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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

ANTIVIRAL DRUGS ADVISORY COMMITTEE MEETING

VOLUME II

Tuesday, November 2, 1999

8:30 a.m.

Holiday Inn Gaithersburg
Two Montgomery Village Avenue
Gaithersburg, Maryland

MILLER REPORTING COMPANY, INC.
507 C Street, N.E.
Washington, D.C. 20002
(202) 546-

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P R O C E E D I N G S

Call to Order

DR. HAMMER: Good morning. I would like to open this session of the Antiviral Drugs Advisory Committee Meeting. We are here today to discuss the application of resistance testing and its utility in the drug-development process. But this symposium over these next two days will cover a wide range of topics related to resistance testing and, really, tell us what the state of the art in the field is.

I am hoping this will have a fair impact on the field as previous meetings, for example, with respect to RNA did in the past. So I look forward to an interesting two days and commend the agency for bringing this meeting together.

I would like to start with introductions. Dr. Charache, please introduce yourself and your institution.

DR. CHARACHE: Patricia Charache. I am Professor of Pathology, Medicine and Oncology and, in Pathology, my primary appointment, I am the Program Director for Quality Assurance and Outcomes Research. In areas related to this meeting, I am Chair of the Microbiology Panel in the Center for Medical Devices. I am a member of CLIAC, the Clinical Laboratory Improvement Advisory Committee at CDC. And I am a member of the Secretary's Committee on Genetic Testing.

DR. FLETCHER: Courtney Fletcher from the University of Minnesota. I am Professor in the Department of Clinical Pharmacology at the College of Pharmacy.

DR. WOOLSON: Robert Woolson. I am Professor of Biostatistics at the University of Iowa.

DR. MATHEWS: Chris Mathews, Department of Medicine, U.C., San Diego.

DR. KUMAR: Princy Kumar, Georgetown University Medical Center.

DR. GULICK: Roy Gulick, Infectious Diseases, Cornell University.

DR. STANLEY: Sharilyn Stanley, Associate Commissioner of Communicable Diseases, Texas Department of Health.

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DR. MASUR: Henry Masur, Critical Center, NIH.

DR. HAMMER: Scott Hammer, Infectious Diseases, Columbia University.

MS. STOVER: Rhonda Stover, FDA.

DR. POMERANTZ: Roger Pomerantz, Infectious Diseases, Thomas Jefferson University.

DR. WONG: Brian Wong, Veteran's Hospital in West

Haven Connecticut and Yale University School of Medicine.

DR. JACKSON: Brooks Jackson, Professor of Pathology, Johns Hopkins University.

DR. PETTINELLI: Carla Pettinelli, Division of Aids, National Institute of Health.

DR. KAPLAN: John Kaplan. I am from the Center of Disease Control and Prevention where some things that I do there--one hat I wear is to try to coordinate activities we have related to HIV resistance and, specifically, an interest in surveillance in antiretrovirally naive persons.

DR. MAYERS: Doug Mayers, Infectious Diseases, Henry Ford Hospital.

DR. ARAS: Girish Aras, Team Leader, Division of Biometrics, CDER, FDA.

DR. IACONO-CONNORS: Lauren Iacono-Connors, FDA.

DR. RHOADS: Joanne Rhoads, Medical Officer, FDA.

DR. MURRAY: Jeff Murray, FDA.

DR. JOLSON: Heidi Jolson, FDA.

DR. HAMMER: Thank you.

I will now turn the meeting over to Rhonda Stover who will read the conflict of interest statement.

Conflict of Interest Statement

MS. STOVER: 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

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the appearance of such at this meeting.

Since the committee's discussions of these issues relating to testing for development of resistance human immunodeficiency virus will not have a unique impact on any particular firm or product, but rather may have widespread implications with respect to an entire class of products, in accordance with 18 United States Code 208, general matters waivers have been granted to each member and consultant participating in the committee's discussions.

A copy of these waiver statements may be obtained by submitting a written request to the FDA's Freedom of Information Office, Room 12A30 of the Parklawn Building. In the event that the discussions involve any products or firms not already on the agenda in which a 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 participants, we ask, in the interest of fairness, that they address any current or previous involvement with any firm whose products they may wish to comment upon.

DR. HAMMER: Thank you.

I would also like to extend my welcome to all the guest speakers that we will hear over the next two days and now I would like to turn to Heidi Jolson who will give the

FDA introduction.

FDA Introduction

DR. JOLSON: Good morning.

[Slide.]

I would like to welcome everyone here today to the first of a two-day meeting that we are really quite pleased to be sponsoring on the use of HIV resistance testing in drug development.

Before I even get into anything specific, I just want to mention, by way of housekeeping, that we have two overflow rooms so, for comfort, if people want to spread out, there are two other rooms that are down the hall and to the right that have monitors that folks can go to as well.

These were set up because we would hope that this would be the sort of meeting that would have a lot of broad interest through the community and through industry.

Our division is extremely excited to be sponsoring this meeting. The goal is to stimulate further development of the science of HIV-resistance testing with **a** focus on how resistance testing should be optimally incorporated throughout drug development.

Our ultimate goal in bringing forward this issue is to work towards and improve our understanding of how to better use therapeutics for HIV and how to translate this information into product labeling for clinicians. Toward

this objective, we are extremely pleased to welcome our guest consultants and also representatives from the NIH and CDC and all of our invited speakers the this week's meeting.

[Slide.]

In the next few moments, I would like to just set the stage for the two days of scientific discussion. In my comments, I would first like to contrast the implications of HIV drug-resistance development from the somewhat different perspective of individual patient management and overall drug development because the latter is really the focus of today's meeting.

I would also like to elaborate somewhat on our goals for this meeting and our expectations and provide a little background regarding how this meeting evolved.

This is a very complex subject. Everyone in this audience and on the committee is aware of that. You will notice that the agenda actually covers four pages. I think that that is probably a record for one of our advisory committee meetings.

Therefore, in my remarks, at least I will provide road map to the logic of the meeting which is organized into modules or sessions. That is a somewhat different format than our typical advisory-committee meetings.

[Slide.]

From the perspective of individual patient

management, the implications of HIV drug resistance are profound. Develop of resistance is a critical factor that undermines the safe and effective use of therapeutics for HIV. Resistance is one of several factors that may be the cause of treatment failure and, in the setting of resistance and cross-resistance, selection of new regimens becomes increasingly limited.

From a risk-benefit perspective, patients who continue medication to which their virus has developed resistance are at risk for drug-induced toxicity without a potential therapeutic benefit to appropriately balance this risk.

The potential for transmission of resistant virus raises further complicated patient-management questions regarding optimal strategies, the treatment of newly infected persons and prevention strategies in perinatal and occupational exposure settings particularly when contact with drug-resistant virus is suspected.

[Slide. 1

From the drug-resistance and regulatory perspectives, additional implications are raised. Our lack of a more complete understanding of HIV drug resistance complicates and impairs our ability to interpret clinical-trial results and limits our understanding of why patients respond or fail combination therapy in clinical trials.

Further, our ability to test new drugs in patients with the greatest need for new options is limited until we can make predictions regarding who is most likely to respond to an investigational agent on the basis of virus susceptibility.

Last, at the present time, we have a limited ability to provide advice on the optimal use of a new drug in product labeling because of our incomplete understanding of viral resistance development.

[Slide.]

Part of the agenda today is to develop a common understanding of the current constraints in our ability to utilize the emerging technologies. Some of the limitations are listed on the slide and include factors such as the diversity of genotypic and phenotypic testing methodologies and the uncertain relationships between different types of tests, the lack of approved, well-validated assays, the lack of uniform requirements for resistance characterization throughout drug development and into the postmarketing period, the lack of consensus regarding the clinical utility of prospective testing and questions about the interpretation of test results and definitions of resistance, and, last, how resistance testing may be impacted by a host of other biological factors.

[Slide.]

Although we acknowledge that the preceding lists an almost intimidating spectrum of hurdles, we strongly believe that this meeting, as a forum, may facilitate progress towards resolution of at least some of these issues. The first step towards these goals is the identification of what needs to be addressed and to make certain that we understand the limitations that are currently preventing the wider use of resistance testing and drug development.

On this slide, we sort of summarize some of the overall meeting goals which I will just briefly go through. But, first, we would like to define what is known or what needs further study regarding actual technologies. That would include the reliability of assays, how to interpret the results and what their strength is for predicting treatment outcome.

We would additionally like to discuss different approaches for defining resistance including mutational algorithms and break points that would be applicable now and in the future as new drugs are developed.

We would like to discuss approaches to standardized methods for analyzing data that the agency can use when it considers new drug applications. We would like to obtain the committee's guidance on use of resistance testing and drug development and we would like to discuss

what future initiatives should be undertaken to encourage progress.

It is really our expectation that this meeting today is the first step towards achieving those goals.

[Slide.]

I would like to say a few words about the evolution of this meeting. Because of the undeniable public-health importance of this issue, our Chair, Dr. Hammer, at least a year ago, encouraged the division to have this meeting as a proactive approach from the agency towards moving the field forward.

At a similar time that we began to consider the Eeasibility of this meeting and tackling these issues, we were also approached by a newly formed industry collaborative group that you will hear about today which was addressed to answer some of the issues that are in common with today's meeting and also some issues that are beyond the scope of today's meeting.

You will hear more about this group on the next slide. The agenda for this meeting was developed collaboratively within our division, with our chair, with the Resistance Collaborative Group and from feedback that was solicited by a Federal Register Notice that I will describe in a moment, because planning the agenda required, first, identifying the important issues.

Completing **the agenda** took many months and was a goal, in itself. In fact, the agenda for this meeting changed so many times, it was described within my division as a living document. But, hopefully, the agenda that is before you is the last iteration.

[Slide.]

When the division was initially approached about participating in the Resistance Collaborative Group, we stated that our participation was contingent upon well-diversified participation by many pharmaceutical companies and assay manufacturers. We believe that the general composition of this group reflects this diversity. Dr. Douglas Richman, who is Chairperson of the Research Collaborative Group and our first invited speaker, will speak more specifically on the composition of this group and its goals and other purposes.

[Slide.]

In addition to working with the Resistance Collaborative Group, we published a Federal Register Notice on August 23 both to announce this meeting and, also, to make a broader request for information and perspectives that would be relevant to designing today's meeting.

In this notice, specifically, we requested interested persons to provide to the agency data on the relationship of HIV mutation development and changes in

susceptibility either prospective or retrospective data on the relationship between genotype or phenotype and clinical outcome, proposals for the use of resistance testing in clinical trials and, also, proposals for how this information could be incorporated in product labeling to support labeling claims.

[Slide. 1

Just a word about the format for the next two days. As noted, this format is a departure from our usual structure of our advisory-committee meetings. We have planned this meeting more as a scientific workshop and, specifically, we do not anticipate that there will be focussed discussions on issues that are relevant only for specific products.

Additionally, there will be no issues that require voting from the committee. Because of the overwhelmingly broad nature of the topic, the meeting's design is modular. Each session has specific objectives. Each session has invited presentations from other academicians or from FDA reviewers that have been selected to provide relevant background to the committee's discussion of the questions that will be posed.

