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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 III

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Pages 1 thru 229

Gaithersburg, Maryland
November 3, 1999

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AT

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

ANTIVIRAL DRUGS ADVISORY COMMITTEE MEETING

VOLUME III

Wednesday, November 3, 1999

8:30 a.m.

Holiday Inn Gaithersburg
Two Montgomery Village Avenue
Gaithersburg, Maryland

MILLER REPORTING COMPANY, INC.
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Joanne L. Rhoads, M.D.

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

2 Call to Order

3 DR. HAMMER: I would like to call into session the
4 Antiviral Drugs Advisory Committee meeting. This is day two
5 of our consideration of HIV drug resistance in the setting
6 of drug development.

7 I would like to begin again by having the members
8 and guests of the committee introduce themselves for the
9 record. I will begin on my left with Dr. Charache.

10 DR. CHARACHE: I am Patricia Charache. I am
11 Professor of Pathology, Medicine, and Oncology at Johns
12 Hopkins.

13 DR. WOOLSON: Robert Woolson. I am Professor of
14 Biostatistics, University of Iowa.

15 DR. MATHEWS: Chris Mathews, Department of
16 Medicine, UC/San Diego.

17 DR. KUMAR: Princy Kumar, Infectious Diseases,
18 Georgetown University Medical Center.

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

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

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

25 DR. HAMILTON: John Hamilton, Adult Infectious

1 Diseases, Duke University and the Durham VA Hospital.
2 DR. WONG: Brian Wong from the West Haven VA
3 Hospital and Yale University.
4 DR. HAMMER: Scott Hammer, Infectious Diseases,
5 Columbia University.
6 MS. STOVER: Rhonda Stover, FDA.
7 DR. POMERANTZ: Roger Pomerantz, Infectious
8 Diseases, Thomas Jefferson University.
9 DR. JACKSON: Brooks Jackson, Pathology, Johns
10 Hopkins University.
11 DR. PETTINELLI: Carla Pettinelli, Division of
12 AIDS, National Institutes of Health.
13 DR. KAPLAN: Jon Kaplan, Division of HIV AIDS
14 Prevention at the Centers for Disease Control and
15 Prevention.
16 DR. MAYERS: Doug Mayers, Henry Ford Hospital,
17 Division of Infectious Diseases.
18 DR. ARAS: Girish Aras, Division of Biostatistics
19 III, CDER, FDA.
20 DR. LAESSIG: Katherine Laessig, Medical Officer,
21 FDA.
22 DR. IACONO-CONNORS: Lauren Iacono-Connors, FDA.
23 DR. RHOADS: Joanne Rhoads, FDA.
24 DR. MURRAY: Jeff Murray, FDA.
25 DR. JOLSON: Heidi Jolson, FDA.

1 DR. HAMMER: Thank you. I would like to turn to
2 Rhonda Stover who will read the conflict of interest
3 statement.
4 **Conflict of Interest Statement**
5 MS. STOVER: The following announcement addresses
6 the issue of conflict of interest with regard to this
7 meeting and is made a part of the record to preclude even
8 the appearance of such at this meeting.
9 Since the committee's discussions of issues
10 related to testing for development of resistant human
11 immunodefiency virus will not have a unique impact on any
12 particular firm or product, but rather may have widespread
13 implications with respect to an entire class of products, in
14 accordance with 18 United States Code 208, general matters
15 waivers have been granted to each member and consultant
16 participating in the committee's discussions.
17 A copy of these waiver statements may be obtained
18 by submitting a written request to the FDA's Freedom of
19 Information Office, Room 12A-30 of the Parklawn Building.
20 In the event that the discussions involve any
21 products or firms not already on the agenda for which an FDA
22 participant has a financial interest, the participants are
23 aware of the need to exclude themselves from such
24 involvement, and their exclusion will be noted for the
25 record.

1 With respect to **all** other participants, we ask in
2 the interest of fairness that they address any current or
3 previous involvement with any firms whose products they may
4 wish to comment upon.

5 **DR.** HAMMER: Thank you.

6 One quick announcement. For those who are
7 interested, we will be discussing regulatory scenarios this
8 afternoon, and copies of those scenarios and the slide
9 formats are out on the desk for you to pick up if you would
10 like at the break.

11 Now I would like to turn to Joanne Rhoads, who
12 will introduce Session 3.

13 **SESSION 3**

14 **Practical Considerations for the Use of**
15 **Resistance Testing in Antiretroviral**
16 **Drug Development and Use**

17 **Introduction**

18 **DR.** RHOADS: Good morning. I am Joanne Rhoads
19 from the Division of Antiviral Drug Products, FDA. I would
20 like to introduce Session 3, which is devoted to practical
21 considerations for the use of resistance testing in
22 antiretroviral drug development.

23 Yesterday's speakers provided an overview of the
24 genotypic and phenotypic assay technology current available,
25 the performance characteristics and limitations of these

1 assays, and evidence from retrospective and prospective
2 studies supporting their clinical utility.
3 Today, before we continue to discuss the role of
4 resistance testing in drug development, we will consider the
5 prevalence of drug resistant HIV-l in selected U.S.
6 populations and also discuss in more depth some of the
7 factors which may complicate the interpretation of
8 resistance data.
9 These factors which were touched on many times
10 yesterday include the presence of naturally occurring
11 polymorphism, sampling issues, the complexity introduced by
12 combination therapy, pharmacological properties of a drug,
13 and anatomic and cellular compartmentalization of HIV.
14 The session objectives are:
15 1. To review the prevalence of genotypic variants
16 and/or reduced susceptibility in selected U.S. populations.
17 2. To illustrate possible limitations in the
18 practical clinical use or application of resistance assays
19 in clinical investigations.
20 3. To examine how cofactors associated with
21 treatment outcome confound interpretation of resistance
22 testing.
23 Dr. Susan Little will present data addressing the
24 prevalence of drug resistant HIV in selected U.S.
25 populations of newly infected individuals, and Richard

1 D'Aquila will provide a review of factors which may confound
2 interpretation of resistance data.
3 Once again, we look forward to an interesting and
4 productive discussion. I will introduce the first speaker
5 and then turn the session over to our chair, Dr. Scott
6 Hammer.
7 Dr. Susan Little will now present Transmission and
8 Prevalence of Drug Resistant HIV.
9 Thank you.
10 **Transmission and Prevalence of HIV Resistance**
11 DR. LITTLE: Thank you.
12 [Slide.]
13 The transmission of drug resistant HIV was first
14 reported in 1992 by Erice and Colleagues at the 32nd ICAAC.
15 In this report, a patient with primary HIV
16 infection had blood samples obtained which showed a reduced
17 susceptibility to AZT and sequence data then showed the
18 presence of a tyrosine at position 215, conferring AZT
19 resistance.
20 Since this first report, there have been numerous
21 reports of the sexual transmission of drug resistant HIV,
22 first in the setting of single drug and more recently in the
23 setting of multi-drug resistant HIV.
24 The initial reports, not surprisingly, documented
25 transmission of single drug AZT resistance and more recently

1 3 TC resistance, while more recent reports, first in the
2 form of isolated case reports and more recently in larger
3 cohort studies document the transmission of multi-drug
4 resistant HIV.

5 [Slide.]

6 The transmission of drug resistant HIV is not
7 limited, however, to the sexual transmission of HIV. The
8 transmission of drug resistant HIV has also been documented
9 by several groups in the setting of perinatal or vertical
10 transmission following injection drug use transmission, and
11 between two children following a presumed unrecognized blood
12 contact.

13 [Slide. 1

14 These studies have clearly shown, however, that
15 the transmission of virus with reduced drug susceptibility
16 is not associated with lower pretreatment viral loads. As
17 was covered yesterday, it is not known what level of reduced
18 susceptibility is reproducibly associated with virologic
19 failure for each drug.

20 As a result, completely arbitrary classifications
21 of reduced drug susceptibility have been adopted by most
22 investigators and they are shown here for the two most
23 common assay types that are going to be discussed today, so
24 I won't review them again.

25 [Slide.]

1 As an introduction to this kind of interpretation
2 or how to interpret these tests, I am going to present a
3 little bit of data from our own cohort study. We evaluated
4 antiretroviral susceptibility using the virologic
5 susceptibility assay among 141 subjects with primary HIV
6 infection from five cities across the United States.
7 The number of patients from each city are shown
8 here - 48 in San Diego, 48 in Los Angeles, 19 in Dallas, 13
9 in Denver, 13 in Boston.
10 We estimated the date of HIV infection in these
11 study patients using the date of high risk exposure or
12 symptom onset in symptomatic seroconverters or the date of
13 the first positive HIV test in asymptomatic seroconverters.
14 [Slide.]
15 The percent of patients with any level of reduced
16 drug susceptibility to the nucleosides and non-nucleoside
17 reverse transcriptase inhibitors are shown here. The dark
18 blue boxes and the dark red boxes indicate the proportion of
19 patients with higher level or greater than IO-fold
20 reductions in drug susceptibility.
21 Overall, the proportion of patients with reduced
22 susceptibility to the nucleosides was relatively low with
23 only 3 percent of our study cohorts showing reduced
24 susceptibility to one or more of the nucleoside reverse
25 transcriptase inhibitors. The lighter boxes, I should say,

1 indicate reduced susceptibility in the 2.5 to lo-fold range.
2 In contrast, the proportion of patients with
3 reduced susceptibility to the non-nucleosides was
4 surprisingly high given that the use of these compounds was
5 not widespread at the these patients were identified.
6 The level of reduced susceptibility, however, that
7 was identified was generally lower than has been described
8 in patients with genotypic resistance. Again, only 1
9 percent of the study cohort had a greater than IO-fold
10 reduction in susceptibility to the non-nucleosides.
11 Similarly, only 1 percent of the study cohort had a greater
12 than lo-fold reduction in susceptibility to the nucleoside
13 reverse transcriptase inhibitors.
14 [Slide.]
15 The percentage of patients with any level of
16 reduced susceptibility to the protease inhibitors varied
17 from 1 percent for saquinavir, 2 percent for indinavir, 5
18 percent for ritonavir, and 9 percent for nelfinavir, again,
19 darker boxes indicating those patients with greater than 10-
20 fold reductions in susceptibility.
21 Overall, 10 percent of our study cohort had some
22 level of reduced susceptibility to the protease inhibitors
23 with only 1 percent again having a greater than IO-fold
24 reduction in susceptibility.
25 [Slide.]

1 We then evaluated reverse transcriptase and
2 protease sequence in the 39 patients in whom we identified
3 some level of reduced drug susceptibility. In the setting
4 of what is now a very extensive list of amino acid
5 substitutions that have been reported to date in association
6 with in vitro or in vivo drug resistance, we chose to
7 identify or report only those well-characterized amino acid
8 substitutions which have been clearly associated with in
9 vitro drug resistance according to the 1998 JAMA consensus
10 guidelines on antiretroviral drug resistance published by
11 Hirsch and colleagues.
12 These guidelines identify a set of so-called
13 primary drug resistance mutations shown here for reverse
14 transcriptase and protease. These primary drug resistance
15 mutations are generally selected early in the process of
16 accumulation of drug resistance mutations, tend to have a
17 discernible effect on drug susceptibility, and are often
18 drug specific.
19 [Slide.]
20 Those, as Dr. Richman introduced yesterday, are
21 these black and white mutations. These are the gray. These
22 guidelines also identified a group of secondary mutations,
23 shown here again for reverse transcriptase and protease.
24 These mutations tend to accumulate in viral
25 genomes that already contain one or more of the primary drug

1 resistance mutations. They may have a more limited effect
2 on drug susceptibility and may, in fact, be selected because
3 they improve viral fitness.
4 [Slide.]
5 So, using population-based sequence analysis on
6 those 39 patient isolates, there were three patients in our
7 cohort for whom we identified a major reduction or greater
8 than lo-fold reduction in drug susceptibility. Among these
9 three patients, primary drug resistance mutations in reverse
10 transcriptase and protease are shown here, the primary drug
11 resistance mutation shown in yellow and/or bold, and the
12 secondary mutation show in white.
13 These were observed in the background of numerous
14 other polymorphisms. There was one of these three patients
15 in our cohort who had an isolated high-level or greater than
16 IO-fold reduction in susceptibility just to the non-
17 nucleosides, and in this patient we did not identify any
18 primary drug resistance mutations.
19 In contrast, among the 36 patients in whom we
20 identified a moderate reduction in susceptibility, we
21 identified one -- and this is the merit of Power Point, I
22 changed these slides this morning -- we identified one well-
23 characterized drug resistance mutation, the presence of a
24 T215Y mutation in a patient who had an 8.4-fold reduction in
25 susceptibility. It didn't make the cut-off of 10 for the

1 major reduction in susceptibility.

2 So, in the setting of only one well-characterized
3 drug resistance mutation, in the background again of
4 numerous polymorphisms, this suggests that these may, in
5 fact, be wild type viruses with reduced susceptibility,
6 which are really of unknown clinical significance.

7 [Slide.]

