AIDS patients on HAART therapy, and again she had four of the children that were responsive to the therapy also showed a corresponding P24 response in the ATP assay, whereas the children that were classified as non-responders did not. So here is an example of specific immune reconstitution.

And then in actively immunized patients, Britt Wharen's group at the Karolinska Institute showed that in HIV-infected patients could mount a GP160 response, which is seen in purple. This is for three different patients. And that this also could be used as a way to look at specific immune reconstitution in response to vaccination.

We believe that it is really unlikely that the functionality of the immune system will be able to be defined, as I mentioned before, by a single test. It is more likely that an algorithm is going to be useful in looking at responses to I would say non-specific immunity mitogens, allo antigens, recall antigens and HIV-specific antigens, and that these could be used as a monitor, both in the pathogenesis of disease, but also in guiding the patient's clinical course.

This particular assay describes a way to interrogate one aspect of the immune system, which is the activation stage here, which is really a requirement for cytokine production or lymphoproliferation. Yet, for any of these types of assays, we recognize that it is important to validate them in the clinical context.

I think that we recognize that any of these types of immune function tests will need to meet certain analytical parameters, and yet at the same time we are challenged by how do we define accuracy in an immune surrogate marker or sensitivity. What is the measure if there is no other corresponding test? Despite these challenges and concurrent with the fact that there are new drugs under development to modulate the immune system, we believe that there is a need for tools for the measurement of immunity.

We would like to take advantage of the fact that the immune system can anticipate clinical changes before the onset of symptoms. And by monitoring functional immunity along with viral load and CD4, we can improve the management of disease.

I'd like to acknowledge our collaborators in this work, including the

scientists at CYLEX, particularly Dr. Rich Kowalski. Thank you for your attention.

CHAIRMAN MASUR: Thank you very much, Dr. Britz, for those comments. The next discussion will be by Thomas Kwyer, M.D., President and CEO of AmmunoMed.

DR. KWYER: Thank you very much for the opportunity to address the committee. While we are getting the first slide, I think it would be helpful to give you an idea as to where we come from by sharing with you our concept that we have at AmmunoMed, and that is that AmmunoMed is focused on the evaluation of metabolic mechanisms in order to discover natural solutions to healthcare challenges. We use an evidence-based decision making process to pursue our goal of developing information into education and discovery.

In this regard, we look specifically at glutathione for this talk. Glutathione is a small tripeptide. A few of the speakers have talked about the concept of tripeptides and signaling. We will explore that because it does fulfill our interest in metabolic mechanisms.

As you can see if you can read that slide, which might do better if we make it a little bit darker in here, there are a number of places in

the metabolic machinery that glutathione shows up. The immune system is just one.

The reason we embarked on this is that there is strong evidence and has been for over ten years that immunonutrition actually does have a significant impact on the outcomes in terms of clinical patients in relationship to immunity.

This meta-analysis of 1,500 patients came up with very significant statistical benefits in terms of infection with a significant reduction in the relative risk of acquired infection, ventilator days, hospital length of stays, and also key is that there was no increase in side effects of feeding that was reported during the studies.

I want to look at just one of the studies of the twelve that were included in the meta-analysis. This is a study on severe abdominal trauma. As you can see, therapeutic antibiotic days, ventilator days and ICU days are all very significantly affected in terms of an immune-enhancing diet compared to an isocaloric diet compared to a control. This study is very well standardized.

Everything that I will say to you is very statistically relevant and the way the groups were chosen is that there are surgeons who choose

not to feed trauma patients. That constituted the control. And then the two arms were simply generated by those who were involved with early elemental feeding, one choosing isocaloric diet -- one being randomized isocaloric diet and the other patients being randomized to the immune-enhancing diet.

As you can see, this has cost relevance. This is the only non-statistical but heavy trend that you can recognize in this particular group. If you don't feed anyone after they have had severe abdominal trauma, they cost you about $140,000.00 to treat. If in fact you treat them early or you feed them early, you will at least cut that by $30,000.00 down to $110,000.00.

And if you use an immune-enhancing approach, you can get this down to $80,000.00. The variance took this out of actual statistical significance. But what is significant is on the next slide.

This is a slide of hospital day in this particular study. You can see that there are about 35 days for control, 32.5 days for the patients who are fed, and 18 days -- a two-week reduction in hospital length of stay. That is really where the

power of this particular method comes up. But the problem is this early generation of immune-enhancing materials are very diverse. They have a large number of constituent amino acids and other sorts of things that are thrown in, and it makes it very difficult for this to be studied. So this is the reason that we decided to abandon this group and in fact look at the immune system itself.

And what we found is that when we looked at the immune system, there actually was -- this is a busy slide, and don't try to worry about it too much, but just pick up the bolded things if you can. We are talking about TH1 pattern, TH2 pattern of cytokine expression. And at the top there it just basically says glutathione levels in antigen presenting cells modulate TH1 versus TH2 response patterns.

And basically what it comes down to is that in the antigen presenting cells, which include the macrophages, the dendritic cells and the B cells, these are all central to the development of either TH1 or TH2 immunity because the antigen presenting and presentation recognition are required to initiate the response. So we are going to start right at the very beginning. What we found is that glutathione depletion inhibits TH1

associated cytokine and favors TH2 cytokine. This is like a switch now. And of course we are evidence-based. So we are just simply pulling this from the existing literature. All of these slides, as you can see, are referenced and they are easy to find.

This is also busy but what is most important is on the bottom, and that is also crucial to the ability of the antigen processing cells to break up the antigens. And this really comes down to the fact that you have to take apart disulfide bonds. Step number one, you take the disulfide bond complex it with glutathione, and the disulfide ends up being one with the protein and the glutathione. Ultimately, that is split and then you end up having the free sulfahdyro. This allows the antigen to be processed. And what it really comes down to is that low intercellular glutathione levels in antigen presenting cells correlates with defective processing of antigen and that is because of this disulfide problem -- disulfide bond problem.

This is also -- we are just going to read the top and we can go on. Lymphocyte proliferation in glutathione depleted lymphocytes, a direct relationship between glutathione

availability and the proliferative response. And basically what it comes down to is the studies done by Hamilos indicate that these studies confirm the importance of intercellular glutathione in lymphocyte proliferation.

This is basically a summary. Glutathione levels in antigen presenting cells modulate TH1 versus TH2 response patterns. Antigen presentation and recognition are required in terms of initiating the immune response. And in terms of interferon gamma, it became clear that this was clearly related to this switching. That it was apparently controlled by glutathione. And on the bottom here, we talk about it being a key role, and it does have an impact on HIV, which of course is the focus of this discussion.

This is a paper that has been around for two and a half years. You may be familiar with it. But this characterizes the glutathione level as something that is studied and measured by fluorescence, and the Herzenberg's have done a study and it does show very specific levels of glutathione -- intercellular glutathione, that actually are predictors of survival.

Two year survival in all HIV positive patients who have a significant amount of

glutathione are approximately 90 percent. And if you don't have enough right at one level, you can drop to 32 percent. It is a very large difference. In fact, if you have the low CD4 cell candidates, you are talking about an 87 percent five-year survival if you have enough glutathione intercellularly, and if you don't a 17 percent -- a very wide swing. Again, it reflects a clinical response you would expect to something that might be considered to be one of probably many switches in the immune system.

This is a paper that really well defines the TH1/TH2 cytokine response, and it is going to be the basis of a quote that is going to come up on the next slide that is a summary.

We are just going to put back on the statistics that we got from the Herzenberg study. This is really a quote. It should have actual quotes around it, from Clerici. "Antiretroviral therapies will not successfully eradicate HIV, and HIV seropositive patients will not be ultimately cured unless therapies aimed at restoring the immune system are associated with the antiretroviral drugs currently employed."

So what I would like to present to you is -- this is a case study. This is a case study

and actually going now to test this out, but in the pulmonary realm -- chronic obstructive pulmonary disease. One patient, and basically what we are dealing with is a patient who did respond to steroids and ultimately continued to have reduced pulmonary function tests.

You can't see that at all. Well, there you go. Basically what that slide is supposed to show you is that at the sixth month, this patient walked into her physician's office and was taking a method of increasing intercellular glutathione with undenatured whey protein. The glutathione level increased by 94 percent and the FEV1 and FVC increased substantially, approximately 30-some percent if I can remember this level. The physician asked her to stop taking the material. She dropped precipitously and then reincreased when on the final month of this study she regained this. She increased her glutathione by almost a factor of two, 94 percent, in approximately one month. So we do know that we can actually modulate. We actually have a material that will be able to be modulating the glutathione levels.

Lots of stuff on here. But basically this is an indication of the undenatured whey protein concentrate. The most important thing from

my way of looking at it is that there are two things we want to get across. Number one is that it appears to be cystine. The disulfide bound two cystines, which of course is one of the two sulfur containing amino acids that makes the difference. And the nice thing about this approach is that this is actually something that is taken from nature. This is essentially extracting the proteins associated with increased immune response from milk.

Just the first few words. We are going to talk about hepatic nitrogen mechanisms, antigen presenting cells and astrocytes, all being directly related to the cystine, cysteine and glutathione.

I actually thought I had left this behind when I got out of my first year of medical school. Biochemistry was not my strong suit, and I thought it was just a flunk-out course that you had to take and then eventually they would let you take care of patients. I have come to realize that is just not the case and in fact while this is a busy slide, it is in fact the diagram of positive versus negative nitrogen balance. We will come back to that. But it is based on proton donation -- the first two words in the title -- proton donation. That is really what glutathione does.

This is a slide of proton donation. You can see that there are two hydrogens out there with no electrons and two little protons sitting out there. This is what is important to actually balance off equations. So we are talking about very, very, very basic principles.

In terms of the pathogenesis of cysteine

-- in terms of the pathogenesis of cysteine, we are talking about wasting, and wasting really has two points to be made in terms of cysteine. And this comes from a paper by Drobe which demonstrates that cystine, which is the two cysteines together -- cystine level is regulated primarily by the post-absorbed skeletal muscle, that which you have already built up, and it has then gone into protein catabolism. So the body is trying to gain cysteine from the muscles. Number two, the cysteine level itself is a physiological regulator of nitrogen balance and body cell mass.

What we are seeing is that in AIDS, as well as in sepsis, major injury, cancer and chronic fatigue and a number of different conditions that are associated with wasting -- a number of features that are essentially consistent through this group -- low cystine, low glutamine, elevated glutamate,

increased urea production and reduced natural killer cell activity.

This is that busy thing revisited. And if we look on the right all we are really saying there is if you increase cysteine, you will increase the proton. The proton availability will neutralize bicarbonate. The bicarbonate will work on the actual switch, carbamoyl phosphate, and you will reduce that. That will mean that the ammonium ions will be saved and you will have positive nitrogen balance. As well if you look on the far right of that slide if you can see it, the glutamate that is in that slide, if you have enough cystine, will go to glutamine. You will make endogenous glutamine. We have heard a lot about that in other realms.

This is just an editorial comment. We got the wrong picture and the bottom says that the ammonium ions are saved when we reduce cysteine. That is not true. They are lost. And when you have not enough cystine, you will lose the ammonium ion into the urea.

Basically the real point of cystine is that it is transported into the cell differently. And that is where I will end.

CHAIRMAN MASUR: Do you want to make a

summary comment?

DR. KWYER: Well, the summary comment basically relates to what has been going on here in that I think that the meeting so far has described a number of measures and a fair amount of confoundation as to how to go to the next level. And I think what I am just simply identifying is that it seems that when we look at other parts of the medical world and we address things just from a straight immune standpoint, we may well be able to find something that may well be adjument. In fact, it almost begs the question as to whether the concept of the reduced immune response that we are seeing after certain therapies or even the toxicity of therapies themselves might not be an expression of deficiency of certain parts of the immune system to respond. And when you either stop therapy or move away from certain drug regimens to others, maybe you are just simply stressing it in another way and not losing as much or allowing it to rebuild. Glutathione might be one of those things to look at.

CHAIRMAN MASUR: Okay. Well, thank you, Dr. Kwyer for those comments. The last comments of this session are by Dr. Clifford Lane, the Associate Director of NIAID.

DR. LANE: So thanks. I had a couple of very brief comments that I didn't feel were appropriate for the other talk, so I asked permission to give them during the public comment.