The common theme for each session is issue identification and how to achieve progress within that given area.

[Slide. 1

I will just briefly let you know about the sessions that we will be going through and a little bit of logic of how they are organized. The first session relates, really, to developing and understanding of the currently available technology to assess resistance. In the presentations this morning, we will cover general principles of resistance testing and an exploration of the performance characteristics of currently available genotypic and phenotypic assays with a commentary on their limitations, on quality-control issues that need to be addressed and on existing data to link genotype and phenotype.

We will also have discussion of the committee of potential roles of assays in drug development and we will hear an update from the Center of Biologics Evaluation and Research on their proposed approach to assay regulation based on an advisory committee meeting that was held in September of this year.

[Slide. 1

This afternoon's session relates to the clinical validation of these assays and will explore the predictive value of knowing baseline genotype or phenotype in treatment outcome. Towards this effort, the Resistance Collaborative Group has assembled a spectrum of studies that has been reanalyzed using a common data-analysis plan. This has been

really a major undertaking from this academic group and we want to thank them for their efforts in terms of reanalyzing the data for the committee's consideration today.

Additionally, you will hear the results of three prospective trials that have evaluated whether knowing genotype or phenotype at baseline is correlated with ultimate treatment outcome.

We will also discuss approaches for characterizing and categorizing and analyzing resistance patterns and we will identify additional clinical research to further define the clinical utility of resistance testing.

[Slide. 1

Tomorrow morning, we are going to devote some time to the practical considerations and feasibility issues of resistance testing. First, we will identify patient populations for whom resistance testing and drug development is most important and most useful and we will explore other factors to consider when resistance testing is incorporated into clinical trials.

To give you a preview, just a few of the factors that will be for discussion will be the complex biology of drug-resistant HIV, the issue of drug concentration, the timing of sampling of specimens, the issue of anatomical compartments and a host of other issues that the committee will be asked to consider as they relate to resistance

testing.

[Slide.]

Tomorrow afternoon, we will spend time devoted to discussing potential roles of resistance testing in drug development and we will specifically ask for guidance from the committee, both on in vitro and clinical data necessary to characterize resistance and cross-resistance potential as drugs come to market.

We will also obtain guidance on postmarketing evaluation of resistance and then we will ask you, in an informal, more interactive session for feedback on potential scenarios for the use of resistance testing to support regulatory claims.

I will just refer the committee, in the slide series, those scenarios are actually at the very last slide packet. We would ask you to take a look at them this evening so that you will be familiar with the scenarios which are just very generic. They are not product specific but they represent scenarios that either we have received through the docket or that sponsors have posed to us.

Tomorrow, we will ask you, in an informal session, some feedback on what sorts of evidence would be necessary to support those scenarios. That is our homework assignment for tonight.

[Slide.]

Last, I want to acknowledge many folks who worked on this meeting. This has been a very complex meeting to develop. First, I want to acknowledge the expertise on the Resistance Collaborative Group. You will be hearing more about this group in a few moments, but there is no question that the agency alone could not have put forward this meeting because of the degree of technical expertise that was required, in terms of formulating the agenda and gathering the relevant data.

I would also like to specifically acknowledge Glaxo Wellcome who has been responsible for coordinating the logistics of when the Resistance Collaborative Group meets. I want to thank all of our invited speakers who are listed in the order of their presentation starting with Dr. Douglas Richman, the first speaker for this morning.

I would like to acknowledge the many folks within our division and outside of our division at FDA who worked on planning this meeting. Specifically, I would like to acknowledge Dr. Jeff Murray who was really the leader within our division and planner of this meeting.

This is one of those no-win slides because I am certain that I have left off other folks' names or, even worse, have misspelled them. But this should just give you a general idea of the effort of many folks in terms of developing this meeting and the importance that our division

has for this issue.

So I will close with that. I would like to next introduce Dr. Lauren Iacono-Connors who is our Microbiology Team Leader in the division and who will introduce Session 1.

SESSION 1

Performance Characteristics and Limitations of Currently Available Genotypic and Phenotypic Assays

Introduction

DR. IACONO-CONNORS: Good morning. I am Lauren Cacono-Connors from the Division of Antiviral Drug Products .n FDA. Before we begin each session of this two-day vorkshop, a member of our division will take a few minute to)rovide a brief introduction to each session.

I am going to introduce Session 1 which is devoted .o the performance characteristics and limitations of :urrently available genotypic and phenotypic assays. As you re all aware, the majority of this workshop is devoted to he critical evaluation of resistance-testing data generated n the clinical setting and how these data could or should e used to support the regulatory development of the drug.

It is clear that the reliability and quality of he resistance testing data are directly dependent upon the eliability and quality of the assays used to generate it. herefore, it as felt that this two-day workshop should open

with a session devoted to summarizing the types of assays that are currently in use along with the presentation of data demonstrating the performance characteristics of selected assays; that is, to literally define the important features of an assay such as an assay's precision, accuracy, specificity and sensitivity.

The session objectives are: to describe the methods and accuracy of genotypic assays in discriminating among wild type and viral virulence; to describe the methods and accuracy of phenotypic assays in discriminating susceptibility profiles; to obtain scientific input on issues related to sensitivity and specificity, reproducibility and quality control, of genotypic and phenotypic assays across a range of HIV RNA levels; to review criteria used for the analytical interpretation of assay results; to explore data that describes comparability between genotypic assays; and, finally, to explore data evaluating the correlation between genotype and in vitro phenotype.

Due to the magnitude of assay information and performance data, the majority of the session will be devoted to a comprehensive overview of the diverse assay technology and the performance characteristics of all predominant assays including data quality and comparability within an assay class.

Since there are a number of diverse genotypic and phenotypic assays currently in investigational use, it was difficult to select a subset of speakers that could complete our session goal. Therefore, it was felt that a single speaker could best provide an appropriate and comprehensive presentation on the subject.

Dr. Douglas Richman from the University of California, San Diego, agreed to tackle this very difficult task.

Since these assays were intended to be used to assess the resistance status of HIV-infected patients, a presentation and discussion of the current policies followed by the agency on the regulation of these types of assays give an important balance to this section. Currently, there are no FDA-approved assays for HIV resistance testing.

The Division of Emerging and Transfusion Transmitted Disease from Center for Biologics, FDA, is the group within the agency who is responsible for the regulation of HIV resistance-testing assays.

A member of this group, Dr. Andrew Dayton, will complete this first session with a presentation on historical perspectives of the FDA's requirements for HIV resistance assay marketing followed by the current regulatory policies which were born out of a recently held advisory committee meeting on assay regulation in September

of this year.

In addition, Dr. Dayton will take a few minutes to provide us with the general assay characterization principles and what we can expect in the near future on resistance-testing regulations.

We look forward to an interesting and productive discussion. I will introduce the first speaker and then turn the session back to our Committee Chair, Dr. Scott Hammer.

Dr. Douglas Richman will now present an overview of performance characteristics of genotypic and phenotypic assays.

Thank you.

Principles of HIV Resistance Testing and Overview of Assay Performance Characteristics

DR. RICHMAN: Good morning and thank you, Lauren. I have, as Lauren mentioned, a complicated task and, hopefully, the areas I have selected to present and to overlook will be considered fair and balanced.

First a few introductory remarks.

[Slide.]

Those of us who care for patients, especially in infectious disease, are familiar with drug-resistance testing. Testing for drug resistance serves to guide the treatment of many infectious diseases. It predicts what

drugs will not work and why they don't work and what drugs might be useful. Everybody is familiar with this principle and it is now being introduced into HIV.

[Slide.]

Before I proceed, I would like to provide a few definitions that I think will help clarify people's perspective. First, the term "susceptibility assay." Susceptibility; we measure the phenotype of replication or its inhibition by various concentrations of drug. It is important to appreciate that resistance is a phenotype. So the terms phenotype and genotype resistance are really somewhat confusing. Resistance is a phenotype and various mutations, the genotype, are what confer the phenotype.

Sensitivity and resistance, to my mind, are value judgments. Sensitivity is a value judgment hopefully based upon data that a drug is likely to work based upon the results of the susceptibility assay.

Similarly, resistance is based upon data that a drug is not likely to work based on the susceptibility result. There are certain susceptibility values that may be sensitive for one drug and resistant for another. Once again, the issues of pharmacology come up so that an IC50 for indinavir, for example, may be resistant for indinavir monotherapy and conceivably could be sensitive for indinavir enhanced by ritonavir.

That is theoretical. There are no solid data at this point but the point is that these various cutoffs and definitions are also impacted by pharmacology and other issues.

[Slide.]

With regard to genotype, wild type is a strain of virus that has not been selected by drug treatment. We obviously need to know the history but that is how we define wild type. A natural polymorphism is a genetic variant present in wild type populations. With HIV, there is an incredible amount of genetic variation and there are a lot of amino-acid usages in various HIV isolates that are also seen in drug-resistant isolates.

It is important for us to appreciate whether it is a natural polymorphism or a drug-resistance mutation. A drug-resistance mutation is an amino-acid change conferring reduced susceptibility selected by drug treatment. I make this distinction because there are lot of changes that occur with drug treatment that are also natural polymorphisms that are seen more frequently following drug treatment.

Their presence, however, cannot be used to make a claim that drug resistance is being transmitted. So these are important distinctions. A drug-resistance is a mutation, or a collection of mutations, associated with the phenotype of drug resistance and best, it is what accounts

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for it.

[Slide.]

So drug-resistance assays for HIV, just as with other microorganisms, cannot be expected to guarantee efficacy-- that is, drug susceptibility is not the only predictor of treatment response and there are other factors drug potency, pharmacokinetics, plasma-protein binding, adherence and all the other things that we all know about, the point being that if one has an isolate that resistance is a better predictor of failure than sensitivity is of success for these reasons.

[Slide.]

So, before I proceeding with the discussion, I just want to make a few remarks about this Resistance Collaborative Group. The background is that phenotypic and genotypic HIV drug-resistance assays are now being used to evaluate drugs. They are now being used to manage patients. There are lots of them. They are multiple in number with limited standardization and their clinical utility has not been well established.

We are all aware of this and we are also aware of what happened with HIV RNA assays where the transition from clinical endpoints to the use of RNA for drug development and its approval for patient management was a little bit of a slow and painful process.

I think HIV drug-resistance testing is a technology that fits into those categories and I think what the various people in the field wanted to do was to make this transition a little more smooth. So there was an existing model. There was a Surrogate Marker Working Group that was established that involved representatives of various groups to help put together the data regarding the use of HIV RNA in the documentation of drug efficacy.

That helped to make the transition for HIV RNA. What we wanted to do was to be a little more proactive with regard to drug-resistance testing.

[Slide.]

So we put together a group that included academic investigators, both from the U.S. and Europe, clinical virologists and statisticians, representatives from industry and virtually every company we were aware of who had an interest was invited, and this included those in pharmaceutical development and the companies involved in development of diagnostic assays.