8 We also evaluated the isolates in our study
9 according to the year in which their baseline sample was
10 identified, beginning in 1989 through 1998.

11 Although 70 percent or nearly 70 percent of our
12 study cohort was identified in 1997 or later after the
13 release of the first really potent protease inhibitors, we
14 did not identify any increase in the proportion of patients
15 identified over time with some level of reduced
16 susceptibility to the protease inhibitors, nor for that
17 matter, for the non-nucleoside reverse transcriptase
18 inhibitors.

19 Again, these numbers are really quite small, but
20 we did not see any trends. Like other of the larger cohort
21 studies, however, the two patients in our study who had high
22 level or greater than IO-fold reductions in susceptibility
23 to the protease inhibitors were both identified in 1998.

24 [Slide. 1

25 So, using this kind of interpretation, I have

1 taken great liberties with the data that has been presented
2 and published by numerous other North American, European,
3 and Australian investigators, and re-analyzed their
4 published and presented data using these guidelines, these
5 criteria for primary and secondary drug resistance mutations
6 and cut-offs for the established two phenotypic assays to
7 try and determine whether there is any degree of consensus
8 among the many published and presented studies now in terms
9 of the overall prevalence of drug resistance in patients
10 with primary or recent HIV infection.
11 To talk through this table, these are the North
12 American studies using our study first as an example, again,
13 141 patients, our population was patients with primary HIV
14 infection of less than 12 months duration. The mean time
15 from seroconversion, approximately two months.
16 We identified again 2 out of 141 patients with
17 primary drug resistance mutations, again with the caveat
18 shown down here, we did not sequence all samples or at least
19 as of the date of this table, had not sequenced all samples,
20 but only sequenced those samples in which we identified any
21 level of reduction or reduced drug susceptibility. So, I
22 think it is unlikely that this is going to be substantially
23 higher when we put in the data from all of those samples
24 that we have now sequenced with wild type susceptibility,
25 but it could change slightly.

1 So, again, 2 out of 141 or 1 percent of the cohort
2 of the primary drug resistance mutations, 31 out of 141 or
3 22 percent with secondary drug resistance mutations.
4 Again, we used the virologic assay using this cut-
5 off of greater than lo-fold, 3 out of 141 or 2 percent of
6 the cohort with greater than lo-fold reductions, 26 percent
7 with 2.5 to lo-fold reductions in susceptibility.
8 The Boden study, published the same month in JAMA,
9 80 patients, their population less than 6 months from
10 seroconversion, again mean time from seroconversion 2
11 months.
12 They identified primary drug resistance mutations
13 the same primary that I used, in 8 out of 80 patients or 10
14 percent, and secondary drug resistance mutations in 48 out
15 of 80 or 60 percent using the same virologic assay, but
16 unfortunately, with a different cut-off that I haven't yet
17 been able to resolve, of greater than 5-fold reduction in
18 susceptibility, they found 8 out of 67 or 12 percent with a
19 greater than 5-fold reduction in susceptibility.
20 So, again, once this number is adjusted to conform
21 to the greater than 10-fold cut-off, this number may
22 actually fall to be more in consensus with the others.
23 In the 2.5- to 5-fold, they identified 10 out of
24 67 or 15 percent.
25 The Wegner study, 114 patients, all with recent

1 HIV infection less than three years duration, mean **time** from
2 seroconversion approximately 12 months.
3 They identified primary drug resistance mutations
4 in 6 of 95 or 6 percent, secondary in 22 percent, using the
5 Virco assay with their cut-offs greater than lo-fold in 8
6 percent, greater than 4- to lo-fold in 19 percent.
7 Bob Grant's study, 89 patients, again primary
8 infection less than 12 months duration, primary drug
9 resistance mutation only for the nucleoside reverse
10 transcriptase inhibitors. That is the only data I had
11 access to for this.
12 10 of 89 or 11 percent with primary drug
13 resistance mutations, they only presented data on the lower
14 level or 2.5- to lo-fold reductions in susceptibility to
15 only the non-nucleosides, but again just to show that they
16 are seeing the same fairly high number in terms of frequency
17 associated with this lower level reduced susceptibility to
18 the non-nucleosides.
19 This study by Weinstock, 99 patients, recent
20 infection less than 24 months, 5 percent of their cohort
21 with primary drug resistance mutations, roughly 21 percent
22 with secondary. 1 out of 12 or 8 percent showed greater
23 than lo-fold reductions in susceptibility, 4 out of 12 or 33
24 percent showed 2.5- to lo-fold.
25 Verbiest, 230 patients. Treatment-naive patients

1 with chronic infection. 5 out of 199 or 3 percent with
2 primary drug resistance, 15 percent with secondary. They
3 used the Virco assay. 3 percent with greater than IO-fold
4 reductions in susceptibility and 11 percent with 4- to 10-
5 fold.
6 [Slide. 1
7 I also reviewed less completely the European and
8 Australian and a few Canadian data. The French study looked
9 at 370 treatment-naive patients with chronic HIV, but I was
10 most interested in this subset of 103 patients within their
11 group that had primary HIV infection or infection of less
12 than 12 months duration.
13 Again, 8 out of 103 or 8 percent had primary drug
14 resistance mutations, 3 out of 103 had secondary, again, the
15 caveat being that in this cohort, the protease domain was
16 sequenced only if the reverse transcriptase domain showed
17 mutations. So, again, this may underestimate. This might
18 move up if they were to sequence more of their isolates.
19 The Spanish study, 150 patient, treatment-naive,
20 18 out of 149 with primary drug resistance mutations, but
21 with the caveat that this is the Mirex line probe assay,
22 which interrogates only primary reverse transcriptase
23 inhibitor mutations.
24 Yerly, the Swiss study, 82 patients, approximately
25 less than 6 months duration of infection, so primary HIV

1 infection. Again, 8 out of 82 or 10 percent with primary
2 mutations, 51 percent with secondary, but they only called
3 secondary protease mutations.
4 She used a different phenotypic assay, an in-
5 house, home brew assay, but it had the same cut-offs
6 actually of greater than 4 to 10, and greater than IO-fold
7 for their different categories.
8 2 out of 14 or 14 percent had greater than IO-fold
9 reductions in susceptibility, but again, they assessed
10 susceptibility only if primary or secondary protease
11 mutations were identified.
12 So, again, this number might increase if they also
13 looked at those patients that had primary or secondary RT
14 mutations. 8 percent with the lower level 4- to IO-fold
15 reduction in susceptibility.
16 Balotta, the Italian study, 37 patients, less than
17 six months duration, 11 percent of this cohort had primary
18 resistance mutations. Again, this was only the RT domain
19 that was sequenced. 30 out of 33 had secondary mutations.
20 The Australian study, 84 patients, very recent
21 infection. Again, 14 percent had primary reverse
22 transcriptase inhibitor mutations.
23 Finally, Veronica Miller's data, 46 patients, less
24 than six months duration, 5 percent had primary mutations,
25 54 percent had secondary mutations.

1 [Slide. 1

2 So, in an effort to try and summarize all of this,
3 at least the North American data, a major reduction in
4 susceptibility, that is, greater than IO-fold reduction, has
5 been observed in 2 percent of subjects with primary HIV
6 infection and 3 to 8 percent of treatment-naive subjects
7 with recent infection.

8 Primary drug resistance mutations have been
9 observed in 1 to 11 percent of subjects with primary HIV
10 infection and 3 to 6 percent of treatment-naive subjects
11 with recent infection.

12 So, if I am permitted to very broadly round,
13 something like 5 percent of patients with primary or recent
14 HIV infection in the United States appear to be infected
15 with clinically relevant drug resistant virus.

16 [Slide.]

17 Many of these larger cohort studies have also
18 identified patients infected with multi-drug resistant
19 virus, that is, virus with resistance, primary drug
20 resistance to greater than one class of antiretroviral
21 drugs. This has been shown in multiple cities now in the
22 United States and Europe with frequencies in the 1 to 4
23 percent range.

24 In many of these cases, there was no exposure
25 history to suggest the transmission of multi-drug resistant

1 HIV with which to promote or advocate a more selective use
2 of resistance testing. Thus, strategies are desperately
3 needed to permit rapid identification of these individuals.
4 [Slide.]
5 As one such example, in our own cohort study,
6 patient 98-1186, shown in blue here, resistance was not
7 suspected in this patient. He was started very rapidly
8 after seroconversion on a regimen of AZT, 3TC, and
9 indinavir, and showed a rather slow virologic response,
10 which is fairly self-evident when compared to a more typical
11 patient, initiating the exact same regimen who shows a much
12 more rapid viral decay.
13 Because of his slow virologic response, sequence
14 analysis was performed and showed primary drug resistance
15 mutations to AZT, 3TC, and multiple protease inhibitors. As
16 a result of this data, his treatment regimen was switched to
17 an entirely new, non-nucleoside-based combination regimen,
18 which resulted in complete suppression to viral load less
19 than 50, which I am told has now been sustained for six
20 months even beyond the follow-up shown in this slide.
21 [Slide.]
22 Thus, moderate reductions in drug susceptibility
23 to certain drugs are highly prevalent and frequently not
24 associated with recognized drug resistance mutations.
25 The presence of moderate reductions in

1 susceptibility may actually, we believe, represent wild type
2 virus that is simply being discerned with greater confidence
3 using these more precise phenotypic assays.
4 However, we cannot exclude the possibility that
5 subpopulations of more resistant virus are present and not
6 being detected by our population-based sequence analyses.
7 Alternatively, there is, in fact, greater, as was
8 shown yesterday, natural variability in the susceptibility
9 of wild type virus to the NNRTIs and some of the PIs, which
10 may explain the variability that we observed.
11 The treatment implications of infection with virus
12 of moderately reduced susceptibility are currently unknown.
13 [Slide.]
14 So, now that we know something about the
15 prevalence of drug resistant HIV in North America, what
16 about the transmissibility of drug resistant HIV?
17 Several groups have identified an apparent
18 selection against the transmission of resistant virus. In a
19 study by Wahlberg, they identified 4 patients or subjects
20 with recent HIV infection and their sexual source partners
21 or donors.
22 Although all 4 source partners were infected with
23 AZT resistant virus, only 1 donor transmitted resistant
24 virus to the sexual partner recipient.
25 Similarly, in a study by Colgrove, 4 mothers who

1 were infected with mixtures of AZT resistant and sensitive
2 virus, 3 of these transmitted only the wild type to their
3 infected infant or only 1 transmitted drug resistant virus
4 to the infected infant.
5 [Slide.]
6 Another issue that is going to be relevant to the
7 issue of transmission of drug resistant virus is that of
8 compartmentalization. A study by Zhu in which patients, 5
9 acute seroconverters were identified and again their
10 respective sexual source partners or donors were identified.
11 They compared gp120 sequences in the
12 seroconverters from the time of seroconversion out to 6
13 months of follow-up, and gp120 sequences in both the blood
14 and genital secretions of the donor as close to the
15 transmission event as was possible.
16 They identified a sequence heterogeneity in the
17 blood and genital secretions of all of the donors and
18 relative homogeneity of the viral population in the
19 recipients from seroconversion out to 6 months of follow-up.
20 In all cases, the transmitted virus was a minor
21 variant present at 0.5 to 27 percent within the population
22 and the semen of the transmitter, suggesting that a
23 selection process occurred during transmission.
24 Somewhat in contrast, a study by Poss showed 6
25 recently infected Kenyan women in whom they also evaluated

1 envelope sequence evolution over time, again, from the time
2 of seroconversion out to 6 months of follow-up.
3 They identified more heterogeneous viral
4 populations that were present in the cervical secretions and
5 blood of these women from shortly after seroconversion out
6 to 6 months of follow-up, suggesting that either less of a
7 selection process occurred or perhaps there may have been
8 more subtle sampling differences which may have explained
9 these discrepancies.
10 [Slide.]
11 The transmission of drug resistant virus is almost
12 certainly going to occur more frequently in patients who are
13 receiving or have received antiretroviral therapy in the
14 absence of complete virologic suppression, however, even in
15 the setting of complete virologic suppression, replication
16 competent virus has been isolated from the seminal cells of
17 2 of 7 subjects that Zhang studied, who were receiving HAART
18 and had plasma viral loads of less than 400.
19 Actually, in those 2 in whom they were able to
20 isolate replication competent virus, both had viral loads
21 less than 50 on all of the days that they were evaluated.
22 So, even with complete virologic suppression, these kind of
23 data suggest that the transmission of virus may at least be
24 theoretically possible although to my knowledge it has not
25 yet been documented in this setting.

1 Both the study by Zhang and that by Overvaugh
2 demonstrate that the viral strain detected in the genital
3 secretions may represent a minor variant of the genotype in
4 the blood, suggesting that even with all of these advanced
5 methodologies that we have access to, we may have a very
6 difficult time predicting who is going to transmit what to
7 whom.
8 [Slide.]
9 So, finally, to summarize, we need additional
10 studies to monitor the prevalence of drug resistance,
11 particularly with an eye toward identifying any possible
12 geographic patterns of variability and differences that may
13 exist among persons who acquire HIV through different risk
14 exposures.
15 We need to better understand the mechanisms and
16 the rates of transmission of drug resistant virus. We need
17 to learn more about the clinical significance of primary
18 infection with resistant virus and learn the most efficient
19 manner of identifying these patients.
20 Finally, we need to understand treatment responses
21 among patients infected with virus showing moderate
22 reductions in drug susceptibility since these virus
23 populations seem to be so widely prevalent among patients
24 with primary and recent HIV infection.
25 Thank you.