I think it is very important to try to look at this whole area of immune-based therapies and surrogate markers and treatment for HIV infection in perspective and try to keep in mind where we are and what we are trying to do, which is really trying to set policy of where things should or should not go.

The whole field of immune-based therapies has had a fairly difficult start, a midlife crisis, and is trying to itself get reborn, and it is very difficult, I think, as you all can tell. I mean, we struggle with all these assays. We know the virus destroys the CD4 T cells, and the lower your CD4 T cells, the more likely you are to get sick. But despite that simplicity, I think we just have different types of approaches -- different philosophies. And this poor tortoise continues to lag behind the hare of antivirals.

I think it really begs the question, and one that I think is good, to see the combination of CDER and CBER advisors looking at the issues of antiretroviral therapies. Because I

think one question is are these two being handled in the same way? Is this fair? As I mentioned earlier, there are a variety of factors determining the relative importance of the immune system and the virus. The range of values at time interval, and I sort of add reporting by the media, which as we all know is just a reflection of the alter egos of the scientists that are involved in the epidemic.

So I put here some press that came out sort of in the middle of the initial enthusiasm for combination antiretrovirals. In fact, I think it was shortly after ACTG 175 looking at the value of combination therapy. It was right on the heels of showing that IL-2 could induce increases in CD4 T cell counts. And the quote says, "CD4 measurements were terrible predictors of prognosis providing wildly misleading information. However, the amount of virus in the blood stream perfectly predicted how quickly the patient would sicken or die." And when you get polarizing views like this based on results from a single study, you set impressions in motion that can be very difficult to break.

So I would just conclude then by looking a little bit at where we are with antiretroviral therapy. We have very good clinical

data. And believe me, I will be the first one to say that combination antiretroviral therapy has been the most wonderful thing to ever happen in the treatment of patients with HIV infection. It has changed, I think, our perspective on what we could accomplish, not just in HIV but really in anything. But when we look really hard, what do we know? We know that we can do great benefit for patients with advanced HIV infection. We know that we can prevent transmission of virus from mother to child. So based upon that, we have licensed drugs for the treatment of HIV infection when antiretroviral therapy is warranted, AZT monotherapy, and Indinavir in combination with antiretroviral agents as indicated for treatment of HIV infection. The problem is I don't know where to go to find out when antiretroviral therapy is warranted. And I think data that have been generated in very limited settings are now being translated to the entire spectrum of HIV infection and I am not sure that is correct.

So what I would say is I am not trying to lower the bar for immune-based therapies. I am actually making the case that I think we should raise the bar for antiretroviral therapies and apply the same standards to antiretrovirals that we

are talking about requiring to apply to immune-based therapies.

CHAIRMAN MASUR: Well, thank you. We were hoping for comments that would make our current and future meetings easier. But we appreciate those comments nevertheless. I think since we would like to spend uninterrupted time on the questions, this might be a good time to take a five-minute break. And then in five minutes we will come back and deal with the questions that have been posed to the committee and its guests.

(Whereupon, at 2:11 p.m., off the record until 2:23 p.m.)

CHAIRMAN MASUR: We need a few more committee members. So everyone in the audience as well as the committee should have a copy of the questions that have been posed to the committee. So I am not going to read the entire text. I guess there is one question on virologic outcomes and one on immunologic outcomes. And I would like to go around the table and solicit both our guests and committee members. I think from the Agency's point of view, they are interested in our comments, not necessarily a resolution of any -- of a consensus, but they would like to get some guidance after we have heard these excellent presentations of the

morning.

So, again, you can see the background paragraph up here. The first question we would like to tackle regarding immune-based therapy is listed as number A there. Immune-based therapies have a different mechanism of action compared to antiviral agents. How likely will changes in viral load during or following an immune-based therapy be predictive of clinical benefit? So I am happy to take volunteers and then we will go in some order. Fred, we appreciate your willingness to volunteer.

DR. VALENTINE: Simply not to solve any questions, but to say that we do, as someone else commented in this morning's presentations, have to separate out I think those immune-based therapies that are attempting to induce anti-HIV immunity, where I would suggest that viral changes are a very appropriate readout if we could figure out a way to do them in the context of potent therapy. We have to separate out those types of immune-based therapies designed to induce specific anti-HIV responses from other types of immune-based therapies which are designed to increase CD4's and to increase antigen presentation and what have you.

So in the first question, I would say that viral load readouts are very appropriate for

the vaccine type or other type of immune response such as the DOP therapy design to enhance anti-HIV immunity.

CHAIRMAN MASUR: Fred, when you say that viral load is appropriate, does that suggest that that is as predictive of clinical benefit as viral load would be for an antiretroviral therapy? Is it, in the year 2000, a validated surrogate?

DR. VALENTINE: We certainly are licensing a lot of drugs under anti-HIV drugs on their ability to decrease viral load. The assumption is that they are having the clinical benefit, which has been demonstrated upon giving anti-HIV therapy on the basis of their ability to lower the viral load. By the strict Prentice criteria, there also perhaps is something else going on. Maybe Tom wants to comment about that. But from my perspective, yes. If you could find a good clinical trial designed to show that the introduction of an anti-HIV immune based therapy dropped viral load in a reproducible and sustained manner that that would be just as good as doing it with the drug. That is my opinion.

CHAIRMAN MASUR: Well, Nancy, we are actually -- it looks like we are tackling a through d simultaneously. So maybe you could --

DR. VALENTINE: That is always the case.

CHAIRMAN MASUR: Well, I mean I think that is a constructive and provocative start. The question is would anybody like to -- why don't we go around and I guess we will bypass the Agency people. Chip, you had said to me at the break that you were eager to answer these questions.

DR. SCHOOLEY: I thought you were an honest man until then. That is a lie too. I think that I would agree with Fred with a yes, but. I think that one of the things that we should really try to take advantage of is what we have learned over the course of the last 20 years and not try to reinvent the entire field every time something new comes up. I think that the -- in the strict context of an immune-based therapeutic that's major focus and goal is to try to decrease viral replication, we are much firmer ground there feeling comfortable than this is of more relevance than with another mechanism of action.

The but part is that because there are multiple ways and approaches to do that, you have to also be careful that the toxicity involved in this particular form of therapy doesn't have some counterbalancing effect. So, for example, if you

have an immune-based therapy that is attempting to decrease viral load by, for example, eliminating CD4 cells by radiation, you have to take that caveat into consideration and not directly extrapolate the two. But I would hope that we don't start out by thinking we know nothing and try to reinvent the entire knowledge base of HIV pathogenesis and its relationship to the disease just because we are starting with a new set of therapies. That does people a disservice as well.

CHAIRMAN MASUR: Well, Chip, let me just follow up on those two comments with you and Fred before we move on to Brian. You know, there have been issues with drugs like hydroxyurea that may enhance antiviral effect without necessarily having a CD4 effect. Given that you brought up the caveat on CD4's, how would you use a virologic endpoint with an immune-based therapy without also looking at other parameters? Would you be -- are you suggesting that you would be less enthusiastic about looking at that as an isolated phenomenon without looking at other issues? And then what else would you look at other than CD4's?

DR. SCHOOLEY: I guess again seeming to -- trying to make this more complicated than you would like it to be, we shouldn't look at any

potential therapeutic in isolation looking at a single variable. Very time we have an antiviral drug come before us, we should be looking at it in the context of what else it does, and the same thing is true for immune-based therapeutics. With hydroxyurea, obviously the counterbalancing effect on CD4 cells is something that causes it to be a bit different in considerations from a strict antiviral agent. And I just think that my plea is that we look at all of this in context and take the whole knowledge base as part of the decision making process and not try to isolate a single thing and having it be if you can just show that your immune-based therapy decreases viral load by X tenths of a log for X number of months, then it is an antiviral agent. That oversimplifies it as well.

CHAIRMAN MASUR: Well, I think that is a good point that we don't look at any drug in isolation. We look at the entire efficacy and safety package. But then again, just to follow up before we move on, is there any -- the second and third questions have to do with what parameters of viral load change you would focus on. Should the focus here be different than with the parameters we have looked at before, 24 and 48 weeks sustained, decreases in viral load, or how might you look at

that?

DR. SCHOOLEY: Well, I think we are trying here, as in antiviral drugs, to demonstrate clinical benefit. And clinical benefit, if we get back to the antiviral drugs, as has for the most part been demonstrated in situations in which as little as a half log change in HIV RNA has been demonstrated for a period of several months. The reason we consider that an incomplete response in antiviral therapy isn't because it doesn't benefit the host. It is because we don't see it as being a durable response because resistance is being induced while we sit there with an incomplete antiviral response. So if you have a patient with advanced HIV disease and floating along with 40,000 copies of the virus and you said to me, I have got this therapeutic intervention that will decrease their plasma HIV RNA level to 2,000 copies, just for argument sake vaccination, I would consider that, taking into account other issues related to toxicity, a successful intervention if it was a durable effect. And not say, well gee, you didn't get to 500 copies, it was a bad thing.

So I think that it really here ought to go back to where we were at the nucleoside days. How much of an effect do you have to have to have a

beneficial clinical effect. Is that part of the consideration paradigm when we are looking at an immune-based therapeutic?

CHAIRMAN MASUR: So you are focusing on magnitude, durability and the price you pay?

DR. SCHOOLEY: Right.

CHAIRMAN MASUR: Fred, before we get around, do you want to elaborate on what you said? At the end we will come back to question D, which has to do with what type of study design one might consider.

DR. VALENTINE: You seem concerned that the immunologist might not go ahead and measure something other than viral load. I can assure you from what we have seen today that they will measure lots of things in addition to viral load.

CHAIRMAN MASUR: Yes, we are concerned about that.

DR. VALENTINE: Not just because they are concerned about their employment. We do want to understand what is going on also, Henry.

CHAIRMAN MASUR: Right. A good part of the NIH budget goes to that. Brenda, I don't want to pass you as we go around the table. You can either comment now or we can come to you at the end, whenever you would like.

MS. LEIN: Going in order is fine.

CHAIRMAN MASUR: All right. Brian?

DR. WONG: I don't think I have anything really to add to what has been said. I think that it is very difficult to --

CHAIRMAN MASUR: Can we turn the volume up over here?

DR. WONG: It is very difficult to postulate in advance what criteria one would have to see when the manipulation or the intervention is not known and the characteristics of the study population are not known. But it is usually pretty clear when you actually see the data whether -- you know, whether it worked or not. And I think the sorts of things that Chip mentioned -- you know, magnitude of effect or ability of effect and the toxic cost are all considerations.

I don't think we can say that anyone has
-- I mean certainly from today -- from what we have seen today, no one has shown that any of these measures necessarily correlate one-to-one with clinical benefit. But I would also advise the Agency not to use that as an absolute standard. I mean, antiviral effect would meet the standard that I would set if it was really believable and

achieved at low toxicities.

CHAIRMAN MASUR: Chris?

DR. MATHEWS: I guess I would make a comment amplifying something that Chip said. When you said --

CHAIRMAN MASUR: Can we turn up the microphone --

DR. MATHEWS: Not trying to rediscover things that we have learned over the last 20 years. I mean, in general I would agree with that. On the other hand, you know I think that there have been class specific effects of various agents that we have looked at over the years where there is genuine ambiguity about how much you can conclude that a homogeneous response, say for example in viral burden, means the same thing if it is produced by one mechanism of action versus another. And in my own mind over the last year or two, for example related to this whole virus fitness issue, continuing regimens when viral load appears to have rebounded because there is evidence of continuing clinical benefit, we know that seems to be the case with protease inhibitor-based regimens, but do we know that in fact that is the case with protease-sparing regimens? And so I am not perhaps as sanguine in concluding that a change in viral load

induced by a vaccine necessarily translates into a comparable amount of benefit induced by current therapeutic approaches.

The other point I would make is that focusing on any laboratory measure, while for purposes of accelerated or even traditional approval with a longer time frame, as the Agency has done now, still really does not provide us with a single integrated summary of overall benefit. If you focus on any combination of laboratory measures, you can still miss what the overall predominant effect on toxicity is. And I recall that in the early hearings on licensing of some of the protease inhibitors where data on lipid abnormalities were presented, many people on the committee kind of looked at one another. We saw triglycerides over 1,000 and said, well that is interesting. I wonder what that is going to mean. And of course much of the discussion that brought us here today was precisely the aftermath of those very early observations of uncertain significance. So focusing on laboratory measures without finding some way to integrate the net benefit or net efficacy of treatment I think is going to be problematic.