We invited representatives from the agency and from various centers because the impact involves them all, the representatives here from CDER, the representatives from CBER who have oversight for the devices in HIV. I am sure Dr. Dayton will get into this more, but devices outside of HIV are under the Devices Center and so we invited them as

well even though they didn't have the oversight for HIV because of their expertise and experience with antiinfective devices in general and also because I believe that any precedence that we set for HIV will, by definition, I think, have implications for hepatitis B and hepatitis C and all of the principles of virology dynamics and resistance in HIV are going to apply there.

So I think the decisions made here are going to nave other implications. We invited European health authorities and members of the community.

[Slide.]

The activities of the group which was formed just over a year ago--at our first meeting, we decided we needed to focus on two issues; one was to set up a Resistance Technology Standardization Subcommittee of which Tom Gingeras was the chair to try to address criteria for assay performance and validation and then we set up a Clinical Validation Subcommittee under John Mellors chairmanship to identify studies characterizing the utility of drug-resistance testing; that is, correlating phenotype of genotype with outcome and to develop a data-analysis plan to standardize definitions of resistance and outcome.

You will hear about this second part later today from Victor DeGruttola and John Mellors. What I will try to do is summarize some of the issues regarding technology

standardization.

[Slide.]

The issues that I want to consider fit under the issue of assay performance characteristics. But, first, I want to mention that we have to think of these in terms of their applications. There are really two different applications and we should keep these, I think, separate.

The first is drug development and evaluation with subcategories of the preclinical development and evaluation or putting together the investigation of a new drug package, and then the clinical evaluation of drugs, the NDA and product labeling indications.

Those should be considered separate from the issues of patient management. Drug-resistance testing needs to be used for both but we may be using different tests in different situations and some tests may be better applied to some than to others.

[Slide.]

So, before getting to the characteristics, I want to remind you of the specific challenge of HIV in terms of developing these tests. No two strains of HIV are identical. Within each individual, an HIV infection represents a mixture of genetic variance or a quasispecies; that is, each individual's virus which is different from any other individual's represents a complex swarm of genetic

variance.

This quasispecies is constantly evolving. Drug treatment selects for additional variation and the drug treatment practices in this country and the world are rapidly changing. What we do with our patients often precedes what is in the published peer-reviewed literature and what we do with patients often includes investigational drugs and this is, obviously, having an impact on the quasispecies in the various patients.

So the drug assays that are being developed have to be useful and have to be valid in the context of this incredible complexity.

[Slide.]

So, in terms of the assays that we are going to use, the first consideration is what is the intended use of each assay. They may not all be the same. First, the specimen; is it useful for plasma or for other specimens like semen, peripheral blood cells or whatever, and, in terms of these issues, one might sort of think, by analogy, to HIV RNA assays.

For example, the extraction methodology for the Organon Technika assay for HIV RNA made that assay useful for semen while inhibitors in semen made the assays developed by Roche and Chiron nonapplicable to semen unless a different extraction method is applied.

What is the target pathogen? Analogous to HIV RNA, the initial assays only work for clade B. They are expanded. It is obviously clear that we want applications to work for other clades circulating especially outside of North America. So, does it work for all of HIV I? Does it work for HIV II? Which agents does the assay work for and has it been validated for?

What information is provided? For a genotype assay, for example, it could state that it is going to provide the sequence of 1500 nucleotides of pol or it might provide just twenty specific codons, a codon-specific assay. Or it might provide some other information.

What is the application? Is it useful for drug evaluation or useful for patient management.

[Slide.]

In terms of validating these assays, and this is the responsibility of each diagnostic company, there is a whole series of issues--and I am sure Dr. Dayton will get into these in more detail--that one needs to consider and one needs to know this information to decide whether that assay is useful for any of the particular applications we have been talking about.

Sensitivity; we want to know, with regard to genotypic assays, what is its sensitivity for specific nucleotides or codons and, because of the sequence context

around each codon, one may have a different ability to pick up different codons with different assays.

Also, what is its sensitivity for plasma HIV RNA levels; in other words, can this assay be applied to plasma from someone who has got a viral load of 5,000 or 500 or 50. We want to know those data.

What is its specificity? Does it get the right answer for specific codons? Does it get complicated by the presence of other agents? The precision. The reproducibility. I will show you examples of each of these types of information.

We also want to have information about clinical conditions. We want know whether the assay is going to provide the same results if a person has HTLV circulating, CMV, hepatitis B, hepatitis C, rheumatoid factor, renal failure, liver failure, hyperlipidemia and various drugs on board, heparin and so on.

So these are all very practical issues but we want to know whether that assay is going to perform under those conditions.

What we do know, with the various genotype assays, is that these characteristics vary for different codons. With the genotype assays, they do have some variability for different drugs for the phenotype assays and the type of answers you get vary with different mixtures and I will show

you some of the data.

For genotyping, a major variable is the performer, and I will discuss this a bit. It is important, I think, with HIV--and I describe some of the issues regarding the challenge of HIV--to not get too discouraged by the complexity. If somebody wanted sort of a fixed, platonic situation in which the validation of a test worked and it worked in perpetuity and there were going to be no changes, they really should be going to another field in HIV.

What we have to do is decide what criteria, what sort of validation, what sort of level of security we feel comfortable with and then realize that we are facing an opponent that is going to be constantly changing and a step or two ahead of us all the time.

We just have to accept what are set-up standards and then proceed. We can't let excellence be the enemy of good.

[Slide.]

So, in terms of the assay performance characteristics, other issues that are important are what reagents are we going to use for control and standardization. With this complex agent that we have to deal with, we have to make certain arbitrary and finite decisions about which codons we are going to use with regard to genotype assays are going to be tested as the standards

for comparison. We can't test all 500 or whatever codons we are interested in as controls for every assay.

Which concentrations of virus are we going to be testing at 1000 copies or 50,000? Which mixtures are we interested in; 50:50, 80:20, and so on? Pure samples? And which samples? Are we going to look at purified nucleic acid, purified virions? Are we going to look at plasma spiked with these?

These are practical issues for setting up standards and controls.

[Slide.]

What I am not going to do is go through the painful details of the technology of each assay and go through their relative merits and demerits. I will try to go over some general principles. It is really, I think, the obligation of each of the diagnostic companies to document their assay and its validation separately. So I will just try to give some general principles.

[Slide.]

With regard to genotype assays, there are sort of two general approaches to genotyping. One is sequencing. Usually, but not always reverse-transcriptase PCR of products from patient plasma and then that is assayed by either chain termination or some other variant of the Sanger sequencing method. Examples of this are the Perkin Elmer

and Visible Genetics type of hardware approaches.

Or one can use a hybridization-based system to ascertain the sequence using the microchip technology a la Affymetrix. Then there are point-mutation assays that are just interrogating for whetner you have a wild type or mutant at specific codons that are known to be important for drug resistance. Examples of this are differential nybridization developed by the Chiron group or the line probe assay developed by Innogenetics.

[Slide.]

There are advantages and disadvantages of everything. With regard to sequencing, the advantages are .t interrogates the complete sequence of the amplified gene :hat you are looking at; thus, it detects the unknown. So, for development of a new drug, this is clearly important. Ve already know what causes AZT resistance but, if one has a iew nucleoside that one is developing, one can't assume that :hat is going to be what one is looking for. So one has to .ry to detect the unknown.

The limitations of sequencing are the magnitude of .he data. If one gets 1500 nucleotide sequences and one starts getting them from dozens or hundreds of patients, .hen one has a lot of data to digest and handle. A software .o deal with this is critical.

Once one has that all put together, one still has

the incredible difficulty of interpretation of the contributions of each genetic variation. With the natural polymorphisms and the mixtures, this is a challenge as well.

[Slide.]

The differential hybridization assays, the codon-specific ones, have certain advantages. They are usually more sensitive in detecting minority species. The sequencing methodologies will often pick up mutant populations if they are in the 20 to 50 percent range, at best. These assays will often be tenfold better, perhaps two to five-fold.

So picking up minority species could be an advantage in mixtures. They are simpler to perform and usually simpler to interpret. Their limitations are they only detect what they look for. So each of these applications may have its benefits and limitations in different situations.

[Slide.]

What I am going to show you now are some data from various studies regarding the comparability of sequence determinations in different laboratories. I will give you sort of the more complicated issue first. Rob Shuurman and his colleagues in Utrecht have sort of put together standard panels and distributed them to interested parties who perform sequencing to see how they compare.

The first panel was published in the Journal of Clinical Microbiology this summer. This is the result of the second panel.

[Slide.]

What they did is they took plasma samples that had been spiked with infectious HIV that contained genotypically characterized compositions of clonal origin. Each of these had mutations both in the reverse transcriptase and the protease. They looked at homogenous and heterogenous samples; that is, pure wild type, pure mutant and various mixtures. They did this, once again, an arbitrary decision, at a viral load of 50,000 copies. One will get different results with different viral loads.

[Slide.]

So this is what they put in there. They put in two different viruses in various combinations that contained either wild type or mutant protease and reverse transcriptase. The mixtures that they selected were 0, 25, 50, 75 and 100 percent of each of the components.

[Slide.]

They let each of the sites perform the assay according to whichever of the many methods that they happened to have and they reported them as differences from a standard reference sequence and entered that electronically into a database.

[Slide.]

Fifty-six laboratories worldwide participated. This is important, I think, to point out that same of these laboratories are academic or commercial labs that do service and have validated their methodology for reporting results. There are some industry laboratories that do drug-resistance sequencing for their own purposes and there are some academic labs that are quite small that are doing this to see how they are just doing.

So we have a wide range of different types of performers. What they looked at was interlaboratory variation looking at five resistance mutations in protease, five in reverse transcriptase and they looked at the various comparisons.

[Slide. 1

There were various approaches using home-brew reagencies in Perkin Elmer using the kits of Perkin Elmer, using the Visible Genetics system and using various other types of systems.

[Slide. 1

The results; what this shows on the left side are the results in which we have 100 percent wild type. You can see almost all the labs got almost all the answers right. On the right side, you have 100 percent mutant and you can see most of the labs got all the answers right. The rest of

the labs, all but two, got nine out of ten right.

As you get various sorts of mixtures, you get various sorts of mixed answers which is part of the reality of this world as well.

[Slide.]

Lookingat the number of correct calls with various mixtures, you can see that some labs get all the answers right all the time. Some get most of the answers right all the time and some need to work on their answers. This is just part of learning how to do all this.

[Slide.]

You can see, here, the frequency of mutation. One thing that is important, both in the ENVA-1 and the ENVA-2 panel, is that the success or failure rate of various Laboratories appears to be independent of which of the kits or assays they used. Those of us who have done sequencing know that having a good, experienced person doing it gets the best results.

Just as if you give someone a recipe and some food, and give ten people the kitchen to do it in, some are going to come out with a better product even given the same materials and the same recipe that others will. This is one of the practical issues that performance characteristics is an important issue here. It is just something that needs to be considered.

[Slide.]

This shows the variation of the resistant ones.

[Slide.]

In terms of more experienced laboratories, the results do seem to be quite comparable. These are the data provided to me by Brendan Larder from the Virco group in which they have looked at reproducibility of their own quality assurance.

They looked at 16 samples on ten different occasions, repeated on ten different occasions. This shows the reproducibility of nucleotide variation and amino-acid variation and we are well over 99 percent. In addition, those patients who had viral loads over 1000, 95 percent of the time, they got a successful result.