1 DR. HAMMER: Thank you very much.
2 Are there questions for Dr. Little?
3 Please, Dr. Kaplan
4 DR. KAPLAN: That was a beautiful presentation.
5 Thanks very much.
6 I wanted to ask you about the very last point,
7 which becomes obviously really important in the data you
8 presented showing the moderate phenotypic resistance
9 particularly to NNRTIs, which appears to be common, and we
10 heard about this quite a bit yesterday.
11 I wonder if, in your studies in San Diego, you
12 will have any opportunity to look at what the clinical
13 significance of what that moderate resistance is, in other
14 words, are any of those patients being treated with NNRTIs
15 or are you using the results of your testing to get around
16 NNRTIs in your treatment.
17 DR. LITTLE: Yes. I tried to look at this in the
18 San Diego cohort, and it is just too small a number of
19 patients with too diverse treatment regimens.
20 So, what I have recently proposed is to the NIH
21 Primary Infection Group, and to the group that has
22 participated in this first study, is a study to look at the
23 clinical responses of most of the North American patients
24 with primary HIV infection, which should give us a much
25 larger denominator to look at despite varied treatment

1 regimens.

2 It will be a retrospective look, but we should be

3 able to look at treatment responses in patients evaluated

4 with the same phenotypic susceptibility assay over time.

5 So, my hope is yes, I will be able to answer that.

6 DR. HAMMER: Dr. Jolson.

7 DR. JOLSON: Dr. Little, thank you for your

8 presentation.

9 Just a quick question. In terms of interpreting

10 the prevalence of mutations of reduced susceptibility, in

11 your cohort, do you know if any of those patients had

12 received prophylaxis to some sort of occupational or other

13 exposure?

14 DR. LITTLE: None in our cohort. I am not aware

15 in the other cohorts, but in most of the presentations I

16 have heard it has never been mentioned. All the patients I

17 presented were either treatment-naive or had had less than

18 seven days of therapy to the best of my knowledge.

19 DR. HAMMER: Dr. Pomerantz.

20 DR. POMERANTZ: Again, Susan, that was a great

21 talk.

22 Thank you for re-analyzing all that data. That is

23 extremely helpful. But my question is, getting back to

24 those moderate resistant strains, because that I think is a

25 unique finding if they really are wild type that have

1 differences compared to your controls, have you had a chance
2 -- you quoted our study in the New England Journal -- we
3 only looked at genotypic markers, so we could have missed in
4 those seminal samples, and we have actually three more
5 patients that go with those -- we could have missed the ones
6 that are moderately resistant, and have you had a chance to
7 look for moderate resistance phenotypically in general
8 secretions in either men or women that were treated?
9 DR. LITTLE: No, we have not. We are collecting
10 genital secretions, but have not yet done any susceptibility
11 assays in those samples.
12 DR. HAMMER: Dr. Mathews.
13 DR. MATHEWS: Susan, could you clarify something
14 for me? I am quite confused about what is actually meant by
15 polymorphism, because nearly all of the studies that you
16 showed, showed a much higher prevalence of the secondary
17 mutations than primary mutations, so are you saying that if
18 the secondary mutations are present along with the primary
19 mutations, they are resistance mutations, and if not, they
20 are possibly polymorphisms?
21 DR. LITTLE: Sort of. My interpretation, most of
22 the secondary drug resistance mutations do occur as natural
23 polymorphisms or genetic variants in untreated patients.
24 So, the presence of one or more secondary drug resistance
25 mutations in an untreated patient, to me does not imply drug

1 resistance or moderate reductions in susceptibility that one
2 should expect that in that population.
3 However, when present in association with primary
4 drug resistance mutations, I think I would more likely
5 predict that they might confer some additional perhaps
6 reduction in susceptibility, but in terms of an actual
7 breakdown of -- I mean lots of people are trying to do this
8 -- which secondary drug resistance mutations, how many of
9 them, in what combination, and appearing in what frequency
10 predict reduced drug susceptibility. I certainly do not
11 know.
12 I believe that most of the secondary drug
13 resistance mutations that were identified or I should say
14 the amino acid substitutions that were identified in these
15 patient populations are more likely to be natural variants.
16 I cannot prove that at this point, but that is my estimation
17 based on the range of reduced susceptibility of the samples
18 that we saw.
19 They were mostly in nelfinavir and the non-
20 nucleosides, which, as I said, are known to have a much
21 wider range of susceptibility. So, my bet is that those are
22 natural polymorphisms, but we are going to be looking into
23 that.
24 DR. HAMMER: Dr. Hamilton, then Dr. Stanley.
25 DR. HAMILTON: Is it possible to determine from

1 your analysis and/or re-analysis whether there is a
2 correlation between viral load and number of resistance
3 mutations?
4 DR. LITTLE: I didn't address that, so, no, I
5 couldn't. Most of the patients in these groups that were
6 identified with primary infection, their baseline isolate
7 was collected fairly recently after seroconversion on the
8 order of two to sometimes six months out. So, in general,
9 their viral loads were quite a bit higher than mean viral
10 loads in many of the chronically infected cohorts, but other
11 than saying their viral loads were in general higher, no, I
12 haven't addressed that.
13 DR. STANLEY: Just a point of clarification, on
14 your slide on compartmentalization, the study by Zhu with
15 the five pairs, are those all MSM or were there some
16 heterosexual?
17 DR. LITTLE: One heterosexual.
18 DR. HAMMER: Dr. Pomerantz.
19 DR. POMERANTZ: I forgot to ask you one question.
20 Again, back to those moderately resistant
21 patients, they were all shown by the virologic system,
22 correct, the phenotypic moderate resistance?
23 DR. LITTLE: In our study, yes.
24 DR. POMERANTZ: Have you confirmed any of those in
25 a nonchimeric backbone doing your typical PBMC resistance?

1 DR. LITTLE: No.

2 DR. POMERANTZ: So, you don't know whether the
3 switch to chimeric backbone might have affected that. Okay.

4 DR. RICHMAN: When we did the original nevirapine
5 studies 10 years ago using the old-fashioned assays, these
6 sorts of observations were also seen, but we never bothered
7 to write up the baseline thing because we didn't know what
8 to make of it, and we didn't have the type of precision of
9 the data, but I think this information does exist. The data
10 with these assays are confirmed.

11 DR. HAMMER: Dr. Gulick, and then Dr. Jackson.

12 DR. GULICK: Did you get a feel for the
13 demographics of the patients? We talk about the North
14 American experience, but it is my suspicion that this
15 represents select cities and select groups of patients being
16 characterized. Do you have a feel for that?

17 DR. LITTLE: It is definitely overrepresented as
18 one would predict in this country. The patients that are
19 most frequently sampled are men who have sex with men, the
20 largest proportion of whom are white.

21 So, certainly trying to look at all of the North
22 American studies, there is some geographic variability, but
23 even in the other studies, they seem to be more weighted to
24 the West Coast than the East.

25 So, I think at this point, it is fairly

1 representative of most study cohorts in the sense that they
2 are probably something in the order of 80, 85 percent male,
3 and the majority of them, men who have sex with men is their
4 primary respector. I didn't break it down, but that is my
5 guess looking at most of the studies having about the same
6 breakdown.
7 DR. JACKSON: Could you comment on Dr. Kaplan's
8 point about the treatment response or perhaps the clinical
9 relevance of these sort of moderately reduced susceptible
10 viruses, particularly to the NNRTIs? I wasn't clear how
11 much reduced it was.
12 For example, with nevirapine, these levels in vivo
13 are typically 200 to 400 times the IC50, and so it is not
14 clear whether these findings are going to have any relevance
15 to treatment response.
16 DR. LITTLE: Agreed. I mean I think the level of
17 reduced susceptibility was quite a bit lower than has been
18 generally associated with virus that carries genotypic
19 resistance. For instance, in the one patient in our cohort
20 who had up to 20-fold reduced susceptibility to multiple
21 NNRTIs, not a single primary drug resistance mutation for
22 the NNRTIs was identified.
23 So, evaluating treatment responses in patients
24 with moderate reductions in susceptibility to the protease
25 inhibitors, I think will be easier than to the NNRTIs,

1 simply because it is going to be impossible I think to
2 evaluate treatment responses within patients in whom
3 moderate reductions in NNRTI resistance or susceptibility
4 are noted, who are then not treated with a primary NNRTI-
5 containing regimen.
6 So, we will have a much smaller subset of those
7 patients, particularly in late '98, '99. Some patients are
8 initiating NNRTI protease-sparing based therapies, and we
9 may be able to address that in a smaller group, but I still
10 think that is going to be the most difficult group even
11 retrospectively with a large number of patients to evaluate.
12 DR. RAMMER: Thank you very much for a superb
13 presentation. I think we will move on now.
14 The next speaker is Dr. Richard D'Aquila, who will
15 discuss factors confounding interpretation of resistance
16 testing.
17 **Factors Confounding Interpretation of**
18 **Resistance Testing**
19 DR. D'AQUILA: I would like to thank the committee
20 for the opportunity to speak today.
21 [Slide.]
22 Many of the factors that confound interpretation
23 of resistance testing have been discussed yesterday, and I
24 think a good subtitle for my talk might be why it might be
25 optimal to have an 800 megahertz multitasking processor to

1 keep track of all the different factors, whether that
2 processor is carbon based or silicon based probably doesn't
3 matter as much.
4 [Slide.]
5 I think everyone is familiar with the fact that
6 drug resistance may not always be the initiator of treatment
7 failure. Drug resistant virus can be present, as we have
8 heard, pre-existing before treatment is started or it can be
9 emerging during treatment, and it can initiate failure, but
10 obviously, some inhibitory drug levels or reduction in the
11 host immune responses against HIV can also lead to
12 persistent viral replication, which allows then subsequent
13 evolution of drug resistance and the final common pathway to
14 drug failure.
15 [Slide. 1
16 This is a partial list of what I consider the most
17 important factors confounding the interpretation of drug
18 resistance tests. In addition to the fact that drug
19 resistance need not be the initiator of failure, I think we
20 have to account for many of these factors.
21 The biology of drug resistant HIV includes very
22 complex interactive effects of the mutations. The issue of
23 detection of minorities of resistant virus has been much
24 discussed. We have also heard a bit about linkage of
25 multiple resistance mutations within the same genome, and

1 issues of latency or persistence of the resistant virus.
2 Another factor is the presence of the selecting
3 drug, which is clearly related to the level of drug or drug
4 concentrations that are present in vivo during therapy, and
5 multiple drug combination regimens, the presence of other
6 drugs in addition to the one to which the virus may be
7 resistant. All of these also have to be taken into account.
8 Then, keeping in mind the issue of what initiates
9 drug failure, timing of sampling blood from a patient who is
10 failing a treatment regimen is very important in
11 interpreting the results of a resistance test from that
12 specimen. The timing relative to viral load rebound when in
13 the process of viral load rebound, the sample is obtained.
14 Finally, I will touch-very briefly on issues
15 related to anatomic compartments and cellular mechanisms of
16 resistance.
17 [Slide.]
18 The mutation effects are very complicated, but
19 indeed we do have pretty good correlations between geotype
20 and drug susceptibility of clinical isolates. These
21 correlations are not perfect and there may be some easy
22 explanations for why they are not perfectly correlated.
23 The mutation effects that we see in clinical
24 isolates may not be identical to the effects that were noted
25 in site-directed mutants that have been studied in

1 preclinical laboratory tests. There are differences in
2 genetic background and other sequences of the virus remote
3 from the mutation of interest that lead to variation in the
4 effect of a given mutation, and we really have not defined
5 these to any great extent. Only a few of these are really
6 defined.

7 [Slide.]

8 I think this is one reason why we see this kind of
9 data where there really is a very good correlation between a
10 genotypic sensitivity score and a phenotypic sensitivity
11 score. This is data from ACTG 372 with a 0.75 correlation
12 coefficient, but there is some splay in the data. It is not
13 perfect. You cannot predict the exact IC50 given a
14 predetermined mutation.

15 [Slide.]

16 I think there are several potential reasons for
17 discordances between phenotype and genotype, and those
18 discordances can be in two directions.

