

1 work is the amount of noise in these laboratory  
2 systems. If we really want to get rapid answers to  
3 biologic questions, then we need to do something to  
4 remove the noise in the system. I think basically  
5 a lot of the things that you have heard today are  
6 strategies that we could use to help us do that.

7 My summary of the criteria that we would  
8 need to really be able to develop meaningful  
9 diagnostic assays where there is less noise in the  
10 system relate to the need that we have to advocate  
11 for consistency of laboratory testing. Without  
12 consistency we really can't make any sense of these  
13 tests at all.

14 We also need to be able to measure the  
15 performance of those tests, and then, finally, have  
16 a method to measure the performance of laboratories  
17 in an ongoing way and validate new laboratory  
18 issues in trying to become part of this procedure.

19 We heard about the assay characteristics  
20 this morning from Drs. O'Leary and Watson. I think  
21 another aspect of this that wasn't really  
22 emphasized is the idea that we need to know when  
23 specimens are adequate to be even used for testing.  
24 In other laboratory tests we do make some  
25 assessment of whether a specimen is adequate and if

1 frozen samples are required, then we certainly  
2 should say that we shouldn't be using fixed  
3 specimens for that particular type of laboratory  
4 test.

5           The issues of specimen handling really  
6 need to be dealt with by having us record the  
7 timing of these different steps in specimen  
8 handling. If we were to record those steps in  
9 specimen handling I think we would begin to  
10 understand what impact they have on our procedures.

11           Finally, I think it is very important that  
12 we have more reliable interpretation and reporting  
13 criteria. This is something that will require the  
14 presence of reference materials in order to train  
15 people how to do that interpretation.

16           The College of American Pathologists does  
17 do ongoing proficiency testing, and has engaged in  
18 these activities for both FISH and  
19 immunohistochemistry assays for HER2. I would like  
20 to summarize those results for you that we have  
21 engaged in over the last two years. Here you can  
22 see the results of the FISH proficiency survey  
23 where, in the year 2000, 35 laboratories  
24 participated and in the year 2001 63 laboratories  
25 participated.

1           There was very high concordance for FISH  
2 amplification or non-amplification, although in  
3 2001 there were some laboratories which did not  
4 return a response. This may have been that they  
5 got no signal; that their assays were technically  
6 inadequate. We did not ask the questions to really  
7 answer that so we don't know why they didn't turn  
8 in a response. But of those that did turn  
9 responses, there was a very high concordance rate.

10           The concordance rate for  
11 immunohistochemistry was not nearly as good as one  
12 might expect, but the number of participant  
13 laboratories was much greater. You can see that,  
14 getting back to the question of false negatives, if  
15 you are using immunohistochemistry 3.7 percent of  
16 laboratories called the FISH amplified case  
17 negative. That might well be within the range that  
18 one might expect. Ten percent of cases called the  
19 FISH unamplified case positive, which I think is  
20 certainly consistent with what everyone is  
21 reporting as being that number of cases which are  
22 going to overexpress protein and not have amplified  
23 gene.

24           A bigger problem is with the  
25 interpretation of these tests. As part of the last

1 survey, photos were provided to the participants  
2 with referee scores. The photo number is shown on  
3 the left. The score given by the referees is shown  
4 next. And, you can see that there is a wide  
5 variation in the interpretation that was rendered  
6 by the pathologists based upon those refereed  
7 scores. This really speaks to the issue of getting  
8 better validation of the criteria for  
9 interpretation than we currently have.

10           If you look at this data, we clearly have  
11 better concordance rates for FISH than we do  
12 immunocytochemistry. Are there any lessons to be  
13 learned from that? I think there are some  
14 significant lessons to be learned. One is that  
15 maybe specimen handling doesn't have as much to do  
16 with adequacy of FISH interpretation than adequacy  
17 of immuno-histochemistry interpretation. Also, the  
18 FISH test that we have has an internal standard for  
19 most of the laboratories doing it. They are using  
20 the PathVysion method which has a chromosome 17  
21 probe which allows the laboratory to know whether  
22 the assay worked or not. That is a very important  
23 internal standard and we don't have internal  
24 standards like that for HER2 testing by  
25 immunohistochemistry.

1           But I think a very important point that  
2 really hasn't been raised yet today is the issue of  
3 training. In order for a laboratory to perform  
4 FISH testing, that laboratory has to use standard  
5 procedures and engage in training. That training  
6 has to be completed before the laboratory can  
7 actually perform those assays. No such requirement  
8 is made for immunohistochemistry because this is a  
9 generalized kind of procedure that is done in  
10 pathology laboratories around the country.  
11 Although with HercepTest it is clear that the  
12 company has made a very great effort to have a  
13 standardized kit and standardized instructions for  
14 how to perform those assays, nevertheless, as you  
15 have heard, there are many assays of being  
16 performed with home brew kits, and with no training  
17 and no standard operating procedures.

18           The interpretation criteria for FISH are  
19 also very quantitative, and for  
20 immunohistochemistry certainly, although some work  
21 has been done to try to educate pathologists about  
22 how to do this interpretation, those criteria are  
23 somewhat ambiguous, partly because the intensity  
24 measurement that we are requiring people to make  
25 for immunohistochemistry is something that the eye

1 is very poor at discriminating. Part of that could  
2 be solved by using imagine analysis systems to  
3 evaluate these systems.

4 Those are really the main points that I  
5 want to make. The other slides that I have here  
6 are answers to some of the other questions that  
7 were raised. I think the College of American  
8 Pathologists, as an organization, is there to help  
9 in these kinds of operations by providing ongoing  
10 proficiency testing, and where there are standards  
11 provided for laboratory performance, those  
12 standards could then be used in the process of  
13 ongoing laboratory accreditation which is handled  
14 on an ongoing basis for a number of laboratories in  
15 the United States.

16 So, I think that this organization can act  
17 to participate in the process of standardizing and  
18 improving the consistency of the way in which these  
19 assays are done around the country. Thank you.

20 DR. NERENSTONE: Thank you. Dr. Taube?

21 DR. TAUBE: I am not going to show any  
22 slides right now. I have some slides for the  
23 discussion if questions are asked. But I just  
24 wanted to say that I represent the Cancer Diagnosis  
25 Program of the National Cancer Institute, and we

1 have recently launched a new program to ensure the  
2 more efficient and effective translation of these  
3 technologies to the clinic. We are focusing  
4 particularly on this issue that was raised earlier  
5 about development of tests along with the  
6 development of drugs, and trying to make it a  
7 smoother process so that we don't end up needing  
8 this time of session every time.

9           What we are doing is we are trying to  
10 focus on the various bottlenecks and barriers that  
11 we have identified in the process, some of which  
12 have been adequately identified here related to  
13 reference materials, and to establishing  
14 standardization in the whole processing of  
15 specimens. There are other problems that occur in  
16 the development of new tests that relate to the  
17 size of the early studies and the lack of similar  
18 techniques being applied. We are trying to deal  
19 with those issues as well. I think that is all I  
20 will say at this point.

21           DR. NERENSTONE: Dr. O'Leary, did you have  
22 anything you wanted to add?

23           DR. O'LEARY: Just sort of a comment that  
24 reemphasizes that point. To a degree, the reason  
25 that we are here is because the development of the

1 clinical laboratory tests and the validation of  
2 therapeutic agents were not carried out in lock  
3 step in close proximity. I think this committee  
4 could do a very great service if it were to  
5 emphasize the importance in the targeted  
6 therapeutics arena of validated the tests that are  
7 going to be used for selecting patients for therapy  
8 at the same time that one validates the efficacy of  
9 the therapy itself.

10 Running a concordance study against a  
11 clinical trials assay is not really coming any  
12 place close to a gold standard. I have had many  
13 conversations with representatives of both the  
14 pharmaceutical industry and the laboratory  
15 diagnostics industry. In general, the laboratory  
16 diagnostics industry is very amenable to being  
17 involved early in the process. There is a great  
18 deal of heterogeneity in the pharmaceutical  
19 industry and perhaps that can be addressed.

20 DR. NERENSTONE: I would like to open up  
21 the discussion to the panel, and I am going to take  
22 the chair's prerogative to ask the first question.  
23 Dr. Hammond, getting back to the survey that you  
24 did, we are all very interested in the correlation  
25 between laboratory size and the results. You

1 showed that there was really 100 percent  
2 correlation with FISH assays. What were the sizes  
3 of the laboratories? Were they all big labs  
4 because that is only who does FISH or were they a  
5 mixture?

6 DR. HAMMOND: I am sure they were all  
7 laboratories certified by the College of American  
8 Pathologists. These are probably, by definition,  
9 larger laboratories since most laboratories just  
10 engaging in FISH in a sort of less intense way  
11 would be less likely to go through that  
12 certification process, although I don't really have  
13 data about that specifically. I am not sure that  
14 question was asked, but that is my assumption.

15 DR. NERENSTONE: Dr. Lippman?

16 DR. LIPPMAN: Just to follow-up on the  
17 last comment, I don't have a copy of your slides  
18 but I thought even in the select institutions that  
19 are highly trained doing FISH, concordance was not  
20 all that great for the non-amplification. It was  
21 49 out of 63.

22 DR. HAMMOND: There was 100 percent  
23 concordance in the responses that were returned.  
24 The remaining institutions did not return a  
25 response.