This is reassuring that an experienced laboratory lets more reproducible results.

[Slide.]

In a study presented at the Drug Resistance meeting in San Diego last June, a blinded comparison of specimens in two experienced labs, one a commercial laboratory and one an academic laboratory.

[Slide.]

What they tried to do is determine if the sequence analysis of complex clinical isolates yielded comparable results from the two different experienced laboratories.

These materials were aliquoted, distributed blinded and the sequences were generated on 44 isolates from heavily pretreated individuals.

The laboratories used different in-house validated ABI-based sequence-analysis protocols.

[Slide.]

Once again, using the sequence of over 4000 protease and 11,000 RT amino-acid residues, the concordance was quite impressive. So I think that this is, once again, an example of experienced laboratories getting relatively reproducible results.

[Slide.]

The issues of genotype, the various of kits and methodologies, are under the purview of CBER. The performance of the various laboratories is an issue that we are all interested in. There are a lot of different assays and different approaches.

The phenotype situation is quite distinctive. Those of us who started looking at HIV susceptibilities as long as ten and twelve years ago, developed and used assays that can fairly be described as expensive, cumbersome, labor-intensive, slow and relatively imprecise.

That type of methodology that we used which helped to identify the phenomenon of drug resistance are really not satisfactory for patient management or for high through-put

data generation.

So two companies have now come up with assays that fulfill those criteria and have pretty much taken over the field with regard to drug-resistance assays. What I will do is try to describe what they are doing. Once again, as with the genotype assays, I will not try to contrast them but describe what they have in common and what they can do and their limitations.

There are two assays that exist, Virco and ViroLogic. What they have in common is they amplify a segment of the gag/pol gene that incorporates protease, much of RT and some of gag from the patient plasma HIV RNA. They put this amplified material into a recombinant virus construct that, then, they use in a standardized high-throughput assay.

Right away, one sees one potential limitation here in that these assays are potentially useful for reverse-transcriptase inhibitors and protease inhibitors which represent all of the approved drugs now but, obviously, there are people trying to develop drugs against fusion inhibitors, chemokine receptors, integrase and so on. These particular assays will not be applicable to those.

These are rapid high-throughput automated and they are in-house assays. They are done at each of their sites and they cannot be put in a little plastic container and

just add water at each site and then run it. The material has to be shipped to them.

So, right now; they are not regulated by the FDA and their accreditation for their performance is overseen by various laboratory-accrediting agencies like CLIA, College of American Pathologists, various states and European agencies. Both of them have fulfilled this accreditation.

[Slide.]

These are data provided by Brendan Larder regarding Virco quality-assurance data. Looking at between 1700 and over 2200 determinations per compound--we are talking about twelve to fifteen drugs per assay--the variation was less than three-fold for each of the assays over a period of a year looking at a wild-type reference strain.

[Slide.]

Lookingat inter-assay reproducibility, looking at sixteen samples on ten different occasions, the variation in IC50, 1.2- to 2.5-fold. The sensitivity; 95 percent of the samples with a viral load over 1000 copies/ml were successfully amplified and assayed. Their subtypes; they get amplification and successful genotyping that has been demonstrated in all the groups and subtypes of HIV-l.

[Slide.]

This is an example of the type of report of the

data that are generated by Virco. It doesn't project ideally but it shows each of the approved drugs. The susceptibility that is generated is then shown with this blue dot here and the data on this scale are the fold difference from a laboratory reference strain. So one would be the same as the reference strain.

They are reporting things as not significantly different from the reference strain as anything less than four-fold different. That is in this green area. Then everything greater than ten-fold difference is in this red area. Then there is also a yellow area between four- and ten-fold.

It is important to appreciate for this assay and, also, for the other one I will describe that this cutoff of four-fold here is what they are confident is statistically not different, that they can reliably say is not different from the wild type.

That does not tell you what is clinically important. Clinically important is the susceptibility that makes the drug work or makes the drug not work. What we don't have with this assay or with the other phenotypic assays or with a lot of genotypic assays is--what we would like optimally is systematic, prospective controlled data saying what level of susceptibility would predict success or failure with monotherapy with that drug.

Those data would be unethical to obtain and so it is going to be real challenge now for us to generate data that will help us to say what cutoff is clinically important. Those data have been, I think, best generated with abacavir now with retrospective studies and you will start to see some data from the group this afternoon that will give some suggestions.

But the precise cutoff is a challenge. From the data that Dr. Little will show you, there are wild-type isolates out there that have eight-fold reduced susceptibility to certain drugs. The question is they are not resistant transmitted virus. That is the wild-type variation and we don't know whether that is clinically important or not.

[Slide.]

The other assay is the one from ViroLogic. I nention there is amplification of segment from the plasma of :he patient containing protease and RT, then put into a test vector which is transvected into cells and the generation of :he test vector which, then, infects a cell line that expresses luciferase is what is measured and what is inhibited by the presence of various concentrations of drug.

[Slide.]

Once again, the type of validation done with this assay, repeated testing of multiple patient plasma

specimens, twenty replicates per sample, looking at multiple operators, assay runs, reagent lots, repeated testing of drug-sensitive and drug-resistant viruses and, once again, the multiple-conditions runs and so on.

[Slide. 1

This is the fold variability that all those repeats generated. You see the fold change is less than two-fold for all of the approved drugs with one exception nere, of course, with AZT now showing a little bit more variability with multiple patient samples in terms of reproducibility.

It is on the basis of this that their reports say that if it is greater than 2.5-fold from the control, it is different, once again, that being a laboratory definition of reproducibility and not a definition of clinically significant difference.

[Slide.]

The assay variability on repeated testing of reference samples; this is a controlled sensitive virus and these are two resistant viruses. Once again, you see the variability.

[Slide.]

None of 80 seronegative samples produced a result and testing of HIV samples containing multiple interfering substances under interfering conditions had no impact.

These included high levels of triglycerides, hemoglobin, bilirubin, bacteria, fungi, other viruses and so on.

Then, looking at various virus concentrations and processing times and so on--so these are important variables that need to be defined.

[Slide. 1

This shows a point from patients who are known to be treatment naive. Many of these samples were obtained in the '80's before the drugs were available so we are confidence they were naive. You see the variation from the control virus. The mean here is pretty tightly around 1, but the 5th and 95th percentiles, you see three drugs here in which over 5 percent of the isolates are greater than three-fold different than the control virus.

That is shown, I think, better on the next slide.

[Slide.]

So these are the means and the 25th, 75th percentiles and then the 95 and 5 percent variation. You see a large number of samples fit within a type range for wild-type virus but there are a few drugs for which there are wild-type viruses circulating that are almost always less then ten-fold resistant.

But the questions are is something between four and ten-fold less susceptible. Is wild type going to respond or not? That is an interesting question and that

will be discussed more by Dr. Little. The genetics that account for these natural variations are now being defined.

[Slide.]

These are data showing the level of plasma RNA that generates a result. 90 percent of patients will generate a result with 540 copies or more, 95 percent with 700 or more. So these are important issues more for patient management, really, than drug development but they are important pieces of information to know.

[Slide.]

The issue of mixtures, putting artificial combinations of mixtures with various--l0 percent, 20 percent and so on--of highly resistant virus to each of these drugs up here with a wild-type virus, you can see the fold change here. This is the 2.5-fold cutoff.

You can see with the various protease inhibitors mixtures of highly resistant virus and wild-type virus. At least if 20 percent of the composition is resistant, it is going to be picked up as significantly different--in some cases, 10 percent--with the protease inhibitors.

With 3TC, the wild type has much better growth characteristics and one has to have the majority of the virus being 3TC resistant before one can see a phenotypic change in the assay.

So whether it reads out as resistant or sensitive

will depend on how much sensitive or resistant is in the composition and also whether the resistant virus is highly resistant or moderately resistant. This is just part of the reality. This sort of issue applies to genotyping as well.

[Slide.]

This is the type or report from the other assay as well. You can see that one gets, with a virologic assay, a result showing that it fits in the same as the reference, decreasing susceptibility. Also, here is an example of another phenomenon, the significance of which needs to be defined.

There are a number of mutations, especially in reverse transcriptase, but it has also been shown in protease, that confer resistance to one drug and make the virus hypersensitive to others. Here, 3TC abacavir resistance has been associated with a shift to greater susceptibility to NNRTIs. The question is whether this is going to have any impact on treatment effects. The answer is we don't know.

[Slide.]

So correlation of phenotype and genotype; the phenotype of drug susceptibility is conferred by the viral genotype. Thus, they must correlate. But how well do they correlate. Basically, the virus has it all figured out. It correlates 100 percent for the virus.

The issue is what our assays do, how well our assays correlate with each other because we are only approximating what the virus is up to. The challenge here is that some correlations of black and white--that is, if you have a mutation at 184, you are absolutely resistant to 3TC and if you are a wild type of 184, you are sensitive to 3TC unless we discover some new drug-resistance mutations which, in fact, some people have--the Virco group described at the last drug-resistance meeting.

But, at least with that mutation, we know that the drug will not work. That is true with a number of drugs, especially the non-nucleoside RT inhibitors and other drugs. If we see certain mutations, we know that drug won't work.

But, unfortunately, there are various shades of gray because, for many drugs, especially the protease inhibitors, multiple mutations are usually needed to generate high-level resistance and there are very complex interactions which can be additive or suppressive and have various effects on each other. Some of these, we haven't figured out yet.

So this is part of the complexity of analyzing genotype data. Dr. D'Aquila, tomorrow, will discuss this in more detail.

[Slide.]

But with the various drug-resistance assays, they

have all shown that, in general, if you have the right mutations, you are going to show the right answer; that is, if you have various mutations known to cause resistance to non-nucleoside RT inhibitors, you are going to have reduced susceptibility to those drugs.

There are some exceptions in that here is one mutation that doesn't confer resistance to delavirdine, another one that has a very small effect on efavirenz. What we need to know is what happens when these drugs are used in patients who have developed these mutations. Can you then use a second NNRTI inhibitor if you happen to have the right mutation or the right susceptibility.

[Slide.]

Similar data with protease inhibitors, but these are even more complicated. These are defined viruses with given mutations. You can see here that there is a significant amount of cross-resistance but there are some exceptions. Most notably, the first mutations seen with nelfinavir do not cause cross-resistance to the others.

You add some more mutations and it may or may not cause cross-resistance. You get various combinations and permutations. So when one looks at a genotype report, it is often very difficult for a non-experienced caregiver--and I can tell you for some of us who think we are experienced, it is still very difficult sometimes to make predictions based

on the complex data.

But one does get reproducible answers with the same virus in these sorts of assays.

[Slide.]

Here is an example of a type of approach from the ACTG 372 data in which a list of drug-resistance mutations known to cause resistance were listed. Then the susceptibility was obtained. There was excellent correlation for almost all the drugs and then this set of drugs for which the genotype resistant situation is a little more complicated, the prediction wasn't quite as good.

[Slide.]

This shows a phenotype sensitivity score was arbitrarily generated and a genotype sensitivity score based on the number of mutations that were considered bad. Then you can see a clear correlation. The size of these dots are the number of specimens in each of those categories.