CHAIRMAN MASUR: And we will go around

and we will eventually come back to David Parenti. One of the things we have learned is that pediatric patients are not necessarily identical to adults. Do you have comments on that or other?

DR. YOGEV: Well, first I have a fundamental problem. It sounds to me that we are trying to suggest that virus (inaudible). We saw this discordance. And for some reason maybe more in pediatric than in adult. And we, two years ago because of suggestion that viral load has to come down spends drugs like there is no tomorrow. That told us very quickly that those (inaudible) at least for the first two years, has been almost (inaudible). Those who got the viral load down to whatever number we decided according to the methodology -- if it was 1,000, we said good. If it was 500, we said fine. If we need 50, we go 50. So I think it would be wrong if virological outcome would be the yardstick that we are going to take the immune system. Not to mention that I think the immune system -- what we see in the blood is not sufficient to what we really need to see how the immune system is responding.

So I for one would say that the virological load is a nice parameter, but I would not use it as a surrogate for the immune system

function because of what we are already seeing. Trying to reinvent our failures and come several years later.

My other problem is from what I heard today -- you know, I came here -- how shall I say it

-- timid, and I am leaving confused. Because there are so many parameters and we didn't identify the major one. There must be something in the immune system we haven't put or finger on that is more simple, like the virus to the antiretroviral. So I am afraid we are going to work by tradition, what we see, instead of trying to pursue what is there else. And I would encourage the Agency not to try to define a surrogate marker, but encourage looking for them. Because I don't think we have them, including the virus.

CHAIRMAN MASUR: I guess as we go around, hopefully everyone will try to specifically address the first two questions, which is again would viral endpoints be reasonable for immune-based therapies and are there parameters other than the ones we are currently using for antiretroviral drugs to look for. And then we will tackle CMV after that. So, Courtney? All right, Courtney will hold for the moment. John?

DR. HAMILTON: Well, as is usual with most complicated things, the devil is in the details. Of course all of us would like to be able to capitalize on the extraordinary data base that has been accrued in the course of the antiviral era, and to some extent I think we are dependent on that data base. There have to be, however, other very salient variables that are brought to bear on the analysis. And whereas I would say that I would agree that some of the virologic measures are among the appropriate measures that should be made in the course of trials of immune-based therapy, they are certainly not the only ones. And in order to find out what the others are, I think what we need are a series of -- and this is probably bad news potentially for some -- but what we need are some very rigorous trials where things are systematically and rigorously examined in detail. So that we can either accept or reject those parameters.

For the person developing it or the institution that is developing these measures, a negative finding, of course, is not good news. But for the scientific community, it is just as important at this stage for us to know what doesn't work. We have to stop casting about. We need to

become much more focused. And I confess I am not knowledgeable enough in the field of immunology to make those discriminating judgments. At this moment, I need some data and I need somebody to provide that for me.

CHAIRMAN MASUR: Okay. Thank you, John. Mike, one of the penalties for being late is that if you look under B up there, in addition your comments about whether or not immune-based therapies ought to use antiviral endpoints, maybe you could make some comments and elaborate on what Chip said. Should we be using any different parameters from the kind of magnitude and durability of virologic response that we are looking for currently with antiretrovirals. Time to response and slope of response -- should there be other things we look for in immune-based therapy a this point, or do we simply not know enough to make those judgments?

DR. SAAG: Oh, I think we maybe know more than what we give ourselves credit for. I would like to frame it in sort of the context of history. When we started off, we didn't have anything to really measure what was biologically happening as we used antiretroviral therapy. We had P24 antigen that was variable and not as

predictive, and that is maybe a little bit of how we are right now in terms of trying to measure immune system responses and not having the technology to really nail down what immunologic interventions are doing.

So what we do have, though, is the biologic outcome in terms of viral load. And I think it is important, if we are going to say that whatever this intervention that we are doing has biologic plausibility and that that intervention -- it is going to be based on an individual assessment. But if an immune-based intervention has biologic plausibility to have an effect on the virus, then, yes, we should be looking at the virologic response. If there is a connection. If you can connect the dots and make some sense out of it. And I think to hold the immunologic interventions up to a different standard is unfair. Because I think as the points were made earlier, you have virologic effects in certain instances, but a patient maybe does worse. And I think what Chip was saying is right. You have to take the entire picture of not just the virologic response but the toxicities into account as well.

But on the other hand, if you have, for example, a subset of patients -- and I think this

is what we heard from the community perspective -- we have a fairly significant subset of patients who can get a virologic response, those who have advanced disease, but their CD4 count response is poor. I think that was panel C on the slide that was shown earlier. And what about those individuals? We just did an analysis of those folks in our clinic. In over four years, 40 percent of them had died. And so that is not a good outcome.

CHAIRMAN MASUR: These are people that are virologically responsive --

DR. SAAG: These are people with virologic responses with no CD4 count increase. And the fact of the matter is that we have all types of information that suggest that, yes, viral load is important, but also CD4 counts are as well. And I think for certain subsets of populations, if we have an intervention that can move them to someplace other than where they are, then I think we should consider gaining access at least to some of those individuals, much like we did having access given to antiviral drugs at an earlier time when it was apparent that they might have some clinical benefit. With that comes an obligation, however, to follow these things up very closely

over time. But to not allow access in some form to this type of intervention I think is wrong, just like it was wrong not to allow protease inhibitors to start coming out when there was enough data to indicate that there was some potential benefit.

So to answer question B, I think it does depend on what the intervention is. But if the intervention has a link biologically to virologic production or the genesis of viral particles, let's say, then I think virologic endpoints can be part of the equation and maybe more interpretable in a sense than a lot of the immunologic assays for which we don't have a lot of good reproducibility or we have difficulty interpreting.

CHAIRMAN MASUR: So, Mike, if I understand you, you would be willing to consider approving an immune-based therapy for accelerated approval based on a virologic surrogate endpoint if there was a sustained decrease in viral load and no other logical prohibitive factor regarding an immunologic or safety parameter?

DR. SAAG: Logical. Yes, to answer your question. I would be -- I think we should hold -- we should hold the immune-based therapies up to the same balance that we do antiviral therapies because the need is there. And I think we should be

in some ways just as progressive as the committees were looking at antiviral drugs in the past because the need is still there. This is not anywhere close to being over.

CHAIRMAN MASUR: As we go around the table, does anybody who has already spoken want to take issue with that? Would anybody not be willing to consider accelerated approval if an immune-based therapy produced a sustained virologic effect and there wasn't some other prohibitive issue?

DR. SAAG: And it was biologically plausible.

CHAIRMAN MASUR: Right. I mean, I see Dr. Schooley twitching a little bit and Brian twitching. Does anybody want to take issue with Mike's proposal?

DR. YOGEV: Just to clarify. You mean that you can identify there is nothing to do with the antiretroviral in it?

DR. SAAG: Right.

DR. YOGEV: Specifically you are able --

DR. SAAG: Let's be concrete. Let's say you had antiviral intervention, as you suggested, that dropped viral load to some amount that wasn't undetectable. Which, by the way, the only reason

that I am aware of that people pushed for undetectable wasn't for clinical benefit directly, but it was more in prevent resistance. It wasn't saying that kids weren't going to benefit if they went from 500,000 down to 50,000. I think everyone figured there would be a benefit there. It is a question of preventing resistance.

DR. YOGEV: (Inaudible) better than 50 or better than 400, but now we are retracting from it? Like a year ago or three years, there was a suggestion that clinically you do better if you are less than 50?

DR. SAAG: Not that I am aware of. I mean, I thought of it in terms of preventing resistance. But let's go back to the analogy. Let's say there was a situation where you could take a kid from 400,000 copies down to 50,000. That is the best you could do. And then you had some intervention X that was maybe immune-based that would drive that viral load down to less than 50, and therefore prevent the development of resistance. And those who didn't get that intervention X did not get that benefit. And then you could show that over one year's time, those who did not get the immunologic intervention, 50 percent of them developed resistance to their

regimen and the ones who did get the immunologic intervention, because there was less reproduction of the virus, et cetera, there was only 5 percent. Then I would say that is pretty strong evidence that there was something going on there. Even if we can't explain it fully. And as Chip said, you don't show a lot of untoward events and toxicities in the big picture. I think that should be considered as a possible agent for approval on an accelerated basis just like we do with antivirals.

CHAIRMAN MASUR: Well, Mike, you are willing to be pinned down, so I will try to pin you down one time further before we move to Tom Fleming. If there were -- let me ask you two questions. If there were an immune-based therapy which without antiretrovirals would reduce your viral load from 500,000 to 50,000 or 5,000, would that demonstration be enough to warrant approval?

DR. SAAG: If I could understand at least in some fashion how that agent was working. Now right now I can't picture that type of drug unless it was causing toxicity to the point where you created somebody to be in morbid condition and there was no biologic activity period. But, yes, I think it should be looked at.

CHAIRMAN MASUR: Well, I think we have

to assume that there must -- one condition has to be biologic plausibility.

DR. SAAG: Right.

CHAIRMAN MASUR: And then if a drug had a virologic effect, would you also demand that you see some immunologic parameter that benefitted, or would virology in and of itself be enough if there again was biologic plausibility?

DR. SAAG: Again, it would depend. And I know you are trying to pin me down. But if I were confident that the assay fit the biology and the assay were reproducible, I would want to see that as well. But frankly -- and I know I missed this morning's presentations, but I have seen a lot of data presented in the past where some of the assays are not as reproducible and their interpretations are more difficult than understanding viral load. I can understand that a little bit easier than I can some of the marker studies, et cetera. On the other hand, if there was consistency in the marker study and it fit the biology as we understand it for how an agent would work, yes, I think that would be complimentary to the package. You need to look at the whole picture.

CHAIRMAN MASUR: All right. Well, I

appreciate your willingness to be pinned down.

DR. SCHOOLEY: I was just going to say, but if your premise is that it is acting as an antiviral agent and obviously there is association between CD4 cell responses and viral responses with antiretroviral drugs, but in groups of patients, you generally see things move in opposite directions. Would you not want to see, at least with a simple parameter of CD4 cell counts, the same type of response if you are going to use the same type of yardstick? I mean, it would make me feel less comfortable if you had disassociation there.

DR. SAAG: I agree. I think there is an assumption that I am making that may not be fully correct. And that is that for the time being, anyway, I have a belief -- underscore that -- that the immune system is an antiviral agent in and of itself. That it does have the ability to control replication. If you can do something to improve that activity, I would think that you would see the other benefits that would go along with suppression of virus as you would see with an antiretroviral drug, which would include rising CD4 count and clinical outcomes in the long run that would be beneficial.

CHAIRMAN MASUR: Okay. Well, I think that is a good point. You wouldn't want to create a discordant patient, as you were describing before.

DR. SCHOOLEY: Well, if you are trying to argue that you are using the data base you had before, the data base you had before says that if the viral load does X, the CD4 cell count should do Y. And if it doesn't, that is a red flag that there is something different here that you need to consider more carefully.

I think the other assays that we talked about this morning remain interesting and could be biologically supportive of an intervention if that intervention was supposed to have an effect on that parameter. But there are things that I would consider deal breakers from the standpoint of trying to consider an antiviral or an immune-based therapeutic.

CHAIRMAN MASUR: Okay. That is an important point. Again, we will get around here eventually. Ram, do you want to have a follow-up point?

DR. YOGEV: Yes. I think we already said that CD4 does not always represent itself going in the other direction. It is only 45

percent of the time. As much as it is devastating to hear that a patient (inaudible) in the pediatric population. That is an extension of life compared to what it was before. So I am not sure that the CD4 are as good with the viral coming down that the immune system didn't respond. There are other (inaudible) in the immune system which you don't understand that are not connected directly to the CD4. I agree that I would love to have it. And if somebody comes to me and shows me viral load is down and CD4 went up, for me it is perfect for what we know today. But if CD4 didn't go up and the question is when it should go up, but it remained and didn't go down, for me it is as good because it depends when I am starting the therapy. But to suggest that if CD4 doesn't change and viral went down it is fete accompli not as good, I think we are not doing fair to this.