1 DR. LIPPMAN: So, the 49 out of 63 is just  
2 a response.

3 DR. HAMMOND: Well, 49 out of 63 said that  
4 the amplified case was amplified. The other  
5 institutions did not return a response so we don't  
6 know if they would say it was not amplified. They  
7 just chose not to respond. It could very well have  
8 been that they over-digested the slide and couldn't  
9 read the result, or something.

10 DR. LIPPMAN: I have a couple of other  
11 questions and a couple of comments on what you  
12 presented. Again, part of the issue is the  
13 concordance between FISH -- the 10 percent that  
14 have an increase in expression, overexpression and  
15 negative FISH. So, in your very systematic, nice  
16 study how did you define a positive protein  
17 overexpression and how did you define FISH  
18 positivity in that concordance study where you had  
19 10 percent?

20 DR. HAMMOND: We used the FDA approved  
21 guidelines for that, the package inserts for those  
22 kits.

23 DR. LIPPMAN: So, you used 2+ as positive  
24 for the protein?

25 DR. HAMMOND: Yes, we did it by the

1 scoring system. So, 3+ is 3+ and 2+ is 2+.

2 DR. LIPPMAN: But when you said that it  
3 had protein overexpression, that is defined as 2+  
4 or greater?

5 DR. HAMMOND: No, it is by the score. I  
6 think you have the slide in your handout. The FISH  
7 unamplified cases, some of those were 2+. Seven  
8 percent of the 2+ IHC positives were FISH  
9 unamplified. Of the 3+, 3 percent were  
10 unamplified.

11 DR. LIPPMAN: Then just a couple of things  
12 to come back to the training and volume issue, you  
13 made the comment, and I agree, that some of the  
14 lack of concordance with the IHC may have been that  
15 there were many more centers, probably some lower  
16 volume centers, not well trained. So, I think  
17 again some of the hits due to immunohistochemistry  
18 may, in part, be due to that. Do you agree?

19 DR. HAMMOND: Oh, absolutely. I also  
20 think that we have a problem with the assay  
21 interpretation. The scoring system was developed  
22 on frozen tissue, with no antigen retrieval, done  
23 with a different assay than the one in which  
24 HercepTest scoring system was sort of used based  
25 upon the FDA approval. That scoring system has had

1 to go forward because of the way in which the  
2 studies were done that allowed for FDA approval to  
3 occur. That scoring system maybe needs some  
4 modifications in order to make it more valuable and  
5 more rigorous. As you heard Dr. Roche talk,  
6 anybody who looks at large numbers of these cases,  
7 your threshold for 3+ is really much more rigorous  
8 than the one that is in the package insert. So, I  
9 think if we could tighten up the interpretation  
10 criteria we would be able to improve the  
11 immunohistochemistry results dramatically.

12 DR. LIPPMAN: Right. So, I think when we  
13 compare generically IHC with FISH it is almost  
14 apples and oranges because the people doing the  
15 FISH studies at this point are much more highly  
16 selected. So, I think we need to take that into  
17 account.

18 You made a comment about computerized  
19 image analysis, and I think we ought to go there  
20 because this would eliminate a lot of the  
21 subjectivity in the IHC analysis.

22 The only other comment I was going to make  
23 now was picking up on what Dr. O'Leary said. I  
24 don't know if we will get there in this discussion,  
25 but this is not only selecting patients for

1 biologic-based therapy but is following the  
2 treatment efficacy as surrogate endpoints, which  
3 may be beyond the scope of this meeting. But you  
4 brought up a very important point.

5 DR. HAMMOND: I would like to underscore  
6 another point that has been made. I don't know if  
7 I emphasized it strongly enough, that one of the  
8 other problems we have with these assays is the  
9 lack of standardized reference materials. Having  
10 those standardized reference materials available  
11 would quickly allow laboratories to do a more  
12 efficient job of evaluating their own performance.  
13 Only by that measurement of performance can we hope  
14 to get rid of the noise in the system.

15 DR. NERENSTONE: Dr. Redman?

16 DR. REDMAN: One of the questions we are  
17 being asked is about the development of diagnostic  
18 tests, not diagnostic therapeutic products. So, I  
19 guess the question to the panel is, you know,  
20 Herceptin is an approved therapy. Somebody in some  
21 lab -- I am not oriented in basic science enough to  
22 understand -- comes up with an assay that they  
23 believe is cheaper and wants to bring it to  
24 somebody so that it can be standardized, brought  
25 forth and become a test that can be used in the

1 community to determine whether somebody is a  
2 candidate for Herceptin therapy. What currently is  
3 the process for that?

4 DR. HAMMOND: Right now it is at the  
5 laboratory's discretion to do that. As long as the  
6 laboratory director follows the process and they  
7 are willing to take the responsibility for the  
8 development and validation of that test. But as  
9 you can see from the data that has been presented,  
10 the fact that that is allowed, that we can bring  
11 tests and use them in that way, makes for a lot of  
12 noise in the system.

13 DR. REDMAN: So, right now if I am a  
14 start-up company and I come up with a better assay,  
15 I don't need to go to the federal government at  
16 all. I can just go to my local hospital and say  
17 please start using my assay.

18 DR. NERENSTONE: Dr. Gutman, if you would  
19 like to respond to that?

20 DR. GUTMAN: It is not quite that easy.

21 [Laughter]

22 DR. REDMAN: I didn't think so.

23 DR. GUTMAN: Let me clarify because there  
24 may be some failure to understand that there are  
25 two ways to commercialize tests in this country.

1 If a company plans to market a test for use in  
2 multiple labs, if that is a marketing intent, that  
3 is a test kit or system that will be in commercial  
4 distribution and the FDA believes it is a medical  
5 device. It would need to come, actually, to a  
6 different group than the one hosting this  
7 particular lovely session. That would be actually  
8 the Division of Clinical Lab Devices, which is a  
9 diagnostic group in the Office of Device  
10 Evaluation.

11 We have seen at least a number of products  
12 directly related to the subject on the table, each  
13 with different designs and different performance  
14 claims, and they have gone through similar panels  
15 like the one being held today. Actually, the one  
16 being held today is germane to a product under  
17 review.

18 There is an alternative mechanism for  
19 entering the commercial marketplace, and that would  
20 be if an academic or, for that matter, an  
21 entrepreneurial commercial lab decided to set up a  
22 system on its own for use at a single site. That  
23 is called a laboratory testing service or an in-  
24 house test or a home brew test. That is a  
25 perfectly legitimate enterprise. The activity,

1 however, must stay at that single site and there  
2 are different rules that apply. The FDA actually  
3 does indirectly regulate home brew tests, but the  
4 regulation is through the active analyte that would  
5 go to the home brew test, and that active analyte  
6 needs to be registered and listed. It needs to be  
7 made using good manufacturing practices. Then,  
8 there are some labeling caveats for both the  
9 analyte and the test being offered off that  
10 analyte.

11 So, an alternative mechanism that is under  
12 indirect regulation, not direct regulation, is home  
13 brew test. As has been alluded to constantly -- it  
14 is almost a leitmotif this morning, there are a  
15 wide variety of both academic and commercial  
16 interests who have become involved in this  
17 enterprise with variable success perhaps.

18 It is worth noting that at the extremes --  
19 a home brew test, by the way, is considered a  
20 medical device. A laboratory which has entered  
21 that practice is actually considered by the agency  
22 a manufacturer, but because there is a corollary  
23 regulation under CLIA and because of our limited  
24 resources, we chose to use enforcement discretion  
25 and not to exert oversight over that particular

1 nature of test.

2           There is one final sort of extreme edge,  
3 and that is if a home brew test is generated so  
4 that the reagent itself is made entirely within the  
5 four corners of the lab, the lab not only develops  
6 the test but, in fact, makes its own reagent on  
7 site for use, then it actually falls outside FDA  
8 regulation entirely. That is considered a home  
9 brew test in its purest form and that falls within  
10 the practice of laboratory medicine.

11           DR. NERENSTONE: Just to follow-up on  
12 that, just for clarification, I guess we don't  
13 necessarily have problems with the test but how it  
14 is being applied, either the procedure up to that  
15 point and perhaps the interpretation. Now, a  
16 pregnancy test that only had 75 percent correlation  
17 would rapidly -- that laboratory would be in big  
18 trouble, I would think, pretty quickly. I guess I  
19 am just amazed that there is no regulation, or  
20 there is no follow-up, or there is no "nothing"  
21 about something, obviously for my patients, that is  
22 as important in terms of therapeutics.

23           DR. GUTMAN: No, perhaps the regulation  
24 isn't perfect yet but it is not that there isn't  
25 regulation. There is regulation through the

1 Clinical Laboratory Improvement Amendments of 1988  
2 so all laboratories do, in fact, need to meet  
3 standards. Whether the standards are appropriate  
4 or not, could be strengthened, could be improved,  
5 that might be arguable but there is oversight of  
6 every test that goes into a human being in this  
7 country. It is a question of degree perhaps.

8 DR. NERENSTONE: Dr. O'Leary?

9 DR. O'LEARY: There is also sort of an  
10 indication for use issue as well that I think we  
11 need to deal with when we consider the home brew  
12 and how it is being used in the laboratory, why it  
13 was originally developed, and how it might be  
14 misused. For example, in my laboratory we first  
15 introduced an immunohistochemical test for HER-B2  
16 long before the time of introduction of the  
17 HercepTest. That particular use was as an adjunct  
18 test for other things. It may be used, for  
19 example, to assist in the determination of the  
20 histogenesis of metastatic adenocarcinoma showing  
21 up for the first time in a pleural effusion,  
22 something quite different than being used to select  
23 therapy.