You can see it doesn't fit perfectly on a straight line but it is clearly statistically significant. The type of endpoint data that these approaches have generated, you will hear more about to reassure you that, in fact, this is meaningful data.

[Slide.]

So how do we use genotype and phenotype? In closing, what I will do is give you my personal opinion. I

haven't run this by the rest of the Resistance Collaborative Group so you can take it or leave it, as you will.

For drug development, I think we clearly need both. For patient management, the most useful test--that is, the test that gives the best predictive value for treatment efficacy considering cost, turnaround time, et cetera, requires data. We don't have the information to say which test to use in which situation and, in fact, it may vary in different clinical situations. It may be different in naive patients and in highly experienced patients.

But our job today is to concentrate on drug development.

[Slide.]

So I will give you my personal opinions about what I think the center and this committee should expect to see at the very least. In terms of preclinical studies, for an IND, I would think that for a new drug, one would want to assay for that drug's activity--that is, get the phenotype--against a series of laboratory strains, both wild type and with well-characterized resistance, well characterized by both phenotype and genotype to other approved drugs, and then look at various clinical isolates, both wild type and well-characterized with regard to resistance to the approved drugs.

Then one would want to ask to see what happens

when one selects for resistance in vitro, characterize the phenotype of that resistant virus against approved drugs so we know what kind of cross-resistance it causes and then sequence the resistant virus that has been selected and identified in mutations that have been generated and characterize these mutations to see which ones are important by in situ directed mutagenesis for the resistance to that drug and to the other drugs.

[Slide.]

Clinical studies for new drugs; what do we want to see? What I would like to see is when a new drug is used, both as monotherapy or in combination, for patients who fail treatment--that is, don't become suppressed--we want to look at the post-treatment isolates and look at their phenotype against that drug and other drugs and the genotype.

Also, importantly, we need a lot more data to test the activity of this drug that we are interested in in patients with resistant isolates that have been well characterized at baseline for genotype and phenotype against this drug and against other drugs.

This is a very important and practical problem. We know that a significant proportion of patients have detectable virus with resistance in them and these patients need help with new drugs. We would like to know how to use these new drugs most intelligently.

This is the type of data that we need. Presumably, this type of data will help in the new product labeling.

[Slide.]

I would like to close and acknowledge the people who have helped me put the talk together. The drug guidelines for validation, the subcommittee that wrote, really, beautiful reports were Tom Gingeras from Affymetrix and Chris Petropoulous from ViroLogic, and then some folks who lent me some slides; Brendan Larger at Virco, Nick Hellman at ViroLogic, Rob Shuurman from Utrecht and John Mellors from the 372 team.

Thank you for your attention.

DR. HAMMER: Thank you very much, Doug.

We have a few minutes, I think, before the next presentation if there are immediate questions from the committee.

DR. POMERANTZ: Are you, or someone afterwards, going to discuss the development of computer programs or algorithmic analysis for this because it looks like maybe some of us need that help as we look at patients.

DR. RICHMAN: Right. There are two types of computer programs. The various sequencing companies have developed and are developing software just to align sequences and identify those sequences that are known to be

at

associated with drug resistance.

A number of the companies that generated genotype reports provide those lists. For many drugs, it is straightforward; 184, 3TC resistance; 215, 41, AZT resistance. But for other drugs, especially the protease inhibitors, you see two or three mutations and you could say, "Well, this person was on indinavir, developed two or three mutations resistant to indinavir and ritonavir." But the average expert can't predict, "Is it going to work against amprenavir or nelfinavir for whatever, or drug X?"

Sometimes, there isn't the database to do that. Other times, there may be but it is very difficult. There are proprietary databases. Virco has correlated its genotypes and its phenotypes and comes up with a prediction that is usually right, or often right, but, once again, not always right because the virus is always a step or two ahead of us and there are mutations we don't always know about.

But that is proprietary. So this is an area that, I think, is in a lot of flux. Just for the fun of it, I will venture an opinion. My own opinion is that, for "salvage" patients, highly experienced patients who have failed many times, it is going to be easier to manage patients with phenotype data than genotype data.

For other situations, that is something else but, for developing a new drug, I think it is complicated. You

probably want both types of data. But the perfect analysis of a genotype? We know this virus is too fast to know everything ahead of time.

DR. MASUR: Doug, one of the things we have wrestled with is how to determine activity of drugs and determine that the activity is really due to one drug rather than a drug cocktail. On your next-to-last slide, you talked about testing activity of drugs in patients with resistant isolates but, since there is so much reluctance to add one drug to an existing regimen or to use monotherapy, if you are going to be looking to add at least two active drugs, how do you actually get any positive data?

You can get negative data if the patient fails to respond, but then you get drug interactions, antagonisms. How do you actually accomplish that second bullet on your next-to-last slide?

DR. RICHMAN: That is a very important question. It is a critical question. You are going to see from John Mellors the type of data that would suggest that these data are predictive of success and failure in combinations but, in fact, from the point of view of developing a new drug, it is a fundamentally important--and I think we do have a dilemma.

I think knowledgeable patients and caregivers are unwilling to participate in studies that use prolonged

monotherapy, are unwilling to do add-on studies if there are other alternatives and are unwilling to participate in study designs to meet "regulatory" purposes in which there is a suboptimal arm.

It has clearly been done in the past and I, personally, don't think that type of approach is acceptable anymore. So, now, if we design--the only right type of study to do is one in which patients are randomized to arms that are acceptable standard of practice.

If that is the case, we have automatically precluded the opportunity to define the activity of any of the components. So, what I have done is I have reiterated your question. I haven't given you an answer.

DR. MASUR: That is a very effective technique.

DR. HAMMER: You are also in the right part of the country to do that, near Washington.

DR. RICHMAN: But I think this is a dilemma that is important for people who are trying to conduct studies, people who are trying to develop drugs and people who are trying to regulate it. So all of us, the investigators, industry and the agency really have to figure out what is acceptable given those constraints without compromising proper patient care.

DR. HAMILTON: Doug, you indicated that certain technologies were applicable, perhaps exclusively, in

different populations, different body fluids. Is there reason to think that different strains exist in one body fluid versus the other?

DR. RICHMAN: There is not only reason, there is data. Yes; there is no question that there is discordance. We showed two or three years ago that both the quasispecies and the drug-resistance mutations could be discordant in the brain and central nervous system.

There are three or four such studies that have shown similar data in genital secretions, primarily semen, which is important for transmission as well. So there is some discordance. I think Rich D'Aquila is going to discuss that to some extent tomorrow.

DR. YOGEV: You talked about how you predict success. Do we talk also length of time? For example, if you define certain drugs are sensitive by phenotype and genotype, then we have a pressure on the quasispecies who are in the minority that we cannot pick up by the sensitivity of the test and are not coming out.

So should we define a success by how long that specific therapy is effective, six months, a year, or--

DR. RICHMAN: Oh, absolutely. It has to be long enough to be clinically significant because we have a chronic disease and, even though we are interested in what is going to happen in five or ten years, we can't wait for a

five- or ten-year study before making decisions. So we have to figure out what duration is acceptable, and whether that is six months or more is--

DR. MAYERS: Doug, some of the most difficult drugs we have to deal with are the drugs that lose their virologic activity without a clear phenotype, the drugs you listed, ddI, ddC and d4T. How should we work on trying to define for the clinic to determine success and failure when you don't have a clean resistance phenotype?

DR. RICHMAN: There are mutations that do confer significant resistance to those agents but we also know that an experienced patient with AZT is not going to have, necessarily, the same response even though they have got wild type virus as an inexperienced patient.

That always raises questions about pharmacologic effects. There are data regarding p-glycoprotein efflux pumps for protease inhibitors. There was a very nice paper in last month's Nature and Medicine from St. Jude showing that MDR-related gene will pump out triphosphates and can be selected for and amplified in cells.

So there may be other drug-resistance mechanisms analogous to those in oncology that may be applying to patients here. They will only add to the complexity. They won't supersede the issue of drug resistance to the virus.

DR. CHARACHE: Is there data for drug development

or would you suggest data on drug combinations in vitro to see .f they may be inhibitory or synergistic, particularly if they have the same phenotypic target?

DR. RICHMAN: I didn't put that on the list, and maybe I could have, of things to recommend in the preclinical package. Clearly, I am a believer in looking at combination studies primarily to show antagonism. I think that is worth doing to provide an indication why some drugs might not be combinable.

So that type of information is useful and I guess it is also useful for looking at resistant virus and whether combinations are useful. There are some practical technical problems that I could get into if you want about problems with doing such assays. They have to be done at concentrations of drugs which are subtherapeutic or they can't be analyzed.

There are some practical issues, but I think those sorts of assays are useful in a package; yes.

DR. POMERANTZ: Doug, you touched on a real difficult question in your talk and that is the group of viruses that are phenotypically mildly resistant, four- to eight- or ten-fold but have no genotypic correlate. It has come out in a few papers, one recently from your group.

Now, one of the things that you can think of is that we just haven't found those subtle genotypic markers

for that phenotypic resistance or it might be something more complex. Clearly, we don't know what they mean clinically. Do you have comments on what may be causing that?

DR. RICHMAN: Yes; there is some preliminary data that Andy Leigh Brown has generated with the data that Susan will show you that there are some sequence explanations with natural variability that account for much of this. so I think that the variability of wild-type strains will account for susceptibility to certain drugs.

DR. POMERANTZ: I'm sorry; so you think that that is natural variability in the wild type and not subtle changes due to pressure from the drug?

DR. RICHMAN: Yes.

DR. KAPLAN: Doug, in your comments about correlation of phenotype and genotype, you mentioned there are some blacks and whites, like, for example, the 184 mutation, but there are a lot that are in the gray zone. I guess there are some data being developed by the drug companies. You mentioned a lot of it is proprietary, but I wonder what the prognosis is for more publicly available information in the future on correlation of genotype and phenotype such that people using genotype assays may have to resort to phenotypic testing less frequently.

I recognize, in some situations, you are always going to have to do it but where are we headed in that

regard?

DR. RICHMAN: This is a moving field and there are usually are not many secrets too long. So my guess is there are going to be lots of data generated by various companies, by various investigators and more sophisticated ways to analyses and correlations. I suspect we will know a lot more in the future than we know now. I certainly hope so.

But I am optimistic that we will learn more. Obviously, looking at the unknown, what the phenotype does is measure the susceptibility while the genotypes are a current approximation of that. But which situations each of those assays will be most useful will--data is what drives it.

DR. GULICK: Doug, you made a big point about the experience of the labs doing the genotypic assays. Could you comment on what is the definition of an experienced lab and how we evaluate the experience of a lab?

DR. RICHMAN: That is a very good question. Experience doesn't necessarily mean size or name recognition. It means performance. So there are some academic labs that are small that do good work and there are some very large labs that may not. But I certainly would not name names.

So this is a practical problem. This may be better addressed by Dr. Dayton, but there are two issues.

ne is the kits and reagents I think the **agency** can oversee. ut performance of laboratcries is more lab practices, ccreditation type thing. I am not really ideally qualified o discuss that.