CHAIRMAN MASUR: Although I think what we are talking about is what surrogate marker situation could we have confidence in. There may be other situations where there would be benefit, but we would have a -- demand a higher degree of rigor before we would be willing to recommend licensing of the drug. Is that your perspective, Mike?

DR. SAAG: I think so. I want to add

this one caveat. And I think again it goes back to the concept of fairness in the direction of immune-based approaches as well. And that is because of these uncertainties, I think anytime there might be any type of accelerated approval, there comes with it an obligation to have follow-up data over a long period of time. And I think that would answer a lot of these questions.

CHAIRMAN MASUR: Yes. Well, I think -- I don't know whether Jay or Heidi would like to make a comment on that, but I think that the Agency has been fairly vigorous and rigorous about assuring that if there is an accelerated approval, there is follow-up studies. Do you want to make a comment on that? I don't know, Heidi, whether you wanted to make a comment from the audience.

DR. SIEGEL: Well, I'd only want to comment that, yes, we would feel strongly about that. I think that to the extent that we -- that there are recommendations that some therapies might be approved using -- immune-based therapies might be approved using viral load markers, it would be recognized that that would be on the basis of reasonable likelihood under the accelerated approval regulations. As obviously we have all discussed, there is not yet validating data. And

part D of this question is, in fact, how to get that validating data. I think we would all agree if we take such a step that it would be nice some years from now to be able to look back and learn the lessons of what works and why and how. So generating that data is important, although not always as easy as it may seem theoretically.

CHAIRMAN MASUR: All right. Well, we are sort of going around, but we will allow a little bit of backtracking. Fred?

DR. VALENTINE: Just a follow-up because it was just discussed. In the context of anti-HIV immune responses, and even more importantly for the next question, we have to keep in mind that the immune system is clonally structured and that it is indeed possible to induce a very large and significant effector function by immunization or another means perhaps without having a great increase in total CD4 cell numbers. And I think that was what Ram was -- the point he was trying to make. And it will be very important when we get down to the second page.

CHAIRMAN MASUR: Okay. Tom?

DR. FLEMING: In addressing this issue of the use of virologic outcomes as a surrogate for immune-based therapy, I guess I go back and track

what we learned from the past. In fact, I might rephrase how this opening paragraph is worded, which states that because of well-established correlation between decreases in viral load and clinical benefit in studies of subjects receiving antiviral therapies, changes in viral load are -- meaningful changes in viral load are accepted as a surrogate. Actually, I would have thought that it was more than just noting this correlation that led us to this acceptance. It was tremendous good fortune that over the past decade we have seen remarkable strides taken through triple drug therapy in terms of achieving profound effects on viral load. And in addition to that, documented profound effects on clinical endpoints. And it is the aggregate of those insights, I think, that has led us to the level of confidence that we have at this point in using viral load as a surrogate for an antiviral agent.

CHAIRMAN MASUR: Well, Tom, you would say we have confidence in that, again, with drugs that have a different mechanism of action?

DR. FLEMING: My point is we have confidence in this not simply because there is a, as Jon was referring to terminology this morning, a Type 0 or Type 1 association, but there actually is

a Type 2 association, at least in so far as the use of this as a marker in later stage. Now going beyond this, actually the stage of infection is important. When one has observed profound effects on viral load of sustained duration in a more advanced disease setting, one can argue that that is the setting in which the surrogate actually is the least helpful because the clinical effect itself is immediate and can be validated. Where the surrogate is of greatest benefit to us is where it gives us an answer much more rapidly than the clinical answer. And even for anti -- even with antivirals, I think it is very -- it remains very controversial how validated viral load is. I think there is a great deal we still don't understand in early stage infection as to the level of effect that we have to see in order to be confident that we have established the proper role for the intervention -- when to start and when to switch, et cetera. So where surrogates are most valuable is often where they are most challenged, and I would simply point out that viral load itself is a measure that has a certain level of validation and that level of validation is where the effects are most profound, most durable, in a most advanced disease setting, where actually the benefit of such

a marker is least.

With this as a backdrop, what can we say about use of these measures of virologic outcomes for immune-based therapy? Well, I think I looked at two criteria. I go to what Fred was saying at the beginning of this discussion. If we are going to use a virologic outcome for immune-based therapy, one needs to very clearly, as best we can, understand the relationship of the marker, in this case viral load, with the overall disease pathophysiology. And certainly it can be stated, it is the virus, stupid, as often is stated. And in fact if the effect is profound, it is in fact a fairly reliable marker in that setting. But as Fred points out then, if the immune-based therapy is targeting an anti-HIV immunity, then it is much more plausible that that in fact is the mechanism that this intervention is going to affect. My worry is that, as he was pointing out, treatment to enhance CD4, CD8 and CDL, we may be substantially insensitive or underestimating the effect of this intervention ultimately on clinical endpoints by not specifically targeting the most sensitive measure.

The other issue --

CHAIRMAN MASUR: Well, right now we are

-- I guess we are going to come back to whether or not there also ought to be other surrogate markers among immunologic parameters.

DR. FLEMING: That is a very good point. In fact, I think the answer to this with an immune-based therapy in nearly any setting it is going to be a multivariate marker. I think we would undoubtedly be better served, although I can't tell you what this multivariate components are -- but better served by a marker that captures both the antiviral effects, specifically viral load effects, as well as what are the plausible immunologic changes that are induced to achieve those virologic effects. So undoubtedly some kind of a multivariate marker will be more sensitive and reliable in predicting benefit, being sensitive to benefit, and in fact reducing the risk of false positives as well.

The other issue, though, is the unintended mechanisms. If an agent is sufficiently potent to generate an immune response that in turn will generate an important viral load effect, it is unrealistic to think that there aren't a myriad of other effects. Immune-based effects as well as other toxicities. And certainly these have to be factored in as well.

Which brings us back to the issue -- it is clearly, i.e., the reliance on a virologic marker will certainly depend in a significant way on what is the magnitude of the effect and the duration of the effect. If we are, in fact, benefitting from having a triple drug effect and we are looking at trying to improve on this, to anticipate an order of magnitude improvement, again over and above what we have seen in the past, as we have seen in other disease areas, is a difficult thing to replicate. Of course if we are saying what we are interested in is looking at immune-based therapies against a placebo control that would be initiated in the early stages of infection in a way to delay the need -- delay the implementation of triple drug therapy, then one may well be able to achieve a very significant reduction in viral load. But again what has to be factored in is what is the duration of the effect and the magnitude of the effect and the time of the overall infectious process. If you are looking at an early initiation, I worry considerably about how to reliably understand the results on the surrogate and what they are reliably telling us about the best way to use this intervention and what its ultimate effect will be on much longer term

clinical endpoints.

CHAIRMAN MASUR: Okay. Those are important considerations. Trip?

DR. GULICK: Well, I, like other speakers, would find it compelling if an immune-based therapy could induce a significant and durable decrease in viral load. Inherent with that I agree that an assessment of the immunologic properties at the same time and very importantly the toxicities of the drug would need to be taken into account. And others have made these points today. I too think that you need to understand the biological plausibility, the mechanism of action of the immune-based therapy in order to evaluate its effect on these endpoints.

One point that I think Dr. Fleming began to reach toward is that an assessment of a new therapy in HIV disease not only can benefit from what we have learned in the development of antiretrovirals but actually has to consider antiretroviral therapy. Or not coming in with a brand new drug and saying, well we don't have anything else, let's try this drug. This needs to be tested in the clinical scenario where we do have antiretroviral agents which are quite capable of positive effects that have been well demonstrated.

So I think thinking about immune-based therapies effects on viral load and other markers, we need to think about that in the context of what we can do with antiretrovirals today.

Some of the previous speakers thought that there might be novel ways to do that or novel populations to study, such as people who don't respond to what we have today or are failing what we have today. So that might be the most appropriate place. I guess inherent in accelerated approval, there is a stated need for a new therapy in that context. And that too draws us, I think, to certain populations rather than trying to apply this to all comers.

CHAIRMAN MASUR: Right. Well, we are going to get -- we will start with you when we get back to question D, which is what kind of study design might be appropriate for this. But let's keep moving. Sharilyn?

DR. STANLEY: I guess I am going to get to the same place that everybody else is at, but I get there a little differently. I don't think the bars are even because -- and I call it the duh hypothesis. With antiretrovirals, you are targeting the virus. With immune-based therapy, you are targeting the immune system. And so I think

that ultimately markers of immune function are going to be more important than viral load, perhaps. But if the ultimate sign of a healthy immune system in an HIV-infected person is the ability to suppress virus by their immune system, then that is an important marker.

So I guess I come around to saying that viral load is important. It is something that should be looked at. But again as Tom was talking about, it is going to have to be in a spectrum of a variety of measurements that also target the immune system.

CHAIRMAN MASUR: All right. Brenda?

MS. LEIN: Well, I know that it was echoed -- I think Fred brought it up first -- that I think we have to separate the discussion between HIV-specific immune-based therapies and those that may enhance other types of immunity. And I think that viral load is really important if you are talking about an HIV-specific immune-based therapy. And I wouldn't disagree necessarily with anything that has been said, although I kind of wonder where we are all living because I don't know of an immune-based therapy that is HIV specific today that sort of inhibits HIV to the magnitude and duration of an anti-HIV therapy. So I think the

discussion is a lot more difficult than what we are having. And perhaps it is in D a little bit, but I think what needs to happen is there needs to be another panel convened to take a look at the viral load data and reassess what we really know. Because I don't believe that it is a really strongly -- viral load is a really strongly validated Type 2 marker. I think it is a Type 1 marker and in advance stage disease, it is probably least useful. I have had too many friends die with undetectable viral load to think that lowering their viral load further would have benefitted them any.

So I think that when we talk about immune-based therapies that are HIV specific, we are talking about something a lot more subtle. We are talking about maybe vaccines that currently -- I mean, the current vaccine products seem to alter HIV-specific immune responses that we don't know what those mean and don't really have any effect whatsoever on viral load that people have noticed to speak of. So the other HIV-specific immune-based therapies are HIV-specific CTL therapies and others. So in the context of reality of where we are at right now, I think that the discussion needs to shift to the subtleties. I don't think that any of the products that are currently available could

be approved on the basis of current guidelines for what we are using to approve antiviral drugs. I don't -- I think that perhaps we have to be changing the discussion to look at added benefit.

I know that it has been repeated a few times that our need to develop immune-based therapies is because the current drugs are toxic and we are in an urgent situation. And I really think that perhaps the response to the current drugs are toxic is to push for the development of drugs that are less toxic that are antiviral in nature. But we need IBTs to enhance the natural ability of the body to control the disease, be it HIV, which it doesn't seem to be able to do very well, or the sequelae, which really seems to be what is killing people. If an immune-based therapy that wasn't HIV specific didn't have an impact on HIV RNA and in fact maybe even HIV RNA was increased, I don't think that would affect me as a patient advocate. The outcome measures and what we are looking for are really different. Certainly it needs to be looked at in the context of other things. I think wasn't it FIAU that was able to so beautifully decrease viral load but killed people? That there is a larger context to look at and those long-term follow-up studies are going to be

critical. But I hope that we would move away to sort of where we are at with immune-based therapies, which isn't a potent antiviral response.

CHAIRMAN MASUR: Right. Well, I think that is going to be -- I mean, those are good points. We are going to get to part 2 of this, which will be to discuss markers that are not virologic in nature and whether or not there is a basis for that. That will be part 2 that will come around again.

MS. LEIN: Well, and so then if we look at question B, I think that the question is really relevant for anti-HIV therapies coming down the pipeline as well. What types of new study designs and what type of responses and effects on viral load are necessary to even approve a new anti-HIV drug over what we have today. You know, all of the different ways that we can think about anti-HIV approaches, be they immune based or other. Okay, so if I went off therapy -- maybe even going off therapy for six months should let you approve a drug because you have been able to sustain viral load decreases and save someone the toxicity of therapies. And boy, shouldn't that receive an accelerated approval based on real clinical care issues and the experience of patients. But I don't

know myself, and I think we really need the data on the table and in front of us to show that decreasing viral load from 5,000 to undetectable -- from a low level to an undetectable level really does something meaningful for an individual. I think that people have been using that approach with anti-HIV therapies. I am on this three-drug regimen. Let's throw on five to get it undetectable. Is that necessary? Is that useful? Let's reevaluate the data and find it out before we say, yes, we would accept that. And that seems to be what we are asked to look at.