24 So, in an adjunctive context, there may be  
25 differences of interpretation and use which are

1 acceptable perhaps, that are not acceptable when  
2 one is looking at decisions on entering patients  
3 into clinical trials. Again, with time these  
4 problems tend to go away. If you look at any of  
5 the CAP surveys, the introduction of the survey  
6 tends to be one of the best things for driving  
7 inter-laboratory concordance, and that 75 percent  
8 that you see now will probably be much smaller a  
9 year from now, if I consider every other laboratory  
10 test for which I have seen a new survey introduced.

11 But it is possible for you to get a report  
12 saying that a breast cancer is 3+ positive for  
13 HER2/neu for somebody who did this test for some  
14 purpose other than selecting patients for a  
15 clinical trial. Unless you know why they are doing  
16 the test and how they are doing the test, you don't  
17 have the information you need to make a therapeutic  
18 decision, in my opinion.

19 DR. NERENSTONE: Dr. Albain?

20 DR. ALBAIN: I wanted to go back to a  
21 comment -- I forget who said it on the panel --  
22 that we need to, in the future, perhaps do things a  
23 bit differently than was done with Herceptin in the  
24 sense of developing the assay in parallel. I  
25 forget how it was worded. But looking back on it

1 all, I think the industry, with exceeding vigor and  
2 robustness, did what they could do at the time.  
3 There was an exciting therapeutic agent and we  
4 didn't have any tests really, except some of these  
5 as you have been alluding to, and the process is  
6 just evolving. So, now take it away from breast  
7 cancer and Herceptin and let's talk about, for  
8 example, the EGFT tyrosine kinases. We have just  
9 conducted some pivotal trials, and others are  
10 ongoing, in lung cancer. Patients were accrued to  
11 those trials regardless of receptor status because  
12 it may be that you don't need either amplification  
13 or overexpression, and we don't really have a test  
14 for that whole pathway and what goes on downstream.

15 So, what I thought we were going to be  
16 spending more time discussing is how to design  
17 these trials as we proceed in the future, in terms  
18 of what should be required on a pivotal trial, such  
19 as what should be collected. You may not have any  
20 assays at all, but you have an exciting new agent  
21 and you can't wait five or ten years to work out  
22 all the assay systems. So, I wondered what the  
23 panel members thought about these scenarios.

24 DR. NERENSTONE: Dr. Taube?

25 DR. TAUBE: Yes, this is a major issue,

1 and I think the question that you have raised is a  
2 very valid one, which is at the very early stages  
3 of development of a drug you may not know what you  
4 need to measure. So, I think it is absolutely  
5 critical that specimens be obtained and stored.  
6 There are problems as well because we don't know  
7 how to fix them, how to preserve them and so on.  
8 But I think that that is a different issue and  
9 there are ways of addressing that.

10 I think there are a number of problems in  
11 terms of the idea that I said before about making  
12 an assay as you go along. There are many risks in  
13 developing a truly standardized assay, risks for  
14 the manufacturers to go down a path that may turn  
15 out not be a very productive one. So, what we are  
16 trying to do is figure out how to do that better.

17 I think that we are going to have to set  
18 up a series of decision criteria that help us know  
19 when we need to really push standardization, and I  
20 think the first step is going to be just having a  
21 lot of tissue so we can go back and look at the  
22 association between different markers that relate.  
23 For instance, in the EGFR situation we may have to  
24 look at the whole pathway, and we may have to find  
25 out which of the genes that are altered in that

1 pathway correlate best with the response to  
2 therapy. So, we have to develop a mechanism for  
3 evaluating that as we go along.

4 This is not trivial but we are working on  
5 it. As I said, we have this new program, and we  
6 have a strategy group, and we are going through  
7 some of these major issues and targets that are  
8 coming down the pike. We have not come up with an  
9 easy answer, but we are definitely working on it  
10 and we are going to have to continue to work on it.

11 DR. NERENSTONE: Dr. O'Leary?

12 DR. O'LEARY: Just to reemphasize, keeping  
13 the tissue available would be very, very useful.  
14 If one looked at the original HercepTest  
15 submission, the greatest source of consternation  
16 was the fact that we were doing a correlation  
17 against the clinical trials assay and not a direct  
18 comparison of the predictive value of the  
19 HercepTest assay in response to therapy. If we had  
20 even been able to do post hoc analysis looking at  
21 the patients who were enrolled in the clinical  
22 trials and how well we might have done in a post  
23 hoc analytic situation using that tissue, I think  
24 it would have given everybody a great deal more  
25 confidence that we understood what was going on.

1 If we had seen that assay then utilized and  
2 performed in multiple sites, in many more sites  
3 than it had been, both at the enrollment level and  
4 the central laboratory level, we might have had an  
5 idea several years ago of the kind of heterogeneity  
6 that we have been seeing in the presentations  
7 today.

8           So, it is really the process of getting  
9 hold of and retaining some degree of control over  
10 that tissue that becomes important. That is really  
11 problematic. There are a lot of reasons why  
12 laboratories would like their tissue back and,  
13 quite frankly, medically-legally, many of us find  
14 themselves in the position where they want to  
15 destroy the tissue and the slides as soon as they  
16 can legally do it lest somebody sue them later on  
17 and uses them against them in a court of law. That  
18 is bad news and I don't know how to deal with it.  
19 But if you can keep control of the tissue, you will  
20 solve a lot of these problems.

21           DR. NERENSTONE: Dr. Blayney?

22           DR. BLAYNEY: I think that Dr. Albain has  
23 alluded to one pathway but I can think of several  
24 drugs -- STI-571 and the ability to use that not  
25 only in gastrointestinal stromal tumors, which is a

1 very difficult diagnosis on an H&E stain to make,  
2 but on any CD-117 positive sarcoma, and if the  
3 agency would allow industry to develop a drug like  
4 that, with those broad criteria, that is a little  
5 risky in our framework but I think that is the way  
6 we ought to go. Similarly, many of the monoclonals  
7 against CD-20 in lymphoma are very difficult  
8 pathologic criteria to subdivide on H&E stain, but  
9 the target is the CD-20 antigen on the cell and  
10 that kind of definition for the study population is  
11 something that the agency ought to allow industry  
12 to go to.

13           We heard some business about  
14 standardization earlier, particularly the College  
15 of American Pathologists standardization against an  
16 assay, and then the National Institute of Standards  
17 is talking about providing reference materials. Is  
18 it possible to provide reference materials reliably  
19 over time of biologics of tissue, the things that  
20 are really based on a piece of tissue? You are  
21 going to exhaust even the largest tumor at some  
22 point. Is it possible to provide standards that  
23 are biologically sound over time and widely  
24 available?

25           DR. BARKER: I think you have gotten

1 exactly to the point that presents somewhat of a  
2 difficulty. NIST standards are primarily those  
3 things that can be regenerated at will and that can  
4 be quantitated to a high degree or precision.  
5 Obviously, a tissue block from one patient would  
6 not be good material as a HER2/neu standard.  
7 However, we had some preliminary discussions with  
8 Dennis Slamon sometime ago, perhaps two or three  
9 years ago, about the possibility of using  
10 characterized cell lines with known levels of gene  
11 amplification, with or without the expression of  
12 the MRN protein.

13           Theoretically, that is possible. I think  
14 that would be an advantage in that we could  
15 potentially grow up a very large amount of the  
16 material and fix it in a standard fashion so that  
17 those sorts of physical standards could be  
18 available. I don't know off the top of my head  
19 what the shelf-life of those kinds of things could  
20 be, but conceivably that is a possibility. I don't  
21 think a standard based on clinical specimens is  
22 really the kind of national standard that would be  
23 useful over a long period of time.

24           DR. NERENSTONE: Dr. Taube, did you want  
25 to add?

1 DR. TAUBE: Yes. Actually, I only  
2 partially agree with that. I mean, obviously you  
3 do exhaust specimens and you can't talk about a  
4 biological standard in the same way as an extracted  
5 chemical standard, or even perhaps a cell line  
6 standard although cell lines change over time as  
7 well. But we have been talking about the  
8 possibility of making tissue microarrays with well-  
9 characterized, well-preserved tissues where we can  
10 have centralized testing to establish the so-called  
11 truth of the diagnosis, whether it is positive,  
12 negative, however it is defined, including in that  
13 cell line standards as well.

14 But I think that the reason for using an  
15 actual tissue specimen is important, and that is  
16 that the pathologist looks at tissue specimens that  
17 have a multiplicity of cells on them and that are  
18 also handled differently. Even if you embed the  
19 cell lines, it is not the same as a tissue that has  
20 been taken out of a patient and fixed and embedded.  
21 So, we are in the process of designing tissue  
22 microarrays and testing them to see whether we can  
23 use these as standards because you can, in fact,  
24 get multiple sections, obviously, from an array  
25 block and, thereby perhaps qualify laboratories and

1 use this as part of a proficiency program.

