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DEPARTMENT OF HEALTH AND HUMAN SERVICES  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH

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ONCOLOGIC DRUGS ADVISORY COMMITTEE  
Sixty-Ninth Meeting

Wednesday, December 5, 2001

8:30 a.m.

Holiday Inn  
Kennedy Ballroom  
8777 Georgia Avenue  
Silver Spring, Maryland

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Guest Speakers (non-voting):

M. Elizabeth H. Hammond, M.D.  
Soonmyung Paik, M.D.  
Patrick C. Roche, Ph.D.

Musa Mayer, patient representative (voting)  
George Ohye, industry representative

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P R O C E E D I N G S

**Call to Order and Introduction**

DR. NERENSTONE: I would like to thank everyone for coming, and point out that this is the 69th meeting of ODAC, and we are certainly hopeful that this will be a less eventful ODAC than our last one.

We always like to start by going around the room and having everybody introduce themselves for the record, and if you will state your name into the microphone for the recorder, Mr. Ohye, if you would like to start?

MR. OHYE: I am George Ohye, industry representative nominee.

DR. O'LEARY: I am Tim O'Leary, from the Armed Forces Institute of Pathology.

DR. WATSON: Mike Watson, from the American College of Medical Genetics.

DR. BARKER: I am Peter Barker, with the Biotechnology Division of the National Institute of Standards and Technology.

DR. CARPENTER: I am John Carpenter, a medical oncologist from the University of Alabama at Birmingham.

MS. MAYER: Musa Mayer, a patient

1 representative from New York City.

2 DR. ALBAIN: Kathy Albain, medical  
3 oncologist, Loyola University, Chicago.

4 DR. GEORGE: Stephen George,  
5 biostatistics, Duke University.

6 DR. KELSEN: David Kelsen, medical  
7 oncologist, Sloan-Kettering, New York.

8 DR. NERENSTONE: I am Stacy Nerenstone,  
9 medical oncology, Hartford Hospital, Hartford,  
10 Connecticut.

11 DR. TEMPLETON-SOMERS: Karen Somers, the  
12 executive secretary to the committee, FDA.

13 DR. BRAWLEY: Otis Brawley, medical  
14 oncologist, Emory University.

15 DR. LIPPMAN: Scott Lippman, medical  
16 oncologist, M.D. Anderson Cancer Center.

17 DR. BLAYNEY: Doug Blayney, medical  
18 oncologist, Wilshire Oncology Medical Group, in  
19 Pasadena, California.

20 DR. TAYLOR: Sarah Taylor, medical  
21 oncologist, University of Kansas, Kansas City.

22 DR. REDMAN: Bruce Redman, medical  
23 oncologist, University of Michigan.

24 DR. GRIFFIN: Connie Griffin, Johns  
25 Hopkins University. I am a medical oncologist and

1 I do cytogenetics.

2 DR. GUTMAN: I am Steve Gutman. I am  
3 Director of the Division of Clinical Laboratory  
4 Devices in the Office of Device Evaluation, FDA.

5 DR. JERIAN: Susan Jerian, medical team  
6 leader, Center for Biologics, Division of Clinical  
7 Trials.

8 DR. KEEGAN: Patricia Keegan, Division of  
9 Clinical Trials, Center for Biologics.

10 DR. SIEGEL: Jay Siegel. I direct the  
11 Office of Therapeutics in the Center for Biologics.

12 **Conflict of Interest Statement**

13 DR. TEMPLETON-SOMERS: The following  
14 announcement addresses the issue of conflict of  
15 interest with respect to this meeting, and is made  
16 part of the record to preclude even the appearance  
17 of such at this meeting. Based on the submitted  
18 agenda and information provided by the  
19 participants, the agency has determined that all  
20 reported interests in firms regulated by the Center  
21 for Drug Evaluate and Research present no potential  
22 for a conflict of interest at this meeting, with  
23 the following exceptions:

24 In accordance with 18 USC, Section  
25 208(b)(3), Dr. Scott Lippman has been granted a

1 full waiver. A copy of Dr. Lippman's waiver  
2 statement may be obtained by submitting a written  
3 request to the agency's Freedom of Information  
4 Office, Room 12A-30 of the Parklawn Building.

5 In addition, Dr. Sarah Taylor's employer,  
6 the University of Kansas Medical Center, has  
7 interests which do not constitute financial  
8 interests in the particular matter within the  
9 meaning of 18 USC, Section 208 but which could  
10 create the appearance of a conflict. The agency  
11 has determined, notwithstanding these interests,  
12 that the interest of the government in Dr. Taylor's  
13 participation outweighs the concern that the  
14 integrity of the agency's programs and operations  
15 may be questioned. Therefore, Dr. Taylor may  
16 participate fully in this morning's discussions and  
17 vote.

18 Further, Dr. George Sledge will be  
19 excluded from participating in the discussions and  
20 vote concerning IHC and FISH assays.

21 With respect to FDA's invited guests, Dr.  
22 Soonmyung Paik and Dr. Elizabeth Hammond have  
23 reported interests that we believe should be made  
24 public to allow the participants to objectively  
25 evaluate their comments. Dr. Paik is an

1 investigator in the National Surgical Adjuvant  
2 Breast and Bowel Program, protocol B-31, and  
3 investigates the worth of Herceptin as an adjuvant  
4 therapy. This is a government-funded study with  
5 additional financial support from Genentech. Dr.  
6 Paik is also a member of Genentech's HER2 pathology  
7 advisory board. The board advises on assay formats  
8 for HER2 testing.

9 Dr. Hammond would like to disclose that  
10 she periodically lectures to clinicians on HER2  
11 testing. She has also been invited to a single  
12 pathology advisory committee meeting to discuss  
13 HER2 testing.

14 Lastly, we would also like to note for the  
15 record that George Ohye is participating in this  
16 meetings as an industry representative, acting on  
17 the behalf of regulated industry. As such, he has  
18 not been screened for any conflicts of interest.

19 In the event that the discussions involve  
20 any other products or firms not already on the  
21 agenda for which FDA participants have a financial  
22 interest, the participants are aware of the need to  
23 exclude themselves from such involvement and their  
24 exclusion will be noted for the record. With  
25 respect to all other participants, we ask in the

1 interest of fairness that they address any current  
2 or previous financial involvement with any firm  
3 whose product they may wish to comment upon.

4 I would also like to note for the record  
5 that our consumer representative, Dr. Jody Pelusi,  
6 had to cancel her participation in this meeting at  
7 the last minute, and there was no time to replace  
8 her. We are, however, fortunate to have Ms. Musa  
9 Mayer as our patient representative to provide that  
10 special point of view. Thank you.

11 DR. NERENSTONE: I just wanted to point  
12 out that today is a little bit different than our  
13 usual ODAC meetings. This morning we are going to  
14 be talking about the development of assays linked  
15 to therapeutic products as opposed to the  
16 therapeutic product itself, and this will set the  
17 stage for our afternoon discussion as well.

18 Dr. Siegel, if you would like to begin?

19 **Introduction**

20 DR. SIEGEL: Sure. I would just like to  
21 take this opportunity to welcome you all and make a  
22 few remarks to set the stage in context for our  
23 discussions this morning.

24 As I think many or all of you know for  
25 about ten years now there has been active

1 discussion in the pharmaceutical industry, the  
2 biotechnology industry, the medical and scientific  
3 communities about the promise of new molecularly  
4 targeted drugs and patient individualized  
5 therapies, this promise arising in significant part  
6 from technological developments, and notably the  
7 ability of powerful genomic tools to characterize  
8 diseases, patient responses to diseases, and  
9 patient genetic status and physiological status.

10           Perhaps this promise has been most acutely  
11 felt and hoped for in the area of oncology because  
12 of the diversity of tumors and the toxicity of most  
13 of our effective therapies, many of which have to  
14 be given to a broader population of patients than  
15 those who will ultimately benefit because of  
16 limitations in our ability to identify those  
17 factors that might predict who will respond and who  
18 will not respond to a therapy. But, surely, these  
19 issues are by no means limited to oncology and  
20 there is significant hope for understanding at a  
21 much greater level variabilities and disease  
22 process, how people respond to disease and how  
23 people respond to and process the drugs that are  
24 used to treat those diseases.

25           Trastuzumab, Herceptin, and a few drugs

1 that are out there represent early examples of  
2 these therapies, but the agency has seen for some  
3 years now many more under development and expect  
4 many more to come. Of note, they raise a unique --  
5 perhaps not unique but a special class of problems  
6 in the development of many of these therapies, and  
7 that is really the type of problem we are going to  
8 be discussing today, that is that at the same time  
9 one is developing a therapeutic modality one is  
10 also developing a test to identify appropriately  
11 how to target or use that modality.

12           The issues of timing of the development of  
13 the therapy and of the testing have been  
14 particularly complex and occasionally problematic,  
15 where the development of a therapy may be steps  
16 ahead of the development of the testing to target  
17 the use of the therapy. Some of the issues that  
18 have arisen, in most cases in preclinical  
19 development of experimental agents, are agents  
20 being ready to go into definitive efficacy trials  
21 before there is a well validated assay used to  
22 target it, leading to situations where there may be  
23 each side doing their own test in ways that are not  
24 well validated and not consistent, sometimes  
25 leading to results that may be different to

1 interpret or reproduce or, even if they are  
2 interpretable, difficult to commercialize or bring  
3 out to the community because of unavailability of a  
4 test that can identify the same population and  
5 ensure that those results might be generalizable  
6 and reproduced in a broader population.

7           Also, issues have been raised in  
8 development of how tightly to restrict entry  
9 populations. There is a desire often by the  
10 investigators and sponsors of therapies to look at  
11 a very narrow population of those people who score  
12 very high on a test. Perhaps that is where you are  
13 most likely to see efficacy but at the end of the  
14 game, if that is all you studied, there are many  
15 unanswered questions about who should be treated,  
16 where a cut point should be, how to make  
17 determinations of how broadly or narrowly to use a  
18 therapy with a potential for either toxic,  
19 ineffective, off-label use, or overly narrow use.

20           Also, raising considerable concerns as to  
21 what happens in any of these situations as the  
22 tests develop and new tests come along. If you  
23 have only tested a limited population or if you  
24 don't have samples from the initial population, it  
25 is very hard to know what the responses would be

1 like in people identified by a new test when all  
2 your data is limited to those identified by an old  
3 test, or who are strongly positive on the old test  
4 as the new test may identify an overlapping but  
5 different population.

6 So, there are a lot of important questions  
7 out there, a lot of questions that the developers  
8 of these therapies have been struggling with, a lot  
9 of questions that the agency has been struggling  
10 with in working with the developers. We are  
11 particularly pleased to have this distinguished  
12 group of experts together to help us work through  
13 this issue and provide your guidance. So, thank  
14 you again and welcome.

15 DR. NERENSTONE: Thank you. Dr. O'Leary?

16 **Immunohistochemistry for Therapeutic Target**  
17 **Identification: The Total System**

18 DR. O'LEARY: I am going to talk just a  
19 little bit about immunocytochemistry,  
20 immunohistochemistry in a general sense and as a  
21 total test system, stealing a term from Clive  
22 Taylor who was really the founder of  
23 immunomicroscopy in surgical pathology and who has  
24 contributed a great deal to this area.

25 The idea here is that in one way or

1 another we have to go from something like this, a  
2 tumor or another tissue, to something like this, an  
3 immunohistochemically stained tissue. And, a lot  
4 has to happen along the way, and not all of this is  
5 at the level of applying the immunohisto-chemical  
6 stain itself.

7           From the time the tissue is first excised  
8 a number of things begin to happen. The first  
9 thing is that this tissue begins to die. In the  
10 process of dying, a whole bunch of stuff goes on.  
11 Nucleases, proteases, lipases, other enzymes are  
12 released. These begin to destroy the tissue, both  
13 the tissue morphology, protein antigens and nucleic  
14 acids. So we have to stop this from happening so  
15 that there is something to look at.

16           That is what fixation is all about.  
17 Fixation finishes off the process of cell death.  
18 It makes cell death occur very, very rapidly but,  
19 to one extent or another, it stops these process of  
20 tissue degradation from going on.

21           After fixation, then we have to do things  
22 like dehydrate the tissue, take all the water out  
23 by moving it through a series of graded alcohols  
24 into xylene; infiltrating it with paraffin to embed  
25 the tissue; cutting it; staining it; make a

1 diagnosis and then finally apply our adjunctive  
2 techniques, such as immunohistochemical studies.  
3 Each one of these has some potential artifacts, and  
4 each of them can affect the process of performing  
5 and interpreting an immunohistochemical assay.

6           Obviously, you can get away from some of  
7 these things if you use frozen sections. In that  
8 case, you stop a lot of these things from going on,  
9 but the expense in most laboratories is for less  
10 well defined morphology, very difficult tissue  
11 handling and a real mess on the part of the  
12 pathologist's time and a very large increase in  
13 expense. So, in a practical sense, this isn't  
14 going to happen. Most of the time we are going to  
15 be talking about paraffin-embedded tissues.