19 The phenotype may indicate drug susceptibility
20 when the genotype indicates resistance. The two most likely
21 reasons for this are that there are mixtures of wild type
22 and mutant basis at a position conferring resistance, or,
23 the second major point down here, that there are interactive
24 effects of mutations causing resistance reversal. The most
25 well defined mutation that does this is the effect of the

1 M184V substitution on AZT resistance, but let's go back for
2 a minute to the issue of mixtures of wild type and mutant
3 basis.
4 A minority of resistant variant may be detectable
5 by genotyping, which generally has a level of detection of
6 at least 20 percent, but that may not be a large enough
7 proportion within the virus population to increase the IC50
8 in a phenotypic assay.
9 Now, this may or may not provide an early warning
10 of resistance, and I think we need more data on that. We
11 need to address the question of whether the resistant
12 minority will become dominant and then demonstrate a
13 concordant effect in a phenotypic test if the same treatment
14 is continued for somewhat longer.
15 [Slide.]
16 Discordance can also occur in the opposite
17 direction where the phenotype may be resistant and the
18 genotype may indicate susceptibility. The most likely
19 reason for that, I think, is that there are previously
20 uncharacterized mutations or combinations of mutations, and
21 indeed we have seen some examples of this just in the past
22 couple of years with the identification of the 69 insertion
23 mutation in the RT and some other, more recently identified
24 mutations, which were identified by screening for samples
25 that would have resistant phenotype without any of the known

1 mutations, and then going ahead and thoroughly
2 characterizing those viruses genotypically to identify the
3 new mutations.
4 I think that is going to continue to occur
5 particularly as we use drugs in different combinations and
6 as we get more new drugs into our patients.
7 The other reason why there might be this type of
8 discordance is that there might be mixtures of wild type and
9 mutant at a position conferring resistance. Again, a
10 minority of a resistant variant may be detected by
11 phenotyping and not by genotyping.
12 Again, it could be related to relative differences
13 in amplification where a minority variant might be better
14 amplified in the genotypic assay than it could be in the
15 genotypic assay.
16 Will any detection of resistance with either
17 method minimize false negatives? I think this is an
18 important question for future research, and one of the
19 rationales for studying for some of the clinical trials that
20 are ongoing or in development that will attempt to use both
21 genotyping and phenotyping.
22 [Slide. 1
23 Now, the complexity of mutation effects also leads
24 to difference in the rule-based algorithms that are used by
25 different laboratories to interpret drug susceptibility or

1 resistance from a genotype. It varies to some extent which
2 mutations are included in the rule for calling resistance to
3 a specific drug, and I think that leads to some of the
4 differences in interpretations that you may see from
5 different laboratories, and different algorithms account for
6 interactive effects, such as resistance reversal, some may
7 account for it, and some may not.
8 Finally, there may be preclinical site-directed
9 mutant data that is incomplete or not directly relevant to
10 the specific mutational pattern seen in a clinical specimen.
11 [Slide.]
12 This leads to some very difficult interpretations,
13 and these are just some examples of that. There may be on
14 mutation which does not confer increased resistance by
15 itself, but may contribute to resistance when additional
16 mutations are added.
17 One situation like that is where you might see in
18 a clinical isolate less than a complete RT 151-complex
19 multinucleoside resistance genotype.
20 Now, this has been studied in site directed
21 mutants in one study, where this constellation of mutations
22 was studied separately, as well as together. The 151M
23 mutation alone conferred some nucleoside resistance in the
24 site directed mutant, but any of the other mutations in 62,
25 75, 77, or 116, any one of those others alone did not confer

1 a detectable level of phenotypic resistance.

2 But if you put several of these together, then,
3 you do get very broad cross-resistance to all of the
4 nucleosides.

5 So, how do you interpret a finding of 151 alone?
6 That is pretty easy. We have some data. We would probably
7 all agree that that is likely to be a nucleoside resistant
8 virus, resistant to most of the nucleosides.

9 But what about if you have 62 alone or 77 alone or
10 116 alone, does that indicate that the virus is still
11 susceptible to the nucleosides, or might there be other
12 genotypes under the surface, below the limit of detection,
13 which have one or two or three additional mutations, and
14 therefore that isolate should be considered as potentially
15 resistant. We really can't give a definite answer to that
16 problem today.

17 The same issue occurs with the RT 69 insertion,
18 which together with the characteristic AZT selected
19 mutations confers broad multinucleoside resistance in site
20 directed mutants, but when it is present by itself, doesn't
21 give as much resistance.

22 The same problem may also occur with many single
23 protease active site resistance mutations. They may not
24 confer much resistance to some of the protease inhibitors,
25 but one always has to wonder, in all of these situations,

1 whether there are additional mutations under the surface in
2 vivo and whether an isolate with just one of the mutations
3 might be predisposed to more rapidly develop further
4 resistance.
5 Again, this is a very difficult interpretation,
6 and I don't know in every case that the answer will always
7 be the same.
8 [Slide.]
9 Again, the biology of the drug resistant virus is
10 what leads to this complexity. As you know, it replicates
11 as a genetically heterogeneous swarm of different quasi-
12 species, and a resistant mutant may be present at any
13 proportion including very, very low proportions in the total
14 population, and the current tests only detect optimally 20
15 percent minority.
16 Indeed, the ENVA panels, which were presented at
17 this past summer's resistance workshop suggested that some
18 academic laboratories might miss a 50 percent mixture of
19 mutant and wild type.
20 There has been speculation in discussion of those
21 data that operator experience is important, and I think we
22 need to investigate that further, but that is clearly
23 another issue in the interpretation - how good is the lab,
24 how low is the actual limit of detection in that lab for
25 that operator on that day.

1 [Slide.]

2 Now, there is some potential to improve the level
3 of detection of minorities. Hybridization-based analyses of
4 a pool of PCR products with one of several methods that are
5 in development by different manufacturers do have relatively
6 lower limits of detection, maybe down at best to about 1
7 percent of a population, but the tradeoff there is that
8 these methods don't give you sequence in every codon, so
9 that they are limited to specific codons rather than giving
10 you the entire sequence of protease in RT.

11 The other approach to improve minority detection
12 might be to evaluate multiple molecular clones of PCR
13 products, and in a study from my laboratory, which was
14 published in the Journal of Clinical Microbiology in
15 September, 60 percent of the 30 specimens we examined with
16 both bulk PCR product sequencing, the current state-of-the-
17 art, and with a clonal analysis looking it up to 15 clones.

18 Sixty percent of these specimens had a minority
19 mutation detected in the multiple clones, that was not
20 detected in the bulk PCR product sequence.

21 So, I think this might incrementally improve
22 things, but again it comes at great cost in terms of the
23 amount of labor and resources that are required to do this,
24 and I think it is not really feasible on any large scale at
25 this point in time, but again perhaps technology will

1 advance far enough, so that this will become feasible.

2 [Slide.]

3 One issue was raised yesterday that it is

4 important to account for the linkage of multiple mutations,

5 and another issue with using bulk PCR product sequencing is

6 that that is giving you the dominant base at each position

7 in the population, and that need not necessarily indicate

a genetic linkage.

9 All those mutations don't necessarily have to be

10 in the same genome. There could be equal proportions of

11 different genomes with mutations at different positions

12 which will give you that same result in a bulk PCR product

13 analysis.

14 But to date, clonal analyses do support the

15 assumption that when there are multiple dominant mutations

16 noted, they generally are in the same genome. This is

17 clearly important when we are dealing with salvage therapy

18 choices, because potentially, if you have mixtures of

19 viruses with different mutational patterns, you might be

20 able to construct a regimen where one drug will get 20

21 percent of the population and another drug will get a

22 'different 30 percent, and thereby try to construct a regimen

23 that is more effective.

24 But to date with really a very small number of

25 specimens from highly experienced patients being reported in

1 the literature, multiple mutations from these heavily
2 pretreated patients have been linked in the same clones.
3 [Slide.]
4 This shows you one example of this from a specimen
5 that we studied, that shows clonal analysis detecting a
6 minority population and the linkage of multiple mutations in
7 this minority population. Of the 15 clones on this slide,
a the two on the bottom -- and I apologize, this really is not
9 very legible -- but the two on the bottom contain multiple
10 resistance mutations including major active site, major
11 primary protease mutations in codons 48 and 82, and primary
12 RT resistance mutation in codons 184, 215, and several
13 important secondary mutations, as well.
14 So, it is not a swarm of different mutational
15 patterns, but they are all linked together.
16 The other important thing about this slide and
17 this specimen -- and I will ask you to remember this pattern
18 as we go to the next slide, please --
19 [Slide.]
20 -- is that that specimen was obtained several weeks after a
21 combination drug regimen was stopped and the patient was off
22 all drug therapy.
23 It illustrates the point that within weeks of drug
24 withdrawal, the virus population may shift from a multiply
25 mutated virus to a dominant wild type virus population.

1 Now, this comes at some risk. The risk is a
2 decline in CD4 count. I don't want to discuss this as a
3 treatment strategy right now. My point today is that
4 resistance testing should always be done when drug selection
5 pressure is present because otherwise it can lead to quite
6 misleading results.
7 [Slide.]
a Those results may be misleading because those
9 resistant mutants may not disappear. This is a very
10 instructive anecdote from John Condra, which he has
11 presented at a meeting several years ago, that indicates
12 that resistant mutants can remain either latent or
13 persistent, below the level of detection, and rapidly
14 reappear.
15 This is a patient who started treatment with
16 indinavir as monotherapy, developed an 82 mutant in the
17 protease that was 100 percent of the virus population. The
18 y axis is the frequency of the mutant in the virus
19 population.
20 Using sophisticated clonal analyses, after
21 indinavir was stopped, this mutant declined to being 0.04.
22 That would be 4 in 100 viruses in this plasma RNA
23 population. But many months later, the clinicians thought,
24 well, why don't we try again to restart indinavir, and very
25 quickly this mutant moved from being 5 in 10,000 up to 80

1 percent of the virus population, indicating, I think, that
2 these resistant mutants may remain archived in one way or
3 another, but I have shown you an anecdote here which is very
4 instructive, and I think probably very common, but we don't
5 have a lot of very hard data on this phenomenon, and I think
6 we need to look for this phenomenon more as we begin to use
7 resistance testing more commonly, because I really don't
a know how commonly this will occur.
9 I suspect it will be common, but I think it is
10 important for us to study how commonly this will occur.
11 [Slide.]
12 I want to now move to another factor, and this is
13 moving into the area of drug concentrations. Not only does
14 the drug have to be present, but its concentration when the
15 drug is being used is also very important, because
16 resistance is a relative, not an absolute, phenomenon.
17 As you heard yesterday, there is still some
18 effectiveness of these drugs even against resistant virus.
19 The inactive drugs, I believe it was in the GART study, John
20 Baxter told us that there was some drop in viral load
21 associated with use of inactive drugs.
22 I think this is also one possible explanation for
23 the lack of immediate decline in CD4 cells when viral load
24 rebounds on triple combination regimens. There is still
25 some ability to suppress.

1 [Slide.]

2 Again, we have said many times at this meeting

3 :hat the level of resistance needed to overcome the drug

4 :oncentration that is present in the blood is unknown for

5 ıny drug. I arbitrarily call this an IC50 cut-off.

6 It is likely that trough blood concentrations are

7 :he relevant measure for the protease inhibitors and the

a ıon-nucleoside RT inhibitors, and perhaps it would be useful

9 :o adjust for protein binding of those drugs.

10 For the nucleosides, the situation is much more

11 :omplicated because we need to evaluate cellular

12 :riphosphate levels, and that is just a whole additional

13 Layer of complexity.

14 Given all of this, monitoring individual drug

15 Levels, as well as resistance, will undoubtedly improve the

16 prediction of drug effect.

17 [Slide.]

1a You saw one example of that presented by Dr.

19 Clevenbergh from the VIRADAPT study yesterday, where

20 patients were categorized into these four groups with

21 suboptimal trough PI concentrations or optimal trough PI

22 concentrations either in the control arm or the arm that had

23 treatment chosen based on genotyping.

24 [Slide.]

25 There was this very nice discrimination where the

1 best viral load responses were seen in those who had
2 genotyping to help guide regimen choices, and who had
3 optimal protease inhibitor trough drug concentrations.
4 I don't want to spend a lot of time on this, but I
5 think clearly, this is where we need to move in the future
6 to combine resistance test results with analyses of drug
7 levels in order to fully interpret the resistance results.
a [Slide.]
9 Can resistance be overcome by higher drug levels?
10 Some drugs which are now in development do achieve higher
11 concentrations in blood relative to the virus' IC50 against
12 that drug than the current drugs.
13 For current drugs, as we have heard, the level of
14 drug in the blood is probably at most 5- to IO-fold above
15 the wild type virus IC50.
16 [Slide.]
17 But Dale Kempf and his colleagues from Abbott were
18 kind enough to provide me with some very interesting recent
19 information looking a new compound ABT-378/ritonavir in PI-
20 experienced patients. Some of this data has been presented
21 at the resistance workshop, but Dale and his colleagues
22 undertook another analysis of the data from their study M97-
23 765, using the data analysis plan or the DAP that you saw
24 presented yesterday, and I am going to show you just a
25 couple slides about that.