2 DR. NERENSTONE: Dr. O'Leary, did you want  
3 to add something?

4 DR. O'LEARY: There are two things. One  
5 positive thing on the arrays is that while you may  
6 not be able to get exactly the same performance on  
7 a tissue by tissue basis, the statistical  
8 performance of large arrays constructed at  
9 different times is likely to remain very, very  
10 constant. So, you can probably get some reasonable  
11 validation information there.

12 Second, the industry is making attempts to  
13 develop other kinds of standard reference  
14 materials. I have seen people that use a  
15 combination of phage display and then biopanning to  
16 try to define tissue antigens very, very carefully.  
17 The idea is then to incorporate them in a matrix in  
18 some fashion or another that simulates that of the  
19 cell. It is early work. It is difficult work. It  
20 is not going to happen today.

21 But it is clear that efforts are being  
22 made in these areas. But it is also clear that we  
23 have to be careful. Not all 117 antibodies are  
24 reacting against the same thing and telling you the  
25 same thing. You know, there are seven or eight

1 different manufacturers of kit antibodies, and used  
2 on the same tissue they will give you different  
3 histological appearances and it may be possible to  
4 tweak them to do the same thing or it may not be  
5 possible. By retaining tissue, at the time that  
6 you do validation in your clinical trials, you can  
7 understand the performance characteristics later.  
8 If you don't retain the tissue, then somebody has  
9 to do a whole new study.

10 DR. NERENSTONE: Dr. Hammond?

11 DR. HAMMOND: I think we are mixing up two  
12 types of uses for reference materials. There has  
13 to be reference material used on every assay that  
14 is performed so that you know that the assay is  
15 working. So, that is one kind of standard for  
16 which a NIST type standard would be a wonderful  
17 blessing. But then there is also the problem of  
18 ongoing laboratory performance or initial  
19 certification of laboratories to perform testing  
20 for which the tissue microarray idea is a much  
21 better plan.

22 DR. NERENSTONE: Dr. Watson?

23 DR. WATSON: I think I overlap a little  
24 with that comment in that it is both disease  
25 specific standards and technology specific

1 standards that are lacking. For instance, a  
2 standard that would be of great value to a FISH lab  
3 would be one that tells them that their  
4 fluorescence microscope is actually working well,  
5 and NIST has considered the possibility in the past  
6 of developing some bead systems on a slide that  
7 would allow you to know that your microscope bulbs,  
8 that have a limited lifetime, are still working at  
9 maximum efficiency. So, there is a range of  
10 standards that aren't just specific to HER2 and  
11 tissues but many of these technologies that are  
12 rapidly evolving that aren't in place either.

13 DR. NERENSTONE: Dr. Lippman?

14 DR. LIPPMAN: My first comment addresses  
15 the home brew because the first time I heard that  
16 was with Dr. O'Leary's paper and talk. My first  
17 interpretation was relatively negative, sort of a  
18 negative connotation, but the clarification here a  
19 few minutes ago of home brew, as I take it, is that  
20 there are certain types of home brew that are very  
21 acceptable, particularly in the setting where we  
22 don't have CBC-like automation for all of these  
23 markers. So, the idea is that an institution that  
24 has a good lab, with good QA and QC monitoring,  
25 could perform their own tests with specific

1 antibodies.

2           But I would like to go back to Dr.  
3 Albain's point because one of the things, as we go  
4 forward, is what should we require of these  
5 studies. Now, in the case of Herceptin the cart is  
6 clearly in front of the horse. The drug is out  
7 there; it is helping people. We are now trying to  
8 find the best way to get the assay. That makes  
9 sense. You know, it was one of the first of its  
10 type of therapy. But now we know that the future  
11 is this type of molecular targeting therapy,  
12 whether it is at the gene level or the protein  
13 level. So, when we get to the point where someone  
14 meets with the sponsor to develop a pivotal Phase  
15 III trial with a molecular targeting agent, one  
16 would presume in this day and age that there is a  
17 hypothesis based on that molecular target. That  
18 may change during the trial. There are examples  
19 where we thought we knew what the target was and it  
20 changes. But at least I think there should be some  
21 sort of requirement that tissue is collected to  
22 assess the ability of this target to predict  
23 outcome.

24           DR. NERENSTONE: Dr. Carpenter?

25           DR. CARPENTER: Wouldn't this be one

1 place, particularly for staining things, where some  
2 kind of standardized image analysis description  
3 could be used? Because that could be sent out as  
4 reference material. It could be generated not only  
5 from the same cell line, but from things that are  
6 shown in a relatively objective way to be  
7 comparable, and also something that certain  
8 institutions could use on their own for continuing  
9 internal validation.

10 DR. HAMMOND: Another kind of standard.  
11 You would have to have interpretation standards.  
12 Absolutely.

13 DR. NERENSTONE: Dr. Brawley?

14 DR. BRAWLEY: May I ask when we use a  
15 standard, is the standard more to determine if a  
16 positive is a positive as opposed to a standard  
17 being used to determine that the test is not  
18 passing up a true positive and labeling it falsely  
19 negative?

20 DR. HAMMOND: It is both. Actually, it is  
21 both things. You have to have standards that help  
22 you know that the test worked. You have to have  
23 standards that help you know what the sensitivity  
24 and specificity of the test is, and then standards  
25 to help you know that you are interpreting the test

1 properly.

2           If we had some rigor in this process, we  
3 could avoid a lot of the problems that we have  
4 gotten into with HER2. In the future, if we could  
5 design that rigor into the system, I think we would  
6 be a lot further ahead.

7           DR. NERENSTONE: What I would like to do  
8 now is to turn to the questions that the FDA has  
9 proposed that we discuss.

10           The first one, use of molecular targets to  
11 select patients for a particular therapy is a  
12 rapidly advancing aspect of therapeutics  
13 development. The FDA would like to facilitate  
14 concurrent development of the molecular assay and  
15 therapeutic while maintaining high scientific  
16 standards. In addition, FDA would like to be able  
17 to respond to advances in assay methodologies.  
18 Please comment on the role of each of the following  
19 aspects of concurrent assay and therapeutic  
20 development. The first is use of a central  
21 reference laboratory during the conduct of the  
22 pivotal clinical trial.

23           I think we have sort of talked about that.  
24 Actually, Drs. Barker, Watson and O'Leary are  
25 supposed to come back to the table for this part of

1 the discussion. The panel part of this is done and  
2 we need you back at the table to help with the ODAC  
3 discussion.

4 While they are doing that, Dr. George,  
5 would you like to comment?

6 DR. GEORGE: Yes, with respect to this  
7 point, I would just like to bring up one issue that  
8 might be overlooked. That is, in the clinical  
9 trials arena we have pushed for several years now  
10 to try to get larger, simpler trials in order to  
11 get more patients on studies, in order to answer  
12 more fundamental questions. This thrust runs  
13 directly contrary to that. I just want to be sure  
14 everybody realizes that because this could mean  
15 delays in process, complicated, more expensive  
16 trials and in such a way that people who might have  
17 entered onto trials may not be entered because they  
18 have to comply with a lot of laboratory samples,  
19 and such. I just wanted to throw that on the table  
20 to make sure it wasn't forgotten. I mean, it is  
21 easy to say, oh yes, this should be done,  
22 particularly when you sit around with a lot of  
23 laboratory people who always want their samples --  
24 in fact, when I deal with laboratory people, they  
25 want to require that all these things get done or

1 you can't enter onto the trial, which creates a  
2 different kind of eligibility restriction on the  
3 patient. So, I am throwing that out just as a word  
4 of caution at this point.

5 DR. NERENSTONE: And I agree with that.  
6 Certainly in the GOG when a study requires samples  
7 to be sent, there is always a question of accrual  
8 of patients as opposed to when it is not. So, I  
9 think all the cooperative groups would agree with  
10 you that that is a problem. Dr. Kelsen?

11 DR. KELSEN: I think that is a good point  
12 but we heard a presentation this morning from two  
13 cooperative groups with a central lab for the  
14 compound under discussion, and the discordance  
15 between the central lab and the referring labs I  
16 thought was fairly striking. We at least got a  
17 hint at the question that people who do a lot do a  
18 better job of it. So, my answer to (a), I mean, I  
19 think having a central research laboratory for a  
20 molecularly targeted compound would be very  
21 important, and there is a model for one of the  
22 studies at least where you could enter the study  
23 and then proceed with the treatment arm. If it  
24 turned out that there was a serious discrepancy for  
25 the target, the patient would then leave the trial.

1 So, the patients accrued but there was a mechanism,  
2 and it is ongoing. It would be interesting to hear  
3 if that has affected their accrual at all.

4 DR. NERENSTONE: Dr. Albain?

5 DR. ALBAIN: I think that approach could  
6 work if you are certain of what you are assaying.  
7 Again back to the other molecular targeting agents  
8 in different solid tumors, we don't know yet. In  
9 that case it may be premature even to use up  
10 materials, if you are not quite sure, as the  
11 pathway is being studied. So, it may be there that  
12 central banking is more important than starting out  
13 with a central assay. There could be a peer review  
14 on what those bank tissues are used for, as we have  
15 done in the various cooperative group settings  
16 wherever the trial is being conducted, so that you  
17 don't exhaust precious material but, yet, you still  
18 have a significant resource as that pathway is  
19 worked out and as you have responses to correlate  
20 activity with.