CHAIRMAN MASUR: Those are important questions. I have the feeling that we are not going to be able to address those here. But you are right, there need to be other forums to look at that. Let's keep going. Bob Redfield?

DR. REDFIELD: My comments are very similar to Michael's.

CHAIRMAN MASUR: Is that microphone working? Try again.

DR. REDFIELD: I don't think it is working.

CHAIRMAN MASUR: All right. So you said your -- all right, try again.

DR. REDFIELD: My comments are similar

to Michael's. For those immune-based therapies that are thought to have an immunoregulatory impact, I think that viral load is totally appropriate. And in terms of the issues related to other additional antiviral sort of readouts, I think in individuals that are on initial therapy or that are suboptimally suppressed, I think having an impact that looks at more optimal suppression, differences in rates of resistance and enhanced durability is, I think, a very reasonable goal line for an immunoregulatory-based immune-based therapy. And in individuals that are optimally suppressed, say viral loads less than 50 copies, just to echo what Brenda said, I think in the post-treatment structured treatment interruption, if one could demonstrate that one could take someone that is antiretroviral dependent and convert them to antiretroviral independent by some thresholds yet to be defined, say 500 or 5,000 -- again, whatever the debate is, based on clinical relevance, that this too would be a very reasonable path for those immune-based therapeutic strategies, again that have a basis underpinning them that they are basically causing enhanced in vivo immune regulation.

CHAIRMAN MASUR: Okay. Doug Fish?

DR. FISH: Well, I would agree with much of what has been said, and certainly I think that the viral load piece would be important to look at. Echoing what Dr. Gulick has said, I think we have to remember that these would be developed in the world where HAART exists and so there is not going to be necessarily a HAART control arm and an immune-based therapy control arm looking head-to-head in a naive patient necessarily. So in as much as several of the immune-based therapies that are under consideration would be in patients who have controlled viral replication on HAART, getting to question B, what could we look at and then time to viral rebound I think would be important there. Also, if patients were coming off of therapy -- say they were on HAART versus a HAART plus immune-based therapy, perhaps resetting their viral setpoint, like has been demonstrated with acute HIV. Perhaps that might be a mechanism for chronic infection. So that would be a place where I could see viral load being important.

And then thirdly, as patients are on immune-based therapies, just making sure that there was not an adverse effect on viral load. That viral load didn't go up compared to standard therapy.

CHAIRMAN MASUR: Okay. Thank you.

David Parenti?

DR. PARENTI: I guess it is hard to disagree with most of what people said. My concerns I guess would be that the bar of dropping viral load is too high for the immune-based therapies, particularly when they are being used with antiretrovirals and that the other measures of virologic response, whether it be delayed rebound and strategic drug interruption or measures of persistent viral replication, that those probably should be looked at as well.

CHAIRMAN MASUR: Well, I think that is a good introduction to sort of our second point of other endpoints. So we will come back to that. Since we are looking for wisdom rather than -- usually we confine these discussions to just committee and our consultants. Do any of the invited speakers in the front row want to make any comments on one of these? Dan? And keep it to less than 30 minutes.

DR. KURITZKES: Yes, I'll do this in two minutes. I think I would -- I have an easier time feeling comfortable with the parameters suggested when you are looking at things going down than I am with things going up. I think it is clear to me that if an immune-based therapy either

enhanced the extent to which virus load was suppressed or increased the duration of viral load suppression that that ought to be treated in the same way that antiviral agents are.

Since I am not certain what it means to interrupt treatment in terms of duration of clinical benefit and since that remains a hypothesis currently that interrupting therapy is of benefit in a global sense, it is less clear to me that a regimen administered to patients which then leads to virus plateauing at some intermediate level following cessation of therapy in and of itself is conferring benefit simply because you plateau at 20,000 copies instead of at 50,000 copies. That benefit implies knowledge that the treatment interruption -- the cessation of the antivirals is of itself of benefit. And so I have more difficulty figuring out exactly how to apply this in that setting. If it allowed you to be totally free of drugs with preserved CD4 count and complete viral suppression, then yes, I think I would accept that. But the intermediate levels, I am less certain how to deal with.

CHAIRMAN MASUR: Okay. Mike, Alan, Larry, Cliff?

CHAIRMAN MASUR: I think that since it

is most likely that the beneficial effect of antiviral therapies is mediated largely if not entirely through some permission of some immunologic restoration, that if you have an immune-based therapy that is going to diminish viral replication to some degree, I think this must be balanced in the context of some plausible evidence of immunologic enhancement, whether it is an increase in CD4 cell numbers or some functional enhancement. Because dropping your CD4 cell count, for example, and blocking viral load a little bit, it is hard to balance those two off as necessarily in the patient's best interest. So you need some enhancement.

CHAIRMAN MASUR: I think we have learned that from a few examples. But I think that is an important issue that Chip brought up that we have to keep in mind. Alan, do you or Larry have a comment?

DR. LANDAY: Well, I think overall what we have seen in terms of the discussion, I would certainly see that with immune-based therapies that can impact the viral load that we could certainly look at that from a point of view of accepting that aspect. But I think in term of that impacted viral load yet we are not measuring CD4, I think we

clearly have to look at that in conjunction. So I think the two are really inextricably linked, that CD4 and viral load have gone together. We have seen those disconnects. We certainly have seen variation in the outcomes or at least some of the indications are that there may be variation in clinical outcome. I think Mike Saag brought the issue of being able to measure. Those are at least two measures that we know can be done pretty well in laboratories at this point.

CHAIRMAN MASUR: Well, I think we will take that as the Schooley hypothesis, that we need to look at the whole package and that there may be deal breakers there and it is hard to define those ahead of time.

DR. FOX: I'd like to point out that if our understanding of why CD4 count rises dramatically with the initiation of HAART is true, and that is presumably because the antigenemia is reduced and that the pro-inflammatory response is reduced and that the adhesion molecule production is reduced and therefore you have less trapping of memory CD4 cells in the immune organs, that we then might see just the opposite with an effective anti-HIV response generated by an immune-based therapy. There might be more inflammatory response and you

might see at least transiently a drop in CD4 cells as more trapping occurs. You would hope that eventually we would see a reduction in viremia and that then we would see the rise that we see so dramatically with HAART, but there may not be the same connection that we see with antiviral drugs if the inflammation increases.

I would also like to underline once more that we need combined endpoints in these studies. If the shift that we are seeing happening in the community continues, as soon as the first study is published that shows at the end of a year or two interrupted therapy is as good in terms of being able to reduce the viral load once you resume therapy and the CD4 count going back to where it was as continual therapy or continuous therapy, I should say, there will be an enormous movement for people to stop using continuous therapy and use interrupted therapy. That could very well become the pattern of antiviral therapy two years from now. We will go from the infectious disease model to the cancer model. You put the patient into remission and then you treat again when you need to.

If that is the context in which we find ourselves a year or two from now, anything that

permits you to prolong that period of remission obviously is going to reduce toxicity and that is what everyone is going to be looking for. So if we have models in which we have combined endpoints of virologic CD4 changes with toxicity measures, then I think an immune-based therapy is likely to win that contest against continuous antiretroviral therapy.

CHAIRMAN MASUR: All right. So I guess the question will be if in two years we will have had enough time to be convinced that there are no deleterious effects, but that is another discussion.

DR. FOX: But we actually -- if take too long, we may be left behind by our patients who will go that way anyway.

CHAIRMAN MASUR: Right. Well then, we have about 40 minutes left and I'd like to make sure we get to question 2. But question C and D here have to do with the type of study and what other endpoints we might look at. We have talked about accelerated approval. Would anybody like to volunteer some comments? Tom?

DR. FLEMING: Maybe a quick comment. I think this distinction that is raised by D is really critical. The comments that I gave before

was really in the context of what level of evidence would be required to establish adequate plausibility of efficacy to yield an accelerated approval. It is certainly another dimension of difficulty to determine whether the plausibility of efficacy as achieved by documented effects on a marker would be adequate to ultimately sufficiently establish efficacy to give a complete approval. So at least my preferred answer to 1D would be that there would be clinical endpoint data. At least in the spectrum of studies that we would be doing looking at classes of agents, there would be some studies that would allow us to determine in a direct way what the effects are on clinical endpoints.

In fact, if we would propose using simply measures that are based on virologic endpoints, I guess I would ask my colleagues to suggest what would be the magnitude and duration of effects on virologic endpoints alone and at what stage in the disease process in order to justify that an effect on such markers is essentially conclusively establishing an effect on clinical endpoints.

CHAIRMAN MASUR: Right. Again, I don't know, Jay, whether you would like to make a

comment. I would again assume that if you were going to use surrogate markers as a basis for an accelerated approval, that you would demand, so to speak, a clinical endpoint long-term follow-up study. Is that a safe assumption?

DR. SIEGEL: Well, I guess we would. I should hope we would. I think maybe Heidi can -- or maybe the committee can comment on this better than I can. I think in the area of antiviral drugs, where of course there is much more validating data, there is some suggestion of using more durable, long-term antiviral effects as the confirmatory data, if you will. Since those may be further validated as predictive of efficacy. I think our current thinking, if that is what you are asking me, regarding immune-based therapies would be, as Dr. Fleming just suggested, if we were to do an accelerated approval based on antiviral, we would want to see a study design that confirmed clinical outcome benefit.

CHAIRMAN MASUR: Right. I mean, I was assuming that we were talking about an as yet unvalidated surrogate rather than a surrogate which given some of the caveats that have been mentioned seems to be relatively well validated. Heidi Jolson, do you want to make any comment or should

we keep moving on? She doesn't want to make a comment. Okay. Mike?

DR. SAAG: There are just -- I think in the ideal sense what Tom has said is right. You want clinical endpoints. But there are some practical problems with that in this day and age. When antiretroviral therapies were first introduced, the concept of prophylaxis wasn't a routine practice. And also I think we have learned a lot more about the conversion between defining a syndrome where X number of opportunistic processes were used to define the syndrome versus other complications that you might consider clinical endpoints now. So I think that the situation we are living in right now is very different and that the frequency of clinical endpoints is going to be much less today in aggregate than it was 10 years ago.

CHAIRMAN MASUR: Well actually, just to -- I tried to make the distinction between clinical endpoints and long-term follow-up. It may be that long-term follow-up is the best you can do.

DR. SAAG: Exactly. Because I think the ultimate -- I mean, the endpoint that you really need to watch is mortality, and that takes a long time fortunately these days for that to happen as

opposed to the past. But I think that someone developing esophageal candidiasis, which would classify as a clinical endpoint, isn't necessarily the same as developing PML lymphoma. So I think those are the kinds of difficulties when you design a clinical endpoint study. It could be driven by the less, if you will, meaningful or the diseases that have less clinical impact in the long run.

CHAIRMAN MASUR: Trip?

DR. GULICK: One way to think about what types of study design should be considered is need. And I think the greatest need in this field right now is for people who have taken and failed all currently available approved antiretroviral agents, the so-called salvage therapy field. So you could make a strong case for the fact although these patients are difficult and challenging and often advanced that that is the place to start with studies of a new novel therapy like an immune-based therapy.

Two other important places to think about are building on comments that other people have made. The fact that virologic failure occurs commonly on our best drugs would make that population interesting to look at to see if a new novel therapy could prolong the good effects that

we have seen. And hand-in-hand with that is that the people who are developing toxicities on our best drugs, could immune-based therapy somehow provide a durable virologic and immunologic effect, even if we needed to discontinue medications that cause toxicity.

CHAIRMAN MASUR: Trip, would you then be less enthusiastic about using these in untreated patients with the idea of trying to forestall their reaching the endpoints that would trigger your starting the currently available antiretrovirals?

DR. GULICK: Well, perhaps the cleanest population to look at are those with early HIV disease, where it is not clear that antiretroviral therapy should be started, and then you could ethically do a placebo-controlled trial, a true Phase II or a Phase I just to document the biological effects of these regimens before you proceeded. But I think that would be a reasonable place to look also.