16           The proteases are of greatest concern for  
17 immunohistochemistry because they destroy protein  
18 antigens. But they don't destroy those antigens at  
19 the same rate. So, a protease may destroy one  
20 antigen very, very quickly; another antigen very,  
21 very slowly, meaning that the development of  
22 internal controls for the effect of protease can be  
23 quite difficult.

24           The nucleases go out and they can wipe out  
25 nucleic acids. RNA tends to be destroyed much more

1 quickly than DNA. Again, these, as well as the  
2 proteases, can be found at different levels in  
3 different tissues. So, in pancreases, for example,  
4 or in eosinophils nuclease concentrations,  
5 particularly ribonuclease, are very, very high and  
6 these can go out and wipe out a nucleic acid signal  
7 from ISH or some other technique very, very  
8 rapidly.

9           Similarly, the presence of one tissue  
10 within another in which these materials,  
11 particularly eosinophils or polymorphic  
12 nucleosides, are present can cause the destruction  
13 of antigens and nucleic acids in adjacent cells.

14           In terms of the overall stability against  
15 digestion by these various enzymes, DNA tends to be  
16 the most stable of the potential targets; protein  
17 next and then RNA is far and away the least stable.  
18 But some of the proteins, for example collagens,  
19 tend to be almost as stable as DNA in practice.

20           So, in fixation we are going to attempt to  
21 stop the degradation of cellular components by  
22 these enzymes, preserving cellular morphology and  
23 then preserving reactivity for these various later  
24 kinds of tests. The ideal fixative is, first of  
25 all, cheap because nobody likes to pay for medicine

1 or laboratory tests anymore. They can rapidly  
2 penetrate through a tissue because these tissues  
3 are, after all, of a significant thickness when  
4 they are first excised. It inactivates these  
5 enzymes without reducing their antigenicity. The  
6 ability to extract cellular components for other  
7 analyses; the ability to use nucleic acids as  
8 substrates for polymerase reactions, RTPCR and so  
9 forth. That fixative doesn't exist.

10           Instead, the most common fixative is  
11 neutral-buffered formalin, formaldehyde, but other  
12 fixatives, so-called precipitating fixatives like  
13 alcohol and acetone are also in use. In general,  
14 the cross-linking fixatives stop enzymatic  
15 degradation more quickly and completely than do the  
16 precipitating fixatives, but they can both do a  
17 pretty good job of that.

18           Formaldehyde actually has some properties  
19 of both because it doesn't exist as the aldehyde  
20 but, rather as methylene glycol and the reactions  
21 are, for the most part, of methylene glycol. So,  
22 certain of the properties resemble those of  
23 precipitating fixatives, but it does form cross-  
24 links.

25           What kind of cross-links does it form?

1 First, intra-molecular and inter-molecular cross-  
2 links in proteins. It will turn concentrated  
3 protein solutions, such as one sees in a cell, into  
4 a gel that is more stable than it was before you  
5 added the protein. It also forms inter-molecular  
6 cross-links and those inter-molecular cross-links  
7 are different depending on the protein structure.  
8 It will also form cross-links between DNA bases,  
9 but pretty much in the areas where the DNA bases  
10 themselves are accessible. In other words, if you  
11 have a pure double-stranded DNA, in general  
12 formaldehyde doesn't attack that very effectively.  
13 However, it can glue proteins and other things on  
14 around those nucleic acids. So, it is not that it  
15 has no effect. Quite to the contrary, you have to  
16 do something about this fixation because it make  
17 even DNA inaccessible.

18 Finally, there are direct adducts formed  
19 between the bases of RNA and formaldehyde, methylal  
20 adducts. These are reversible, and they probably  
21 also result in the formation of peptide adducts to  
22 RNA and nucleic acid adducts can form as well, in  
23 other words, RNA adducts. Finally, lipids and  
24 other structures can be affected.

25 This is not meant to be read but simply to

1 say that we have lots and lots of different  
2 fixatives that are out there in common use.  
3 Formalin is the first one but there are  
4 modifications which are better for some purposes.  
5 In fact, if you go to the literature, probably  
6 hundreds of different fixatives have been used in  
7 histology. But five or six fixatives are commonly  
8 used and in a particular hospital it is possible to  
9 find a preserved fixative. You will find hospitals  
10 that don't use formalin. You will find hospitals  
11 that don't use the five. It is not that we always  
12 tailor everything to the precise application for  
13 which it is best suited.

14           Still and all, most pathologists do prefer  
15 formaldehyde for most tissues. It gives relatively  
16 crisp tissue morphology, relatively good  
17 preservation of antigenicity. In addition, it is  
18 really cheap.

19           But the process of fixation, needless to  
20 say, reduces antigen reactivity. It can mask the  
21 epitopes in one way or another or, alternatively,  
22 it can just make the tissue impenetrable. If you  
23 are cross-linking proteins one to another, then  
24 getting a protein such as antibody in for an  
25 immunohistochemical assay becomes more difficult.

1 It doesn't destroy the secondary or tertiary  
2 structure of the proteins and it is partially  
3 reversible.

4 Well, how do you deal with the problem of  
5 formaldehyde fixation? Historically one approach  
6 was to use protease digestion to help improve  
7 reactivity. Nowadays, most of the time we  
8 basically heat the slide in some kind of a solution  
9 to a high temperature and incubate it for a while.  
10 This removes formaldehyde which, in fact, forms a  
11 relatively weak bond with proteins. When the  
12 formaldehyde comes off at high temperature, the  
13 protein does become denatured so you are not left  
14 with the same thing with the usual antigen  
15 retrieval processes.

16 Other approaches that are used are  
17 protease digestion. In terms of  
18 immunohistochemistry, it has been largely, and in  
19 some places completely, replaced by high  
20 temperature antigen retrieval approaches but it is  
21 still a major component of many in situ  
22 hybridization protocols.

23 When it comes to doing  
24 immunohistochemistry, what are we up to? The first  
25 thing we are going to do, we are going to incubate

1 the tissue with an antibody directed to the antigen  
2 of interest, whether that be the HER-B2 protein,  
3 collagen or whatever. We are going to bind that  
4 antibody. We are then going to wash it off and  
5 bind it to a detection system. The detection  
6 system I am showing you now is actually the one  
7 that is used in the DAKO test, the so-called  
8 Invision system. It basically is a signal  
9 amplification system in which, after binding the  
10 antibody, a series of a colorimetric development  
11 steps can be used to give you a nice brown, red or  
12 some other color signal on a slide.

13 But there are lots and lots of so-called  
14 immunoperoxidase methods. I list a number of them  
15 here, and this is only a general set of  
16 categorizations of the immunoperoxidase methods.  
17 In fact, every one of these has many, many variants  
18 on the theme.

19 I will just show two of them. One is  
20 probably the most common immunoperoxidase method,  
21 the ABC method, and it is a method in which we take  
22 an antibody. We bind it to the tissue. We take a  
23 secondary and react that to the primary antibody.  
24 Then we take a compound called streptavidin or  
25 avidin that binds biotin and we bind it to the

1 biotin. Then we use a horseradish peroxidase  
2 enzyme which has been complexed to develop a  
3 colorimetric reaction with diaminobenzidine or some  
4 other colorimetric reagent.

5 The result is, again, a color  
6 corresponding to the locations in the tissue where  
7 that antibody originally bound. If you have a good  
8 specific antibody, then you have something that is  
9 pointing up the antigen of interest.

10 We compare that, in the lower panel, with  
11 an amplification method that is a proprietary  
12 amplification method in which a much higher degree  
13 of amplification is possible. What you have here  
14 is a very similar idea but one in which now the  
15 color developed with a particular amount of antigen  
16 is much greater than it would have been with the  
17 original ABC technique. So, by changing  
18 immunoperoxidase techniques you can get different  
19 levels of sensitivity to the antigen that is down  
20 there. It can vary by orders of magnitude in  
21 sensitivity. Immunoperoxidase is not  
22 immunoperoxidase, is not immunoperoxidase, is not  
23 immunoperoxidase.

24 The process of doing this in the  
25 laboratory is actually reasonably complex. Again,

1 I don't want you to actually try to read any of  
2 this but I will just note that, first of all, there  
3 is a lot of stuff going into it. These are the  
4 materials. Then, if you just count the number of  
5 steps on an RABC procedure, I am up to 14. I have  
6 21 different steps being taken in the laboratory  
7 and every one of those steps is a place where a  
8 mistake can potentially be made.

9 Most importantly, there are lots of  
10 technical artifacts even outside these steps that  
11 can affect the result of an immunohistochemical  
12 test. For example, with many antigens, after  
13 floating the tissue sections, so before you even  
14 begin to do your immunoperoxidase assay, you take  
15 the tissue section; you prepare it on a slide; and  
16 then you dry it. If you warm it up wet to 55 or 60  
17 degrees, you will destroy many antigens. The  
18 mechanism of that isn't well understood but the  
19 fact is that the immunogenicity goes down  
20 substantially. Alternatively, if after embedding  
21 and cutting and before you do your  
22 immunohistochemistry you don't get all the paraffin  
23 out, well, paraffin is not a very good solvent for  
24 antibodies and you don't get very good things  
25 happening with your immunohistochemical reaction.

1 So, there are many, many ways in which your  
2 antigens can get screwed up.

3 We already know that studies in other  
4 areas show that variation in an immunohistochemical  
5 procedure can make therapeutic outcome differences.  
6 Let's consider this in the case of HER-B2. The  
7 HercepTest assay differs substantially from the  
8 immunohistochemical assay used to select patients  
9 in clinical trials. I suspect that is one of the  
10 reasons we are here. It is likely, however, that  
11 therapeutic decisions regarding the use of  
12 Herceptin are made on the basis of home brew assays  
13 that haven't even gone through that indirect  
14 validation that was done in the clinical trials.

15 These home brew assays can differ  
16 substantially in their performance characteristics.  
17 In many cases the differences may not be known to  
18 anybody. Furthermore, the results of these assays  
19 can vary a great deal depending on what has  
20 happened to the tissue before you ever get it to  
21 the assay. Did the surgeon put it in formaldehyde  
22 to begin with? Was it cut properly so it could be  
23 fixed throughout? Or, was it, indeed, fixed in  
24 saline and incubated on the space heater for three  
25 hours before you got to the specimen?

1 Well, there have been lots of attempts now  
2 to improve standardization in immunohistochemistry,  
3 going back to 1977 and most recently Dr. Hammond,  
4 from whom you will hear later, is leading a work  
5 group to push this process ahead further. What  
6 does this mean in practice? I have stolen here  
7 from DAKO actually their web site for the  
8 interpretation for HercepTest.

9 Here is the question that needs to be  
10 asked when you interpret these tests. Is the  
11 difference I see, from left to right, the result of  
12 different amounts of antigen, which is what I am  
13 really attempting to look for here? Or, is it  
14 reflecting different amounts of antigen degradation  
15 due to late fixation; different effectiveness of an  
16 antigen retrieval process, particularly if you  
17 varied from the recommended process in the  
18 performance of the test? Different assay for the  
19 same analyte? You know, maybe one wasn't in fact  
20 using the HercepTest assay but a home brew assay.  
21 Is the difference of not staining because of a  
22 complete or partial assay failure? Finally, what  
23 is 2+ anyway? Our individual pathologists may  
24 differ in interpretation.

25 So, the point to be made here is that

1 immuno-histochemistry is a total test system that  
2 includes everything from the time the tissue is  
3 excised up to and including the pathologist's  
4 interpretation. Variations in any part of this  
5 test system can result in a different  
6 interpretation. Very importantly, preanalytical  
7 issues are often much more important than the  
8 analytic procedure itself or variations in the  
9 assay execution. Typically speaking, if you have a  
10 test system that is well developed on tissue that  
11 is treated consistently within your laboratory and  
12 before your laboratory sees it, you can get the  
13 same results every time. If the tissue is treated  
14 in radically different ways, or if you are  
15 inconsistent in your use of the test system, then  
16 you are going to get differing results.

17           In order to get past this, I think  
18 ultimately the community believes that there are  
19 improvements that we need to make in our overall  
20 practice. One that I am sure would be dear to  
21 Peter Barker's heart is development of certified  
22 reference materials for common immunohistochemical  
23 stains; the development of reference methods. We  
24 don't have any traceable methods for this.

25           There is no gold standard. There are no

1 gold standard materials. We have, at best,  
2 probably cast white metal, not even brass  
3 standards. Finally, validation of commercial  
4 staining instruments and test systems ultimately  
5 needs to occur against these reference materials  
6 and methods.

7           Keeping in mind these caveats, this is in  
8 no way or another to suggest that this is a fly-by-  
9 night technology. In fact, when carefully and  
10 consistently executed, immunohistochemistry is a  
11 very, very powerful tool for analysis. It can give  
12 excellent results, but you have to have excellent  
13 control of the tissue from the time that it is  
14 originally excised until the time that you make an  
15 interpretation. You have to deal with things the  
16 same way time after time, after time. When you  
17 have variation from laboratory to laboratory, from  
18 procedure to procedure it becomes difficult to  
19 understand exactly what those differences in  
20 staining intensity really mean. Thank you

21           DR. NERENSTONE: Thank you very much, Dr.  
22 O'Leary. Dr. Watson?

23           **FISH Technology and its Application to**  
24           **Gene Amplification Analysis**

25           DR. WATSON: Thank you. You would almost

1 think Tim and I had coordinated our talks because I  
2 am really going to pick up where he left off. He  
3 covered well most of the issues about getting this  
4 piece of tissue to the point where a laboratory  
5 begins to consider doing FISH-based testing. Many  
6 of the issues that apply to IHC equally apply to  
7 the application of FISH technologies. So, what I  
8 was asked to do is actually take you through the  
9 FISH technologies themselves; give you a sense of  
10 their applications to various kinds of testing,  
11 straight through to cancer applications and their  
12 use in amplification detection systems.