21 DR. KELSEN: It would still require tissue  
22 though.

23 DR. ALBAIN: Correct. The large, simple  
24 trials we are looking at, of course, are in many of  
25 our chemo-therapeutic and/or hormonal areas but are

1 we ready to conduct large, simple trials with these  
2 agents in a whole new class? Perhaps we have to  
3 give up some of that and realize that to go on  
4 these trials there needs to be a commitment from  
5 the investigator to provide tissue.

6 DR. NERENSTONE: Dr. Lippman?

7 DR. LIPPMAN: Kathy, you stole my thunder  
8 there. I think the point is great, the large,  
9 simple trial, but I think we can't do that here. I  
10 think, for the reasons I mentioned before with  
11 these kinds of molecular targeted agents there is a  
12 hypothesis, we need to collect tissue to be able to  
13 test that. So, then when the trial is done, in  
14 some cases there will be a closer relation between  
15 the cart and the horse and we will have the kind of  
16 information we would have liked to have had with  
17 Herceptin and HER2.

18 So, I think Kathy's point of just banking  
19 it so you are not talking about real-time  
20 turnaround to determine whether to treat the  
21 patient is another issue. But I think tissue needs  
22 to be collected. It needs to be banked. And, that  
23 makes it not a large, simple trial in some people's  
24 minds and I think we can't allow that generally  
25 with these kinds of agents.

1 DR. NERENSTONE: Dr. O'Leary?

2 DR. O'LEARY: I think there are a couple  
3 of interesting issues here. If you are going to  
4 ultimately use this in the community, then the  
5 true-use trial requires that the testing be done in  
6 the community, and you need a process to use your  
7 central laboratory to help bring the community  
8 laboratories up to snuff so people are getting the  
9 right results in the sense of the way that drug is  
10 going to be used long term.

11 At the same time we have another  
12 challenge. In breast cancer now many of the  
13 diagnoses we are getting are on the basis of thin  
14 needle biopsies and that is the only tissue there.  
15 So, there may not be a lot to deal with. You may  
16 have to be careful about designing some flexibility  
17 in the study and in the tissue accrual, and  
18 recognize that you are not going to bet perfect  
19 answers on tissue accrual because the tissue just  
20 is not there to accrue in some cases.

21 DR. NERENSTONE: Dr. Brawley?

22 DR. BRAWLEY: Dr. O'Leary in some sense  
23 stole some of my thunder, but let me try to put it  
24 in simple words from a simple man and maybe I can  
25 help the rest of the committee understand some of

1 what Dr. George was saying. If you do a large,  
2 simple trial you are actually addressing a number  
3 of questions. One of those questions is how well  
4 does the assay work with the therapy, but implied  
5 in that question is how well people use the assay.  
6 If you use a centralized laboratory, you are  
7 defining your question with much more focus to ask  
8 about the assay and the therapy with almost all  
9 elements except how well the assay actually works  
10 being controlled. Is that correct?

11 DR. O'LEARY: That is certainly an  
12 interpretation that I would be inclined to apply.

13 DR. BRAWLEY: So, if we have a centralized  
14 laboratory in a clinical trial we are asking a  
15 simpler question. If we have a large, simple trial  
16 we actually have a lot more variables that we are  
17 dealing with. It is actually the old efficacy  
18 versus effectiveness discussion in epidemiology. A  
19 large, simple trial may actually tell us much more  
20 of what we can expect in the United States as a  
21 whole if this thing goes forward, but the focused  
22 trial tells us what is scientifically possible.

23 DR. O'LEARY: By accruing the tissue you  
24 make it possible to answer both questions.

25 DR. NERENSTONE: Dr. Taylor?

1 DR. TAYLOR: But I think when you know  
2 more, then you can allow more variables, and until  
3 you do know the answer about some of these drugs  
4 and how they work and biologics, I think that by  
5 using a central lab you reduce the number of  
6 variables until you know. When you know the answer  
7 which tests can do it, and when you are going to do  
8 it, then you can give it back to the community to  
9 see how well it is used in the community. But  
10 right now, when you throw in all the community labs  
11 you throw in an extra variable that I don't think  
12 we need at a time when we don't know how the  
13 biologics are all working.

14 DR. NERENSTONE: Dr. Carpenter?

15 DR. CARPENTER: I think the large, simple  
16 trials are helpful but they are not the only kind  
17 of trials that are helpful. I think what we are  
18 going to distinguish here is where to start and  
19 where to go with it. If we are going to start with  
20 a biologically-based therapy, we are going to need  
21 to collect tissue and understand the target,  
22 understand what it takes to find that and use it in  
23 a smaller number of people. If we get an answer  
24 and we get a therapy that is active, then some of  
25 these things should move from there. That is when

1 you can then begin to move toward the large, simple  
2 trial. That is when you can begin to diffuse the  
3 technology or the laboratory portion of it out  
4 beyond the central lab into the community and see  
5 how it is going to play out. But I think the idea  
6 that we are going to get the definitive answers by  
7 mixing these two kind of things is probably a  
8 misnomer, and we are going to have to have a couple  
9 of stages of testing for biological studies, just  
10 in the same way we do for clinical testing of  
11 chemotherapy drugs but they may be somewhat  
12 different.

13 DR. NERENSTONE: Dr. Griffin?

14 DR. GRIFFIN: Along the same lines, I  
15 think it is probably important to differentiate  
16 between diseases which are relatively rare yet have  
17 important biologicals coming along, like for  
18 glivack. There would have been no difficulty,  
19 relatively speaking, getting enough specimens I  
20 think since there were only five centers to give  
21 them out, and we immediately saw people starting to  
22 mix FISH and PCR and cytogenetics about how we were  
23 going to detect if somebody was a whatever. There  
24 was no standardization which potentially could have  
25 been. Those were highly motivated patients.

1           There are other things coming along for  
2 leukemias. Leukemias are rare. I think there is a  
3 big difference between what kinds of standards we  
4 might want to apply for biologics and something  
5 like a colon cancer or breast cancer that would be  
6 logistically different than what you would perhaps  
7 be able to extract, at least initially and perhaps  
8 indefinitely, for rare tumors.

9           DR. CARPENTER: Where those patients are  
10 treated is going to be somewhat different too.

11           DR. NERENSTONE: Dr. Watson?

12           DR. WATSON: I think, adding on to what  
13 Dr. Griffin suggested, the idea of a centralized  
14 reference lab is one where the manufacturer or  
15 whoever is driving the trial is essentially  
16 establishing a certain standard that that  
17 laboratory is going to meet to initiate the trials  
18 within its laboratory. That is not uncommon to  
19 what happens if it is broadly distributed in a  
20 trial. Certain standards are met for the  
21 laboratories participating in the trial.

22           But my sense is that the devices world  
23 operates differently from the therapeutics world.  
24 In devices you are looking for that broad inter-  
25 laboratory comparison of performance to understand

1 how well that test works when dispersed as front-  
2 line criteria. Apparently, it is in the context of  
3 therapeutics that one might look at the centralized  
4 lab as a mechanism of controlling those variables  
5 to get to the endpoint of the therapeutic. There  
6 may be two somewhat different perspectives that are  
7 trying to get melded together.

8 DR. NERENSTONE: Dr. George?

9 DR. GEORGE: I just wanted to clarify that  
10 I wasn't speaking against this point here. I think  
11 that a central reference laboratory can be very  
12 useful, but I just wanted to be sure that we  
13 understand that this could very well be an  
14 impediment in clinical trials and in the kind of  
15 inferences that are drawn therefrom.

16 Another thing is that there is an implied  
17 model here, I think, that I would like to try make  
18 explicit. That is, you develop some targeted  
19 therapy based on the molecular biology or genetics  
20 of the disease in question. That implies that you  
21 need some way to measure the target reliably.  
22 Then, there is this implied implication that it is  
23 only those that express this target that will be  
24 benefited by this. I think we have to be careful  
25 with that in the sense that we don't necessarily

1 know that that is going to be the only pathway or  
2 the way this is going to work. What might have to  
3 happen is a broader-based eligibility requirement  
4 for trials, combined with some kind of storing of  
5 samples in which you can test these kinds of  
6 hypotheses.

7 DR. NERENSTONE: Dr. Lippman?

8 DR. LIPPMAN: Right, and just to follow-  
9 up, I agree with you and I thought I sort of made  
10 that point. You know, you go in with a hypothesis  
11 because, by definition, you are targeting a certain  
12 molecular event. But there are certainly some high  
13 profile cases now where the initial target you  
14 would have predicted is, in fact, not only the  
15 major one but not even involved in that so-called  
16 molecular targeted therapy. But I still think that  
17 by the time you get a pivotal Phase III you have  
18 this hypothesis based on a target, and that should  
19 be analyzed during the trial.

20 But getting back to Dr. Brawley's point  
21 about large, simple and issues on that, when I was  
22 referring to the central reference laboratory I was  
23 referring really more to the issue of collecting  
24 tissue. I mean, that is really the major sort of  
25 thing that takes a large simple trial to the next

1 level. The appropriate tissue needs to be  
2 required, and stored in a bank. It doesn't have to  
3 be analyzed right away to get back for real-time  
4 treatment decisions because we don't know. But I  
5 think that tissue should be collected, and that  
6 tissue can be analyzed centrally and locally to  
7 analyze the question how community standards  
8 correlate with central review.

9 DR. NERENSTONE: Dr. Keegan, do you have  
10 enough? Do you really want us to vote or do you  
11 really want the sense of the committee?