CHAIRMAN MASUR: Let's have two more comments and then I am going to ask Jay or Karen if they want any more clarity here. But I would like to have at least the half hour before some of the panel members go to discuss the second point. But Chip, Ram and someone else here had a comment.

Let's start with Chip.

DR. SCHOOLEY: Well, I agree with Trip about the fact that people with advanced stage disease who have been through many or all of the drugs we have available are where the most immediate medical need is present. I think we have to be careful not to impose a one size fits all approach to evaluating these agents as well. Go back to the mechanism of action. If you have a passive immunotherapy like monoclonal antibody, that might be a patient population in which that form of therapy would be very appropriately targeted. On the other hand, if you are talking about active immunotherapy with a vaccine, that may not be the place to go. So just as in the antiviral division where I think it is important not to have every drug evaluated in the same way, you have to think about what niche you are going to use it in, I think here we really should keep an open mind about what the mechanism of action is and what the intended clinical niche is and not decide that -- it may be that passive immunotherapy isn't going to work, but if somebody showed me a vaccine that would forestall the need for therapy for 15 years, I would love it too.

CHAIRMAN MASUR: All right. Let's have

the last comment from Ram. Then either Bill, Karen or Jay can let us know if they want more clarity before we move on to issue 2.

DR. YOGEV: I for one would like to see study stock in a population where the immune system is less (inaudible). We are asking too much (inaudible). As we know today, we don't have this great one. So I would like to see a continuation of in effect, for example, all antiretroviral therapy. And I would suggest -- it is surprising to me that we really agree that triple therapy is the right thing to do when a lot of data suggests that at least 25 of the population do very well with dual therapy. Many issues apply to this group because of the toxicity, compromising work happening in real life. Identify those which are working and then put them against -- on dual therapy with an ADT to see an elongation of effect. I also think the study should be much longer because we see -- and we learned the hard way that viral load, we need to wait -- if you recall it was 8 weeks, 12 weeks, 15 -- I would say 20 or more. Just recently we did a drug that is still doing well at 24 to find out at 48 it is not good. The immune system is so much lagging in its response, that we need at least to have before we even start

saying it is good somewhere around a year or 48 weeks before we can even say it. Because then to go to less than 50 immunity to start the ABT and ask for a longer one before you go. And then even longer to see if it continues. But I think the salvage will kill potentially too many potentially good weak ones because the immune system is already overburdened.

CHAIRMAN MASUR: That is an important. I would rather not get into the issue about dual therapy right now because I know that there are some members of the panel that could probably debate that for hours. A quick comment from Courtney and Fred and then let's -- or, Mike, did you have a quick comment?

DR. FLETCHER: Just in terms of trial design. I really pick up on a comment that Dr. Siegel made this morning about pharmacokinetics not being exactly as useful for these immune-based therapies as they perhaps have been for antiviral drugs. I think what it means is in the Phase I/Phase II environment in terms of trial design, there is going to have to be particular attention paid to if you are going to use let's say viral load as a surrogate marker, to developing a quantitative understanding between the dose or

doses of this therapy and that reduction in that surrogate marker so that you can have a rational framework for then what you are going to study when you get into your pivotal trial. The caveat here is a drug in which you can demonstrate a 28-day half-life for example may not at all be able to be used on a once monthly basis. So I think in terms of trial design, that Phase I/Phase II area to develop the doses for the Phase II/Phase III area is going to be particularly important.

CHAIRMAN MASUR: That is an important issue. I would presume that also one has to be very careful about what the effect of immune-based therapy is on the kinetics of traditionally retrovirals and that we not overlook that.

DR. FLETCHER: I think that is exactly right. If you have the possibility of affecting any of the major organ systems involved in clearance, you really are going to have to know that and that really needs to be done early on in the Phase I/Phase II and not wait until some surprise comes up during Phase III.

CHAIRMAN MASUR: Fred? Last comment?

DR. VALENTINE: Four types of study designs. Patients who are early in their disease, as Trip suggests, who we don't feel we have to

treat. Secondly, time to virologic relapse in patients who are on current therapy. Third would be people who are on therapy, who received a vaccine, for example, stopped therapy. And the fourth would be people who are incompletely suppressed in spite of everybody's best efforts but who have a sufficient viral load in which the immune system might suppress but whose immune system is sufficiently intact that they might respond. I think there is a real question as to what type of virologic endpoint and what cutoff you put into each of those study designs, however. We don't have any idea, at least I don't, as to what level of viral load the immune system might be expected to control, and somebody else earlier eluded to that as well. Certainly the initiation of an immune-based therapy faces the same dilemmas, as someone pointed out, that the initiation of a new antiretroviral therapy does, if you are giving it in the context of current potent therapy in the sense that you are trying to fish out an additional increment of benefit in people who may be doing fairly well and that is why the time to virologic relapse or stopping therapy might be particularly appealing.

CHAIRMAN MASUR: All right. Well, thank

you for those concrete recommendations. Bill, Karen or Jay, do you want to make some final comments here before we go on to number 2?

DR. SIEGEL: Well, I would just like a quick clarification on that issue. I guess we heard much of the committee comment on the -- what is it, the Schooley hypothesis -- that sustained significant viral reduction in the context of considering toxicity and other aspects could well be likely to predict benefit. And I guess perhaps it is fair to say somewhat more mixed comments about what we know or don't know about measuring viral levels during treatment interruption and what that might mean.

One of the other areas though under B in talking about how we look at viral levels is the one Dr. Valentine just mentioned, and it has really only to my count been commented by three people, but is one that there is a lot of interest in looking at, which is taking people who have good virologic control on HAART, adding on an immune-based therapy and looking at the time to relapse to recurrence of virus or whatever. Is there a general consensus that that is also a rather useful virological measure likely to be predictive of clinical benefit with the various caveats that we

have given regarding lowering virus load?

CHAIRMAN MASUR: Well, I guess whether one could be confident that it is likely to be predictive. I guess I'd be a little hesitant, but I saw Chip raising his hand.

DR. SCHOOLEY: I didn't mean to buy the pony. I just moved my fingers.

CHAIRMAN MASUR: I thought that was all the energy you had left.

DR. SCHOOLEY: That is right. I think if the premise again is that your intervention is going to have an antiviral effect and by doing so will delay the time to relapse, it is likely also to be able to be demonstrated to have an antiviral effect in the dynamic range you can measure it. So I don't see those necessarily being disassociated.

CHAIRMAN MASUR: No. The question is will that be beneficial in the long term -- will that predict benefit in the long term.

DR. SCHOOLEY: Well, I guess I would say one would hope so. But I have less certainty about that than I do with antiviral drugs. The reason I think it is is that in general if you are at a point in your disease in which one feels that antiviral therapy is indicated with all the caveats that we have heard before, the longer one can go on

regimens without burning through a bunch of drugs, the more likely you are to have options available later. So increasing durability in the overall life of the patient I would see as a good thing. Now that is something that take a long time to demonstrate with clinical events, which is the good news today, and will take longer and longer as new agents come along. So I think it is plausible. If you in fact are seeing more durable response, you should also be able to demonstrate an antiviral effect in people who have a dynamic range in which you can measure it.

CHAIRMAN MASUR: Let's see if there are one or two more comments. Nancy has already put up issue 2. Maybe we will start with Bob Redfield and go around from there on issue 2 after we have our last comment there.

DR. SAAG: Well, I just wanted to say regarding that last comment -- maybe Bob said it exactly right. If the trend continues, and that is a big if -- but if it does, it is not hard to imagine a year from now -- if safety of stopping therapy in certain patients is demonstrated and it becomes the standard, then the ability to show that some other intervention can prolong that time of durable suppression off therapy buys more time for

a patient without exposure to certain drug toxicities, I think that would be a benefit and I think that would be a role for immune-based approaches in the future.

CHAIRMAN MASUR: Would that be enough to get you to vote in favor of an accelerated approval if that was the endpoint that was shown?

DR. SAAG: If it were a significant difference. And now you are going to ask me what is that.

CHAIRMAN MASUR: No. I won't pin you down that much. So now we are at issue 2. The last three lines there, please discuss the potential utility of specific tests for specific types of immune-based therapeutic intervention, including approaches to facilitate selection and/or validation of such measures. So, Bob, you are in the hot seat.

DR. REDFIELD: Well, I think I would echo both what Cliff brought up and what Mike Lederman brought up earlier today. I think if we are going to start, and I am obviously an advocate of looking how to assess the immune function in the setting of HIV infection, I think initially we need to do that in the context of functional in vivo immune function. And in that regard, from a

clinical perspective, I would say that the best way to do that is to assess the ability of the host to respond to a novel or a recall antigen in the form of immunization. And probably secondarily would be the ability to recall antigens in the form of a functional delayed hypersensitivity skin test. Because again to try to develop these strategies originally that are thought to have some clinical relevance, and I think that is the real issue here, I think we are fairly restricted at this point. I mean, in terms of what functional human immunity is. So I would be an advocate of trying to standardize and assess the ability to determine whether the human host is functionally immune competent and to try to define that in the context of their ability to respond to a novel and a recall antigen. I think that is what Cliff said. I think that is what Mike Lederman said. They may want to comment themselves. But I think that is where I would come off at this point. And then validate over time, and it may be a more accelerated way to assess that, which would then alleviate the necessity to go through some type of active immunization process with a known or several known antigens to determine functional immunity.

CHAIRMAN MASUR: All right. Now are

you referring to trying to assess biologic plausibility or developing a reproducible surrogate that predict favorable outcome?

DR. REDFIELD: Well, in terms of -- you mean initially? I think at this point to determine whether or not whatever the intervention modifies the functionality of the human immune system. So it would be the biologic plausibility to determine whether there truly is a clinical modulation or clinically potentially relevant modulation of the human immune system from unable to respond to an immunogen to being able to respond to an immunogen. Because the issue really is going to come down to what Tom asked before in terms of the antiviral, what is relevant. And I think I want to start there and say if I am going to start there with what is relevant, it is going to be

-- you know, anergy is relevant and the lack of anergy is relevant. I think there is clinical precedence for that. The ability to respond to an immunogen and the ability not to respond to an immunogen I think is the way to assess in vivo function. So I think that is where I would start and then try to build these other functional assays that we have heard about and validate in the context of having demonstrated functional

relevance.

CHAIRMAN MASUR: Okay. Doug?

DR. FISH: Well, certainly I think what we need is something that is simple and something that we can do in the clinic that is relatively reproducible and has a reasonable cost. Those would be things that come to mind in terms of design. I would agree with Dr. Redfield in terms of looking at DTH, because that is something that we have experience with and can readily be done and measured.

The other thing that I am intrigued by is the lymphocyte proliferation assays, and specifically I am thinking of Dr. Walker and Dr. Rosenberg's presentations at IDSA at their assays which they I think now have what they said was down to 24 hours. The problem is the length of time it takes to do some of these assays. But a stimulation index looking at HIV specific immunity. A concept like that that if it could be done on a large scale and proves to be valid would have great utility. It is a long ways from that, but it is a concept that I think from the clinician's standpoint is relatively easy to understand in this complicated field.

CHAIRMAN MASUR: Okay. Thank you.

David?

DR. PARENTI: I think that obviously there are lots of problems with standardization and validation of the different assays. We don't have answers for the in vitro assays which antigens are best for stimulation, et cetera. I think that Dr. Redfield's comments about using immunization and DTH as markers for immune response are important ones as well.

I think from the standpoint of someone who does clinical trials in this area, assays that we can do in the field that either require less preparation in terms of separation of cells, et cetera, are ones that will be more feasible -- particularly the Phase III clinical trials as you move into that stage of development.

CHAIRMAN MASUR: Okay. Thanks. Chip?

DR. SCHOOLEY: I guess I would try to divide my comments into two different areas. One is when your immune-based therapy is supposed to affect a parameter that we already are using in part to grant either approval or accelerated approval, i.e. CD4 cell elevation. So if you have adopted immunotherapy with CD4 cells or a cytokine that is supposed to raise CD4 levels, it would be one category. The other would be when you are

trying to stimulate some other aspect of immune response that we don't have any experience with at all in terms of its clinical implications. Proliferative responses to toxoplasma antigens and so forth.