13 My experience in this comes from having  
14 been director of clinical and molecular  
15 cytogenetics at Washington University for fifteen  
16 years, doing a lot of these types of tests, and  
17 have since moved to Washington to do this kind of  
18 thing, I suppose.

19 What I want to do is really try to do two  
20 things, put genetic testing into context because,  
21 as Tim said, we would like gold standards in most  
22 areas that we are going to be talking about today.  
23 Really what we have in the literature is just  
24 concordance kinds of tests between these different  
25 tests rather than a clearly established gold

1 standard test that we say is the bottom line  
2 against which all others are compared. So, much of  
3 our literature is this concordance/discordance sort  
4 of perspective. So, what I thought I would do is  
5 sort of put the technologies into perspective, both  
6 FISH and those that are coming and competing with  
7 these technologies for the applications we are  
8 going to be talking about today.

9           Within genetic testing, classically we  
10 have had chromosome-based analysis, biochemical-  
11 based genetic testing and molecular diagnostics.  
12 Molecular cytogenetics was a new area that really  
13 bridged the gap between looking at chromosomes at a  
14 very gross level of resolution versus a classic  
15 molecular test that looked at the base pair level.  
16 This really bridges that and allows you to look at  
17 large chunks of DNA for their presence, for their  
18 absence and for their quantity. It can be done in  
19 a number of different formats and for a number of  
20 different clinical indications.

21           The FISH technologies themselves use  
22 molecular probes to ask the same kinds of questions  
23 IHC is asked to do, that is, the presence, absence  
24 and dosage of materials that are present inside  
25 cells. FISH can be applied to either a non-

1 dividing cell, which we can call an interface cell  
2 and is usually that which you see in a tissue  
3 section, or it can be applied to dividing cells or  
4 chromosomes where one has a very different kind of  
5 target. The target is clearly intact as opposed to  
6 a section where cells are being cut and nuclei are  
7 being cut. We will come back to that when we talk  
8 about scoring of these technologies. But, as I  
9 said, we lack many of the gold standards for these  
10 tests since they are often the first ways by which  
11 we can detect some of these changes in the genome.

12 FISH can be done in two different sort of  
13 general ways. One is called an indirect FISH  
14 assay. In an indirect FISH assay the DNA probes  
15 are pre-labeled with a hapten such as dioxygenin or  
16 biotin, as Tim talked about, and you are  
17 essentially now sandwiching antibodies to bring  
18 fluorochromes into this sandwich to be able to  
19 visualize them through a fluorescence microscope.  
20 The methods that allow you to turn this into a  
21 colorimetric reaction are not very clean right now  
22 for these applications. So, for the most part, the  
23 FISH tests are done through a fluorescence-based  
24 system.

25 After one has hybridized the sequence of

1 interest to which this hapten is attached to the  
2 DNA in the cell, you then come back with antibodies  
3 to that hapten that have the fluorochrome attached.  
4 This particular type of test has a number of  
5 additional steps in it that are not present in the  
6 direct FISH assay, which I will discuss, and to  
7 some people's minds those are deficiencies; in  
8 other people's minds they are advantages. Although  
9 there are more steps, they give you a little bit  
10 more flexibility in amplifying signals, for  
11 instance, whereas there are more steps so there is  
12 more room for difficulty to arise in the assay  
13 itself.

14           The direct FISH tests are the ones in  
15 which the DNA probes themselves are directly  
16 labeled with the fluorochromes, and that probe is  
17 then hybridized to the chromosome or to the cell  
18 and the signal is directly attached by that  
19 hybridization reaction. Then one detects it  
20 through a fluorescence microscope, with other steps  
21 along the way to clean up background, to highlight  
22 the background against which this signal is showing  
23 up over a cell. It requires fluorescence  
24 microscopy and does require fewer steps than the  
25 indirect FISH-based assays.

1           To just sort of generally put this into  
2 perspective for you, this is a slide showing some  
3 of the highlights of FISH-based assays themselves.  
4 As you see, this is two chromosomes here. One  
5 starts with a DNA probe that is specific to some  
6 region of interest. DNA probes come in a whole  
7 wide range of varieties, highly repeated sequences  
8 which are relatively easy, big targets and cells.  
9 You can get a lot of a fluorochrome on them and you  
10 can very clearly see them in fluorescence  
11 microscopes; all the way down to unique sequence  
12 genes that can be quite small and more difficult to  
13 get high amounts of fluorochrome into, though the  
14 technologies are very powerful now and none of  
15 those are real important technical issues based on  
16 size of probes really. When we talk about  
17 amplification signals we are talking about  
18 relatively large probe systems and signal detection  
19 systems that are fairly powerful.

20           You can see that in this indirect assay,  
21 which we show here, we have fluorescent antibodies  
22 that are recognizing the DNA probe on which the  
23 hapten has been attached. Then, we are following  
24 up with the fluorochrome itself in an antibody  
25 reaction to detect that hybridization target in the

1 cell.

2 FISH applications are not new. I started  
3 doing variations on FISH probably in 1982 or so  
4 when the very first systems were being developed  
5 for in situ hybridization as we moved out of  
6 radiolabeled systems into fluorochrome-based  
7 systems. Its applications are very broad in  
8 clinical laboratories now, wide application in  
9 prenatal diagnostics. Probably the largest area of  
10 application now I guess is cancer diagnostics, not  
11 just amplification but all sorts of cancer  
12 abnormalities of leukemia cells. Then, the  
13 molecular cytogenetics of birth defects and mental  
14 retardation, which is really the area in which  
15 these methodologies developed to the point where we  
16 are using them now. They also have extensive  
17 application in the research sector, very useful in  
18 gene mapping. They can be used in gene expression  
19 assays now on microarrays that are coming down the  
20 pike. They can be used to interrogate tumor  
21 biology, and they answer questions about how the  
22 nucleus is organized and how chromosomes and DNA  
23 within a nucleus is organized.

24 The FISH probes themselves come in a wide  
25 variety of products. One can buy FISH probes that

1 are essentially DNAs that recognize an entire  
2 chromosome. They are an agglomeration of unique  
3 sequences that are chromosome specific that allow  
4 one to visualize an entire chromosome inside a  
5 cell, or a subset of a chromosome inside a cell.  
6 They can also be used to detect repeated sequences  
7 in cells. For instance, if one wanted to detected  
8 centromeres or the ends of chromosomes that are  
9 highly repeated, these FISH assays are very  
10 commonly used in those areas now in the clinical  
11 laboratory, and they can be used against unique  
12 sequence targets.

13           So, the basic approach to FISH really --  
14 this shows you its application to chromosomes  
15 through the detection of trisomy in cells. You can  
16 see that on the left we have two chromosomes. Each  
17 chromosome has two arms so we actually have two  
18 signals on each chromosome, and in an interface  
19 cell those can get a little bit separated during  
20 this replication process. You see that another set  
21 of chromosomes shows the three signals that are  
22 unique being detected on a chromosome within a  
23 cell.

24           When one looks at an interface cell, if  
25 one was probing for both of the different

1 sequences, whatever they were, you would visualize  
2 it as the three dark signals or the two light  
3 signals, with some variability depending upon  
4 whether or not the cell might be dividing.

5           This is the sort of thing you see. Here  
6 we see application of that technology for trisomy  
7 detection to a set of chromosomes in a cell, and  
8 then to interface cells, on the right. You already  
9 begin to see within that interface cell some of the  
10 variation that you have to deal with when you are  
11 doing these sorts of assays. Those cells each have  
12 three signals in them. Some might overlie some  
13 because you have a three-dimensional object. So,  
14 when you are setting up your assay reference ranges  
15 you take into consideration that possibility,  
16 unless you have the capability of really  
17 visualizing throughout the planes of a cell. In  
18 most labs that would be dangerous.

19           As this technology has evolved, here you  
20 see that we can apply this technology with a wide  
21 range of fluorochromes to look at virtually all  
22 chromosomes within a cell being labeled differently  
23 based on proportions of perhaps five different  
24 fluorochromes, and they allow that ability to  
25 detect individual chromosomes within a cell. This

1 is a spectral karyotyping approach to FISH.

2 Here you can see that same sort of  
3 scenario where you will see some chromosomes that  
4 are individually labeled; others where the  
5 individual arms of chromosomes are distinguishable  
6 from one another based on the fluorochromes that  
7 have been combined to detect those particular  
8 regions.

9 Here you see the application to  
10 chromosomes telomere detection in a dividing cell.  
11 Also, these are among the most useful kinds of  
12 systems for detecting the absence of material from  
13 a chromosome or a cell. Most molecular methods are  
14 very poor at detecting large deletions in  
15 chromosomes, mainly because a PCR reaction will run  
16 across a normal chromosome, detect that material,  
17 and unless you have a quantitative assay you are  
18 not able to really detect the other signal very  
19 well.

20 So, here you see that that stippled  
21 chromosome has lost its signal on one, and when you  
22 look at an interface cell you are seeing what might  
23 be the control in this particular assay, having two  
24 signals, showing that both of those chromosomes are  
25 present and you are able to detect the signal.

1 From the other chromosome of interest the deletion  
2 was demonstrated.

3 This shows that general kind of  
4 application on the left side. The chromosome  
5 target or reporter is in the white arrow, and the  
6 region of interest that we are detecting for  
7 deletion is shown by the red arrow. In the right  
8 cell you see the deletion of that material in one  
9 of the chromosomes.

10 As you begin to move into cancer  
11 applications of FISH though, you begin to look at  
12 rearrangements which are classical, gains and  
13 losses in entire chromosomes, regions of  
14 chromosomes or rearrangements. The FISH probe  
15 systems now have become very useful in  
16 rearrangement detection. They can detect  
17 translocations of chromosomes where one might bring  
18 two regions together by showing those two probes  
19 coming together. One can detect inversions in a  
20 system that either brings two things together or  
21 takes a target to a particular chromosome region in  
22 which an inversion occurs and breaks that probe,  
23 essentially, into two parts and dissociates them  
24 from one another. So, you see the dissociation of  
25 that gene itself by way of inversion.

1           This shows an example of that sort of an  
2 application in a leukemic cell, with a chromosome  
3 11-6 translocation, among the more difficult  
4 actually to detect cytogenetically in leukemia.  
5 The FISH technologies have become very useful.  
6 This looks a little big ugly but, frankly, for a 6-  
7 11 translocation that is a pretty nice cell. But  
8 in many labs this kind of an abnormality might go  
9 undetected, but with a FISH assay, which is now  
10 independent of that kind of resolution we saw in  
11 the chromosome where greater length provided  
12 greater band detection and greater ability to see  
13 rearrangements, this technology avoided that  
14 problem.

15           This shows you use of the MLL probe that  
16 is on 11-Q23 associated with acute lymphoblastic  
17 leukemias. Down in the bottom corner, a normal  
18 chromosome 11. You can see up in the top corner  
19 though a chromosome 11 that now has a much smaller  
20 signal, and you can see that that signal has been  
21 relocated to the end of the chromosome 6. So, here  
22 you are able to visualize the translocation by the  
23 detection of fluorochromes having moved to  
24 different locations from where you would have  
25 expected them in the normal situation for the

1 chromosome 11, in the bottom right-hand corner.

2 Inversion is the same sort of situation, a  
3 normal chromosome 16 at nine o'clock in this  
4 particular cell; chromosome 16 with an inversion of  
5 the region involved in the core binding factor  
6 rearrangements in leukemia, showing the inversion  
7 at about ten o'clock.

8 As we move into the amplification  
9 applications of FISH -- I didn't want to talk a  
10 whole lot about HER2 detection as an amplification  
11 system, but there actually aren't that many  
12 examples of clinical application of amplified gene  
13 detection. One that I am reasonably familiar with  
14 is the neuroblastoma system in which the mic gene  
15 is clearly amplified and reflects a particular  
16 stage of a neuroblastoma. That is a test that is  
17 often used now in neuroblastoma clinical testing,  
18 but there is a wide range of conditions in which  
19 gene amplification has been demonstrated to be an  
20 event that occurs as part of the cancer process.

21 What we know in genetics about gene  
22 amplification actually starts quite a few steps  
23 back. In the early days we used to induce gene  
24 amplification by cell culture methods perhaps. If  
25 we put methotrexate on a cell, for instance, and

1 blocked dihydrofolate reductase, if we let those  
2 cells grow for a real long time, over time the  
3 dihydrofolate reductase gene will began to make  
4 extra copies, amplify itself, to override that  
5 particular stress on those cells induced by the  
6 presence of methotrexate. This is probably a  
7 biological mechanism that is common to  
8 amplification methods. For some reason, a cell is  
9 finding a reason to amplify a particular sequence  
10 for some sort of a selected advantage.