12 DR. KEEGAN: What we really want is the  
13 sense of the committee.

14 DR. NERENSTONE: For my two cents I would  
15 say that maybe you could even do an amalgam of both  
16 approaches, which in fact is being done in these  
17 two large clinical trials. A subset of patients  
18 are being analyzed, and that can certainly be  
19 written into the study. I just think you have to  
20 be very careful when you are doing an adjuvant  
21 trial of 1000 patients that you are going to really  
22 have problems accruing if all of them need a large  
23 tissue block, for all sorts of the reasons we have  
24 said, including cost, of having all 1000 patients  
25 be part of that when, in fact, your answer can

1 usually be gotten on a subset of patients, however  
2 you want to define that, prospectively.

3 DR. KEEGAN: Right. I think that we would  
4 define central testing based on a specific  
5 hypothesis and the sample size would be based on  
6 that hypothesis generally speaking.

7 The other point I wanted to make just real  
8 quickly was that the comment that Dr. George made  
9 regarding enrolling a broad population and then  
10 subsequently testing those who have the target or  
11 the target at a certain level or a certain  
12 threshold or cut point, is an idea that we are very  
13 comfortable with evaluating, and the level of  
14 comfort is probably somewhat contingent upon the  
15 degree of risk of enrolling patients who might not  
16 express a target and what they might be exposed to  
17 in terms of risk.

18 But in an initial sense there is no a  
19 priori opposition to studying in a broader sense  
20 and also confirming the utility of the targeted  
21 cell in that hypothesis. We do often have broader  
22 trials where the actual efficacy subset is a  
23 population containing the target rather than  
24 perhaps the broad population. We do endorse that  
25 concept as a way of getting more information about

1 the utility of the drug in some of these other  
2 populations.

3 DR. NERENSTONE: Dr. Blayney?

4 DR. BLAYNEY: I just want to point out  
5 that there is a possibility for bias to creep in  
6 there. If some centers supply tissue and others  
7 don't, there is a possibility for biasing the  
8 result in that way. Secondly, the medical legal  
9 business perhaps could be addressed by scrubbing or  
10 de-identifying the samples and identifying them  
11 only as to response so that some of these potential  
12 medical-legal objections could be dealt with up  
13 front.

14 DR. NERENSTONE: Dr. Albain?

15 DR. ALBAIN: I think when we are talking  
16 about pivotal trials, it is going to be almost  
17 impossible, if you only collect on a subset, to go  
18 back and get tissue because we have tried to do  
19 that in the groups and you just don't  
20 retrospectively get the blocks or the materials.  
21 We are going to be finding various subsets that are  
22 responding to one pathway targeting agent versus  
23 others and, given that the supply of these new  
24 agents is always limited, I don't think it is  
25 unreasonable to ask for tissue submission on all

1 patients going on to the pivotal trial, then using  
2 microarrays or whatever else to store them and then  
3 send the blocks back.

4 DR. NERENSTONE: To go on to the next  
5 question, the assessment of assay performance  
6 across multiple laboratories as part of either the  
7 pivotal trial or separate trial.

8 I think that is the best of all possible  
9 worlds, if you have something that is important you  
10 try and get it out and you assess to make sure that  
11 the other laboratories are, in fact, doing what you  
12 think they are doing. Any other comments? I think  
13 we have sort of covered that.

14 DR. REDMAN: I just want to make sure I am  
15 interpreting the question right. If a pivotal  
16 trial has been done, I am assuming that is the one  
17 that is going for the license application, and if  
18 it becomes approved and some other laboratory  
19 commercially comes up with a better assay, are we  
20 saying then that they have to attach their assay  
21 onto or get a clinical trial of an already approved  
22 agent to verify their assay? Or, is the assay  
23 going to be verified against some standard that  
24 exists, either archival tissue or some kind of  
25 standardization? I don't have a good grasp of what

1 the conclusion is even from the panel on that.

2 DR. NERENSTONE: Dr. Keegan, did you want  
3 to clarify your question?

4 DR. JERIAN: That is really a separate  
5 question than what this question is asking. This  
6 question is asking for the particular assay at  
7 hand, expanding it out to other laboratories.

8 DR. CARPENTER: So the answer would be  
9 that ideally you would try to do both.

10 DR. NERENSTONE: Dr. Lippman?

11 DR. LIPPMAN: I haven't looked at all the  
12 questions but I think Dr. Redman hit on a critical  
13 question. So, are we going to address that?

14 DR. JERIAN: The committee can address any  
15 questions they choose to. You are not limited to  
16 the questions here.

17 DR. NERENSTONE: Do you want to bring that  
18 up at the end so that we get through the questions  
19 you thought were important?

20 (c) Consideration as to when to study  
21 treatment of patients whose tissues are assay  
22 negative or weak, subset 1) if the relationship  
23 between the analyte and the efficacy of the drug is  
24 not definitive.

25 You are asking when should we allow this

1 new drug to patients who test weak or negative for  
2 the target if there is not a definitive  
3 relationship between efficacy and that specific  
4 target. Can you give us an example?

5 DR. KEEGAN: Well, I think that if one had  
6 a hypothesis that might not be backed up by an  
7 extensive amount of preclinical data, for instance,  
8 or a well-developed animal model and so the  
9 relationship was not entirely direct. Or, for  
10 instance, in this particular instance, FISH  
11 amplification which is downstream from the actual  
12 protein expression to which the antibody binds for  
13 Herceptin, if there wasn't a direct relationship  
14 that you felt was backed up by preclinical  
15 information and early clinical data, would the  
16 committee feel comfortable with suggesting that  
17 patients who test negative for the target could be  
18 enrolled and randomized in pivotal trials even if  
19 they may not have the analyte in question? And,  
20 what level of evidence should we have where we  
21 would no longer want to require that? What should  
22 be the cut point at which we would say, no, this is  
23 so definitive that we should never test this in  
24 people who are assay negative or don't express the  
25 target or the proposed target.

1 DR. NERENSTONE: I am not sure if that is  
2 an answerable question at this point. I think it  
3 is really going to depend on the toxicity of the  
4 drug you are talking about, the stage of the  
5 patient you are talking about, whether other  
6 medications are available to the patient.  
7 Obviously, if it is a metastatic patient who has  
8 blown through every other medication known to have  
9 an effect and who still has good performance  
10 status, you would feel differently than if you were  
11 looking at an adjuvant treatment, with a lot of  
12 toxicity, where it hasn't been proven yet to be  
13 effective in the strong marker positive patient.  
14 So, I think really has to be tossed back. I am not  
15 sure we can definitively answer that.

16 DR. KEEGAN: Right. Well, I would, for  
17 instance, refer back to Dr. Albain's discussion  
18 about enrolling a broad population of patients with  
19 lung cancer that would fall in this more extreme  
20 category of minimal alternatives where the testing  
21 will be done after the fact rather than as an  
22 eligibility criterion. Is there a level of  
23 discomfort with that, or what things should we  
24 consider in designing a trial of that nature? And,  
25 your comments were helpful.

1 DR. NERENSTONE: Remember, we are coming  
2 from a discipline where we have given  
3 extraordinarily toxic treatment and we have no idea  
4 why it works or how it works, and we have gotten  
5 comfortable doing that.

6 [Laughter]

7 We are sort of going backwards, and so our  
8 level of comfort -- you know, I think you sort of  
9 have to look at the specifics for us to feel that  
10 it is not worthwhile. Dr. Blayney?

11 DR. BLAYNEY: I think Stacy's point is  
12 well taken. The example I would say is breast  
13 cancer where, based on clinical observations, if  
14 you make a woman without hormones then she  
15 responds, and then the study population on which  
16 some of the drugs were approved did not take into  
17 account whether the tumor was ERPR positive. So,  
18 it is a moving target. I think the danger is that  
19 we rely on what we used in the last war to fight  
20 the next war and the war after that against these  
21 tumors, and this is a moving target and you need to  
22 look at the biology and allow the sponsor to  
23 demonstrate the biology of what they are trying to  
24 prove, and give them some latitude to be able to  
25 get their drug into the hands of doctors to treat

1 their patients.

2 DR. NERENSTONE: Dr. Lippman?

3 DR. LIPPMAN: No, I agree 100 percent.

4 That was precisely the point I was going to make.  
5 I mean, terms like "definitive relationship" are  
6 like the difference between 1+ and 2+ staining. I  
7 think it is on the sponsor to show the biology  
8 behind this and make a compelling case based on  
9 that, that this relationship is important. I think  
10 it is almost a case by case sort of evaluation,  
11 except that that is an important part of the  
12 approval of molecular target drugs being based on a  
13 compelling case of the biology.

14 DR. KEEGAN: But to that extent then, in  
15 order to make that compelling case in the clinical  
16 trials it would require that you enroll patients  
17 without the target to look at that effect as well.  
18 So, it would be the sense of the committee that  
19 that is an appropriate approach in some settings?

20 DR. TAYLOR: Like Stacy said, it is going  
21 to depend on the stages of disease and the type of  
22 patient. It has to be very individualized.