I think in terms of the former, in some ways what I said earlier about antiviral therapy holds, and that is you have to look at it in the context of the overall effect of the intervention, realizing that there certainly could be counterbalancing effects that could negate or even make the CD4 cell rise in the context of other things that happened not beneficial or detrimental to the host. And I think we are on less firm ground here in being able to extrapolate than we are in the inverse situation with viral changes.

The other parameters -- I think if one is looking, for example, at an agent that would enhance toxo-specific immunity, I think it is easy to do Phase I/II studies to see if you are doing that. To decide whether it should be an approved product, I think you would have to show that you prevented the occurrence of toxo as opposed to just manipulated immunity just like you do with other vaccines that are available in other settings. There are a number of assays that have already been

assessed in terms of Type 0 and Type 1 markers in those settings. Jerry Quinin showed 15 years ago that CMV specific CTL activity in the context of renal transplantation is a very good predictor of who is going to get CMV disease post transplant. And those sorts of assays I think give us a very nice early look at the biological activity of these interventions, but the clinical in fact still has to be demonstrated once you have, I think, demonstrated the biological activity.

CHAIRMAN MASUR: So in other words demonstrating that it correlates doesn't necessarily mean that if you alter it with some kind of therapy you will benefit the patient?

DR. SCHOOLEY: I think that is right. It is a lot like -- in developing some of these products, you want to establish what you think you are doing and establish a series of milestones. And if you don't achieve them -- if you don't affect CTL activity, for example, and that is your premise that you are trying to affect, then you shouldn't proceed until you have a way to do that. Once you have done that, then you can move to the next stage. I think sometimes we set these parameters up and then when you don't see something, you find something else like, well gee, there is an

elevation in IL-27 and that must be good for you. Now we ought to go ahead. So I think that that kind of fuzzy thinking gets you in trouble as well.

CHAIRMAN MASUR: Okay. Chris?

MS. LEIN: Can I ask Chip a question?

CHAIRMAN MASUR: Yes.

MS. LEIN: So do you think that -- you know, when you were talking about pathogen specific immune based therapies that may alter responses, is there a space in there where you think accelerated approval is appropriate?

DR. SCHOOLEY: Yes, I do. I think that we now are in a series of niches as opposed to a global sort of comment, and one of the problems we have is the event rate with any specific opportunistic pathogen is so low right now that you'd be treating many, many, many patients with almost any of these to prevent a specific infection. So let me turn it around to you. Which specific infection would you like to target just for argument sake?

MS. LEIN: Say CMV.

DR. SCHOOLEY: CMV. Okay. You know CMV is something that we still see, but it has been very difficult to even accrue patients to trials -- therapeutic trials of CMV. Would I approve

something -- a CMV vaccine, for example, that decreased CMV anergenemia? Until we establish that say anergenemia is a predictor of disease, I would probably want to apply the same standards to that that I do an antiviral intervention like gancyclovere, realizing of course the risk/benefit ratio may be quite different. But I am not going to say I can't envision any scenario, but I just think we have to think very carefully about which patients we are trying to benefit and how many people we have to treat to have that benefit given the rarity of the individual events.

CHAIRMAN MASUR: So, for instance, for CD4 counts, assuming there were no red flags about function distribution, you would be more sanguine about then anything else that we have discussed this morning?

DR. SCHOOLEY: I think we know more about that. Again, I think I have more concern about that than the inverse from this morning from question 1. But I feel more comfortable about that than looking at intercellular cytoplasm staining of interferon gamma when you expose peripheral blood cells to CMV antigen, for example. That is just my own conservatism here, I guess.

CHAIRMAN MASUR: Okay. Chris?

DR. MATHEWS: I don't have any comments about the specific assays, but more on validation issues. The first part is that -- well, a comment was made this morning that none of the available therapies that we have have produced complete immune reconstitution. From where we are right now, I don't think that we should -- unless a particular therapy has promised to restore HIV specific immunity that would ultimately lead to eradication or long-term control, complete immune restoration is probably not important. I think most patients would be satisfied without being sick. And so this relates to what should be the endpoint for validation of any particular marker or assay.

And secondly, I think that the paradigm for endpoints to validate markers against is changing, and I am actually not sure what the endpoints should be. I mean, we have already talked about how it used to be opportunistic events and mortality, and in general what we assumed previously was mortality meant HIV-related mortality. However, I think now we are faced with a situation that the rising mortality rates that we are seeing are increasingly not historical HIV endpoints, and so we should be looking more at all cause mortality and all cause morbidity. And the --

I think that that is going to force us to start looking at patterns of response as opposed to what we have traditionally done, which is look at average responses in clinical trials -- the proportion non-detectable, the percent with a particular CD4 rise and so on. I think increasingly we need to ask questions about what proportion have a particular pattern of response using multiple markers and what is the prognostic implication of a particular pattern of marker responses involving both biologic markers and immune-based markers in terms of subsequent clinical endpoints which are now loaded with a whole variety of events, not just CDC category B or C events.

With regard to validation of the newer markers, the immune-based markers, I think the same rigor has to be used as was used to validate the virologic markers, except it is going to be much more difficult because you may get to the situation where you have to trade off. In other words, what proportion of a clinical benefit is attributable to -- can be explained through an immune-based marker chain versus a biologic marker change, and if there is more toxicity attached to getting a particular magnitude of virologic change, can you trade that off by using changes in an immune-based marker to

get the same degree of clinical benefit.

So I think that the validation issues for clinical benefit are really much more complex than they were when we had only antiviral therapies with limited toxicity and shorter life expectancy for patients.

CHAIRMAN MASUR: Well it certainly is more complex. I guess that gets back to the overall risk/benefit ratio for any of these interventions. Ram?

DR. YOGEV: Taking into account the degree of CD4 and viral load as a marker, I think we have to admit we don't have a good marker except for clinical endpoint and we should go back to what we have been when we started antiretroviral and go to a clinical endpoint. And as a suggestion, for example, if it is true that 40 or 50 percent of the population stop therapy and are looking for structured interruption, maybe a Phase I/II should be done in this type of population to see if you add this whatever immune model that is tested, does it make any difference, for example, in the timing of viral load coming back and the height of viral load coming back on the population. And then when you identify some clinical parameter working, that is the one you should continue validating

obviously. Whatever they ask for is working. I, for one, would be a little bit less conservative. If they find out that IL-27 is there elevated and they do show the difference, it is a nice marker to follow. But I think we have to go into the clinical endpoint that we can get faster in a certain population before we go to other populations that are much harder. But I would not prepare any test today on immunologic that would tell me if it is elevated that is okay.

CHAIRMAN MASUR: We wish we had such a thing. Courtney?

DR. FLETCHER: Just a quick comment that I think kind of picks up on Chris's on validation and that is for these tests, I would certainly look for a test that will best discriminate the effects of the drug. So if, for example, you were going to look at your therapy from a no-effect dose to one that produced the maximal effect possible, I would look for one that can discriminate those responses to that drug.

CHAIRMAN MASUR: Okay. Good point. Mike?

DR. SAAG: This morning I was at the meeting of the Acute Infection Research Network and there was an initial discussion of immunology and

immunologic responses where somebody who was new to the field went to the microphone and apologized for being new to the field saying that if his question sounded naive, he apologized but he hadn't really kept up with the field that much over time. And which Dr. Fauci ran to the microphone and said, "Don't worry, you haven't missed much." So I think the point is that we don't really know that much and I think that makes us obliged to just sort of keep an open mind. I think it is all going to have to be done individually period. There is no way that we can proscribe an answer to this question without knowing the specifics of what is there. But I think things will develop over the next three to five years that will be quite interesting.

CHAIRMAN MASUR: Well certainly question 2 is harder to pin everybody down, but I guess for good reason. Tom?

DR. FLEMING: I see question 2 as being particularly critical. It starts off by recognizing that what we really want to target here are biomarkers that will be -- that have several properties. The first that is listed is sensitivity to the drug effect. And I go back to I think it was Chris's earlier comments that with the array of interventions that we are looking at right now in

immune-based therapy, in high likelihood it is going to be an immunologic outcome as opposed to a virologic outcome that will be most directly sensitive. And so we are faced then in all likelihood with the challenge of understanding how to proceed when we are looking at immune-based therapies when the most sensitive measure is going to be an immunologic outcome. And that then leads us to, as the question appropriately reflects, understanding the reliability of the measurements and very critically the relevance of the measurements to the pathophysiology of the disease. And this is complicated by the myriad of different measures and the myriad of different time frames.

I go back to one of the comments in the open session was recognizing the urgency here. And as I see it, the urgency should motivate us toward a strategy of good science and good science involves quality clinical trials. And our urgency then should be to ensure that we are following a pathway that will obtain reliable answers in as efficient and timely way as possible.

So as I think through this strategy, the first step -- you have to walk before you can run. And as I see the first step is to rely on basic science and empiric research to help us

identify Type 0 markers or at least biologic measures that are likely to be sensitive to the intended mechanisms of action of the intervention but also correlated with clinical endpoints, so it is at least plausible that achieving these effects will be a good signal that we may well be achieving clinically meaningful endpoints. And that should then lead us and that should be the motivation to then assessing the effects on these biomarkers in Phase I and II trials. And with those Phase I and II trials that yield encouraging results, I hope we would aggressively pursue Phase III trials. I don't know that we have always done so. Some of us have been frustrated, for example, in the HIV vaccine for prevention arena at the reluctance to move into Phase III trials without a more high level of certainty from basic science as to what the effects are on the intended mechanisms of action, humoral and cell mediated immune responses. And in my view, we need a balance of basic science and empiric research to have those best insights, and that means we have to be aggressive at points to move into Phase III trials.

Now here is the tough question. What are the endpoints in Phase III trials? Is it adequate in those Phase III trials to simply

address effects on these targeted immunologic mechanisms of action? And that is where unfortunately, because the answer yes would give us a much more timely answer to the overall process, I don't see that we have the data at this point to be able to reliably state that an effect on an immunologic outcome is going to reliably predict an effect on a clinical endpoint. So I see that the Phase III trials at this point, if we are targeting immunologic outcomes, must be designed in ways that it provides us some direct evidence on clinical outcomes. And the comment was made early about all cause mortality and all cause morbidity, and I would second the thought that any clinical endpoint should incorporate all of those consequences of the disease process as well as consequences of the interventions used to address the disease process. So that definitely means that those are endpoints that go beyond simply an HIV infection specific related outcome. It may be in fact that one of the best things that we can do is sustain the effects that we have with current therapy but reduce important morbidities associated with those therapies. And that reflects the fact that the outcomes here are more comprehensive.

So essentially in closing, I would

argue that -- I would argue for an aggressive strategy of moving promising interventions through Phase I and II and into Phase III trials, but at this point in time that experience in Phase III trials needs to provide direct evidence about what the effects on these immunologic outcomes is reliably telling us about effects on clinical endpoints.

CHAIRMAN MASUR: I think we have heard from a number of people of the importance of looking at comprehensive benefit and comprehensive risk. Trip?

DR. GULICK: Well, thinking about this from the clinical trials point of view, the presentations this morning the clearest thing I think that was said repeatedly was the need to validate clinically these immunologic endpoints. But I was both impressed and encouraged by a number of the presentations this morning. The array of markers that people are looking at today that we heard so much about. The efforts to standardize assays across many different laboratories that have been going on for the past several years. The ability to perform some of these immunologic assays, not just on fresh specimens but actually on stored cells I think lends itself to take the next

step, which is to go ahead and validate either using cohort-based studies like John Mellors did with the viral load test so long ago, or clinical trials-based efforts. And I think the ACTG and other groups are doing this now. So perhaps one of the byproducts of a meeting like this is really to focus the energy on this particular issue. And I guess I was encouraged by hearing what is going on in the field.

CHAIRMAN MASUR: Fred?

DR. VALENTINE: My thoughts in this are really based on the clonal organization of the immune system really. Because different clones, as everybody knows, respond to different epitopes and different antigens and different pathogens, there has been considerable anxiety, I think, as to how to evaluate the ability of an immune-based therapy to just increase globally CD4 cell numbers. And clearly we know from the antiretroviral therapy that among those CD4 cells that increase in that context, why certainly there are cells that protect you from getting OI's, because that is how we are seeing a clinical benefit.