11           When these amplification events first  
12 occur, they appear as what we call double-minutes  
13 or just rings really of the particular sequences  
14 that have been amplified. As that becomes  
15 stabilized within a cell, those integrate into the  
16 chromosome, and not necessarily where the original  
17 signal was; they may integrate in a number of  
18 different places within the cell, at least in  
19 neuroblastoma of which this is an example.

20           But those can be visualized as what we  
21 call homogeneously staining regions by looking at  
22 chromosomes. But if one wants to look at  
23 chromosomes, you have to sort of impose the cell  
24 culture selection method, which is not often good  
25 for tumor biology and tumor types of testing.

1           The various kinds of genetic tests that  
2 might be applied to gene amplification can be DNA-  
3 based for gene copy number. Southern-based assays  
4 are ones in which there are some restrictions.  
5 They require very, very clean high molecular weight  
6 DNA, which is often difficult for laboratories to  
7 do. Quantitative PCR reactions are another method  
8 by which one can detect the amplification of a  
9 particular DNA sequence, or the FISH technologies  
10 that we have looked at a bit already.

11           However, other methods of detecting  
12 amplification can involve looking at RNA itself  
13 through Northern assays, where one is really  
14 looking at the expression of the gene through the  
15 RNA itself, and quantify RNA as an assay, or  
16 looking at the product of the messenger RNA, the  
17 protein itself, which is what the IHC assays take  
18 advantage of. Those can also be done through  
19 Western blots or through enzyme immunoassays.

20           As Tim mentioned earlier, fresh frozen  
21 tissue actually can be the best tissue for many of  
22 these types of tests but is actually quite  
23 difficult to get in a good form, and is very  
24 expensive to try to obtain routinely in hospital  
25 settings. It is best actually for Southern,

1 Northern and Western. The downside though is that  
2 it assesses entire tissues when one takes a tissue,  
3 dissociates it, extracts its DNA and then looks at  
4 it. In many tumor situations one has other types  
5 of normal cells that have invaded that can lower  
6 the detection of that signal, and other things that  
7 dilute out one's signal. The FISH assays have the  
8 advantage of actually targeting individual cells,  
9 which is a unique capability of section-based  
10 analysis.

11 As I said at the outset, so many of the  
12 papers we read on these topics are really  
13 concordance comparisons between the different types  
14 of tests used, and I thought I would just quickly  
15 go over some of the limitations and benefits of the  
16 various methodologies used to detect these  
17 amplification events.

18 Southern-based testing, the benefit is  
19 very highly precise assays, very reproducible and  
20 can be highly quantifiable. The limitations though  
21 are as we just said, whole tissue is looked at and  
22 can be diluted with normal cells. These types of  
23 Southern assays require a very large amount of  
24 tissue to get enough DNA for the reactions one has  
25 to run, and it is costly, and its dispersion in

1 laboratories around the country is still somewhat  
2 limited still.

3           As we have talked about IHC, which Tim has  
4 gone over quite well already, benefits are widely  
5 available methodologies out there in most  
6 hospitals. It can be done on a very small amount  
7 of tissue. The tissue can be frozen or a fixed  
8 tumor tissue. The evaluation is at that cell  
9 level, with the retention of the histology of the  
10 area that one is looking at. The limitations in  
11 IHC are the variable antibody reactivity and  
12 interpretation is often highly subjective,  
13 especially in that 1+, 2+ region that we talk about  
14 with the HER2 tests.

15           The benefits and limitations of the FISH  
16 tests are a little bit variant from ones seen in  
17 the other assays. Similar to the prior, small  
18 amounts of tissue can be used for a FISH-based  
19 assay on a tissue section. The tissue can be  
20 either frozen or fixed. The evaluation again is at  
21 the cell level, with retention of histology, and  
22 that retention of histology is something that is  
23 important to think about with these assays because  
24 of the way these technologies have evolved. FISH  
25 technologies have arisen in classical cytogenetics

1 laboratories that aren't common part of a pathology  
2 department. So, their expertise is in looking at  
3 intact cells and intact tissues, not tissue  
4 sections. Nor are they highly expert at histology.

5           So, I think it has led to two things. One  
6 is a slow uptake in the pathology laboratory  
7 community because the assays are already  
8 established in cytogenetics laboratories within  
9 many hospitals. That has limited its sort of  
10 translation into the tissue section-based area,  
11 though it is rapidly moving there now. So, now we  
12 often end up with a partnership between the  
13 histology lab like Tim's that defines the part of a  
14 tissue section that a non-histology laboratory  
15 might apply an analytical technique to, to ask  
16 questions of the presence, absence or dosage of a  
17 particular gene sequence.

18           It is a highly reproducible and  
19 quantitative assay. The limitations are that it is  
20 far more poorly distributed than is Southern-based  
21 testing. FISH testing is not broadly distributed  
22 in hospitals around the country, and is often in  
23 tertiary medical centers. And, it doesn't detect  
24 overexpression without gene amplification. So, if  
25 one is doing a FISH test for a DNA sequence, that

1 can detect amplification of that DNA sequence but  
2 cannot necessarily detect an increase in the  
3 expression of that gene, which is another way by  
4 which an amplification type of mechanism can arise  
5 in a cell.

6 As I alluded to earlier, one of the  
7 limitations, once we get past all the fixative  
8 issues which are very critical to getting a very  
9 good FISH detection system, are additional issues  
10 about these types of assays when one gets around to  
11 scoring. That is the fact that the cell nucleus is  
12 about ten microns in diameter, and we cut sections  
13 for FISH-based testing at 4-6 microns. So, when  
14 one is cutting the sections, one is clearly cutting  
15 into nuclei, can remove a signal from a cell by  
16 having cut it out, which means that when labs are  
17 establishing their analytical validity and  
18 reference ranges against which they are going to  
19 say something is normal or abnormal, this is the  
20 kind of issue that is being taken into  
21 consideration.

22 In HER2/neu amplification, which is one of  
23 the more specific and few amplification type of  
24 assays in clinical laboratories right now, when we  
25 look at invasive ductal carcinomas, about 20-30

1 percent, with an enormous range being reported from  
2 various laboratories and centers around the  
3 country, of these breast tumors have HER2  
4 amplification. Gene amplification itself accounts  
5 for 90-95 percent of those amplification events,  
6 whereas the increase in gene expression, which  
7 won't be detected by the FISH assay, is seen in  
8 some 5-10 percent of patients with amplification.

9           When one thinks about scoring  
10 amplification events, it actually varies with type  
11 of assay system that one is working with. As I  
12 said, there are two basic systems, the indirect  
13 FISH and the direct FISH, but they are inherently a  
14 bit different also in the way they are constructed  
15 with what some consider to be internal controls  
16 within cells.

17           In one particular system that is  
18 available, one actually does the assay based on the  
19 ratio of the amplification signals. So, we are  
20 looking at the amplification of the HER-B2 gene,  
21 and if it is amplified to four copies we compare  
22 that against a part of that same chromosome that is  
23 not amplified. They are labeled with two different  
24 fluorochrome colors. The non-amplified region will  
25 show you two signals in a normal cell. The signal

1 that could be amplified could be two signals in a  
2 normal cell. As that signal amplifies though, it  
3 is not often that the chromosome number increases,  
4 though it can. In tumor biology extra chromosomes  
5 is not uncommon. So, one is often establishing the  
6 ratio between the number of chromosome 17 signals  
7 that are present and the total number of  
8 amplification signals that are being detecting and  
9 setting cut-offs for what is clinical validity  
10 based on your detectability of those two events.

11 In most systems the ratio of two normal  
12 chromosome 17 signals to two HER2/neu signals is  
13 1.0. Amplification is anything greater than a  
14 ratio of two to one though one should apply caution  
15 in that sort of 1.8 to 2.2 region in the way one  
16 interprets these test results.

17 There are other systems that do not  
18 include an internal control. They are purely based  
19 on the detection of the HER2 signal itself. Those,  
20 obviously, have to set a somewhat higher level.  
21 So, somewhere in the neighborhood of four or five  
22 signals may be the place at which clinical validity  
23 is established with a relationship to HER2  
24 amplification in that sort of a test system.

25 Now I am just going to show you a handful

1 of cells from a series of HER2/neu assays, just to  
2 give you a sense of what these things look like,  
3 and perhaps touch on some of the issues that arise  
4 in analyzing these sorts of sections.

5 Here you see a normal situation, two red  
6 signals, two green signals, a one to one ratio of  
7 probe to control within that chromosome.

8 Here you see again a normal result. You  
9 will actually see in most of these cells two green  
10 signals and two red signals. You will see that  
11 somewhere we have a signal out because a nucleus  
12 has been cut, or perhaps because we are not looking  
13 deep enough into the section. And, there are other  
14 cells mixed in here that could be perfectly normal.

15 Here is a normal result, but this is one  
16 in which we have a variable number of HER2 signals  
17 and a variable number of chromosome 17 signals.  
18 So, our ratio of chromosome 17s to HER2 is still  
19 one. You can see that in some of these cells with  
20 three green signals or three red signals.

21 Here we begin to move into the  
22 amplifications of these signals. The four red  
23 signals are the HER2 signal, the three green are  
24 the chromosome 17 target that is not amplified.

25 Here you see another cell in which we see

1 four HER2 signals versus the two control signals.

2 Now we are moving into an area where some  
3 of these assays can be somewhat complex to  
4 quantify. Once you get into this highly amplified  
5 area, you start getting sort of agglomerations of  
6 signals, and it makes really definitively scoring  
7 them difficult though they are clearly associated  
8 with an amplification event. You can see that down  
9 in the right-hand corner, a cell with multiple red  
10 HER2 signals versus the one or two chromosome 17  
11 control signals.

12 Increasing levels of amplification, six to  
13 ten HER2 signals within a cell versus two  
14 chromosome 17s. You see that in a couple of these  
15 cells with multiple red signals in them. Up to  
16 five HER2 signals in these cells.

17 Just to touch quickly on some of the  
18 issues of validation of these assays, as with IHC,  
19 these are also home brew assays to a large extent.  
20 Some of them are available in kit but not all  
21 completely in kit form. Others can be put together  
22 by a laboratory essentially if they were foolish  
23 enough, I guess, these days to try that.

24 Just yesterday, an NCCLS document came out  
25 on FISH-based testing, discussing standardization

1 and control of these assays for a wide range of  
2 applications, all the ones I have talked about not  
3 just this particular application. We have just now  
4 introduced HER2 FISH-based proficiency testing into  
5 the College of American Pathologists survey program  
6 so that laboratories can independently assess  
7 themselves in an external PT program. There are  
8 now standards and guidelines being established.  
9 The American College of Medical Genetics has  
10 standards and guidelines that are generic for FISH-  
11 based testing, but is now also developing them for  
12 many of the cancer areas.

13 That is all I have today. FISH is broadly  
14 applicable.

15 DR. NERENSTONE: Thank you very much, Dr.  
16 Watson. We have some time left over and I would  
17 like to open to open the floor from the committee  
18 to the presenters, if there are any questions. I  
19 have one and I guess it would be for Dr. Watson.  
20 As a clinician, could you tell us what it means now  
21 when we get a report 1+, 2+, 3+, FISH positivity?  
22 Is it appropriate now to tell us how that fits into  
23 what you have been talking about?

24 DR. WATSON: Well, it depends on what kind  
25 of test system you had. What has happened is that

1 people have begun to standardize their calling of  
2 FISH signal systems based on the IHC system of 0,  
3 1+, 2+ and 3+, with the 1+, 2+ range being that  
4 sort of 1.8 to 2.2 FISH range that we talked about  
5 where some caution in interpretation is useful.  
6 Both IHC and FISH are quite concordant I think at  
7 that 0 range and at the 3+ range. It is in that 1+  
8 to 2+ range where the FISH assay I think can bring  
9 additional information to the pathologist  
10 interpreting the results.

11 DR. NERENSTONE: And one other question,  
12 you mentioned that FISH may perhaps miss five to  
13 ten percent of patients who have protein  
14 overexpression because it is not gene  
15 amplification. Does the immunohistochemical  
16 process pick up those patients, or is there any  
17 available way now to pick up those patients, or are  
18 those patients just going to be lost in the process  
19 to us?

20 DR. WATSON: Well, it depends. You know,  
21 people approach these tests differently. I was  
22 involved with this kind of testing from its  
23 evolution in the laboratories. So, we were  
24 routinely doing IHC and then comparing results with  
25 FISH and, over time, evolved to a point where all

1 of our 1+, 2+ were getting FISH to try to sort out  
2 that grey zone based on what could be a more  
3 specific quantitative assay than IHC allows. I may  
4 have forgotten the rest of your question already.

5 DR. NERENSTONE: Well, you said that FISH  
6 misses a small percent.

7 DR. WATSON: Yes. Well, I don't call that  
8 a miss. Clinically it is a miss of an  
9 amplification of something, but the FISH assay was  
10 not designed to detect that amplification. So, as  
11 with most genetic etiologies, multiple methods may  
12 often be required to detect the various mutation  
13 mechanisms that are available to cells and their  
14 genome. Even in cystic fibrosis and any of a  
15 number of other diseases, multiple methods often  
16 have to be applied to the very many different kind  
17 of mutation mechanisms that may be detected there.  
18 IHC is independent of most of those because it is  
19 detecting the product of the gene, though until the  
20 antibody systems get less variable has that  
21 inherent difficulty I think.