1 This compound is coformulated with ritonavir and
2 it produces steady-state trough levels that exceed the
3 protein binding corrected IC50 for wild type virus by at
4 least 30-fold. The activity of this drug plus nevirapine
5 and two nucleosides was studied in PI-experienced
6 individuals. The virologic responses at 24 and 48 weeks
7 were analyzed retrospectively according to the DAP.
a [Slide. 1
9 This shows you a schema of screening for PI-
10 experienced patients who changed their PI on study day one
11 to 378 ritonavir at one of two doses. They made only that
12 change for two weeks, and then at study day 15 added
13 nevirapine and also changed their nucleosides with one new
14 nucleoside being new. The study was evaluated at weeks 24
15 and 48.
16 [Slide.]
17 There was something there yesterday. We did look
18 at it yesterday.
19 Well, what it shows is that there is some
20 resistance to all of the other protease inhibitors, and I
21 can actually see it a little bit. It is a mean of 8-fold to
22 23-fold resistance for indinavir, nelfinavir, saquinavir,
23 and ritonavir.
24 [Slide.
25 But the point is that the responses were not

1 predicted by the baseline drug susceptibility phenotype to
2 APT-378 ritonavir. The wild type IC50 levels listed here,
3 4-fold, the IC50 of wild type virus to 378 is also listed
4 there. The dots indicate the IC50s of the isolates from
5 these patients at entry to the study, and the patients who
6 failed, the six patients who failed really all cluster down
7 here. This is at week 24, failing by week 24, dropouts as
a censored.
9 You can see that there is a wide range of IC50 for
10 the viral isolates, and what is indicated on top here are
11 the mean drug levels in this study at this dose. So,
12 really, even the highest IC50s appear to be below the levels
13 of drug that are present in vivo.
14 [Slide.]
15 This is similar data at week 48. Again, the
16 failures do not all cluster at the high end of IC50.
17 [Slide.]
18 This is looking at the number of mutations.
19 Again, there is no relationship between at week 24, the PI
20 mutation score in either success or failure. The failures
21 are in yellow here. Some have no mutations.
22 [Slide.]
23 This the same analysis at week 48, dropouts as
24 censored.
25 [Slide.]

1 So, what Dale and his colleagues concluded from
2 this was that at both week 24 and 48, there were very good
3 responses. The response was not associated with a 4-fold
4 change in phenotypic susceptibility or the number of PI
5 mutations at baseline.
6 In these DAP analyses, only the baseline RNA level
7 was identified as potentially associated with virologic
a responses in these subjects, and these findings could be
9 consistent with the high sustained plasma concentrations of
10 378.
11 Now, I would like to add just some of my own
12 personal opinions about this. I think it certainly does
13 suggest that we need to evaluate this phenomenon more. I do
14 not take from this the conclusion that there will be no
15 resistance to this drug, and I think no one should walk away
16 from this thinking that. I think we need further studies
17 looking at patients who have PI experience than those who
1a were studied here, and potentially looking at different
19 regimens.
20 I will remind you that here, everybody had
21 nevirapine added to their regimen at two weeks, so that
22 clearly has to be taken into account when looking at these
23 effects, but I think the general point that I would like to
24 make from this is that there may be some drugs which will
25 have high enough levels, so that it will be similar to

1 intermediate resistance of pneumococcus.
2 One can still use penicillin against a
3 pneumococcus which has intermediate resistance to
4 penicillin. That does not indicate that there are no
5 pneumococci out there which have higher levels of resistance
6 for which penicillin will not be an adequate treatment.
7 I think it also suggests to me that it will be
a 'very important in drug development to perhaps -- and this
9 should be discussed later -- perhaps ask sponsor to design
10 studies where, in fact, the resistance pattern can be
11 identified, where some viruses that are resistant can be
12 selected on study and the resistance mutational pattern
13 identified in those viruses.
14 [Slide.]
15 Looking at multi-drug combination regimens adds
16 further complexity, and you have already heard about
17 genotypic and phenotypic sensitivity scores, and the data
18 from 372 are what suggested this, and that was what was used
19 in the DAP.
20 [Slide.]
21 This just shows you those data. If you look at a
22 genotypic sensitivity score, it very nicely predicts time to
23 failure.
24 [Slide.]
25 There is another issue. That first regimen

1 failure may not give you the same mutational pattern as
2 later failures. There are several studies listed here
3 showing that early during failure of the first triple
4 combination regimen, you can see RTI resistance without PI
5 resistance.

6 [Slide.]

7 But the timing of sampling during rebound is very
8 important here. Most commonly early on, only resistance to
9 3TC is seen, but in these first regimen failures it is
10 possible that PI resistant minorities may be present at a
11 lower level and might eventually become detectable if the
12 failing drug regimen is continued.

13 That remains a research question, and I think we
14 need trials that either continue or intensify the PIs to
15 address the issue about how to use resistance testing in
16 this specific setting. Again, it may differ in those
17 failing a second or a later regimen.

18 [Slide.]

19 This shows you just one isolate from a patient we
20 studied early in indinavir, AZT, 3TC failure, and this
21 patient had prior AZT treatment, and they showed up with
22 resistance mutations to AZT, 3TC, and depicted on this
23 slide, PI resistance mutations, as well. Trip Gulick has
24 presented data from the Merck 035 study that also shows
25 pretreated patients presenting with mutations to several of

1 the drugs.

2 [Slide.]

3 Anatomic compartments are another complexity. If
4 resistance testing does not identify a resistant mutant in
5 blood, could it already be present and rapidly emerge from a
6 compartment such as CSF or semen where virus can evolve
7 independently?

8 To date, resistant virus has rarely, if ever, been
9 present in semen in the absence of detection of resistance
10 in blood. Only a subset of patients in general, only a
11 subset of patients with PI resistant virus in blood have PI
12 resistant virus in semen, and I have cited here some work
13 that we presented last year, and there are several other
14 groups that have similar data.

15 I think there are fewer studies of CSF. Joe Wong
16 has some very interesting studies on this, and I think that
17 merits further study because I think there may be greater
18 potential for resistant virus to be present in CSF, if not
19 in blood.

20 [Slide.]

21 Cellular mechanisms of resistance are also
22 necessary to be considered. There may be no virus
23 resistance and yet patients may be failing. We know of
24 several mechanisms for this. PIs are substrates for the P-
25 glycoprotein multi-drug resistance gene 1, encoded P-

1 glycoprotein, and there is another MDR transporter, the MRP4
2 that is induced by adefovir in vitro and effluxes nucleoside
3 monophosphates out of cells.
4 Obviously, there are also other enzymatic
5 mechanisms possible for the nucleosides including changes in
6 the cellular enzymes involved in phosphorylation, and this
7 is still largely uncharted territory.
a [Slide. 1
9 In conclusion, the current resistance tests are
10 imperfect predictors of drug responses, and one needs to
11 take into consideration, in addition to the resistance test
12 result, many viral and drug-related biologic factors.
13 Improved essays may be developed in the future.
14 Interpretation criteria can be better standardized, but I
15 think perfection shouldn't be the enemy of the good.
16 [Slide.]
17 I think these limits shouldn't stop us from using
1a resistance testing. The IAS-USA Resistance Testing
19 Consensus Panel, that published recommendations in JAMA in
20 1998, is updating those recommendations. That paper is
21 being submitted, this week I believe, and much broader
22 recommendations will be outlined in that update based on the
23 retrospective and prospective studies that you heard
24 summarized at this meeting.
25 In general, the recommendations will be to

1 consider resistance testing for pretreatment screening for
2 the recently infected patient, not for the patient with
3 long-term, established infection, and to consider resistance
4 testing at the point of regimen failure if you are about to
5 change drug regimens. Regimen failure is defined as viral
6 load rebound. This is reccmmended either when the first or
7 later regimen is failing.

a Thank you very much for your attention.

9 DR. HAMMER: Thank you, Rich. That was a terrific
10 talk. These first two talks have set quite a standard for
11 our second day, but a great kickoff.

12 I have a couple of questions to start this off,
13 and I think there is no better person to answer a couple of
14 these questions.

15 The first is that resistance mutations are not all
16 created equal. One of the questions that has come up is the
17 functional consequences of some of the mutations,
18 particularly some of the work you have done in RT, but also
19 the issue of protease mutations and replicate of capacity
20 and functional consequences.

21 Can you make some -- perhaps it is unfair --
22 general comments about that and what you think because one
23 of the issues we dealt with yesterday, and we will deal with
24 again later today, is resistance in relation to outcome, and
25 if a virus is impaired, the outcome may be different

1 irrespective of the virus load.

2 DR. D'AQUILA: I am very glad you asked that

3 question because it didn't quite fit into my topic, but I

4 want to address it because none of the current resistance

5 tests assess the replicative fitness, replicative capacity

6 of the virus. I think that may be something we will be able

7 to assess routinely in the future, but my read on it is that

a it is not predictable in advance to say that mutation A will

9 lead to a less fit virus, and mutation B will not lead to a

10 less fit virus.

11 I think it is quite complicated and, in part, can

12 vary based on the genetic background that the mutation is

13 present in. So, I think we can't make assumptions that a

14 particular class of mutants will always be relatively unfit

15 or relatively fit.

16 I do think again we need much more study. It has

17 largely been studied only in vitro, but I think that fitness

1a is probably a relevant factor. It is probably another

19 factor that has to be taken into account in looking at viral

20 load responses to drug regimens.

21 I think a virus that is less fit and grows less

22 well in the absence of drug may be better able to be

23 inhibited by any drug. So, I think that is something that

24 again we can't factor in easily right now.

25 DR. HAMMER: Let me ask you a different question,

1 and that relates to looking at different points on the virus
2 inhibitory curve, and there are pros and cons to looking at
3 IC50 or IC90 or 95 depending upon how you want to look at
4 the virus population, but this also gets to the issue of
5 sort of uniformity and analyses particularly in drug
6 development issues and what this committee might see.
7 Do you have opinions about whether it is best to
8 look at an IC50 or an IC90 or 95 in relation to a drug's
9 activity and particularly in relation to looking at the
10 pharmacologic/pharmacodynamic interactions and the results?
11 DR. D'AQUILA: I think many of the people in the
12 room will recall when we first started working on this, we
13 chose IC50s for a completely technical reason, because the
14 measurement of an IC50 is a bit more robust. In the
15 laboratory, you can really have greater confidence in that
16 measurement.
17 But I think biologically, our goal is to maximally
18 suppress the virus, so that I think a measure, such as an
19 IC90 or IC95, if it could be as reproducible as an IC50
20 would be a better measure, but that means doing much more
21 expensive testing and many more replicates, and I think for
22 practical considerations, an IC50 is probably a more
23 relevant measure to use for clinical purposes.
24 DR. HAMMER: Questions from the committee?
25 Dr. Pomerantz.

1 DR. POMERANTZ: I want to actually continue, Rich,
2 on what Scott brought up because I know you left out some
3 slides on fitness, and clearly, there is data that there may
4 be differences in fitness, at least in vitro, between
5 different viruses, but fitness is really, as you know,
6 reaching a local peak on an evolutionary landscape, and that
7 is associated with replication. It does not say anything
8 about the pathogenesis or the effects on the host.
9 So, my questions are twofold. One is do you think
10 that fitness, as defined or as it is being evaluated, will
11 always correlate with virulence or the destruction of CD4
12 cells either by direct or indirect methods and mechanisms?
13 Secondly, isn't it formally possible that a less
14 fit virus without drug is actually quite fit in the presence
15 of drug, a little bit like some bacteria are now?
16 DR. D'AQUILA: I will address your second part
17 first. Absolutely, that is why you see them. I think we
18 have to be very careful when we use the term. Resistance
19 mutation allows the virus to have better fitness in the
20 presence of the drug, and it can only lead to decreased
21 fitness in another situation, when another drug is being
22 used or no drugs are being used.
23 DR. POMERANTZ: I don't want to go too far on
24 that, but don't you, though, have to measure the replicative
25 capacity of that resistant virus in the presence of drug and

1 compare it to the wild type without drug, not both without
2 drug?
3 DR. D'AQUILA: That would be a more direct
4 reflection of the in vivo situation, but it is much harder
5 to do, yes.
6 Just to address your other question about
7 virulence, I don't think there is an absolute correlation.
8 I think what we are looking at here, particularly for
9 protease and RT, are viral replicative enzymes.
10 So, what we call fitness really relates only to
11 replication. There clearly can be effects of other genes
12 that cause more or less CD4 depletion or other virulence
13 effects.
14 DR. HAMMER: Dr. Wong.
15 DR. WONG: I have two questions. I think one is
16 pretty easy, and one might be a little harder.
17 The easier one is you showed the proportion of
18 protease inhibitor resistant virus that reappeared when a
19 protease inhibitor was reinstituted in one case, but you
20 didn't give the absolute number. So, I wonder if you could
21 give that in addition to the proportions.
22 DR. D'AQUILA: I am sorry, I don't understand.
23 DR. WONG: You showed that it went from less than
24 1 percent to 80 percent when a new drug was instituted, but
25 it was only the proportional data you gave as opposed to the

1 number of --

2 DR. D'AQUILA: I don't have access to that data,
3 but my recollection of John's presentation -- I don't know
4 if John wants to comment on this, I think he is here -- is
5 that that variant was dominant in the plasma RNA at the
6 point that was shown on that slide.