DR. HAMMER: Thank you very much, Doug.

With that segue, I would like to introduce Dr. ndrew Dayton from the Center for Biologics and Evaluation.

CBER'S Policies on Assay Regulation: Definition of Assay Performance Characteristics

DR. DAYTON: Good morning. That last talk of Dr. ichman's is going to be tough act to follow but, on the ther hand, since he has covered most of what I want to say, ou can be forgiven if you begin to lapse into **a** little bit f late-morning sleepiness.

[Slide.]

I am Andrew Dayton. I am in the Division of merging and Transfusion-Transmitted Diseases at CBER. I am going to discuss our policies on assay regulation primarily locussing on definitions of assay performance characteristics.

[Slide.]

The theme of my talk today is the regulation of in vitro diagnostics, or IVDs. It is not rocket science; that is, the regulatory handling of it is not rocket science. I think coming up with the data maybe does qualify **as** rocket

science but there is an inherent logic to how we regulate these IVDs.

There are two centers at the FDA which regulate in vitro diagnostics, the CDRH and CBER.

[Slide.]

In CBER, we currently have IVDs on the market which are basically blood-borne pathogen tests for hepatitis B, HIV and HTLV, hepatitis C and syphilis.

[Slide.]

The statutes that cover IVD regulation are the Public Health Service Act, Biologics Regulation, 24 CFR 600 to 699, the Food Drug and Cosmetic Act, the Medical Device Amendments of 1976 and intercenter agreements.

[Slide.]

The intercenter agreements are critical to how the IVDs get divvied up between CDRH and CBER. The Intercenter Agreement of 1991 basically established that CBER would get the in vitro diagnostics for all blood-donation screening and for all HIV diagnostic applications. CDRH gets the IVDs for almost all other applications.

[Slide.]

The current CBER or FDA-licensed or approved HIV tests include tests for the following: HIV antibodies detected by ELISA or immunofluorescence analysis for screening or diagnosis--screening meaning screening for

blood donors or plasma donors; HIV antibodies by Western blot of immunofluorescence analysis for confirmation of screening results; HIV p24 antigen screening for screening and for prognosis; and HIV-l RNA for prognosis and management.

[Slide.]

One of the general regulatory requirements for HIV tests--basically, they are what you would expect and, actually, Dr. Richman did a very excellent job of introducing you to them and covering them in considerable detail. The key areas that you have to pay attention to are manufacturing consistency; can some of the manufacturers' tests give us some kind of guarantee that they are going to be able to continue to manufacture at the same level of assay performance that they used to test it or to develop it with.

Obviously, sensitivity is of concern, specificity, reproducibility, and clinical utility.

[Slide.]

Manufacturing consistency, as I said, means basically can you continue to produce an assay that performs the same as the one you developed. For us to be assured of this, a sponsor needs to give a full description of the manufacturing process. The sponsor needs to give a demonstration that the manufacturing process is adequately

controlled.

This basically means quality control tests and specifications with supporting data. We need such quality control tests and specifications, for instance, for acceptance criteria for reagents that come into the manufacturing organization. In other words, the manufacturer has to put limits on what condition he will and will not accept reagents under and the sponsor has to test those limits to show that they are reasonable.

We need quality control for critical manufacturing steps and we also need quality control for lot release. It is important to remember that quality control for lot release is necessary but it is not sufficient. Someone who is making an assay can't just introduce one quality control step at the very end--in other words, measuring how the assay works when you are finished manufacturing it and then say, "Hey; it works. It's good."

That is not enough. In order to be assured of manufacturing consistency, we need to know that all the critical steps are also adequately controlled. We also need data on consistency of the assay over time. This includes obvious issues such as reagent and overall assay stability on storage and shipment.

We also need data on lot-to-lot variability. No assay is perfect. It is going to vary from lot to lot and

we certainly are not going to just take a look at the results from one single lot and approve a product based on that. We also need insurance of long-term availability.

[Slide.]

Sensitivity, in general, basically is the question of showing that assay measures the analyte. This can, obviously, take different forms and, in this case, with a different codons, different codons may have different sensitivities.

We are very interested in knowing the range of the measurable analyte and also the effects on sensitivity of interfering substances and conditions.

[Slide.]

Specificity, in general, is the issue of false positives. We want to know how often do you get false positives under ideal circumstances and, also, under situations where you have interfering substances or conditions.

[Slide.]

Reproducibility; this was very well handled in the previous talk. I think all I will say here is that we are interested in run-to-run variability, in site-to-site variability and, again, we see that is a very major issue; operator-to-operator variability. Again, that has already been described as being a major issue; and day-to-day

variability.

Finally, in general terms, clinical utility; I don't have a separate slide for that, but, basically, does it do clinical good. Let me discuss some of these general terms with specific relevance to HIV drug-resistance genotype tests.

[Slide. 1

Again, as I mentioned, we need to know the sensitivity; how often do you get a mutant or a codon that is there? How does it vary from codon to codon and, in particular, how sensitive is the assay at various levels of viral burden? Does it always pick up 20,000 copies per ml, or 5,000 or 2,000 or 200?

We need to know that. We need to know how sensitivity varies across a range of expected viral burdens. The same is true for the range of mutant proportions. Do you pick up a mutant that is there 25 percent of the time, 10 percent of the time? We need to know that.

Reproducibility has been extensively discussed this morning. As I said, lot-to-lot, site-to-site; all very important. We particularly want to know how the reproducibility varies under challenging conditions such as low mutant proportion or low viral burden.

Finally, clinical utility, which is the most complicated issue of all; does the assay benefit the

patient? We are currently wrestling with just how to handle this as a regulatory agency. Basically, the two principles I want to introduce are the predicate device and special controls.

We are seeking to regulate these genotype drug-resistance assays at a Class II level. This usually involves reference to a predicate device, something that is already out there and approved. But it doesn't have to. In lieu of a predicate device, if none exists, we can still regulate it at a Class II level or the 510(k) level by reference to what we call special controls.

[Slide.]

For Class II devices, special controls can obviate the need for a predicate device if the knowledge of the field is such that adherence to the special controls can adequately insure that a device is safe and effective.

[Slide.]

One type of special control can be a guidance document or, actually, not the guidance document but criteria specified in a guidance document. As many of you know, the Blood Products Advisory Committee has recently recommended that HIV drug-resistance genotype assays be regulated as Class II medical devices.

In this case, the special control would be the criteria laid down in the guidance document which is

currently under development.

[Slide.]

I only have time to very briefly go into the guidance document that is under development and I really can only highlight a couple of the concepts that are being entertained by us for putting this guidance document together.

We may list several well-documented loci, resistance loci, with the expectation that the intended use would be limited to the listed mutations. So intended use could be limited to certain codons, certain drugs, as well as being limited to certain body fluids.

Extension to other mutations may require additional data. This can, perhaps, be cited in the literature or, perhaps, be provided as new data by a sponsor. This is all up in the air. This is something I want to emphasize. And extension to other mutations may be handled at a later time by amendments to the original application.

At the bottom, I have listed examples of some of the loci which are probably the most well accepted as having definable phenotype, well-defined phenotypes.

[Slide.]

Clinical utility can be looked at in several ways. One way might be to demonstrate premarket by correlating

virus responses to assay predictions during trials. This would involve going to archive samples or prospective samples from clinical trials correlating the disappearance of certain susceptibility loci, amino acids of susceptibility loci, correlating that with drug therapies and institutions that do drug therapies.

This may be sufficient but it also may not be necessary. Again, I am not giving you the full answer because we don't have the full answer. As an alternative, clinical utility may be established premarket by certain in vitro studies. For instance, a mutation that confers greater than, let's say, an eight- or ten-fold increase in the IC50 or 90 contractions might be considered validated.

[Slide.]

What I want to emphasize is that the draft guidance document that I referred to HIV drug-resistance genotype assays is currently under development and will be released as a draft for public comment.

At that time, we expect a significant input from the entire scientific community and we encourage a vigorous debate and, at that point, we will intend to incorporate the results of the debate into the document.

So when we get this out as a draft document, don't think we are sitting on high and saying, "This is the way it is," particularly for an incredibly complicated field like

this. We expect a vigorous debate and, at that point, we will have to see what we can do to best incorporate comments from the scientific and medical community into the document.

[Slide.]

So, if I could summarize, CBER regulates all HIV tests. HIV drug-resistance assays pose unique interpretations of some regulatory criteria but these interpretations are not counterintuitive and, absent a predicate device, HIV drug-resistance tests may be allowed to go to market with identifiable special controls instead of extensive Class III or PMA-level clinical trials.

Thank you very much.

DR. HAMMER: Thank you.

Let me start with one question or a comment to see if I understood this correctly. In one of your slides, and I understand this is all a draft issue, but you indicated that one of the possible scenarios is regulation or approval based on known loci or determined loci or drug specificity.

For certain techniques of genotyping, those, for example, that don't interrogate just certain regions but, as Doug outlined, give you lengthed sequencing over a defined region, it just seems to me that making something loci-specific or drug-specific means it will be out of date and need to be continually updated on a nearly quarterly, if not monthly, basis.

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Did I understand that correctly or am I misinterpreting?

DR. DAYTON: This is a difficult issue. We would like that it weren't that way but this is a continually evolving field. From a regulatory standpoint, we are always going to be behind the cutting edge. So this is an issue we are dealing with.

If you look at the situation that you have now, they are out there and being used and, yet, they are not really FDA approved and cleared. We are always kind of behind the times. There is no other way to do it in such a complex and rapidly evolving field.

That is one of the concepts, what I put up there, possibly starting out with a certain set of defined loci and approving, or clearly submissions on that basis. That is a concept we are considering. Again, I expect that to feed into the debate. We don't know yet whether that is going to make it into the final guidance document.

DR. HAMMER: I don't know if you wish to comment on this, but I would bring up for discussion because it is, I think, important in the larger context that this committee deals with, not just the specifics of development or approval or recommending approval for a specific drug.

But Class II approval, while deregulating to some extent these kits, it then does not require, if I

understand, a clinical validation study in some fashion, or application of that particular kit or whatever it is to an outcome or whatever it might be. What is, then, the implication, and if you don't want to comment on this, I would understand, for access as far as federal funding for that kit for application in the clinical context?

As we saw, for example, with RNA assays, it was very important to have FDA approval in order to gain reimbursement for populations with limited access. I think the same holds true and we are on the threshold of that for resistance testing.

Does Class II or Class III approval generally, if you want to answer this in a generic fashion, have an implication for whether federal assistance will be Forthcoming to improve access for patients?

DR. DAYTON: I am close enough to be inside the beltway even though I am technically outside it that I could say, "No comment." It is certainly a valid question. Really, all I can say at this point is that the government has to be petitioned for that. I can't make policy here.

DR. HAMMER: Okay; at least I got it on the record.

Let me open this up.

DR. KWEDER: I have a question. Do you review any applications for these--do you consider them priority

application reviews or standard? I think that speaks to the issue of always feeling like we are a step behind.

DR. DAYTON: These applications get put through, at least they are done in a fairly quick time scale. They are not the PLA time scale which takes a year, or which takes six months expedited. These are much faster than that. They are a high priority for us.