22 DR. NERENSTONE: Dr. Kelsen?

23 DR. KELSEN: With either technique, is  
24 there any data on relationship between volume of  
25 test in a given laboratory and their expertise? Is

1 there any relationship between doing a lot of a  
2 test with someone dedicated that gives you  
3 significantly better concordance with outcome?

4 DR. WATSON: I don't know. I mean, my  
5 inherent bias has always been that large volume  
6 tends to reduce the rate of errors in laboratory  
7 testing. You know, we don't have much data,  
8 frankly. We have clinical trials and I have been  
9 involved in some reviews previously of some of  
10 those trials, and one can see wide discordance  
11 between laboratories because, largely, the FISH  
12 methodologies themselves are not well standardized.  
13 In IHC, I think a similar problem with the  
14 variability of the antibodies, leads to a similar  
15 type of problem of variability between  
16 laboratories.

17 DR. NERENSTONE: Dr. O'Leary?

18 DR. O'LEARY: I don't believe that there  
19 is any breakout that has been done based on CAP  
20 surveys in these particular areas. However, in  
21 every area of the laboratory with which I am  
22 familiar where they have broken out concordance  
23 with surveys against laboratory volume, higher  
24 volume laboratories have done better, at least up  
25 to a certain level. The relatively moderate to

1 high volume laboratories tend to do better in  
2 virtually all concordance studies in which that has  
3 been measured.

4 DR. KELSEN: But there is no data on a  
5 learning curve. You know, in certain fields there  
6 is a learning curve and you can say that after X  
7 number of cases the surgeon has reached expertise  
8 that is consistent with other surgeons doing that  
9 number of cases. That is a hard number to get at,  
10 it sounds like.

11 DR. WATSON: This is a slightly different  
12 phenomenon I think because high volume allows one  
13 to have more accurate reference ranges, for  
14 instance, and an accurate reference range in a  
15 method that is not well standardized is a valuable  
16 tool that a small volume laboratory may not have  
17 access to. In home brew assays it is establishing  
18 a reference range around the way you do the test in  
19 your laboratory that is important for the reference  
20 ranges against which you interpret your results.

21 DR. NERENSTONE: But your implication is  
22 that these difficulties could be obviated by having  
23 reference materials that are available to even the  
24 smaller laboratories.

25 DR. WATSON: Yes, well, what is an

1 appropriate reference? When I wanted to develop  
2 this test in my laboratory, my reference material  
3 was getting a cell line in which these signals were  
4 highly amplified or normal. Dissociating that,  
5 embedding it in paraffin, tissue cutting it,  
6 sectioning it so that you would really be  
7 controlling all the same steps in your assay, which  
8 is de-paraffination which has some impact on signal  
9 detection, hybridization -- you know, it is very  
10 difficult to develop a reference which exactly  
11 mimics the situation that one is testing in the  
12 laboratory for some of these tests.

13 DR. NERENSTONE: Dr. O'Leary?

14 DR. O'LEARY: That is a true statement,  
15 but if I can drop back 25 years to when I was doing  
16 analytical chemistry as a profession, when we work  
17 in things that have standard reference methods and  
18 standard reference materials available, we are able  
19 as a community to get much higher concordance than  
20 we are able to without. And, we are orders of  
21 magnitude less precise in immunohistochemistry and  
22 in all forms of genetic analysis than we are in  
23 doing typical inorganic analytical chemistry, for  
24 example. Certainly a part of that is due to lack  
25 of reference methods and reference materials.

1 DR. WATSON: With a significant overlay of  
2 just phenomenally rapidly evolving technologies,  
3 with already several technologies doing  
4 quantitative assays that are competing with the  
5 FISH-based tests now.

6 DR. NERENSTONE: Dr. Blayney?

7 DR. BLAYNEY: Yes, two things. One, I was  
8 a little bit surprised by your answer to Dr.  
9 Kelsen's question about correlating volume with  
10 accuracy or reliability of results, in that one of  
11 you represents an organization which may represent  
12 standards. Do you see any role for standard  
13 testing, as promulgated by various organizations,  
14 to allow high volume or reference laboratories, or  
15 even smaller laboratories as you refer to them, to  
16 meet accuracy standards?

17 DR. WATSON: I mean, that is the goal of  
18 this standardization process, to be able to more  
19 broadly disseminate these technologies, and I think  
20 that is what is happening. I mean, when I say that  
21 a large volume lab -- my bias is that on average it  
22 is going to do a more consistent job with the test.  
23 That does not mean that every small laboratory  
24 cannot do a very good job with the test. I  
25 wouldn't want to suggest that.

1 DR. BLAYNEY: A question to Dr. O'Leary,  
2 the last or next to the last slide you showed,  
3 where you had three criteria, I presume, for  
4 immunohistochemistry, talking about development of  
5 certifiable reference materials, etc., if you were  
6 in a position to advise the regulatory bodies, is  
7 that what you would, at the end of the day, like to  
8 see?

9 DR. O'LEARY: I believe it is one of many  
10 types of steps that we can take to help improve the  
11 process of laboratory testing. It has been very,  
12 very helpful in laboratory testing in other areas,  
13 and I think the work of both professional  
14 organizations and the National Institute of  
15 Standards and Technology in this area has been  
16 very, very valuable and should be encouraged.

17 DR. BLAYNEY: The development of reference  
18 materials, traceable reference materials and  
19 validation? That is sort of your manifesto?

20 DR. O'LEARY: Reference materials and  
21 reference methods are one useful set of tools.

22 DR. BLAYNEY: Thank you.

23 DR. NERENSTONE: Dr. Lippman?

24 DR. LIPPMAN: In the literature you see  
25 these kinds of scorings on these assays, what

1 determines a positive and negative, and in some  
2 cases it is based on number of cells that are  
3 positive that you can see and in others it is based  
4 on the intensity or some combination. I wonder if  
5 you could comment, both of you, on your  
6 interpretation of differences, in other words,  
7 intensity versus the number of cells positive, and  
8 how you would view that in some sort of scoring  
9 system?

10 DR. O'LEARY: Well, I think it depends on  
11 the precise assay and what you are going after. I  
12 think a classic case for seeing this is in the use  
13 of immunocyto-chemical methods for estrogen and  
14 progesterone receptor scoring, in which a  
15 combination scoring system or one based on numbers  
16 of cells has proven, in the hands of at least some  
17 investigators, to be more useful than simple  
18 intensity-based scoring.

19 I think whatever scoring system you use,  
20 the important thing is that it be validated against  
21 clinical outcome. It is difficult, outside the  
22 context of a clinical validation to really say,  
23 well, one is better than the other. I think the  
24 proof of the pudding is in the eating.

25 DR. WATSON: I completely concur.

1 Certainly the FISH test is not an analysis of a  
2 single cell. The assays tend to run in the 50-70  
3 cell range that are analyzed and one is  
4 establishing a ratio within all cells analyzed  
5 within a particular field in which the cells meet  
6 all the criteria, the scoring criteria. You can't  
7 score a cell that clearly looks ragged around the  
8 edges, that may have lost DNA. But at the end of  
9 the day, it is an average within a group of 60  
10 cells. So, one cell with mild amplification will  
11 not give you a final result that suggests  
12 amplification, but there may be information there  
13 in the long-term that has yet to be sorted out.

14 DR. NERENSTONE: Dr. Redman?

15 DR. REDMAN: Before we go on to standards  
16 that don't exist, and one of the things we complain  
17 about is the lack of standards; I understand that,  
18 but, Dr. O'Leary, in the best of worlds, if you  
19 were in the preoperative holding area, following  
20 the patient through to the time the surgeon  
21 harvested the tumor, and you were looking for a  
22 specific membrane-bound protein, how would you  
23 handle that tissue?

24 DR. O'LEARY: In the ideal world, I would  
25 get hold of that as quickly as possible afterwards

1 and probably cut frozen sections to make a  
2 determination, assuming that we had studies or  
3 something else to verify that our test system  
4 afterwards performed on frozen sections actually  
5 correlated with outcome.

6           Given that that is not a likely real  
7 scenario, the second option would be to have it get  
8 to a grossing lab very, very quickly to be examined  
9 by a pathologist, to be cut into thin sections --  
10 not thin in the sense of histology but a couple of  
11 millimeter thick sections that will be fixed  
12 rapidly, and then fixed for a well-defined period  
13 of time in a well-defined fixative system so that  
14 each tissue gets handled the same way.

15           I think consistency is more important in  
16 some ways than the precise technique because it is  
17 consistency that will allow you to compare across  
18 different sites. We tend to like it quick, but  
19 consistent may be more important than quick.

20           DR. REDMAN: So, it is more the operator  
21 than the assay.

22           DR. O'LEARY: Well, the assay is  
23 important, but if you have standardized assays,  
24 because of the use of automated immunostainers or  
25 kit type systems, the assay standardization right

1 now is, in many cases, better than the  
2 standardization of the pre-assay tissue handling.  
3 The basic message here is that we would like to get  
4 control of the entire test system, and the test  
5 system starts the minute that tissue is excised  
6 because the tissue is changing from the minute the  
7 tissue is excised.

8 DR. REDMAN: One other question then, in  
9 your standard best world, is there a difference in  
10 the handling of that tissue whether you are looking  
11 for a membrane-bound protein or antigen versus a  
12 sub-membrane versus cytosolic versus intranuclear?

13 DR. O'LEARY: It depends on an antigen by  
14 antigen basis. While my experience suggests that  
15 many of the membrane-bound proteins are less  
16 amenable to sample mishandling at various stages  
17 than, for instance, many of the cytoplasmic  
18 proteins. This depends on the specific antigen.  
19 When we talk nuclear, it is the same thing. There  
20 is a difference between progesterone receptor and  
21 PCNA for example. So, you really have to  
22 understand the particular analyte and what is going  
23 on with that particular analyte. I think if you  
24 were to apply a generalization, depending on the  
25 point at which it presents, you will probably make

1 mistakes. For example, I don't have studies but my  
2 personal impression is that mishandling of kit CD17  
3 is less of an issue than mishandling of HER-B2.  
4 But, again, I don't have good standards on which to  
5 state that; that is sort of an impression. The  
6 point is that they are both membrane-bound proteins  
7 which are in some ways similar but which seem to  
8 behave differently.

9 DR. NERENSTONE: Dr. Griffin?

10 DR. GRIFFIN: I just wanted to point out,  
11 in case people are perhaps getting the wrong  
12 impression with this discussion of standards which  
13 we clearly need, that the economics of many  
14 laboratories doing tests in low volume I think is  
15 going to obviate some of these problems, in that  
16 you can't afford to run a low volume test, at least  
17 at many institutions. So, that is good because --  
18 I mean, that could be good because that should  
19 force the specimens into laboratories that do a  
20 larger volume. It will obviously add in the  
21 vagaries of shipping and additional handling, but I  
22 think it is a rare laboratory that can afford to  
23 run a test that needs multiple standards, etc. on a  
24 regular basis unless they have an adequate volume.

25 DR. NERENSTONE: Go ahead.

1 DR. O'LEARY: I would also like not to  
2 give the impression that we are, you know, lacking  
3 standards or lacking progress in this area. I  
4 mean, for example, NCCLS has published a set of  
5 guidelines recently which address a lot of these  
6 preanalytic issues. It is not a perfect document  
7 by any means, but it moves us in the right  
8 direction. The committee that Dr. Hammond is  
9 chairing will probably move us further in this  
10 direction. Efforts by a number of professional  
11 organizations are getting us there.

12 So, it is not that this is an area that  
13 has been blown off, or an area in which even where  
14 there is routinely poor practice. I don't think  
15 so. If one looks at concordance in the CAP  
16 surveys, in general things are getting to be pretty  
17 good in laboratories in terms of at least agreeing  
18 on immunohistochemical staining, or in the area of  
19 molecular genetics in obtaining the same results in  
20 the vast majority of laboratories in molecular  
21 genetics tests. So, it not that we are dealing  
22 with a fly-by-night operation but, rather, that in  
23 all areas of medicine there are always areas for  
24 improvement.

25 DR. NERENSTONE: Dr. Siegel?

1 DR. SIEGEL: In the specific area of HER-  
2 B2, the analyte that we think we are interested in  
3 is a surface protein. So, in measuring gene  
4 amplification that raises issues. You addressed  
5 one side of it, Dr. Watson, which was the fact that  
6 in five or ten percent of cases of protein  
7 overexpression there is not gene amplification.  
8 But what about the other side? How common is it in  
9 this system to have gene amplification which does  
10 not increase surface protein expression?

11 DR. WATSON: That is a hard question. If  
12 you accept the ratio of detection, the clinical  
13 correlations are quite good. It is a very  
14 sensitive assay for increase in amplification of  
15 the signal. I don't know specifically -- are you  
16 talking about how often do I see a false-positive  
17 where it looks amplified with either background  
18 signals or some other problem?