7 DR. HAMMER: John, please come to the microphone
8 and identify yourself for the transcriptionist.

9 DR. CONDRA: John Condra, Merck Research Labs.

10 Unfortunately, we don't really know the absolute.
11 We can't really interpret the absolute viral load in that
12 circumstance because the patient was on and off RT
13 inhibitors during the time that the protease inhibitors were
14 used, and withdrawn and reinstituted.

15 So, there is really no simple way to interpret the
16 relationship between the viral load in that patient and the
17 proportions of that mutants in that particular situation.

18 DR. WONG: Thank you.

19 The second question I guess goes to the data you
20 showed about analysis of individual clones in people
21 receiving or not receiving drugs. I guess it worries me
22 that -- as I am sure it worries you -- that looking at bulk
23 genotypes or even bulk phenotypes may not accurately
24 represent what is happening.

25 I understand that it is going to be a tremendous

1 increment in the amount of work that would be required, but
2 should we, at this point, really focus on trying to
3 introduce the idea of clonal analysis and resistance testing
4 and characterizing the individual members of populations as
5 opposed to bulk populations as a goal down the line. I mean
6 is this something that we really should be focusing
7 technology development on?

8 DR. D'AQUILA: Well, I would say yes, but in
9 follow up, I would say we have spent more than a year trying
10 to do that, and to date, have not found viruses that look
11 tremendously different in a clonal analysis than they look
12 in a population analysis.

13 Yes, you get more information, but the take-home
14 message, the important bottom-line message is the same.
15 That is based on study of a dozen or so viruses in this kind
16 of detail, and this is our unpublished ongoing work. I
17 still think it is important to develop that area, but I
18 don't think -- I mean the take-home message that I would
19 like everyone to leave with is that so far the bulk assays
20 do appear adequate. They do not seem to be missing major
21 minor species.

22 DR. HAMMER: Dr. Yogev.

23 DR. YOGEV: A comment and a question. The comment
24 is about the IC50. Both in the study you showed us, for
25 example, the 378, and non-anecdotal data, the IC50 or the

1 wild type is almost the lowest level of the virus tested
2 during the study. YOU also said the IC90 and 95 are very
3 difficult to get, but maybe a factor of 4 of 10 of IC50
4 should be added now, especially for the experienced patient,
5 because every time we use the IC50, upfront giving 50
6 percent of population sensitivity.
7 The question I have this is the second time I see
8 on a study of experienced patient that those who failed have
9 much lower mutation than one would expect. For example, the
10 study you present was more than 50 percent had no mutation,
11 and yet they failed.
12 Are there factors of those which you mentioned as
13 a reason for the failure should be now included when a drug
14 is developed, and not only the genotype, phenotype
15 presentation?
16 DR. D'AQUILA: I certainly would think that
17 information on levels of the drug in the blood are relevant
18 to the drug development process. I am not sure that one
19 would need to go very far beyond that. Obviously,
20 tolerability, toxicity is a concern, and that is relevant
21 for adherence. I think the major issue in my mind would be
22 again merging the pharmacology and the resistance data.
23 DR. YOGEV: You pulled the rug under my foot when
24 you showed the data of the 378, because that is exactly what
25 I thought, but all the patients were below the level unless

1 the individuals were not, but the level you showed versus
2 the failures, they were all below. About half of them have
3 IC50.
4 DR. D'AQUILA: That is correct.
5 DR. YOGEV: So, you would expect that those would
6 not fail.
7 DR. RAMMER: Rich was meant to confound us. That
8 was the point of his talk.
9 DR. D'AQUILA: I can't explain why they failed.
10 DR. RAMMER: Dr. Pettinelli.
11 DR. PETTINELLI: You mentioned that the new IAS
12 Resistance Consensus Panel is recommending pre-therapy
13 screening for recently infected patients, but not for
14 patient who has been infected for a longer period of time.
15 Can you explain the rationale for that?
16 DR. D'AQUILA: The rationale is based on a
17 theoretic concern that in a patient with established
18 infection where the virus has had a long time to replicate
19 in the absence of any drug selection pressure, it may be
20 possible for a more wild type virus population to become
21 dominant.
22 The committee was concerned that we didn't have a
23 lot of data in that setting and we did have good data in
24 recently infected patients, as you heard from Susan's
25 presentation, so that we went there.

1 I think the issue is are the tests currently
2 sensitive enough to detect the mutants if they have declined
3 over time to be now a minor proportion of the total
4 population, and I think we just need to study that before we
5 recommend very broad screening.
6 I mean the other point that I would say is
7 something that came out in the case that Susan showed, that
8 one doesn't have to always screen before starting if one is
9 concerned that the viral load is not dropping, that is a
10 very appropriate point at which to get resistance testing.
11 DR. HAMMER: I would also just make the important
12 point related to the recommendations of the consensus panel,
13 that they haven't been -- they are about to be submitted,
14 they haven't been peer-reviewed yet, so I don't think they
15 should be widely, publicly disseminated as clear cut.
16 The other thing is that I don't think we should
17 infer a black or white recommended or not recommended. Each
18 population is dealt with independently in the draft paper,
19 and there are different levels of "consideration and
20 recommendation" that are being put forward by the panel.
21 So, I think we need to say it is not recommended
22 or not recommended for particular populations. Each one is
23 being handled with the level of data that the panel thought
24 was available.
25 DR. D'AQUILA: Thank you, Scott.

1 DR. HAMMER: I think we need to move on. Thank
2 you very much, Rich.

3 **Questions to the Advisory Committee**

4 DR. HAMMER: There are three questions for the
5 committee in this session, which I think we can deal with
6 relatively efficiently, hopefully. We have a long agenda
7 today, I just would remind the committee members, so we need
8 to stay focused and on time, and some of the members need to
9 leave early.

10 The first question is: Please comment on the
11 types of patient populations in which HIV resistance testing
12 might be useful in drug development.

13 Again, let's focus on the drug development issue.
14 That is why are here.

15 Who would like to comment?

16 Dr. Pettinelli. Thank you.

17 DR. PETTINELLI: My opinion, you know, there are
18 several point of drug development. One aspect is that
19 patient experienced a first failure, because I think at this
20 point, we are dealing with more understandable pattern of
21 mutation, so that is definitely a patient population that
22 will be interesting to study.

23 DR. HAMMER: Dr. Yogev.

24 DR. YOGEV: I think it is a difficult question if
25 you want to exclude any population because now we are

1 getting to the point that you can get an infection with a
2 resistant strain although it is only 25 percent of your
3 patients, but in the pediatric population, for example, we
4 start seeing patients where they are resistant to the drug,
5 we want to use like AZT, so I think when a drug is
6 developed, we should basically go to any population where
7 they use initial therapy and later they will be different,
8 no question, but I think we need to have some data how the
9 drug is really going to do, which is going to increase
10 percentage 'of resistance in so-called naive patients.
11 DR. RAMMER: Dr. Mayers.
12 DR. MAYERS: One patient population that I have
13 been very concerned about is the heavily pre-exposed
14 population, and currently at Ford, 48 percent of our
15 patients have failed two PIs and a non-nuke and have
16 detectable virus.
17 The problem that you see is that most of the
18 pharmaceutical development is focused on naives and first
19 failures, and so what you have is the drugs are coming out
20 with data on very early treated populations, but are
21 immediately then going to be placed into extended access
22 into the very sickest, latest stage patients we have with
23 almost no data.
24 There has to be some mechanism early on in the
25 process to get some data on what the impact of these drugs

1 are going to be on heavily pretreated patients even though
2 that may not be on a direct line to the early accelerated
3 approval because that is where these drugs go almost
4 immediately in the clinical arena.
5 DR. HAMMER: Dr. Wong and then Dr. Hamilton.
6 DR. WONG: I guess I have been very impressed by
7 the data that we have seen over the past couple of days, and
8 although it is not the case that -- I mean Dr. **D'Aquila** is
9 perfectly correct that no one has demonstrated precise
10 correlations between resistance testing results and the
11 results of therapy of any drug.
12 It would be my opinion for any new drug that is
13 coming along, I would want to see these sorts of data on any
14 new drug in all populations that are studied for
15 registration purposes at least, at least in patients in whom
16 we are going to be asked to assess was the drug efficacious
17 or not.
18 DR. HAMILTON: I think the sponsor is going to
19 want to know what the various reasons are for success or
20 non-success in the conduct of their clinical trials, and
21 this is but one variable. So, I think they are going to
22 want to use these parameters in virtually all patient
23 populations.
24 DR. HAMMER: Dr. Kumar.
25 DR. KUMAR: Again, we know very little on the

1 resistance profile in the inner city patient population
2 because everything that has been published so far, and even
3 as Dr. Little said, has all been in certain areas and among
4 white men and whose main factor has been different from what
5 we are seeing in the inner city.
6 So, I think it should be done for any newly
7 developed trial. That is the only way we are going to be
8 able to see whether it is going to be effective in all
9 patient populations.
10 DR. HAMMER: Dr. Kaplan may wish to comment, but
11 there are studies underway to look at, as was mentioned
12 yesterday, naive populations in at least some of the inner
13 cities around the country.
14 DR. KAPLAN: Yes, I would agree. I think it became
15 apparent this morning that most of the data that we have so
16 far are mostly in white men who have sex with men, and I
17 think we would like to see more demographically diverse
18 data.
19 Since I have got the microphone on, I guess I
20 would just concur with what we have been hearing from
21 others, that I think we want to see data in all populations
22 in which the drug that is being proposed would be used, and
23 that would include not only experienced patients, but like
24 Dr. Mayers said, people who have been heavily pretreated
25 with other drugs or people with acute retroviral infection

1 or other recently acquired infection.
2 DR. HAMMER: Dr. Pomerantz.
3 DR. POMERANTZ: Just to join the band, I think
4 Brian said it very well in that you have to -- and just to
5 reiterate -- you have to do a different analysis if you are
6 thinking about drug development versus how you are treating
7 patients in the clinic when it comes to resistance testing,
8 and we have had trouble even on this committee separating
9 the two things.
10 I think Brian is right. For drug development now,
11 it would be very important to test virtually everyone in the
12 drug studies, all the patient populations, especially up-
13 front, because unlike some patients in the clinic, where up-
14 front testing is still somewhat controversial, this will be
15 a unique way to not only develop the drugs, but give the
16 information for other drugs that are still out there,
17 especially looking at these moderately resistant strains
18 that may be wild type out there. Studying during drug
19 development may be key to getting an answer to those and
20 will help with the development of the particular drug.
21 DR. HAMMER: Dr. Jolson.
22 DR. JOLSON: I am going to ask the committee to
23 take this question just one step further. It sounds like
24 there are potentially many populations, if not everyone, in
25 whom it would be useful to have baseline genotypic or

1 phenotypic information for purposes of future data analysis.

2 My next question to you would be for which patient

3 populations would you recommend either prescreening and

4 eligibility based on knowledge of genotype or phenotype and

5 consequently exclusion because of failure to have

6 susceptibility at baseline.

7 DR. HAMMER: I might start since no hands are

8 raising immediately.

9 I think it partly depends, of course, on the drug

10 you have got and its target populations are and where you

11 are studying the drug. For example, if it is a drug that is

12 not specifically with a high profile to go after drug

13 resistance, and so it is a standard sort of drug

14 development, you are looking at, let's say, a naive

15 population for activity, then, I think the epidemiologic

16 data -- that we are accumulating, and we saw this morning --

17 should help drive the need to test all patients before those

18 patients enter the trial.

19 We should also recognize that some drug

20 development is going on outside of the United States with

21 trials that are going to be evaluated here in populations

22 that are truly naive, so I think we have to be careful about

23 recommending drug susceptibility testing in every patient in

24 every trial.

25 So, I think one has to factor in what the

1 likelihood is of resistance and then determine whether it is
2 a subset or the entire population that needs to be evaluated
3 for resistance testing in a "**standard**" drug development
4 process.

5 If, however, it is a drug that has a particular
6 profile against drug resistant virus, and is to be tested in
7 that fashion, then, I think we should move to prospective
8 testing, in which case testing should be done at baseline
9 with the knowledge of that going into the trial, and either
10 randomizing on the basis of that or stratifying for analysis
11 on the basis of that or whatever, but that is a situation in
12 which the entire population, I think, should have resistance
13 testing if, in fact, you are going after the indication that
14 is going to be safe and effective in a treatment drug
15 resistant population.

16 Did that raise questions? Good.

17 Dr. Mathews.

18 DR. MATHEWS: This was something we sort of
19 touched on yesterday. My feeling is that if you know, for
20 example, somebody has K103, and there is a trial that could
21 randomize them to an NRTI, it would be absurd to put
22 somebody in that if you had that information.

23 On the other hand, if the question is does a
24 particular mutational pattern enhance the response to the
25 drug, then, you -- I mean to handle it up-front by

1 stratifying the randomization, so that you could actually
2 examine it with adequate numbers of patients rather than to
3 have to stratify retrospectively where you wouldn't
4 necessarily have balance.