DR. STANLEY: You talk about reproducibility, operator-to-operator, site-to-site. You are talking about licensing devices, basically. What kind of authority do you all have for a certification of the actual lab or the actual performers?

DR. DAYTON: In something like this, the sponsor usually will come up with certain training protocols and proficiency panels. It is up to them to institute. That becomes part and parcel with using the assay. You use the assay when you have been trained and demonstrated to be proficient.

That becomes a part of the assay. The sponsor says, "Here is the panel we are going to test the lab on. Here is the training we give them. Here is how well they have to do." When they pass this, then they are allowed to use the kit.

DR. STANLEY: So that is part of the QC package that you would have to approve.

DR. DAYTON: Yes; we want to see training and proficiency studies.

DR. JACKSON: From a manufacturer's point of view, will you be requiring that sensitivity, specificity, precision and clinical utility be demonstrated for different subtypes besides subtype B that may have different drug susceptibility patterns or mutation-dependent pathways?

DR. DAYTON: We certainly want to see it out there for as many subtypes as possible, but the way that can be handled is in the intended use or the limitations for us. We will only clear you or approve your application based on what data you submit. So, whatever subtype data you submit, we can clear you for use with those particular subtypes.

If certain subtypes are not covered in the data you submit, then it has to be very clear in the product labeling that use of this kit for those subtypes has not been validated. Really, your biggest worry there is market forces rather than FDA.

DR. JACKSON: But, in the past, for example, with blood screening, FDA CBER--

DR. DAYTON: Blood screening is a different story.

DR. JACKSON: I understand. But, in that instance, CBER has required that antibody assays, for example, must be able to detect HIV 2 and group O--

DR. DAYTON: Right. This is a very different

story, but you may be faced with a predicate device which does do those so you have to be careful.

DR. FLETCHER: Dr. Dayton, on the question of reproducibility, if the candidate assay, in the best of all possible worlds, had a between-day reproducibility of two-fold, does that meet--do you have regulations or standards for reproducibility? Would that kind of variability meet it? Second, would it be consistent with other assays that are presently on the market?

DR. DAYTON: We would have to make that decision on an ad hoc basis but, from the data Dr. Richman supplied today, it looks like two-fold is not an unreasonable amount of variability from application of the assay to application of the assay.

DR. HAMMER: Perhaps I could just interject, though. I think what was discussed this morning, at least the state of the art as of today, is that the phenotyping, which measures two-fold change, is not coming under these regulations because they are in-house commercial assays and what we are discussing are genotyping methodologies that would come to the FDA for approval and which fold changes are not really obviously measured but the sensitivity and specificity and reproducibility of picking up the codon alterations is really what we are discussing.

DR. DAYTON: Yes; that is a good comment. I am

glad you made that. We are currently trying to, or hoping that we can, regulate the genotype assays as Class II. The phenotype assays may eventually go that pathway of regulation but currently we are regarding them as basically home-brew situations at the moment.

DR. CHARACHE: I would like to pick up, Dr. Dayton, on the question of clinical utility. As that term is currently used and defined, it refers to how a given drug or product works in the patient.

If we are to use a different concept and say that an in vitro assay equals what is going to happen in the patient, would this not set very difficult precedence for all susceptibility testing including bacteria, fungi and other viruses in which we do not, at this time, accept an in vitro test as meaning it will work in the patient?

DR. DAYTON: This was examined at length by the Blood Products Advisory Committee who did actually recommend that we regulate this as Class II. In the regs, for a Class II regulation, if the knowledge of the field is sufficient or if you have a predicate device, you can go ahead and do it this way.

So it is not without precedent and it is well established in law.

DR. PETTINELLI: Regarding the issue of sensitivity, I was wondering if there has been internal

discussion in the agency regarding what would be an acceptable level of sensitivity when you are dealing with a mixture of wild and mutant virus.

DR. DAYTON: There is lots of discussion and no answer. We really have to get applications submitted to us and make decisions on what we see.

Let me just interject. FDA has, in some ways, an easier task and, in some ways, a harder task than practicing physicians. We need to show that something is safe and effective. It needs to be proven to us that something is safe and effective but you don't have to prove to us it is perfect.

A physician in the field, I think, is really striving for perfection but, to get past us, we just have to know that it works. After all, nothing works perfectly.

DR. HAMMER: Physicians are not perfect either, I think we all recognize.

Other questions?

DR. MAYERS: Dr. Dayton, it was clear in front of the Blood Products Advisory Committee that there were significant concerns about the fact that four companies could get the same genetic data and give four different interpretations back out to the field.

My understanding is that the current regulations would lead to, potentially, standardization of kits that

were submitted for licensure but would not address any commercial laboratories that use an in-house assay and did not apply for kits. So is there any intention to try and get standardization across the whole field or is this just going to be for licensure?

DR. DAYTON: Don't forget home-brew assays actually are subject to FDA regulation should we decide--based on the analyte-specific reagent concept, a home-brew assay needs to be ready for an inspection and they need to have data that is adequate for us, if we decide to go and inspect them.

At the moment, we are waiting for sponsors to come in and apply to us. Those who stay on as home brews, we can't make any representation to this point.

DR. HAMMER: Can I ask a follow-up question to that, then? Would the regulations include--I was assuming it would be the technical performance of the assay, but does it also include an applicant's interpretation of the results, because that is a very slippery slope in that question.

DR. DAYTON: I am not really sure what you are asking.

DR. HAMMER: I am asking, for example, two different applicants could get the same results and show the same performance characteristics on defining X number of

codon alterations but the interpretation of that can vary substantially depending upon resistance mutational interactions, expertise, et cetera.

The Class II regulations really are the technical performance of these kits, not necessarily the interpretation of what a particular incorporation of mutations means.

DR. DAYTON: I think the answer that is going to come to that is we will probably--and this is just probably--we will probably approve or clear these assays for a limited set of loci in which the data seems fairly clear. Then, to extend that to more complicated loci, we would need additional information and additional clarification.

It would seem to me that this kind of situation you are talking about would involve a controversy in the field as to how to interpret something. We would hope that we would know enough about the resistance testing that if a sponsor does claim an interpretation that is controversial in the field, we would know it is controversial.

Certainly, if a sponsor wants to claim a controversial mutation, or any mutation, they have to back it up even if it is just literature citations. At that point, we will review the data. If it is controversial, then that would not part of the intended use.

DR. HAMMER: Thank you.

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I think we will take a fifteen-minute break and return at 10:50.

[Break.]

DR. HAMMER: I would like to call the committee back into session. The committee has three questions for it to consider. Before we do that, I just would like to ask Dr. Dayton for one clarification quickly just for everyone's sake and that is if you could quickly define the difference between Class II and Class III and what the level of clinical data is that is required for a Class II approval. It is less but is there still some. I think this may help us understand some of the issues related to codon-specific approvals, et cetera.

Could you just quickly define that for us so we know what we are talking about?

DR. DAYTON: To put it very simply, a little bit over-simply, the Class II regulations generally refer to situations where there is a predicate device, there is already something on the market which does that and all you have to do is show that you are like that.

Class III, or PMAs, have been, typically, a more stringent level of regulation. It is generally considered that there is not enough information in the field out there to approve a Class III device in the absence of clinical trials.

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So, typically, for Class III, you have to run clinical trials. For Class II, you don't. But the regulations are not quite that simple. Under Class II, we have the right to require clinical trials if we feel that there is a gap in the knowledge.

Also, instead of using a predicate device, we can use special controls which we identify. Basically, those would be typically what you would think of as preclinical studies. If we feel the knowledge is so far along that we can guarantee a device would be safe and effective based on general knowledge in the field, we can establish a criteria that the device has to meet such as the ability to perform at a certain level on certain analytes in a certain panel or something like that.

That is the basic division.

DR. HAMMER: Thank you.

DR. HAMILTON: Dr. Dayton, could you clarify for me--

DR. DAYTON: I thought you said one question.

DR. HAMMER: I did, but I take the Chair's prerogative to recognize Dr. Hamilton.

DR. HAMILTON: Could you clarify for me, please, when and how the authority is transferred from the FDA to a subsequent regulatory body that monitors and evaluates on an ongoing basis the adequacy of performance of a test such as

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think occurs in microbiology where a clinical lab--I don't now the name of the group but there is ongoing monitoring f that.

How does that happen in this case?

DR. DAYTON: What we envision happening in this ase is we will probably require certain postmarket studies r postmarket surveillance and the results of that would ave to be delivered to us and then we would have to pass on t.

We haven't started to examine other possible echanisms but I'm sure--we definitely plan to follow these p postmarket.

DR. HAMMER: Thank you very much. That is the ast question.

Questions to the Committee

DR. HAMMER: There are three questions posed for discussion by the advisory committee for this morning's first session. We will take them in order. The first is, what are the relative strengths and limitations of genotypic versus phenotypic drugs in assessing resistance to antiretroviral drugs through the stages of drug development. Please comment on the potential roles of these two types of assays throughout drug development.

I think, rather than going one-by-one around the table, I will open this up for people who want to comment in

an ad hoc fashion. Who would like to start? Not everyone at once.

DR. PETTINELLI: Actually, I would probably more like to comment on the second part of the question which is the use of the two assays in drug development. I think that, really, both of the assays, when we are talking in terms of preclinical drug development and try to determine the genotypic- and phenotypic resistance to the compound, that they both should be used in preclinical-trial development as well as we should evaluate the clinical isolates.

In terms of, now, clinical development, I think that probably we could target the use of such an assay. For example, from the preclinical, we are now developing a pattern in which the new compound appears to really be sensitive to all other resistant viruses, and then maybe we can do some target study and try, really, to look at a patient who now has the genotypic mutation and how to respond to drug.

I am not sure--it would be more like complementary to what we have as a rationale development until now, but just target this specific aspect.

DR. MATHEWS: I want to just comment on a specific aspect of their use in clinical trials. It seems to me that, in the circumstances where it is known with a high

degree of confidence that a particular mutational pattern confers resistance, or if you are going to use a phenotypic assay with a certain fold change with a high degree of confidence, that we should not be conducting clinical trials that expose patients to agents for which there is a high degree of confidence up front that they will not respond.

I think it is a very different situation where the significance of the mutational patterns is uncertain. I know some trials are already being formulated in which results of resistance assays are made available up front for the selection of certain concomitant drugs but not for others.

I don't know if everybody agrees with that or maybe they don't, but that would be my opinion.

DR. STANLEY: I guess if I were going to try to simplify things or break it down, if you can, I would think you would start with genotypic assays to try to understand if there are specific correlates of resistance with specific mutations. But then you have to show whether that is relevant with phenotypic assays.

The other use for phenotypic assays, I think, is going to be in drug combinations. We don't do monotherapy. So you are going to have to try to somehow understand that even if there is a mutant genotype, does that still confer resistance in a specific drug combination. I think you are

going to need phenotypic assays for those.