19 DR. SIEGEL: I was just wondering if you  
20 looked at your optimal conditions for testing in  
21 both ways, where you had a true positive gene  
22 amplification how common would it be to have true  
23 gene amplification but not any increase over normal  
24 in protein surface expression?

25 DR. WATSON: Yes, that is the gold

1 standard problem I think. These assays correlate  
2 very well at the high end and the low end,  
3 amplification/no amplification. It is in that grey  
4 zone where the variability in the antibodies  
5 doesn't give you that warm and fuzzy feeling that  
6 you are actually interpreting what you are seeing  
7 very well. Whereas, the FISH assay is highly  
8 specific, numerical, and seems to have a good  
9 correlation with amplification in that grey zone, 1  
10 to 2+ region.

11 DR. NERENSTONE: Ms. Mayer?

12 MS. MAYER: So, in light of what you have  
13 told us about the variability of test results and  
14 the current lack of standards, I wonder with what  
15 degree of confidence can you reassure patients that  
16 their test results, if they fall within that grey  
17 area, can be used as basis for treatment?

18 DR. O'LEARY: To follow-up on the last  
19 question, I suspect that one of the other speakers  
20 will talk a little bit about the concordance  
21 studies that have been done in the NSABP so that we  
22 may learn a little bit about that.

23 The second issue I am not sure how to  
24 address. Maybe that will be presented as well. I  
25 think it is pretty clear from the data that was

1 presented before this committee and before the FDA  
2 hematology and pathology panel at the time of the  
3 HercepTest presentation that in the range of 3+  
4 there was an extremely good prediction of clinical  
5 response. My personal interpretation of the data  
6 that was presented, which may certainly not  
7 correlate with that of other panel members or the  
8 agency, was that that was not so clear for those  
9 patients who were 2+. So, how much assurance or  
10 lack thereof to give I think depends on your  
11 interpretation of those studies and other studies  
12 that have been done that have directly looked at  
13 that.

14 This is even using the clinical trials  
15 assay, and I will note that the HercepTest assay  
16 was not the clinical trials assay but was a  
17 correlative assay in which the correlation between  
18 0 and 3+ with the clinical trials assay was quite  
19 good, to my memory, and in which in the 1+ and 2+  
20 arena there was more crossover. The correlation  
21 wasn't perfect any place. I think, again, we are  
22 going to hear more data about that later.

23 DR. NERENSTONE: Dr. Kelsen?

24 DR. KELSEN: Just to follow-up on the high  
25 and the low volume, just to give me an

1 understanding of all the caveats you said, I mean  
2 breast cancer is a very common disease but there  
3 are lots of labs. If low volume means they did X  
4 number of tests per year and high volume is greater  
5 than Y -- we have done this with surgery, for  
6 example in pancreatic cancer and a number of other  
7 tumors, is there a number that sticks in your mind  
8 for low volume, or is that just not defined and it  
9 is impossible to say?

10 DR. WATSON: There is not a number that  
11 sticks in my mind for what discriminates low volume  
12 from high volume. I think, as with any specialty  
13 area, volume, expertise and training are sort of  
14 running one against another and volume won't always  
15 determine quality, and experience and training  
16 won't always but there are advantages to both I  
17 think.

18 DR. NERENSTONE: But it is even more  
19 complicated than that because there are  
20 immunohistochemistry tests that are being done in  
21 all sorts of cancers. So, it is not just that you  
22 have to do ten breast cancers a month because you  
23 are doing a hundred lymphomas and they also use  
24 immuno-histochemistry, but it is whether you have  
25 an immunohisto-chemistry setup that allows you to

1 evaluate it to make sure things are being done the  
2 right way.

3 DR. O'LEARY: I think that is a very  
4 important point. There are labs that are probably  
5 doing a few hundred immunohistochemistries a year  
6 and there are labs that are doing hundreds of  
7 thousands of immunohistochemistries a year. Your  
8 ability to trouble shoot an assay depends to some  
9 degree on the specifics of that assay, but because  
10 there are lots of problems across assays, it also  
11 depends on your general experience doing  
12 immunohistochemistry. So, that really accentuates  
13 the point that you just made, which is you learn  
14 something from both. But we are really not in a  
15 position, based on any data, to say that your lab  
16 should be doing 5000 a year, or something. That  
17 would just be taking numbers, as far as I can tell,  
18 and making them up.

19 DR. NERENSTONE: Dr. Lippman?

20 DR. LIPPMAN: We are focusing a lot on  
21 what the lab does and how they quantitate an assay  
22 -- and this is addressed to Dr. O'Leary -- again, I  
23 have the impression that you think the major issue  
24 is the pre-analytical phase. Did you say the NCCLS  
25 had suggestions about how to handle or how to

1 standardize the preanalytical phase, and if you  
2 could summarize what some of the suggestions were?

3 DR. O'LEARY: I think it talks more about  
4 issues associated with the preanalytical. It has  
5 been very, very difficult when many, many  
6 laboratories have their own home brew assays and  
7 each believes that their home brew assay is  
8 superior to every other to make rapid, rapid  
9 progress on standardization. So, we wouldn't go to  
10 the point of saying that all tissues should be  
11 fixed within, you know, ten minutes of the time  
12 they were excised. I don't think we are at that  
13 point yet. I think it is really in the context of  
14 clinical trials where one has the potential of  
15 gaining a much greater control over the way  
16 patients and tissues are processed so that we can  
17 actually begin to gain that kind of information. I  
18 think it is really quite clear that a lot of  
19 people, both in the community, and Dr. Taube at  
20 NCI, and others are working very, very hard to push  
21 this process forward.

22 DR. LIPPMAN: Just following up on Dr.  
23 Kelsen's comment, you know, there is no number that  
24 you can give for volume but in clinical trials and  
25 operations and patients and a number of clinical

1 settings, there is a minimum volume that one would  
2 need to do as part of an annual standardization  
3 assessment. Do you envision that becoming part of  
4 the recommendations, that a lab would have to have  
5 a certain volume to show proficiency, references  
6 and so on?

7 DR. O'LEARY: I think in the clinical  
8 trials that is certainly a reasonable suggestion to  
9 consider. I think it would be good to do in the  
10 context of actually trying to gather some  
11 information on whether that approach was meaningful  
12 so, not setting the numbers so high that you can't  
13 stratify your data and actually use some form of  
14 either peer assessment or some other approach by  
15 which to try to get a better handle on what  
16 constitutes enough.

17 DR. NERENSTONE: Dr. Redman?

18 DR. REDMAN: Again regarding the  
19 standards, I got the sense from you, Dr. O'Leary,  
20 that the major thing was the preanalytical handling  
21 of tissue, and we are talking about trying to set  
22 up standards and we are still in the situation  
23 where the vast majority of patients have their  
24 initial biopsy done in low volume hospitals, as far  
25 as cancer is concerned, and I think the major

1 problem is going to be the preanalytical handling  
2 of those specimens.

3 DR. O'LEARY: I think you are absolutely  
4 right. Even in high volume places, I can show you  
5 places, significant cancer centers, that have a  
6 total volume of fewer than 200 breast cancer  
7 patients a year. So, you know, high volume is not  
8 necessarily that high. But I don't want to say it  
9 is the most important thing. Having the same assay  
10 done in all of the laboratories has much to  
11 recommend it too.

12 As I said, different assay formats can  
13 give you wildly differently intensities of  
14 staining. So, if you have a whole bunch of home  
15 brews as opposed to a standardized assay format,  
16 you will also be in trouble. You gain much by  
17 gaining control of the assay itself. You will gain  
18 more if you can gain control of the tissue handling  
19 before the assay.

20 DR. NERENSTONE: Dr. Watson, did you also  
21 want to reply?

22 DR. WATSON: In the context of talking  
23 about volume and requirements, it is actually sort  
24 of a two-tiered system where, for instance, my  
25 laboratory did thousands of various types of FISH

1 assays of each year, many of which would tell me  
2 something about what might happen in my HER2 test,  
3 for instance -- issues related to whether my  
4 fluorescence microscopes are working, all those  
5 things fall out in the context of the generic  
6 technologies that one is using, and it applies to  
7 IHC to some extent as well. However, you also need  
8 some level of experience specific to this signal  
9 detection system itself.

10 DR. NERENSTONE: Dr. Albain?

11 DR. ALBAIN: I wanted to come back to a  
12 couple of points. First of all, Dr. Lippman  
13 touched earlier on the estrogen and progesterone  
14 receptors, and I am struck by seeing multiple path  
15 reports from different labs each week still being  
16 highly variable in what is termed positive versus  
17 negative, for just our prototypic estrogen and  
18 progesterone receptor positivity and, in  
19 particular, in the range of low positive where  
20 these patients may very well benefit from an  
21 endocrine manipulation. Why is it that we do not  
22 yet have a national standard for reporting based on  
23 cell count and intensity, or some version thereof,  
24 that is correlated with outcome? I think it is  
25 just going to snowball with all of these new

1' targets if we still don't have an agreement  
2 nationally on what is a positive ER and a positive  
3 PR on IHC.

4 DR. O'LEARY: I concur with the editorial.  
5 I think in many cases it is really in the context,  
6 again, of clinical trials where the oncology  
7 community can gain a great deal of influence in  
8 helping to drive this process. I think, again,  
9 that NCI is working very hard to drive this process  
10 forward too. But, at the same time, pathologists  
11 are engaged in the practice of an art as well as a  
12 science and, just as a patient enrolled on a  
13 clinical trials protocol, they don't have exactly  
14 the same dosage; may not have exactly the same  
15 liver function tests when they were admitted as  
16 everybody else does. There is heterogeneity there.  
17 We will probably remain with some heterogeneity.

18 Understand, however, that we have still  
19 made progress from the point of the dextran-coated  
20 charcoal assay which would also give you different  
21 results when performed by different laboratories.  
22 Heterogeneity has been reduced, and I think over  
23 time will continue to be reduced. In part it will  
24 be pressure that we put on ourselves, and in part  
25 it will be pressure that you put on us.

1 DR. ALBAIN: Yes, just to push that a tad  
2 though, you know, I will get positive versus  
3 negative, or I will get 0 to 5+, or I will get  
4 intense but low number. It is just very difficult  
5 to make clinical decisions sometimes. I am  
6 wondering if your national standards are going to  
7 move in a direction to establish some general  
8 guidelines for how the pathologists report  
9 positivity.

10 DR. O'LEARY: I have no doubt that they  
11 will and, in fact, comments like this are very,  
12 very helpful in enabling those of us who have a  
13 passion for doing this to be able to justify to  
14 other members of our own professional community  
15 that this is an effort that will be appreciated and  
16 worthwhile. So, we appreciate those comments.

17 DR. NERENSTONE: Any further questions or  
18 comments from the committee? Seeing none, what I  
19 would like to do is take a break and have us back  
20 at the table at 10:30. Thank you.

21 [Recess]

22 DR. NERENSTONE: We are going to start  
23 this part of the session with Dr. Paik, who is  
24 going to discuss the NSABP B-31 data.

25 **National Surgical Breast and Bowel Project:**

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**B-31 Data**

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DR. PAIK: Good morning, ladies and gentlemen. What I would like to do this morning is to give you a kind of brief presentation about the results from a central review of an initial subset of cases that have entered into the NSABP trial B-31.

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NSABP B-31 is a trial for node positive and HER2 positive tumors that are randomized to chemotherapy or chemotherapy plus Herceptin, given for one year. In doing this trial we had determined the eligibility, determined by 3+ by the HercepTest result observed by any lab in the U.S. or Canada, or in case other antibodies are used, or if the assay was kind of modified from the FDA format for the HercepTest, and we required strong membrane staining of one-third of the tumor size. This was based on the preliminary data that shows that there is a correlation between the staining intensity and the scoring of the tumor size. Antigen amplification determined by any FISH method was also acceptable.

23

24

25

In the protocol there was a planned central review of the initial 100 cases to make sure that we were capturing the real HER2 positive

1 patients for B-31. The rule was that if more than  
2 20 percent of the cases were negative by both  
3 HercepTest and FISH, then we would consider  
4 changing the eligibility criteria.

5 The method we used, to get away from the  
6 bias of the lab which is essentially responsible  
7 the clinical trial itself to do these tests, we  
8 collaborated with LabCorp of America to function as  
9 a central lab and do blinded testing. So, results  
10 were available at the time of reporting from 104  
11 cases where we had tissue blocks available. To  
12 make sure that the results from LabCorp were  
13 reliable, we did an independent confirmation of  
14 these results by my lab, NSABP, on a subset of 81  
15 cases.

16 If you look at the results from the  
17 central assay from 104 cases, these are all cases  
18 that were entered based on an immunohistochemical  
19 result. There was 94 percent agreement between the  
20 central FISH by LabCorp and central HercepTest by  
21 LabCorp.

22 To confirm the results from LabCorp we  
23 generated microarrays from 81 cases. If you  
24 compare the FISH results from LabCorp and the FISH  
25 results from my lab there was 95 percent agreement.