5 DR. HAMMER: I agree about the 103, but I think it
6 gets very complicated as we have talked about this morning
7 with data that Rich showed from the ABT-378 trial, that it
8 may be that pharmacologic factors will overcome a particular
9 level of what was previously thought to be resistant, and we
10 have also seen some data where a measure of susceptibility
11 of the regimen and response was statistically significant
12 and correlated, but individual drug susceptibilities
13 phenotypically or genotypes were not.

14 So, we need more data in that regard, but it is
15 very complicated.

16 Mr. Harrington.

17 MR. HARRINGTON: I just think that we have seen a
18 lumber of mutations and their complexity. There is going to
19 be huge sample size issues in trying to get statistical
20 significance out of things that might be biologically very
21 significant, but for a relatively small number of people, so
22 you want to know the answer, but I don't really know if you
23 would want to exclude people on the basis of certain
24 mutations except for in the cases where they were extremely
25 common and extremely well characterized like, you know, a

1 drug active against people with 184 or something like that.

2 But I would think in many cases, you would be
3 denying yourself information about how the drug really works
4 in the clinic if you excluded people, but you in some cases
5 would want to gather the information at baseline.

6 DR. HAMMER: Dr. Woolson, did you have a comment?

7 DR. WOOLSON: I think this is a follow up to a
8 comment that you made earlier, Dr. Hammer. I think we really
9 need to remember the importance of sampling in particular in
10 clinical trials. It is not going to be necessary for us to
11 do this in every single patient entering a trial, but
12 perhaps subsets of them.

13 I guess I am particularly concerned that if we
14 only sequence individuals who are treatment failures, that
15 it is going to be very difficult to sort a lot of the data
16 out , so I think we do need to have a mechanism where we are
17 actually getting a broader base sampling, but it doesn't
18 have to be again on every individual.

19 DR. HAMMER: Although I would take Dr.
20 Pettinelli's statement, take it a little more broadly.
21 Basically, the populations need to be well defined and they
22 can be naive by history, but we need to document that now I
23 think by testing.

24 First failures are the clearest picture in many
25 ways as to what is happening and it taught us a lot about

1 what patients are failing, whether virologically, and those
2 have to be separated from multiple failures, because that
3 level of complexity is more than one order of magnitude
4 higher.

5 Dr. Gulick.

6 DR. GULICK: It just occurred to me during the
7 discussion that one unique population to look at would be
8 the expanded access programs, perhaps requiring that
9 resistance be part of any expanded access program given that
10 that is the clinical situation where you have the most
11 advanced patients and that may be a patient group where the
12 clinicians are willing to take the chance of just using one
13 drug at a time, which we all think would be a problem, but
14 here you sort of get out of the ethical dilemma because the
15 primary care doctor and the physician have made that
16 decision.

17 That would be a unique opportunity, I think, to
18 really assess what the resistance pattern is in that
19 population.

20 DR. HAMMER: That raises a huge question, (a)
21 financially, where is that burden going to fall, and it also
22 raises a question that this committee has dealt with
23 historically, and that is, trying to turn expanded access
24 data into some sort of experiential data that can be
25 analyzed in a quasi-clinical trial sort of fashion, but we

1 probably can't go up on that right now.
2 Dr. Stanley, did you have a comment?
3 DR. STANLEY: Well, it was really to reiterate
4 something that I think you already said, which is we have
5 asked for preclinical in vitro data on resistance patterns
6 and where a drug might have an advantage, and so you are
7 clearly going to want to validate that in clinical studies
8 where you target patients that appear to have the resistance
9 pattern that you think your drug is going to overcome.
10 DR. HAMMER: Dr. Wong, and then we will move on to
11 the next question.
12 DR. WONG: I just want to echo what Mr. Harrington
13 said. I would be very careful about trying to mandate that
14 certain patients be excluded from trials unless it has been
15 demonstrated in advance that the drug being studies would be
16 ineffective in that patient, and it is unlikely that that is
17 going to be known before it is tried.
18 So, I wouldn't exclude people, but I would expect
19 chat the sponsors collect the data and analyze the results
20 in light of the pre-randomization susceptibility results.
21 I can well envision a situation in which the
22 overall results of a drug might not be that great, but
23 within a population, for example, that has susceptible
24 virus, that the drug would be effective, and in those
25 circumstances, I could see voting for approval even though

1 the drug overall was less impressive.

2 DR. HAMMER: Dr. Mayers.

3 DR. MAYERS: I think the one thing that has really
4 changed in the last few years is the fact that you used to
5 have to go to **PBMCs** and grow them up, and it was very
6 expensive to expect a sponsor to sock cells down in every
7 patient at baseline.

8 I think things have changed. Now that we are
9 sequencing plasma virus and we are doing RVA assays out of
10 plasma virus, I think it is not unreasonable to expect the
11 sponsors to put plasma down on every patient going into
12 their studies including expanded access potentially, so that
13 they can go back and look at these issues, and then for the
14 failure patients, pare that baseline sample of a failure
15 sample.

16 This is a very inexpensive requirement. They are
17 getting HIV-RNAs on these patients, and this I think would
18 allow them to do the studies that need to be done if it was
19 just simply collected.

20 DR. HAMMER: I think it is fair to state that for
21 the most part -- and I may be wrong -- that, in fact, that
22 is being done. Plasma storage is going on in almost every
23 Phase I, II, and III trial that I am aware of, and I think
24 that is appropriate.

25 You can prospectively test whatever sample you

1 wish. It allows you to retrospectively test the remainder
2 if you need to, so I would echo that. I just wanted to make
3 it clear that I don't think we should glibly state every
4 patient in every study for drug development should have
5 resistance testing at baseline.
6 I think it basically needs to be evaluated on a
7 population, drug, and study basis. We have to be careful
8 what we say because sometimes these statements are
9 overinterpreted.
10 Just to summarize this question, I think we have
11 answered it. On the types of patient populations in which
12 drug resistance testing might be useful in development,
13 essentially, it is very population that may be studied.
14 I think for the most part, you know, drug
15 resistance testing is going to be useful in primary
16 infection, established infection, first failure, multiple
17 failure, and in pregnancy. For the most part, as far as
18 drug development, we are talking about established infection
19 and failure patients.
20 I think the committee's consensus is that all of
21 those are relevant populations in which drug resistance
22 testing is useful. You need to factor in again the activity
23 of the drug, its target population, its target indication,
24 and the epidemiologic data that tells you about your
25 population at that time.

1 So, I don't think we really have excluded any
2 population from resistance testing, and I think that is
3 probably the answer. I wouldn't do it on every patient in
4 every trial at this point necessarily.
5 For example, if you had a 700-patient Phase III
6 trial in a drug that wasn't going to be particularly active
7 necessarily drug resistant virus, and you selected your
8 population for a very low risk prevalence in the community,
9 or, in fact, it was an international trial where there were
10 no drugs, I don't think you have to test everybody.
11 2. Please comment on the timing of HIV resistance
12 testing in the setting of a clinical trial.
13 I would just ask that we make our responses
14 targeted here, so we can stay on time.
15 Dr. Mayers.
16 DR. MAYERS: I think that probably the most
17 efficient way to do it is to get the baseline and when they
18 fail, get a failure sample and test it against the paired
19 baseline sample, and for naives, that would take care of
20 most of your requirements. For the experienced, you are
21 going to have to do some of the baselines, as well, but I
22 think that pairing your failure sample with your baseline
23 gives you most of what you need and allows you to find out
24 what mutations are being selected by the drug.
25 DR. HAMMER: Dr. Mathews.

1 DR. MATHEWS: I was just wondering, do you think
2 there is a role for systematically testing at least a subset
3 of the population irrespective of failure, so that the
4 patterns of evolution of these resistance mutations and
5 their correlation with phenotypic resistance could be better
6 characterized.
7 DR. MAYERS: Can I answer that, Scott?
8 DR. HAMMER: Absolutely. I think it was directed
9 at you, so please do.
10 DR. MAYERS: The problem becomes how because if
11 you are defining failure as a viral load coming up to 500,
12 and people were defined as successful when they are below
13 50, you are not going to be able to sample with existing
14 technologies the patients who are doing well, so I think it
15 makes sense in a statistical way, but I am just not sure how
16 to do it technically.
17 So, I think the answer is in most patients, you
18 are going to have to assume that if they are below limits of
19 detection of your ultra-sensitive assay, they are probably
20 still drug susceptible.
21 DR. HAMMER: I may have missed this, but I think
22 testing, if you are going to do baseline and follow up on
23 the failures, you need some proportion of the successes at
24 baseline to compare in a case cohort or some other design.
25 Dr. Yogev.

1 DR. YOGEV: I just need to add to a similar
2 situation, for example, if a company come around and said
3 that drug X is working in resistant strain, what combination
4 of drug do they want to use, for example, addition of a drug
5 similar to DDI or the ABT, that a test has to be done
6 prospectively to identify this population as a subset to
7 work with, and make sure that it does work, because, for
8 example, into the study introduce people who are not
9 involved in the study at week 12, for example, the
10 contention is really working to that specific group is not,
11 which was identified as resistant is not suffering from the
12 study if it goes for 24, 48 weeks, so I think there are some
13 studies that you have to do it prospectively as part of the
14 inclusion criteria.
15 DR. HAMMER: I think there is consensus about
16 that.
17 Dr. Stanley.
18 DR. STANLEY: And then just to state the obvious,
19 that we were told this morning the example we were given
20 that if you are testing at the time of drug failure, they
21 need to still be on the drug when you collect the sample.
22 DR. HAMMER: Right. I think actually, there were
23 maybe two parts to this question, the timing in the setting
24 of the clinical trial. I think one is the broader question
25 whether it is prospective testing or retrospective testing,

1 and I think the sense is that increasingly we are moving
2 towards prospective testing and clearly baselines, depending
3 upon the relevance of the population and whether you are
4 going to randomize on the basis of that or not, and follow-
5 up specimens at the time of failure, I think have all been
6 recommended by the committee. I think this is fairly
7 obvious based on the discussions over the last day.
8 The third question. I am going to take the
9 Chair's prerogative here. The question is: Please comment
10 on factors that may confound the interpretation of
11 resistance testing in the setting of clinical trials and
12 what may be done to reduce these effects.
13 At least the first part of that question, I
14 personally think Dr. D'Aquila answered better than any of us
15 can answer, but I would state that just personally and let
16 the rest of the committee comment, but also what may be done
17 to reduce these confounders is potentially where we might
18 contribute something.
19 So, Question 3. Does anyone disagree with my
20 premise? Dr. Charache.
21 DR. CHARACHE: I agree with your premise. I think
22 in terms of reducing confusion, high on my suggestion list,
23 as has been pointed out by Dr. Jackson, as well, is that the
24 IC50 has clearly proven very useful as an interpretative
25 point, but I think with new drug development, it would be

1 very helpful to also relate the interpretations to the
2 pharmacology of the sponsor's drug.
3 So, I think it would be very important to provide
4 this information, not only as it pertains to comparison with
5 the lab strain of virus, but also in terms of achievable
6 blood levels and trough levels and area under the curve, and
7 I think that data should be provided.
8 I think there should be similar information for
9 all drugs a patient is on, since they are going to be on a
10 scrap basket of drugs, and that might help make it easier to
11 interpret the results that you are looking at in terms of
12 clinical failure or success.
13 Finally, I think it would be very important for
14 the sponsor to be able to validate the competency of any
15 laboratory that is used to derive this information. I think
16 the Virco and virologic models are outstanding, but I think
17 also they may want to, if they use other units or even those
18 to have testing done between laboratories because this is
19 going to be absolutely critical to make sense out of this
20 very difficult area.
21 DR. HAMMER: Thank you. Well stated.
22 Dr. Hamilton.
23 DR. HAMILTON: It seems to me to evaluate the
24 results of genetic and/or phenotypic testing in the context
25 of a clinical outcome, one needs the simultaneous collection

1 of additional data that can easily or alternatively explain
2 the clinical event, namely, drug exposure, drug adherence,
3 'and I would strongly recommend that if genetic and
4 phenotypic testing is to be done, and it appears that there
5 is a move that it should be in virtually all drug
6 populations, I think there should be simultaneous collection
7 of those other parameters.
8 DR. HAMMER: Dr. Kaplan.
9 DR. KAPLAN: This is part of Question 3, and I
10 guess it also relates back to 2 a little bit.
11 If a company is going to propose use of a drug for
12 recently infected persons, then, an interesting issue that
13 has come for discussion is whether the best timing of an
14 initial specimen is before treatment or maybe after the
15 person has been on drug for a couple of weeks, because then
16 perhaps anything that has partially reverted to wild type
17 will have a chance to pop up.
18 That may be less of a factor for recently acquired
19 infections where there hasn't been that much of an
20 opportunity to revert to wild type than for people who are
21 farther along, but I would be interested in what others
22 think about the timing of that initial specimen if the drug
23 is going to be proposed for those conditions.
24 DR. HAMMER: I think it is quite difficult
25 although in some trials that have been designed have