So how then can we ferret out and look other than by the clinical endpoint studies that Tom points out would be really the convincing way

to do it but yet are so difficult to do. And there may be a way that we could at least begin to approach this. And there are two things that come to mind. One of them Cliff Lane emphasized or has been emphasizing for the past few years, to look at the repertoire itself by perhaps even molecular biologic techniques to see if you can get the same distribution, particularly in the naive cells is where you would want to see it, of T cell receptors that you would anticipate seeing in a normal person of the same age. Now I can't implement this into what experiments should be done now, but that is a way of assessing the breadth of clones present as opposed to just the total number of cells. I mean using the sports analogy, it is not just the number of players on the field that determines the outcome of the game, but rather how well they play and what they are trained to do and so forth. And I think the same thing applies to CD4 cells.

Now another way is to look at responses to specific pathogens, and Chip alluded to this in his remarks. There are studies underway, and there were two posters at the recent retrovirus meeting or the one this past January, that look at lymphocyte proliferative responses to specific pathogen OI's -- or antigen-specific opportunistic

pathogens. There is substantial literature to suggest that the lymphocyte proliferative response does correlate with delayed type hypersensitivity and there is a much longer literature to suggest that the presence of delayed type hypersensitivity in fact correlates with some level of immunologic control against that particular pathogen.

The two posters at the February retrovirus meeting each described two patients, or maybe one of them was three patients, in whom an opportunistic infection occurred at surprisingly high CD4 cell level, simply pointing out that there are occasional, very rare individuals, who do get an OI. In each of those cases when the patients were examined, they did not in fact have a proliferative response to that particular pathogen, but they did to many, many other pathogens. It was a little surprising in a sense because you would guess that some clones might be present against some epitopes in that pathogen, but it seemed to be a pathogen-specific death. Perhaps for some of these -- three of the patients were CMVs and the other one was PCP. That perhaps for some of these a given individual may have relatively few clones that do recognize an epitope in that pathogen.

There is an ACTG study that somebody

else referred to -- Chip, you may remember all these numbers. I can't remember 50-something or other. Which in fact is attempting to evaluate this by looking at cells frozen away and then looking for the rare individual who develops surprising OI and seeing if they have this functional response. But I think clearly the way to go is lymphocyte function. Because the development of function that is associated in other context with protection against the pathogen does not necessarily appear in everybody at the same time with rising CD4 cells.

And one of the many functional measurements of lymphocyte function, particularly of memory function, CD4 memory function that we were presented this morning, might be a way to assess the completeness of an increase in CD4 cells so far as their ability to recognize specific pathogens. You still might well have holes in the repertoire of the sort that Cliff Lane has been emphasizing for the last two years.

CHAIRMAN MASUR: Okay. Thank you for those comments. Brenda?

MS. LEIN: Yes. You know I think when I look at this page, the first thing I think of is that the patient population desperately needs a

validated marker of immune function to help them themselves figure out how to guide their therapy decisions more wisely. Something more than CD4 and viral load is really critically needed for the patient and clinician communities. So that may or may not have something to do with drug discovery or with immune-based therapy development.

I think that it would certainly help it along. But as Michael said, even if CD4 cell counts were shown to explain IL-2, that wouldn't necessarily make CD4 a validated surrogate marker for other immune-based therapies. So I don't know that one answer is going to answer everything.

But I agree with Fred intensely that we need markers of immune function and DTH has been really validated, although Debbie Burkes would say that in order to incorporate the use of DTH in that setting, it took thousands and thousands of dollars of training and she wasn't so sure it was worth it. But looking at some of these newer assays, when we say which assays need to be moved forward, something that would really measure antigen-specific responses with technologies that are widely available like flow cytometry I think is the most practical thing in front of us. Something that is more widely accessible that could be

utilized in clinics and multi-center types of settings. And along those types of lines for what needs to be looked at and developed. But I think that in keeping the bar similar between antivirals, I keep thinking of the criteria for approval of ddi and ddc, and say, oh so then we need 10 T cell counts and no viral load data and we can approve the drug because that is what those drugs were approved on.

And I know there is more information available today, but I think that the bar that we are creating for some of the immune-based therapy studies are way too high. And while I agree that we need to have clinical endpoint data to validate the approval of the potential immune-based therapies on the table, I also think that we have to be talking about an interim criteria for accelerated approval.

I am thinking about some of the approaches that are on the table and if we see a CD4 cell increase, if by all measures that we can look at that these cells look functional, at what point do we give an accelerated approval in recognition of the urgency of the epidemic and the need of people and then have reasonable criteria for long-term follow-up to validate those endpoints with clinical endpoints. I agree that we need

clinical endpoints, but I think that we also need accelerated approval endpoints -- discussions of what would be acceptable, what constellation of criteria would be acceptable. And I think that would really need to include some unvalidated immune function markers.

CHAIRMAN MASUR: Well, do any -- I think that obviously is the crux of the issue is whether or not, for instance, CD4 cells would be an adequate marker for accelerated approval in a situation where there is biologic plausibility and no red flags. We have had a little difficulty coming up with other specific tests. The question asked were there other specific tests. Do any of the invited speakers in the front row want to make a quick comment? Michael, Alan, Larry?

DR. LEDERMAN: You know, I think that the likelihood that we are going to have a highly active immune-based therapy that will enhance immune functions in a general way is greater in the near to immediate future than that we will have an immune-based therapy that will specifically target and enhance HIV-specific immune responses.

So if I had to focus my energy on something now, I would focus that on giving some consideration as to what it is going to take to

help develop and make next steps in terms of developing these agents. That said, how much immune competence is enough is really not clear. And I think Chris made the point that he is not really sure how much immunologic enhancement you really need in order to have a long life. I don't think any of us really know. I think clearly even a little bit of a blip seems to be enough to get people through the night in terms of protecting them from opportunistic infections. But whether or not you can go on for 15, 20 or 30 years with subclinical immune deficiency at this degree isn't really certain. That said, there are all these folks who don't really rise very much and folks in whom the prognosis, even in terms of opportunistic infection outcomes, as Mike pointed out, is pretty poor in terms of people who don't get a CD4 rise.

So what I would like to see happen at the end of this session is that we have some sort of sense as to where we can go to help develop or at least decide upon what kinds of studies or what kinds of immunologic assays are going to be the most likely ones that will give us some sense or some reflection as to the general overall immune competence of the host. And we have heard a whole bunch of assays presented, but I think it would be

really nice to get some sort of focus direction and some suggestions from this group.

CHAIRMAN MASUR: One of the -- I guess one of the difficult problems is that the FDA needs to establish some working rules, even if they are known only to them, about how to proceed with these drugs. And we won't ask them what those rules are, but I guess this is what we are struggling with. It is very easy to give generalities. The question is how do you then come up with specifics that are reasonable. But, Alan, maybe you have an answer to that.

DR. LANDAY: Well, I think in my last slide this morning I kind of summarized the in vivo and in vitro correlates of immune function, which we have heard a lot about. I would agree that DTH responses, either through the immunization or skin test responses both can be used and the in vitro responses that look at an integrated view of the antigen presenting cells, CD4 and CD8. I think those assays which we do have measurements for, we can integrate them and develop them. I think they can be useful, at least initial paradigms to try to define mechanisms of action and move towards the question of whether or not these are going to be eventual correlates.

And I would agree with Brenda that we need to move quickly to understand if we can impact immune function. Are these ways of doing it? Are these going to be effective ways? And then look for the clinical outcome measures. Because I have been sitting here and struggling with this since I started working in HIV clinical trials for over ten years and we are still no farther along in validating, as you saw, because I have worked with Donna Mildvan and Jon Kagan on that list and helped put all those question marks with Donna. She called me and I had said to her, you know I do this in my daily life and here is all the question marks that we still don't have an answer for. So I hope that the Advisory Group and the FDA can at least put forth perhaps to the industry folks here the challenge to try to fill in those points with things that could help us move that field ahead. And I think that would be a valuable contribution of today's efforts. So I could come back next time without the question marks.

CHAIRMAN MASUR: I think on behalf of the committee, I think probably appreciate the fact that there has been a very useful discussion and presentations in the morning. Brenda, before we conclude, do you want to have a final comment?

MS. LEIN: Yes. You know one of the things that the Agency could do to help facilitate selection and the validations of markers is really I think the biggest problems are resources coordination and collaboration, and collaboration including collaboration from industry and perhaps providing incentive. And I don't just mean industry developing IBTs, but industry developing antivirals. To share the samples so that even the assays could be run.

And, Bill, I know when we were talking last year, you had talked about models that the industry had worked in the context of other diseases in really playing a central role in helping coordinate the validation of certain surrogates in other diseases and exploring the possibilities of similar types of things in the context of HIV may be extraordinarily helpful.

CHAIRMAN MASUR: Well, I think with that, Bill, Karen and Jay, we turn it over to you to -- either for your final comments or to ask us for more clarification, which you may or may not get.

DR. SCHWIETERMAN: Let me just address the comment that Brenda made. I agree with you entirely, Brenda, that this is an important

measure. We did discuss this a year-and-a-half ago. The Agency is in fact -- it has a unique -- it is in a unique position oftentimes to do the sorts of things that you've mentioned -- coordinate and foster collaborations and so forth. And I am not exactly sure tangibly here now today how we can effect that. But I will say that I believe that this field is on -- is very much on the verge of some sort of a central body or some sort of a central organization of the sort that you described. And perhaps the Agency could participate in something like that. So we are definitely open-minded about that.

I guess, Dr. Masur, if I could just get to -- are there specific -- along these lines, are there specific sorts of recommendations or guidance or thoughts or perspectives on this issue of collaboration and coordination and sharing of information that this committee has? Perhaps it is too general a piece of advice to ask the committee. But on the other hand, it might nevertheless be helpful to hear what the perspective of this group is as to how the Agency can advise sponsors or whether there is a role for other organizations to take the lead here. It is a bit unusual to do this, but on the other hand this is an unusual

situation.

CHAIRMAN MASUR: I am sorry, take the lead in terms of proposing criteria for approval or to propose study of biologic markers?

DR. SCHWIETERMAN: Studies of biologic markers.

CHAIRMAN MASUR: Well, I open that up to anyone. I mean, I would assume that there are many groups that are certainly heavily involved in that. But, Chip, what --

DR. SCHOOLEY: One of the things that the ACTG has been doing is developing a library of cells and plasma from people in a longitudinal cohort, the so called ALLRT study and that will be linked to clinical events that we hope can both prospectively and retrospectively be used for this specific sort of analysis where case control studies can be put together with low frequency clinical events to let you get to the heart of the matter quickly. So if someone came and said we have an assay we think might be predictive of disease X, we would like to be able to collaborate to use this kind of a sample base to try to explore that without having to start out and recruit 6,000 patients and following them for 7 years.

So I think to the extent that people

come to you with diagnostics, certainly feel free to send them our way and we can tell them at least what we have and whether we have things we think would help.

CHAIRMAN MASUR: Other comments? Mike?

DR. SAAG: Yes, I would echo that. Not only the ACTG, but there are a number of large cohorts that have been established over the last several years that you could perhaps link with. I think that is how MACS ended up getting the data on viral load and I think that was the catalyst to getting viral load appreciated as a meaningful marker. I think the same thing would be true in this situation. So I think that would be one sort of common theme.

CHAIRMAN MASUR: Other comments? All right. Well let me turn this back over then to Bill and Karen and Jay.

DR. SCHWIETERMAN: I would just like to thank the committee and thank the speakers for what I think has been a most informative and I believe productive discussion on a complex issue that isn't easily addressed in a single day. I have been frankly impressed with the degree to which we have been able to clarify the issues and I believe make more transparent, if not completely clear, some of

the hurdles and challenges that we all face. So to the extent that we have done that, I think we have met some of our central objectives. So thank you very much.

CHAIRMAN MASUR: All right. And we look forward to biologics and virologics working together more in the future and perhaps we can have a follow-up on this after a period of time to see what progress we have made. So thanks very much to the audience and to our guests and consultants as well as our committee members.

(Whereupon, at 4:22 p.m., the meeting was concluded.)