1           If you compare the central results from  
2 LabCorp and the results from the original testing,  
3 then overall 18 percent of the cases were negative  
4 by both central FISH and central HercepTest. There  
5 was an interesting trend in the data set. So, if  
6 you look at the HercepTest or other  
7 immunohistochemical assays, 8 cases entered into  
8 our trial were based on HercepTest 3+ staining  
9 originally, and there was an interesting trend in  
10 the data that those assays done by larger volume  
11 labs, in this case arbitrarily defined by at least  
12 100 cases per month, the test looked very reliable.  
13 Whereas, if it was done by a small volume lab, 19  
14 percent of the cases were negative by both assays.  
15 The trend for other immunohistochemical assays was  
16 even worse because all of them were essentially  
17 done by a small volume lab and there was 35 percent  
18 false-positive cases there.

19           Because CB-11 is approved by FDA, we also  
20 categorized according to the other antibodies into  
21 CB-11 or other tests. Although CB-11 is a small  
22 number, it shows a similar trend of 20 percent of  
23 false-positive results. Actually, when you look at  
24 other home brew assays, there was a 50 percent  
25 false-positive rate.

1           There is one publication by Dr. Isola,  
2 from Finland, that shows that chromogenic in situ  
3 hybridization, which is essentially similar to  
4 fluorescent in situ hybridization except that  
5 eventual color labeling is not done by fluorescence  
6 but chromogenic DAB -- here is one example where  
7 there is clear amplification of the tumor size,  
8 these kind of brown dots in the nucleus. So, we  
9 wanted to check whether chromogenic in situ can  
10 replace the FISH, and from a subset of cases here  
11 you can see that there is 92 percent agreement in  
12 the scoring.

13           To confirm that, we looked at another set  
14 of cases from another trial, 123 cases with, again,  
15 exactly 92 percent agreement between FISH and  
16 chromogenic in situ hybridization.

17           In addition to the 104 cases that were  
18 entered by immunohistochemistry, we were able to  
19 get tissue blocks from 27 cases that were entered  
20 based on FISH done by membership labs. Seventeen  
21 of them were from small volume labs and 10 were  
22 from larger volume labs, and 15 labs contributed to  
23 the 17 cases here and 6 reference labs contributed  
24 to the 10 cases here. All of these 27 cases were  
25 confirmed by PathVysion test. Just for your

1 information, in the original test 26 cases were  
2 done by PathVysion test and only one case was done  
3 by Inform test.

4           The conclusion from central review was  
5 that HercepTest, provided by a larger volume lab,  
6 seems to be reliable in determining 3+ cases, with  
7 about 95 percent agreement with FISH. According to  
8 a kind of informal survey on the telephone, we  
9 think that all larger volume labs that perform  
10 both immunohistochemistry and FISH, and this may  
11 contribute to better quality control, these labs go  
12 through kind of close validation of the assays and  
13 have longitudinal experience. So, that might be an  
14 important factor in future quality control  
15 programs.

16           We conclude that immunohistochemistry  
17 performed by small volume labs tends to be less  
18 reliable. Although there were only 27 cases, the  
19 PathVysion FISH test seems to be a very reliable  
20 test and CISH is not a good alternative because the  
21 problem is the detection of the low level  
22 amplification cases.

23           Because of these data, we decided to  
24 change the eligibility for our trial. So, if the  
25 original test was done by any FISH, then it was

1 eligible regardless of the immunohistochemical  
2 result. If the original immunostaining was done by  
3 an approved lab, then it is eligible. If it was  
4 done by a non-approved lab, then it needs to be  
5 confirmed by an approved lab.

6 We have a real-time approval process. Up  
7 until yesterday we had 22 labs in this country  
8 approved for entering patients into our trial. If  
9 they process more than 100 cases per month with the  
10 FDA approved HercepTest, they get automatic  
11 approval. If they process less than 100 cases or  
12 use a different antibody, then we individually  
13 determine the quality of the lab for the approval  
14 based on their concordance rate with FISH, and  
15 their previous track record for entering patients  
16 into our protocol. If they are using FISH it is  
17 automatic approval.

18 Currently we don't really know the  
19 reliability of real-world FISH testing for small  
20 volume labs because we have only looked at 27  
21 cases. It is questionable whether the small  
22 pathology labs, which cannot do immunohisto-  
23 chemistry, can really do FISH. That is a big  
24 question. So, our plan is to conduct another 100  
25 cases after the full implementation of the protocol

1 amendment, which has been in effect for three  
2 months now. Because it is a large trial and some  
3 pathology departments require each of the blocks  
4 immediately, in those cases we actually have kind  
5 of a real-time process to generate triplicate  
6 tissue arrays so that we can do validation assays  
7 on the material that we have. Thank you.

8 DR. NERENSTONE: Thank you. Our next  
9 speaker is Dr. Roche.

10 **N9831: Intergroup Trial for Node+,**  
11 **HER2+ Breast Cancer**

12 DR. ROCHE: I will just spend a few  
13 minutes now talking to you about another trial that  
14 is ongoing for looking at Herceptin in the adjuvant  
15 setting. This is a breast intergroup trial, N9831,  
16 including all members of the breast intergroup, and  
17 it is a Phase III trial to look at doxorubicin and  
18 cyclophosphamide, followed by weekly paclitaxel,  
19 either with concurrent Herceptin or with subsequent  
20 Herceptin therapy.

21 This is a schema of the study design. We  
22 plan to enroll 3000 patients for this study and  
23 Edith Perez, my colleague at Mayo Clinic,  
24 Jacksonville, is the PI for this study, with the  
25 Northcentral cancer treatment group as the

1 coordinating center.

2           Similar to the B-31 trial, the eligibility  
3 criteria in terms of HER2 status for this trial was  
4 a 3+ HercepTest by local testing. You could use  
5 another immunohistochemistry test but it had to  
6 have more than a third of the cells that showed  
7 strong membrane positivity. If a FISH assay was  
8 used to qualify patients for this trial, then they  
9 had to be FISH amplified with HER2 to centromere 17  
10 ratio of greater than 2. If the Vysis test was  
11 being used, and if the Oncor or Vysis Systems FISH  
12 assay was being used, then they had to have more  
13 than five copies of the HER2 gene.

14           Similar to the B-31, we stipulated in the  
15 protocol that there would be central testing of the  
16 first 100 patients that were enrolled onto the  
17 study. This is being done at the Mayo Clinic in  
18 Rochester, using the HercepTest  
19 immunohistochemistry to look at protein expression  
20 and the Vysis PathVysion assay to measure gene  
21 amplification. If there was more than a 20 percent  
22 discrepancy between the local HER2 evaluate and  
23 central HER2 status, then consideration would be  
24 given to modifying the eligibility for patients for  
25 this study, based solely on the results of their

1 local testing.

2           So far, we have had and I will present  
3 data today on 119 patients where there has been  
4 central testing for HER2 status. We have had 65  
5 different labs evaluate these initial 119 samples.  
6 So, we aren't able to draw any conclusions yet  
7 regarding, at least statistically, whether or not  
8 large volume labs do better at this HER2 testing  
9 than small volume labs. For the 65 different labs  
10 there are only two labs that accounted for more  
11 than three percent of the samples that were tested.

12           The distribution of the different types of  
13 testing that has been used, 50 percent of the local  
14 labs have used the HercepTest; 43 percent have used  
15 other immunohisto-chemistry tests, primarily CB-11  
16 either in a home brew assay or on a Ventana System;  
17 and 7 percent, or 9 of the 119, have used a local  
18 FISH assay to qualify patients for this trial. So,  
19 there have been 110 patients that have been  
20 evaluated by immunohistochemistry and 9 patients  
21 that have been evaluated by FISH.

22           If we look at the results then for the 110  
23 patients that have been evaluated by  
24 immunohistochemistry in the local labs, only 74  
25 percent of those were confirmed to be 3+ by a

1 central HercepTest assay. It didn't seem to make  
2 too much difference whether or not they used the  
3 HercepTest for their local testing. There was 75  
4 percent of those that were confirmed by central  
5 HercepTest. Or, if they used another  
6 immunohistochemistry assay, 72 percent of those  
7 were confirmed to be 3+ by central testing.

8           If we look at the nine patients that were  
9 entered on the trial based upon local FISH assays,  
10 seven of those nine had a 3+ immunohistochemistry  
11 by central HercepTest and only six of nine were  
12 confirmed to be amplification by central FISH  
13 assays. With about twice as many patients on trial  
14 now, close to 250 patients, this trend has  
15 continued. There have been 22 that have been  
16 entered based upon local FISH testing, and 14 of  
17 those have been confirmed to be amplification and  
18 15 of those have been 3+. Similar trends have also  
19 been seen with the HercepTest and with other  
20 immunohistochemistry tests, about a 75 percent  
21 concordance rate.

22           So, for the 119 patients that have been  
23 entered on the trial, 66 percent of those have been  
24 confirmed to be amplified by central FISH. There  
25 has been 74 percent that happened to be 3+ by

1 central immunohistochemistry. Similar to what was  
2 mentioned earlier regarding the discordance between  
3 FISH and immunohistochemistry, we have seen 10  
4 percent of patients who are 3+ by central  
5 immunohisto-chemistry but do not show amplification  
6 by central FISH. Again, this trend has continued.  
7 Actually, we have dropped a little bit. I think it  
8 is 6 percent of patients that are 3+ by central  
9 immunohistochemistry but don't show gene  
10 amplification.

11 This is a breakdown of the scoring that  
12 compares central HercepTest versus central FISH for  
13 the first 119. We have had a fairly even  
14 distribution of the 0, 1+ and 2+ cases by  
15 immunohistochemistry. So far we have not seen any  
16 of those to be amplified, although in our  
17 experience at Mayo we would expect to see about 12  
18 percent of the 2+ cases to show amplification, and  
19 around 1 percent of the 0 and 1+ cases. So as we  
20 accumulate more and more numbers, we do expect to  
21 at least see some amplified samples, especially in  
22 the 2+ category and one or two in the 0 and 1+  
23 category.

24 Looking at the concordance between the  
25 central HercepTest and our central FISH assay, 90

1 percent of the specimens have been 3+ by central  
2 HercepTest and amplified by central FISH. None of  
3 the specimens of this first 119 that were scored as  
4 0, 1+ or 2+ by immunohistochemistry were FISH  
5 amplified. The overall concordance for central  
6 immunohistochemistry and central FISH is 92  
7 percent.

8           So, our proposed modifications now for  
9 N9831 are that the patients can be registered on  
10 the trial based upon local testing if they are node  
11 positive and 3+ or HER2 amplified. But while they  
12 are receiving their AC therapy, a block or slides  
13 must be submitted for central testing to confirm  
14 HER2 status. If the patients are 3+ or amplified,  
15 they will then be randomized to one of the three  
16 arms of the trial. If they are not 3+ or  
17 amplified, then they will be cancelled from the  
18 study.

19           Although we don't have data to draw  
20 conclusions regarding large volume laboratories  
21 being able to do this type of testing better than  
22 small volume laboratories, we do feel it is  
23 important that if you are doing HER2 status  
24 evaluate you have access to both types of testing;  
25 that there are HER2 amplified cases that on

1 occasion do not show overexpression and there are a  
2 significant number, ten percent in our hands, of 3+  
3 cases that do not show gene amplification. If you  
4 are going to establish this type of testing in your  
5 laboratory, you need to have feedback between your  
6 immunohistochemistry and your FISH assays. Thanks  
7 very much.

8 DR. NERENSTONE: I will open it up for  
9 some brief questions from the committee for the  
10 last two speakers. Dr. Kelsen?

11 DR. KELSEN: I think that these two  
12 presentations are very interesting, at least to me.  
13 They do begin to address some of the issues that we  
14 were talking about earlier this morning. I have a  
15 question for the NSABP presenter. I take it that  
16 the patients were treated despite the fact that  
17 some of them may, in fact, not have overexpression.  
18 I wonder if we will have enough patients -- it is  
19 going to be a really small number -- to draw any  
20 clinical trials implications at the end. But they  
21 were treated, these first 100 patients despite the  
22 fact that when you looked again, in retrospect, you  
23 are not convinced that they really were  
24 overexpressing HER2?

25 DR. PAIK: From the initial about 500

1 cases that were entered onto our trial, half of  
2 them were randomized to the Herceptin arm, and some  
3 of them were getting Herceptin and some of them  
4 were not on Herceptin treatment yet. According to  
5 our DMC recommendation, we contacted individual  
6 patients who belong to the Herceptin arm, whose  
7 initial testing was done by a smaller volume lab by  
8 our definition, and we informed them about our  
9 result. If they signed a consent form for  
10 retesting, then we provided free retesting by a  
11 central lab. So, some of them may decide to go off  
12 the treatment. So, it is not clear at the end of  
13 the trial that we are ever really going to be able  
14 to find out. But it is planned in the central  
15 review as a secondary aim of the trial to stratify  
16 the patients according to the central review result  
17 and see if there is a differential treatment  
18 effect. But I don't think our trial will be  
19 powered enough to see a real difference.

20 DR. KELSEN: But this type of data though  
21 would ultimately answer the question of the  
22 hypothesis whether small versus large volume has  
23 clinical meaning.

24 DR. PAIK: Yes, sir.

25 DR. NERENSTONE: Dr. Lippman?

1 DR. LIPPMAN: I wonder if you could, Dr.  
2 Roche, give some comments about how you determined  
3 the 1+, 2+ 3+ of the HercepTest test, the IHC  
4 assay.