DR. HAMMER: I think one thing we might do, to frame the discussion, is think about this in the preclinical development and the clinical development and, just to put something forward and an extension of what Dr. Richman said, certainly in the preclinical development, what this committee would like to see--I can't speak for the agency but can infer--would be assays that determine genotypes of viruses that are passaged in vitro and then to follow that up with what the phenotypic pattern is, also in vitro studies that then look at the activity of this drug, of the particular drug, against viruses with known resistance patterns.

That would be sort of the minimum issue. I think the issue of looking at what combinations might do in vitro is something that would be a nice addition to what we have seen thus far. Maybe we should sort of talk a little bit about that stage and then quickly frame the discussion for the clinical development issues which Dr. Mathews introduced. I think going back and forth becomes a little heterogeneous in the discussion.

DR. YOGEV: I would contend that the genotypes would be at the Phase I portion of the development of a drug. I was quite impressed by the correlation between the genotype and phenotype. I think we understand better the

genotype today in certain mutations that can be defined.

We don't understand the phenotype well enough to really accept certain data coming to us that this is in this range, what really are the clinical implications. so I would suggest that, in the drug development, I would rely on the genotype which would suggest the strains, as you suggested, into the drug and see what change in genotype is happening because I am very concerned about the quasi-species.

I think a major problem in those two methods is the variant which is already there, but in a lower percentage. I didn't hear anything--hopefully, it will be clarified later--what does that mean 20 percent? 20 percent of a million is 200,000. 20 percent of 1000 is 200.

There must be sensitivity of how low you can go when you bring it up that we can define in the genotype. So, for me, the genotype is much more scientific at this point to understand that I would like to understand that I would like to see the drug developed on that level and then we move to the clinical, move into the phenotype also.

DR. HAMMER: I would just add that I think genotype and phenotype are important both at the preclinical and at the clinical level but maybe we can define that better later.

DR. MASUR: One of the issues that it would be

actually interesting to get some clarification from some of the people who do the phenotypic assays is what the practicality would be of looking at combinations because certainly--and Pat, before, was talking about other susceptibility testing that we do.

In most areas of infectious disease, we have gotten away from asking the laboratory to do synergy or antagonism studies and we take, on faith, the fact that if we use several active drugs, that is better than using one active drug alone although there is often not good information.

It would be nice to know that if the four drugs that you are using are really better than some combination of three of those drugs or two of those drugs, but that becomes extraordinarily complicated.

Is that technically and financially feasible? As we look at these assays, no one has yet mentioned what the prospect is that these could be done at an affordable price even in Montgomery County. Is that something we could get some comments about?

DR. HAMMER: Would anybody like to comment from the audience? Dr. Larder?

DR. LARDER: Brendan Larder from Virco. I get asked this question all the time. It comes up. I guess the guys from ViroLogic do as well. The short answer is no, it

is just not feasible to do it. But we have done some studies where we have looked at just two drug combinations. In those studies, we didn't really see any advantage of adding two drugs into wells over and above deriving separate phenotypic IC50 or resistance levels for individual drugs.

We have looked at it but, as you said, it is not two drugs we are interested in; it is three or four. Actually, to derive any meaningful synergistic data from that would be just impossible, a three-D checkerboard. If people think assays are fairly expensive now, then, to do that, would be just prohibitive and I don't think it would add very much to the information.

DR. MASUR: Then, just to follow up, Brendan, are you suggesting that, as in other areas of infectious disease, we are just going to have to take on faith that multiple drugs are likely to be better than a single drug? Obviously, you can do viral-load studies but it is getting harder and harder to break that down into segments.

DR. LARDER: I think that is right. I think you would want to look at the reports and try and pick out the most susceptible drugs in that report to build a three-drug regimen that you can try and use.

My understanding is, in the antibody field, that people have been struggling in that field for years and years with combinations and it is not really done as well.

DR. HAMMER: I think one thing that could be said here is that although in the clinical application of looking at all these two-, three- and four-drug combinations and requesting from a commercial phenotyping firm those results on a fee-for-service basis is impractical.

What is not impractical, I think, is to request, as a drug goes through development, that certain in vitro studies be done to look for synergy and/or antagonism in two and three and possibly four drug combinations within limits. I think, as Dr. Richman said, there would have been certain antagonisms in vitro that have been confirmed in in vivo studies.

So I think that there are issues on the commercial side and from physician or the physician/investigator side that make it impractical. But there are ways to look at some of this in a more limited fashion as a particular drug goes through the development.

DR. LARDER: I think you are right. But getting to three drugs is going to be difficult. One of the cases that Doug was probably alluding to is AZT and d4T. But, actually, going back to the preclinical in vitro data, there was controversy about whether there are additive effects or antagonistic.

It was only really until it got to the clinical trials that it showed up pretty antagonistic.

DR. HAMMER: Dr. Richman would like to comment.

DR. RICHMAN: I agree that it would be potentially dangerous to require too much in terms of these combinations for several reasons. One, the practicality of combinations is, in reality, much more dictated by issues of pharmacologic interactions than antagonism.

To my mind, we haven't had enough pharmacologic interaction data when we have been using these drugs in people which, to me, would be a much more important issue in terms of safety and intelligent use than these theoretical issues on antagonism because we really only want to not use a combination if it is antagonistic.

That is the only question because additivity or synergy isn't really that important. We clearly need combinations and we don't want to use them unintelligently or dangerously.

DR. HAMMER: Thank you, although--just a last comment--some focused studies are helpful because sometimes they reopen our horizons. For a long time, people were nay-sayers about dual NRTI combinations and maybe that won't evolve. But, in fact, now they are being investigated clinically. There had been in vitro data to suggest we would think about them from the past and not just assume, because they bind to the same region on the RT, that it would not be worth looking at them together.

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So focussed developmental approaches may help expand the horizon.

DR. POMERANTZ: I would like to continue on what Dr. Masur said or what was the discussion between him and Dr. Larder because I think it is important to look back at some of the knowledge we do have from other infectious diseases, to put on my ID hat. We have, at times, flown by the seat of our pants on a number of different treatment regimens and have not had as much data in other classical infectious diseases that we have even now for HIV.

But, that being said, it is important to realize that there are organisms, we have used the pneumococcus as one, where you have partial resistance in vitro and yet you can still use that drug in vivo to treat the infection.

I think that comes to bear on HIV in the group that I had asked Doug about before, and that is the development of understanding phenotypic resistance in the modest range. Being close to eight-fold or ten-fold resistant, it is not clear to me that, as Doug was saying, those are all truly resistant, both in vitro nor in vivo.

So it is important that when we get these studies together that we realize that modest in vitro resistance may still not obviate you to not use that drug especially in certain combinations.

So I agree with Doug. I think that, up front, you

are going to do genotypic and phenotypic analysis in both preclinical and clinical drug design. But then it is going to get far more complicated unless it is a cut-and-dry issue. If you look at some of the resistance data that has been coming out, there are a number of different phenotypic analyses that are not clear how they are going to fall in vivo.

DR. MAYERS: I think one area that Doug showed in his slides earlier is the issue of what is sensitive. Previously, we would take a group of untreated patient isolates and define the sensitive range for a drug. Now the companies are taking the standard of one reference strain and that reference strain could fall anywhere in the high or low or intermediate range of susceptibility so that there is an hundred-fold difference in AZT resistance in the range of susceptible.

If your isolate references lands high, you are likely to call many things resistant that are probably in the normal range. If it is low, you are likely to call things sensitive that--so I think it is really important that there be some standard of what is the normal range for a drug and, perhaps, a panel like Doug has put out where you have isolates before 1985 in untreated patients is the type of panel you need to get to define "normal" for a drug.

I am very concerned that the phenotyping companies

are calling resistance because it is two-fold greater than their reference strain for isolates that are probably in the normal range of untreated patients.

DR. HAMMER: I think this discussion will also come up in Dr. Little's presentation.

Dr. Mellors?

DR. MELLORS: Doug mentioned this but it needs to be restated. We are talking about a couple here, and we are only talking about the husband and not what is wrong with the wife. Whenever we discuss drug susceptibility, we have to discuss--

DR. HAMMER: Are we in the right committee session?

DR. MELLORS: It is a byproduct of counseling here. Whenever we talk about drug susceptibility, we have to talk about drug exposure. To think that we can predict clinical response from the fold variation over reference strains is ludicrous.

So these two days are devoted to resistance testing but we have to keep in mind that susceptibility is determined by many factors in vivo. One important quantifiable factor is the concentration of drug, free drug, active drug, that can be achieved and maintained.

DR. HAMMER: Very important point.

DR. WONG: Just with respect to this question, I

actually like Dr. Richman's list very much about what should be expected of a sponsor, particularly in the preclinical develop package. I think both phenotype and genotype results should be shown. I might also say, for the sake of perspective sponsors, that, over the past year, we have seen a few presentations in which the virology data were provided only in kind of gross summary form.

I, personally, would prefer to see the populations of viral isolates tested and see the results so that we can evaluate for ourselves what the range of susceptibility to various drugs was for the new drug.

So I would like to see a thorough preclinical evaluation of drug-resistance testing both at the phenotypic and genotypic level in susceptible and resistant strains with a full presentation of those data.

DR. MAYERS: The only difference I would have between the list that Doug gave in clinical studies and my own personal bias is that I think we need to define the genotypes and phenotypes associated with loss of activity of a drug, and genotypes and phenotypes in experienced patients that predict a lack of response on the next round of therapy.

A concern that I have, if that is the standard you move to, is that, for many of the drugs that we currently have in practice, this has never been done. The problem the

company is going to have, if we have never defined d4T and ddI resistance cleanly and you use that in a combination, how do you define the component that is related to the new drug.

I think there has got to be an association between genotype and phenotype and loss of activity in naive patients and genotype and phenotype and lack of activity in pretreated patients because that is the data you would need clinically to use these drugs.

DR. MASUR: Although it is getting a little bit ahead of where we are right now, one of the things in other infectious diseases, to put a couple of people on the panel on the spot, it is very useful when governmental agencies do surveillance and provide information as to what the activity of various drugs is against certain isolates.

When you get data about a particular drug, it is very hard to put that into context with other drugs as to whether this is active. It is active, but it is active compared to what, if you accept what John Mellors said that this is only one aspect of treating a patient.

Is it conceivable that, at some point, the CDC or some other agency is going to do surveillance so that we can put all these drugs into some kind of regional or national or international context?

DR. KAPLAN: Actually, as I mentioned when we went

around the table at the beginning with our introductions, we have a great interest at CDC in doing surveillance of resistance. But the population, I think, we are mainly interested in is antiretroviral-naive persons because I guess it is our perception that there is a lot of this work going on in experienced patients and other networks, particularly NIH-funded studies such as an ACTG.

We do have a system now where we are funding various state and city health departments to recruit antiretroviral-naive persons. We are playing around the idea of using the detuned assay to try to further define which people are recently infected.

In fact, one of the reasons I am here at the meeting is to look at possibilities for expanding our system. So this is a priority for us. I think our focus right now is in naive persons.

DR. GULICK: Just to take a step back from a clinician's point of view on question No. 1. It needs to be stated that probably one of the great needs in drug development right now is to develop new agents which have activity against viruses which are resistant to the agents that we have.

It seems, with these new tools, that we have really turned a corner in sort the encouragement and the development of new drugs given that we have fifteen approved