23 DR. NERENSTONE: Dr. Redman?

24 DR. REDMAN: Yes, I think the important  
25 point in the question is efficacy. I mean, if

1 there is some preclinical evidence that the drug is  
2 going to have efficacy and you are not sure what  
3 the relationship is to a specific target, as in  
4 most things in oncology, I don't think any  
5 oncologist is going to say, well, no, not until you  
6 guys go develop the assay. Come back to me in five  
7 years and maybe we will look at it then. In lung  
8 cancer, in colon cancer and breast cancer advanced  
9 diseases where the effective therapies are minimal,  
10 it doesn't have to have a real strong correlation  
11 but there ought to be some method to bank the  
12 tissue, as much as we possibly can, to try to  
13 answer the question because if it is effective it  
14 would be nice to know why it is effective. The  
15 corollary that we don't do much in oncology is also  
16 if it is not effective, it would be really nice to  
17 know why it is not effective so we don't go down  
18 the same path again.

19 DR. NERENSTONE: Ms. Mayer?

20 MS. MAYER: I think it is important that  
21 we don't prematurely shut down our understanding of  
22 just what the patient population who may respond to  
23 any given drug is. We are still discovering new  
24 applications for Herceptin. We are still looking  
25 at new combinations that may be effective, and the

1 more we narrow the patients who can enter into the  
2 trials, the less we know about the broader  
3 applicability. I think that has to be weighed in  
4 relation to the potential toxicity. We also may  
5 move trials further along if there is a broader  
6 eligibility among patients. We may be able to  
7 accrue them more rapidly.

8 DR. NERENSTONE: Dr. Lippman?

9 DR. LIPPMAN: I think the issue is do you  
10 mean that when you have a molecular target therapy  
11 you select patients based on expression of that  
12 target, and only those patients? I think in most  
13 case, Herceptin aside maybe, that should not be the  
14 case. It should be broad-based because the more we  
15 look into it the more we understand the complexity  
16 of these molecular target agents and we can really  
17 miss something. So, I think we can all agree, if  
18 that is your question, that broad-based trials in  
19 many cases with these newer targets are  
20 appropriate.

21 DR. NERENSTONE: And I think we have  
22 touched on part two, if the assay is not  
23 dichotomous, as well as if the assay method is not  
24 validated. Dr. Kelsen?

25 DR. KELSEN: I was ready to go on to (d)

1 because (d) looks to me like it is a different  
2 point. I agree and I can certainly understand why  
3 you would treat patients who may not express the  
4 target, express it weakly, the assay is not fully  
5 defined in Phase I and II trials. I assume that  
6 question (d) is you did that and now you are ready  
7 to do the pivotal trial, and now presumably there  
8 is enough information for a strong hypothesis that  
9 X has to be at some certain level in order to see  
10 benefit and now you want to prove that that is  
11 true.

12 If that is correct, it would certainly be  
13 highly desirable to make sure that you don't enter  
14 a trial until you have validated the assay. On the  
15 other hand, you don't want to be in a position  
16 where you have an enticing compound and the assay  
17 hasn't yet been developed. You want to move  
18 forward. Or, are you going to wait the one to two  
19 to X years to do that? So, I am not sure that you  
20 can say put it on clinical hold indefinitely until  
21 the assay is developed versus we have a  
22 standardized assay; it is the best assay we  
23 currently have available. We are all doing to use  
24 this assay. We understand its flaws, and there  
25 will be some patients who are truly negative. I

1 think we saw that from the NSABP trial. This may  
2 be the crucial point.

3 DR. KEEGAN: It will definitely be a  
4 crucial point for advice that we would give a  
5 pharmaceutical manufacturer. That is basically an  
6 issue that has to be dealt with before one begins  
7 enrollment, or that is an issue that could be  
8 evaluated during the conduct of the clinical trial.  
9 Obviously, it is an issue that has to be dealt with  
10 before the analysis of the trial.

11 DR. KELSEN: Sure, but I would wonder,  
12 from all the things we just heard this morning from  
13 the two cooperative groups -- that sort of  
14 information makes me think, boy, it would be  
15 extremely desirable to have a set of central labs  
16 or a central lab so that at least you know that in  
17 this pivotal trial everybody is getting the best  
18 assay done in the same way, and not have the  
19 situation where you have multiple labs allowing  
20 entrance into the trial.

21 DR. NERENSTONE: Dr. Lippman?

22 DR. LIPPMAN: David, to follow-up on your  
23 point, I think if you have Phase I and Phase II  
24 data like, let's say, Glivec, the magic bullet.  
25 Then I think by the time you get to anything you

1 know exactly what is going on. I don't know that  
2 we are going to have a lot of other situations like  
3 that, or probably not the majority. I think short  
4 of that, between Phase I and Phase II trials, you  
5 need broad eligibility criteria because if you  
6 select just based on a marker that you think is  
7 important with reasonably positive Phase II trial  
8 data I think you may miss some important targets.  
9 So, I think that is where it comes down to how  
10 compelling it is in terms of how the drug works  
11 from these earlier phase trials. I think in most  
12 cases we are just not going to be that confident  
13 that that target is the one and only answer to this  
14 drug.

15 DR. KELSEN: I just want to respond. I  
16 see the point. I am just thinking about some of  
17 the data where patients who have a certain level of  
18 "expression", from 0-49 or from 0-50 in a Phase II,  
19 how comfortable are you entering patients into a  
20 trial?

21 DR. LIPPMAN: Well, it is based on 12  
22 patients. I think your question is how compelling  
23 it is.

24 DR. KELSEN: Yes, how compelling?

25 DR. NERENSTONE: Dr. O'Leary?

1 DR. O'LEARY: I think the point here is  
2 that you can establish before the trial inter-  
3 laboratory reproducibility, intra-laboratory  
4 reproducibility, and we reference to some external  
5 standard perhaps, whether or not it is measuring  
6 the analyte, but the important thing for  
7 consideration is whether it is predictive of  
8 therapeutic response and it is the trial itself  
9 that answers that and the patient's tissue. The  
10 hope is that you will get out the answers as part  
11 of the trial itself or using the tissue that was  
12 accrued afterwards.

13 DR. NERENSTONE: Dr. Carpenter?

14 DR. CARPENTER: I think reasonable advice  
15 to a manufacturer in this situation would be that  
16 the better validated the assay is at the time of  
17 the clinical trial, the more helpful it is. But  
18 the less well validated the assay is, the more  
19 critically important it is going to be collect  
20 tissue as you go so that if you can't answer it up  
21 front you still may be able to approach the answer  
22 when you get more knowledge.

23 DR. NERENSTONE: I think one other of the  
24 issues is a statistical one, and I think any  
25 company has to be made well aware of the fact that

1 if one of their endpoints is muddy, for instance  
2 the correlation between an assay result and a  
3 clinical response, when you do subset analysis, if  
4 that is not pre-described in the application, you  
5 can't go back and say, well, it didn't work for the  
6 big group but here, in this subset, it did work.  
7 That is no longer a valid post hoc analysis to  
8 justify approval for the new drug. I think that is  
9 really the danger, and I would think that the  
10 sponsor should be apprised of that problem because  
11 that is always what we hear time, after time, after  
12 time, sitting through these meetings.

13 We will go on to number (e), the use of  
14 specimen banking in order to have material  
15 available to address advances in the molecular  
16 target assessment pre- or postmarketing. We have  
17 discussed that.

18 DR. KEEGAN: Yes, I think you have  
19 discussed that really for the premarketing. I know  
20 Dr. Albain has expressed a preference that we  
21 gather enough samples to have available for  
22 postmarketing innovative assays that might come  
23 down the line; that more tissue specimens be banked  
24 so that they might be available from patients. No?

25 DR. ALBAIN: My comments had to do with

1 the pivotal trials, and having tissue available on  
2 all patients that go on pivotal studies.

3 DR. KEEGAN: Right, primarily for purposes  
4 of additional assays or other assays that might  
5 become available. I wasn't sure if the committee  
6 uniformly felt that that should be done because of  
7 the tension in the large, simple trials and the  
8 complications --

9 DR. ALBAIN: But if you are going to have  
10 a response rate of X that is not a Glivec level  
11 response in CML, smaller but yet in a common solid  
12 tumor still potentially meaningful, you are going  
13 to need to have samples on the whole trial  
14 population in order to get down to which of the  
15 target profiles need to be positive to see those  
16 responses. Can we ask what some of the  
17 pathologists think about that?

18 DR. NERENSTONE: I know Dr. Watson wanted  
19 to make a comment.

20 DR. WATSON: Only that I think it is not  
21 "if" new technologies come. There are already four  
22 or five competing technologies for amplification  
23 coming down the pike, and having these resources  
24 available -- I mean, we are still to some extent  
25 stuck in that circular what is the gold standard

1 argument, and the fact that you have multiple  
2 mutation mechanisms by which amplification can  
3 occur -- but I think having the tissue available is  
4 the shortest route to those analytical comparisons  
5 on the front end that can rein in the wide  
6 dispersion of things while they are being sorted  
7 out.

8 DR. NERENSTONE: But I am not sure that  
9 you can require a drug company sponsor to bank  
10 tissue for further hypothesis generation. I think  
11 what you are asking is two separate questions. One  
12 is the link between a marker, which is the  
13 hypothesis they are proposing, and a response and  
14 the scientific importance of going forward. But I  
15 don't know that we can link approval of a Phase III  
16 definitive study to the promise that they are going  
17 to do that for us. I mean, that is more a  
18 regulatory issue.

19 DR. KEEGAN: I guess the question was  
20 would it be appropriate to advise manufacturers to  
21 take into account that there will be advances in  
22 assay methodology, and that the best practice would  
23 be to bank specimens on all patients enrolled in  
24 the trial so that one can do the kinds of assays  
25 that were done for the FISH testing, that Dr.