1 specifically stated that eligibility criteria include having
2 remained on the failing regimen until a specimen is
3 obtained, at least a plasma specimen is in the freezer, and
4 I think if one wants to ensure that, that it is the best way
5 to ensure the selective pressure, then, that is the way the
6 inclusion criteria should be.
7 However, it excludes populations that come on and
8 off drugs, and I think one thing that is difficult, at least
9 for me, I will just state it, is a patient has come off a
10 failing regimen, asking a patient to go back-on a failing
11 regimen to reselect in order to make them eligible for a
12 trial, I personally find difficult to do, and have not done
13 that in trials I have been involved in where the question
14 has come up.
15 So, I think the point is well taken, and it is an
16 issue of again carefully defining your population for the
17 trial, and so that you can analyze it in the best way
18 possible, and then what the broader applicability is to
19 populations who had come off.
20 I think that what that is going to be is a
21 compendium of trial results that will tell us, again with
22 all the interest in salvage therapies after treatment
23 interruptions, and there will be a flurry of data over the
24 next year and plus that will tell us more about whether that
25 is a reasonable strategy or not, I think we will be able to

1 put this stuff together, but I think the selective pressure
2 question can be handled by the eligibility criteria.
3 Dr. Yogev.
4 DR. YOGEV: I think you might consider around
5 three months or 16 weeks, if you are not less than 400,
6 those should be also tested because even if you have the
7 wild type, and you got the reduction, did you get a
8 reduction of the wild type alone or in combination of the
9 resistant one, you can pick it up at that point in time, so
10 I would not wait until the end of the study. I would do it
11 at that point, but I would not exclude patients for entering
12 the study.
13 DR. HAMMER: Dr. Mayers.
14 DR. MAYERS: I think that as the issues of PK come
15 up and potentially adherence come up, that it is clear the
16 companies aren't going to do PK on each patient in the
17 study, but I think that what you can do now is get a time on
18 your drug levels, your last dose at each visit, and with the
19 new technologies of plasma, you can, with 2 mL of plasma get
20 a genotype, a phenotype, and drug levels for all the drugs
21 that your patient is on.
22 So, if the effort is made to attain the time of
23 the last dose of each drug at the visit, they can then go
24 back and actually map out whether the patient had a low
25 exposure, no exposure by that level or not, and if you don't

1 get that data, you simply can't do it.

2 So, I think if you we are going to try and relate
3 resistance and drug levels to failure, there is going to
4 have to be an attempt to be able to at least pair the timing
5 of the last dose with the plasma sample.

6 DR. HAMMER: Let me just summarize. The
7 confounding factors I think as far as trying to reduce them,
8 I would reiterate that what they are was well stated in
9 Richard's talk. To reduce them, as one suggests, the
10 simultaneous obtaining of particularly pharmacologic
11 information and drug adherence information, that may vary
12 trial to trial, but they are the two major factors in trying
13 to determine what failure is all about, and since resistance
14 testing is related to failure on study in at least one
15 circumstance, I think that is the critical issue.

16 Up front, I think it is the issue of defining the
17 population that you are studying and then deciding whether
18 you are going to do resistance testing at baseline, which we
19 have talked about several times.

20 Anything else? Dr. Mathews.

21 DR. MATHEWS: A couple of other things just having
22 to do with conduct of the trials, and it is not a problem
23 right now, but one of the scenarios related to this, that
24 people could start using resistance testing during the
25 conduct of a trial and drop out on the basis of resistance

1 testing.

2 Now, you could say, well, they wouldn't get it if

3 they were nondetectable, but it is easy to just withdraw

4 from drug for several days, rebound, measure it. I don't

5 know whether these things will happen, but they are

6 certainly within the range of possibility.

7 So, selective dropout for that basis could affect

8 the interpretation of the resistance assay.

9 Another issue relates to what is the appropriate

10 control for interpreting the response to a particular

11 mutational pattern. It may not be the particular site or

12 codon that is the issue, and if the trial wasn't randomized

13 on the basis of the mutational pattern, then, there will be

14 a fair amount of heterogeneity.

15 So, mutation A in the setting of several other

16 mutations may not have the same effect as in another

17 context, and so I think it will require quite a bit of

18 thought to figure out what is the appropriate comparison

19 group in a trial.

20 DR. HAMMER: That is a very important point, and

21 it brings up a corollary point, and that is the

22 interpretation of resistance testing, how that factors in to

23 any clinical trial design, and trying to create balance in

24 interpretive skill and recommendations for regimens that may

25 be as a result of some strategic intervention with the

1 results of resistance testing since we have seen that
2 imbalance in certain trials so far, and we have also seen
3 the complexity of interpreting these tests.
4 So, I think that is a factor we haven't talked
5 about in trial design, but it certainly confounds the
6 interpretation of how resistance testing is applied in
7 trials because it is no better than who interprets it.
8 We are running 15 minutes behind, but I think we
9 should stop for a break and reconvene at 10:45.
10 Thank you.
11 [Break.]
12 DR. HAMMER: I would like to officially reconvene
13 and turn to Dr. Jeffrey Murray, who will introduce Session
14 4.

15 **SESSION 4**

16 **Potential Roles of Resistance Testing**

17 **in Drug Development**

18 **Introduction**

19 DR. MURRAY: Session 4 is entitled Potential Roles
20 of Resistance Testing in Drug Development. From our
21 perspective, this is the mother of all sessions because this
22 is where we are expecting the most lush feedback from the
23 committee. That is why we also wanted to get this session
24 started, get the presentations out of the way in the
25 morning, so there would be a lot of chance to talk on the

1 regulatory scenarios which are coming up.

2 The objectives for this session are: to obtain
3 committee recommendations on the amount and type of in vitro
4 resistance data sufficient to initiate and to support a
5 clinical development program;

6 To obtain committee recommendations on the amount
7 and type of in vitro and clinical resistance data sufficient
8 to characterize the clinical activity of an antiretroviral
9 drug against resistant viral isolates;

10 To obtain committee recommendations on the amount
11 and type of clinical resistance data appropriate to
12 determine an antiretroviral drug's potential to induce
13 resistance and cross-resistance;

14 To obtain committee recommendations on testing can
15 be optimally incorporated into Phase II/III clinical trial
16 design.

17 Our two presentations, the first one is Dr. Gary
18 Chikami, who is Division Director of Anti-Infective Drug
19 Products. He will talking on Susceptibility Testing in Drug
20 Development from an Anti-Infectives Perspective. I think
21 this will give us good groundwork to then talk about how it
22 would be applied in the HIV arena.

23 **Historical Perspective from the Antibacterial**
24 **Analogy and Contrasts with Virology**

25 DR. CHIKAMI: Thanks, Jeff.

1 On the original agenda, my sort of charge was to
2 sort of talk about the historical perspective the
3 development of resistance testing, and I think that is sort
4 of a daunting task, so what I want to do in the few minutes
5 that I am going to be talking, about 20 minutes, is to
6 provide sort of the framework that has been developed over
7 the years for susceptibility testing and how it is used for
8 the development of antibacterials, and touch on two
9 regulatory issues with regard to how susceptibility
10 information is included in produce labeling.
11 A related concept which has really come to the
12 fore recently with the rise of the importance of antibiotic
13 resistance, the development of products specifically for
14 resistant indications, and how we have attempted to deal
15 with those in terms of drug development and how those would
16 be included in package labeling.
17 [Slide.]
18 With respect to antibacterials, the goal of
19 susceptibility testing is to predict the likely outcome of
20 treating a patient's infection with a particular
21 antimicrobial agent. This would be useful and important for
22 organisms that are not particularly predictably susceptible
23 to drugs of choice either because of an acquired resistance.
24 These tests should do a couple of things. One is
25 detect frank resistance. Moreover, they can also be useful

1 for the quantitative measurement of susceptibility to
2 antimicrobial agents with some species where there may be
3 direct therapeutic relevance.
4 For example, the magnitude of penicillin,
5 cephalosporin **MICs** for Strep pneumoniae, and that was
6 referred to by one of the talks this morning where, in
7 certain circumstances, an intermediate Strep pneumoniae in
8 fact may be treatable with higher doses of penicillin.
9 [Slide.]
10 Over the years, a framework for the
11 standardization of susceptibility test methods have been
12 developed by numerous organizations. The NCCLS, for
13 example, which provides standard protocols, **and also reviews**
14 data to send interpretative criteria.
15 Those sorts of activities are also important at
16 the FDA as we review drug development and approve drugs for
17 marketing.
18 [Slide.]
19 In regard to the regulatory responsibility for
20 **antibacterials**, in vitro diagnostic tests are reviewed and
21 approved in the Center for Radiologic Health, Medical
22 Devices and Radiologic Health or CDRH, with consultation of
23 CDER, the drug review divisions.
24 Sort of the two steps involved in standardization
25 of the test methods are defining optimal assay methods and

1 development of the interpretive criteria.

2 Later in my talk I will touch on again these two

3 regulatory issues, that is, the inclusion of susceptibility

4 information in product labeling and then specific claims of

5 effectiveness for the treatment of infections due to

6 resistant bacteria.

7 [Slide. 1

8 In regard to the development of test methods, the

9 goals are to develop reproducibility and reliability in the

10 test method. These include a number of sort of physical,

11 chemical, and other specifications including standard assay

12 conditions, sort of a caveat in this or sort of the driving

13 force is measurement of a minimal inhibitory concept is not

14 a physical or chemical measurement.

15 It is a measurement of the interaction of the test

16 drug with the test organism that may be affected by a number

17 of sort of conditions of the test, temperature, ion

18 concentration, inoculum effects. All of these things, in

19 fact, may affect the observed test results, and it is

20 critically important that all of these be optimized and

21 specified in a standardized way, so that the measured

22 results again are reproducible and reliable.

23 Secondly, specification of quality control

24 parameters are very important. Again, this relates to

25 standardization of the assay conditions, but also a standard

1 battery of test microorganisms and their expected results.
2 Again, these tests are done in multiple laboratory
3 conditions, different labs, different quality of
4 laboratories. So, having built into the system an ability
5 to assess the performance of the test, not just with the
6 target organisms, but standardized organisms is very
7 important in being able to develop interpretable results.
8 The third point in terms of developing
9 reproducible and reliable test methods is correlations of
10 different methods that may be used under different clinical
11 conditions for bacterial susceptibility testing, common sort
12 of methods that are used include dilution methods. They may
13 be macro or micro dilutions or disc diffusion methods.
14 In the course of developing these assays, and
15 standardizing them, correlations of these two different
16 methodologies are very important in being able again to
17 develop interpretable results that can then be used in the
18 next step of the process.
19 [Slide.]
20 And that is the development of interpretive
21 criteria. Interpretive criteria relate the quantitative
22 results of susceptibility testing to again that overall goal
23 of the testing methodology, which is to predict the likely
24 outcome of therapy.
25 There are a couple of caveats here.

1 Susceptibility in vitro does not necessarily predict
2 successful therapy. Host factors are often more important
3 in terms of eventual clinical outcome.
4 Secondly, we would like to have methods where
5 resistance in vitro should predict therapeutic failure, and
6 again in terms of understanding why a patient may not
7 respond to therapy or being able to guide therapy in
8 patients in which it may not be obvious what is the optimal
9 choice of therapy.
10 [Slide. 1
11 From that follows these three definitions which
12 are commonly used or which are used for development of
13 interpretive criteria for bacterial susceptibility testing.
14 A strain is called susceptible to the test drug if it may be
15 appropriately treated with a dosage of the antimicrobial
16 agent recommended for that type of infection.
17 An intermediate classification are strains with
18 MICs that approach usually obtainable blood levels or tissue
19 levels and for which response rates may be lower than that
20 for susceptible isolates.
21 There are several factors built in or other
22 considerations built into this intermediate category. There
23 are also conditions, for example, where an intermediate MIC
24 may be appropriate or use of a drug with an intermediate MIC
25 may be appropriate at a tissue site where the drug is

1 concentrated, for example, the use of quinilones in the
2 treatment of urinary tract infection.
3 Some other considerations are also for drugs with
4 a narrow therapeutic index where, in fact, inaccuracies in
5 the assay may, in fact, have great therapeutic import.
6 Finally, the third category is resistant, and
7 these are strains which are not inhibited by the usual
8 achievable concentration of the agent with normal dosage
9 schedules and/or or fall in the range where specific
10 microbial resistance mechanisms are likely and clinical
11 efficacy has not been reliable in treatment studies. An
12 example of that is, for example, an organism and **beta-**
13 lactamase.
14 [Slide. 1
15 With these definitions in mind that have been
16 applied to anti-infectives, what are the sorts of
17 information that are considered in setting these
18 interpretive criteria?
19 Just parenthetically, breakpoints as they are
20 called, or interpretive criteria, are developed early on in
21 the course of development of an antibiotic. Much of the
22 information, for example, the in vitro activity, animal
23 model data, and early pharmacokinetic information which
24 would allow the setting of tentative breakpoints, say, at
25 Phase I or Phase II within a drug development process.