5 DR. ROCHE: Could you repeat the question?

6 DR. LIPPMAN: I was really struck by the  
7 dramatic difference between 3+ HercepTest and the  
8 correlation with FISH and 2+ and below. I mean,  
9 zero out of 31 had FISH amplification.  
10 Biologically, the difference between 2+ and 3+ is  
11 sort of what we are talking about. It seems to be  
12 not a clear-cut difference. So, can you explain  
13 how you determined 3+ versus 2+, and maybe explain  
14 why the correlation was so striking at 3+ and was  
15 zero at 2+ or lower?

16 DR. ROCHE: Our criteria for  
17 differentiating 2+ from 3+ is based upon what is in  
18 the HercepTest kit and the instructions for doing  
19 that. So 3+ is strong membrane staining in more  
20 than 10 percent of the cells. That is the  
21 criterion. Now, anyone who does a lot of this  
22 immunohisto-chemistry knows that when you see a 3+  
23 case it is usually more than 50 percent and it is  
24 in the range of usually 60-80 percent of the cells  
25 that show strong membrane staining, as opposed to a

1 2+ case where the criteria, according to the  
2 HercepTest for 2+ staining, is weak to moderate,  
3 complete membrane staining in more than 10 percent  
4 of the cells. A 2+ case is oftentimes in the 40-50  
5 percent range, but there is heterogeneity in terms  
6 of those cells that show complete membrane  
7 staining.

8 Now, we would expect that there would be a  
9 fairly even distribution between the 0, 1+ and 2+.  
10 I think in the data I showed here there were only  
11 12 2+ cases. In our normal clinical practice we  
12 see that about 12 percent of our 2+ cases show gene  
13 amplification. We routinely FISH all 2+ cases that  
14 come through the clinical lab. So, with the  
15 numbers doubled now to more than 250 cases, we have  
16 seen I think three 2+ cases that do show gene  
17 amplification. But just with the small number  
18 here, with 12 cases, just statistically we wouldn't  
19 expect to see any.

20 DR. LIPPMAN: Because one of the issues we  
21 are talking about is 2+ or not 2+ issue and I can  
22 tell you it is just very difficult to imagine that  
23 moderate greater than 50 percent versus strong  
24 greater than 50 percent would be so striking in the  
25 difference of FISH amplification. So, I think if

1 probably is a small numbers phenomenon.

2 DR. NERENSTONE: I have a question and I  
3 am not really sure who to address it to, but  
4 perhaps the folks from the cooperative groups have  
5 some idea. This data is trying to get at the  
6 false-positive rate. But, as clinicians, we are  
7 very concerned about the false-negative rate as  
8 well. Do either of you have any thoughts about  
9 what we are doing to try and identify that?  
10 Because I think that is a very important patient  
11 population that may benefit from a treatment that  
12 we are not offering to them.

13 DR. ROCHE: What do you mean by false  
14 negative?

15 DR. NERENSTONE: That by the clinically  
16 used tests, done at local levels, we are being told  
17 that these patients are HER2 negative, but by a  
18 central review they would potentially really be  
19 positive.

20 DR. ROCHE: Again, I think it gets to the  
21 issue of standardization and quality of control.  
22 If you are going to do HER2 status evaluation you  
23 need to have feedback between both techniques so  
24 that a lab that is going to set up either one of  
25 these techniques needs to know what their

1 correlation is between immunohistochemistry and  
2 FISH to avoid both false negatives and false  
3 positives.

4 DR. NERENSTONE: We now have a framework  
5 for looking at the false positives. Is there any  
6 similar framework to look at false negatives? Not  
7 that you know of?

8 DR. ROCHE: Not that I know of.

9 DR. NERENSTONE: Maybe this is a question  
10 for the panel later on. Dr. George?

11 DR. GEORGE: I have sort of a follow-up to  
12 that. All diagnostic tests will have false  
13 positives and false negatives of various kinds.  
14 The strategy you seem to be following here is doing  
15 a number of tests. Now, if you do a lot of tests,  
16 whether you do the HercepTest test, some other  
17 immunohistochemistry test, you do FISH assays, more  
18 than one type of FISH assays, and you have the  
19 real-world situation thrown in with people doing it  
20 out in their labs, what are you actually after? If  
21 you do enough tests you are going to get some of  
22 them positive. Are you really after trying to  
23 definitively eliminate all those that would test  
24 negative by all tests, or are you doing something  
25 else? I am a little concerned. If you do enough

1 tests, we will probably get everybody positive in  
2 the long run and we don't have do these tests at  
3 all.

4 DR. ROCHE: What is the question then?

5 [Laughter]

6 DR. GEORGE: What are you trying to do?  
7 Are you trying to make the eligibility criteria for  
8 the trial such that you know the patient is, in  
9 some sense, definitively negative, although there  
10 are a lot of questions about that because we don't  
11 have any gold standard? Is that your real goal?

12 DR. ROCHE: The goal is to select the most  
13 appropriate patients for this trial that will  
14 benefit from Herceptin. From the previous studies  
15 that have been done, we know that those are  
16 patients that show overexpression of the protein,  
17 in the 3+ category and/or are gene amplified who  
18 seem to respond best to Herceptin therapy. So, the  
19 goal is to do laboratory testing that has the  
20 greatest sensitivity and specificity for selecting  
21 those patients. In addition to trying to see what  
22 is going on out in local community testing for  
23 doing central review, the other translational  
24 component of this study was to look at the  
25 correlation between immunohistochemistry and FISH

1 assays.

2 DR. NERENSTONE: Dr. Brawley?

3 DR. BRAWLEY: My question is do you want  
4 something that is really highly sensitive,  
5 something that is really highly specific, or can we  
6 sacrifice sensitivity for specificity?

7 DR. PAIK: For our trial purposes,  
8 obviously, we would like to have a highly sensitive  
9 but highly specific assay for any studies. But in  
10 this particular study, because of the concern for  
11 potential cardiotoxicity and especially because we  
12 are trying this drug in a relatively early stage of  
13 disease, we wanted to make sure that the test is  
14 highly specific.

15 DR. BRAWLEY: So, specificity is more  
16 important than sensitivity.

17 DR. PAIK: That is correct.

18 DR. NERENSTONE: Dr. Lippman?

19 DR. LIPPMAN: I have a question to follow-  
20 up on Dr. Nerenstone's questions and what was just  
21 raised, you know, what are we looking for, what  
22 kind of test. Well, you know, we are talking about  
23 biologic therapy so it has to be based on the  
24 biology of the treatment. So, here we are talking  
25 about protein-based treatment and I think, sort of

1 based on your data, the 2+ HercepTest with negative  
2 FISH would be negative. Unless we had very solid  
3 prospective data to show that, that, to me, would  
4 be a false negative because it is hard for me to  
5 imagine biologically that a tumor that has greater  
6 than 50 percent of the cells that are moderate, not  
7 strong but moderate, which is difficult to define,  
8 would not potentially benefit from a therapy  
9 directed at that protein.

10 DR. PAIK: Yes, when you design a trial  
11 you have to kind of balance the potential benefit  
12 over risk. So, based on the data that was  
13 available from the pivotal study from Genentech,  
14 although that correlation between the CTA, the  
15 original immunohistochemistry, and the HercepTest  
16 and then FISH is a little bit difficult to follow,  
17 still, you have to kind of weigh the risk.  
18 Probably entering the patient for 2+  
19 immunohistochemistry and FISH negative wouldn't  
20 have been justified in this early stage of disease.  
21 So, probably what we need to do is generate as much  
22 data in an advanced disease trial for 2+ but FISH  
23 negative patients before thinking about looking at  
24 the adjuvant setting. Obviously, in this trial we  
25 are trying to generate as much data as possible to

1 look at that issue and to generate the hypothesis.

2 DR. NERENSTONE: Dr. Blayney?

3 DR. BLAYNEY: The way I look at this, this  
4 is information moving down a noisy channel, and the  
5 generation of that information is the events that  
6 take place in the patient which lead to malignancy  
7 and the end of that channel is our ability to  
8 deliver a therapeutic intervention. The intergroup  
9 and the NSABP presentations have I think done a  
10 great job of showing that one way to reduce noise,  
11 or cut down on some of the noise is by having  
12 central testing. Another way, as Dr. George  
13 alludes, is to do a lot of tests so that you  
14 average out the noise generators and the real  
15 signal comes through.

16 I hope the morning's discussion and  
17 advising the agency will allow them to take a  
18 broader view as they look at drug development and  
19 say the biology of the HER2/neu gene and that  
20 system is one thing; the biology of estrogen  
21 receptor is quite another where there may be a dose  
22 response. And, the things that we are going to  
23 develop in the next generation of therapies really  
24 depend on the biology, and for that signal that we  
25 need to detect the developers of those drugs need

1 to have some freedom to reduce the amount of noise  
2 that gets into that channel. So, I hope that we  
3 can take these examples as a way of broadening our  
4 thinking down the road the rest of this morning.  
5 Then, this afternoon, it sounds like there are  
6 going to be some more focused discussions.

7 DR. NERENSTONE: Yes, Ms. Mayer?

8 MS. MAYER: Back to Dr. Lippman's point,  
9 it seems to me that by eliminating from clinical  
10 trials those patients who would be 2+, FISH  
11 negative responders we don't get a direction for  
12 the clinic for treatment for patients who may fall  
13 into that possibly false-negative category. In  
14 other words, we aren't able to say to patients in  
15 any definitive way we think you will or will not  
16 respond. It becomes an unknown that will then be  
17 governed by clinical practice.

18 DR. NERENSTONE: Last comment?

19 DR. LIPPMAN: Just a clarification because  
20 I may have missed this, you made the comment, in  
21 response to my comment, that you thought it would  
22 be inappropriate to treat 2+ HercepTest, negative  
23 FISH patients. Is that based on 12 cases here? I  
24 mean, what is that comment based on?

25 DR. PAIK: We just have to stick to

1 whatever data is available. Although that data was  
2 from a very small study, still that is what was  
3 available. When you design a trial in an adjuvant  
4 setting where the major concern was the potential  
5 toxicity, then it became necessary to kind of  
6 restrict our trial to 3+ cases. At the initial  
7 stage when the cardiotoxicity data was not  
8 available we wanted to include any positive cases.  
9 That would have been a most interesting study.

10 DR. LIPPMAN: In my opinion, that is a far  
11 too strong statement to make on 12 cases.

12 DR. NERENSTONE: Dr. Albain, and this is  
13 the last comment.

14 DR. ALBAIN: Yes, just to follow-up with  
15 Dr. Lippman, I think we are mixing two issues here.  
16 As I understand it, the purpose of this was to  
17 point out the discrepancies where it was determined  
18 that these trials would have 3+ or FISH positive,  
19 and they are adjuvant trials. I think this  
20 afternoon we may hear a lot about what you are  
21 addressing, Scott, that being this category of 2+  
22 and what it means when you are using it in the  
23 metastatic setting for active therapy.

24 DR. ROCHE: I just want to clarify for the  
25 12 cases that you are referring to that were 2+ but

1 not amplified, the entry criteria for this study,  
2 which I didn't design, are 3+ and/or amplified. In  
3 the metastatic setting Herceptin is approved for  
4 patients who are 2+ by the HercepTest. I am not  
5 saying those patients should not be treated.

6 **Panel Discussion**

7 DR. NERENSTONE: The next part of this  
8 morning's session is going to be discussion with a  
9 group of experts in a panel, and that panel is to  
10 the left of our table. Dr. Barker, we would ask  
11 that you join the participants around the table, if  
12 that is okay. I know that some of the discussants  
13 did want to address the committee and have some  
14 slide preparations. Dr. Watson, do you have  
15 anything you wanted to add at this point?

16 DR. WATSON: Nothing in particular, no.  
17 The study sizes were the issues of variation and  
18 concordance and discordance.

19 DR. NERENSTONE: I am going to ask you all  
20 to talk into a microphone because we do need to  
21 have this recorded. Dr. Barker, would you like to  
22 lead off? Did you have anything prepared?

23 DR. BARKER: I just wanted to briefly  
24 mention that the National Institute of Standards  
25 and Technology does have a specific program for

1 development of national standards in a number of  
2 areas that range across many different kinds of  
3 technology. The work at NIST on standards for DNA  
4 sequencing is included in some of the paperwork  
5 that was handed out. We do not as yet offer a  
6 national standard for histology type analytes,  
7 although that is a possibility. So, people should  
8 be aware that there is a specific program that  
9 might do that at some point in the future. NIST  
10 works on consensus in the private sector, academic  
11 sector, as well as the medical community. So, if  
12 we were to be involved in that sort of thing we  
13 would have to have a consensus from the community.  
14 We would have to have a specific request from the  
15 community that needs these sorts of standards. So,  
16 there is that program.

17 DR. NERENSTONE: Dr. Hammond, I believe  
18 you have slides you wanted to present.

19 DR. HAMMOND: Yes, I do. Thank you very  
20 much. I am here really representing the College of  
21 American Pathologists, which is involved in doing  
22 proficiency testing for laboratory tests.

23 I would like to start off by really saying  
24 that I think in the deliberations that we were  
25 involved in this morning, really the enemy of our