1 O'Leary had been available at the time that he  
2 reviewed the HercepTest data. It may not rise to  
3 the level of a requirement, but is this important  
4 enough that we should bring this up routinely as a  
5 strong recommendation, for instance, to the  
6 pharmaceutical industry so that they would be able  
7 to keep up with the advances in the new technology,  
8 and be able to make statements included in their  
9 labeling, and so on?

10 DR. NERENSTONE: Dr. Lippman?

11 DR. LIPPMAN: I have a comment to address  
12 that, and this may address in some way Dr. Redman's  
13 point. I think we should strongly encourage that  
14 they bank tissue because, clearly, if it were  
15 banked and a new assay came up -- I am not talking  
16 about a new gene but a new assay for the same test,  
17 you could do it on a subgroup. It is post hoc but  
18 at least you could do some sort of analysis to see  
19 if that assay also correlated with outcome. That  
20 would really help. But I agree with Stacy, I don't  
21 know that you can mandate that they collect it for  
22 this reason.

23 But I think what you can mandate  
24 premarketing is if the hypothesis is based on  
25 antigen X or protein X, and they are collecting

1 tissue for that, then I think it should go to the  
2 market until they have analyzed whether in fact  
3 that works, because that is what has to be in the  
4 label. I think that should be mandated  
5 premarketing, and if the tissue were available Dr.  
6 Redman's question would be answered postmarketing  
7 with new assays and new genes, for that matter.

8 DR. NERENSTONE: Dr. O'Leary?

9 DR. O'LEARY: For the reasons that you  
10 gave, I think it would be hard to mandate that they  
11 do that for post hoc analysis, but I think that  
12 strong encouragement is quite worthwhile, and I  
13 think if manufacturers think it through they will  
14 find it is in their commercial interest to do it.  
15 One can again just look at the issues at hand now  
16 to see why. So, I think making particularly small  
17 manufacturers, who do much of the innovative work,  
18 aware of those issues could be a very, very useful  
19 thing and could make Steve's job easier down the  
20 line.

21 DR. NERENSTONE: Dr. Blayney?

22 DR. BLAYNEY: Well, I disagree a little  
23 bit with Dr. Lippman. I think that we need to move  
24 these compounds to market as quickly as they are  
25 shown to be efficacious. But I think to the extent

1 that the agency enforces these promises that  
2 sponsors will do Phase IV or postmarketing studies,  
3 the sponsor would be well advised to collect tissue  
4 so that these kinds of studies could be done  
5 expeditiously and really demonstrate the results  
6 from the Phase IV studies to the extent you hold  
7 them to those promises.

8 DR. NERENSTONE: Dr. Watson?

9 DR. WATSON: I think the problem is that  
10 there are different kinds of manufacturers. I  
11 think from the therapeutics perspective the  
12 interest is in having all those technologies sorted  
13 out as to what works well and best and how they  
14 compare. But if you are the manufacturer of one of  
15 the devices for testing, I am not sure you are  
16 going to be collecting specimens for the other  
17 manufacturer to demonstrate that their device is  
18 better than yours. So, I think it is sort of  
19 figuring out how our government can better work  
20 together because I see the collection of specimens  
21 as an NIH responsibility probably for some issues  
22 that cannot necessarily be dumped on the  
23 manufacturer. I think the specimens are valuable  
24 for sorting out a lot of these issues, but there  
25 are different interests for different manufacturing

1 communities in doing those sorts of things.

2 DR. NERENSTONE: Dr. Lippman?

3 DR. LIPPMAN: This isn't the first time  
4 that Doug and I have disagreed, but I will say that  
5 I think if you have compelling data and you are  
6 targeting a certain molecule, and you have a  
7 response rate of 30 percent in the whole trial, and  
8 the whole hypothesis is based on the expression of  
9 this particular molecule and it is built up front,  
10 you need to know whether it is 60 percent in one  
11 group and zero percent in the other. So, I think  
12 that it is important in that primary hypothesis to  
13 do this premarketing to help guide physicians on  
14 the labeling.

15 Again, we have talked about the banking  
16 and postmarketing, and that is very complicated. A  
17 strong recommendation would be great for all the  
18 reasons we have talked about, but it is hard to  
19 mandate.

20 DR. NERENSTONE: Dr. Brawley?

21 DR. BRAWLEY: Just very briefly, if we  
22 encourage companies to do postmarketing research  
23 there will be some instances where a company will  
24 not want their product to be evaluated because they  
25 wouldn't want negative findings. So, I really

1 think we have to do this up front. I am supporting  
2 Scott in my comments.

3 DR. NERENSTONE: If we could go on to the  
4 next question -- that was all question one, guys,  
5 so if we want lunch we have to get going. I am  
6 going to consider question two in its entirety.  
7 Oncologists will generally not have access to the  
8 package insert, or PI, for a molecular assay. For  
9 that reason, FDA feels it is prudent to provide  
10 information regarding molecular assays in the PI  
11 for the therapeutic product. Please discuss what  
12 types of information would be appropriate for  
13 inclusion in the therapeutic package insert.  
14 Specifically, discuss the following: The cautions  
15 regarding use and interpretation of these assays  
16 and about inter-laboratory variability; the  
17 information on assay validation; the information on  
18 assay performance characteristics; the information  
19 on tissue handling, for instance formalin fixed or  
20 frozen; the comparisons between assay methods when  
21 available; and the information on clinical outcomes  
22 as a function of the assay result.

23 You really don't want us to read the PI.  
24 Right? Because I think the more information you  
25 put in, the less likely it is that anybody is

1 really going to spend the time looking at it. So,  
2 I think you have to be very careful about what you  
3 really want in the package insert. I understand  
4 your concern.

5 DR. KEEGAN: Do you have an alternative  
6 suggestion, as you discuss this, for where people  
7 might look for the information.

8 DR. NERENSTONE: Dr. Redman?

9 DR. REDMAN: I think they should look to  
10 their pathologist. I do not know what the  
11 characteristics of CEA or PSA in my laboratory are  
12 to the degree that is put here, and quite  
13 truthfully, I think probably the package insert for  
14 Herceptin should recommend it for HER2 positive and  
15 leave it there, and leave it to the pathologists  
16 and the American College of Pathology to define  
17 what those parameters are in the assays that they  
18 use for those tests.

19 DR. NERENSTONE: Dr. O'Leary?

20 DR. O'LEARY: Your pathology department  
21 probably has a long book, web site or something  
22 with test characteristics and what is going on. I  
23 would think it might be worthwhile to consider  
24 putting in the package insert a general statement  
25 to the effect that differences in assay

1 characteristics, and so forth, can be important,  
2 and then suggesting or at least making people aware  
3 that it might be a good idea to talk to their  
4 laboratory about how that influences what they are  
5 doing.

6 Any way of encouraging a clinician-  
7 laboratory dialogue I think is a very good thing,  
8 but I think it would be very difficult to write a  
9 finite length patient package insert which we not  
10 risk being misleading to a substantial percentage  
11 of the people who would actually read it.

12 DR. NERENSTONE: We also depend on our  
13 pathologists. As oncologists, when they tell us it  
14 is breast cancer we believe them. So, I think that  
15 dialogue is extraordinarily important. Dr.  
16 Brawley?

17 DR. BRAWLEY: Yes, once again Dr. O'Leary  
18 has stolen my thunder, but I deal with a lot of  
19 especially urologic oncologists with PSA where  
20 there is just not an appreciation that there is an  
21 inter-assay variability among physicians, among  
22 patients and so forth. So, in my mind, it actually  
23 is very important that there be at least a mention  
24 that there is this validation variability issue  
25 because then it justifies it as a legitimate issue

1 in a lot of people's minds.

2 I think if you put too much data there,  
3 you are just going to confuse people even more but,  
4 you know, encouraging people to look to  
5 pathologists, to others who might understand the  
6 variability and that there should be caveats in the  
7 interpretation of any test I think is a very  
8 important thing to do.

9 DR. NERENSTONE: Dr. Kelsen?

10 DR. KELSEN: I would support that. I  
11 think if I was looking at the package insert I  
12 wouldn't want a tremendous amount of detail because  
13 I think it would be confusing. But the fact that  
14 clinical outcome varies depending on the assay  
15 result would be of some significant interest, and I  
16 think we heard a lot about that this morning. So,  
17 a general warning to be aware that there are  
18 different assays and how the test is done may  
19 substantially affect what you actually see in your  
20 patients and guide them to talk to their  
21 pathologist.

22 DR. NERENSTONE: Dr. Lippman?

23 DR. LIPPMAN: I agree with that. But I  
24 think if you are talking about a FISH so you are  
25 looking at a gene-based assay versus protein, I

1 think in the package insert you have to indicate  
2 the study that was done, the types of assays that  
3 were done to come up with the approved drug, and  
4 then some recommendations, however we couch it,  
5 based on discussions of this committee and how CBER  
6 takes that, but some recommendations on how to talk  
7 to your pathologist, and at that point concordance  
8 data and other issues will be looked at.

9 In other words, I don't know that the  
10 pathologist in every case knows about the pivotal  
11 clinical trial and what assay was used there. So,  
12 I think that kind of information would be useful to  
13 have in the package insert, but not limited to that  
14 assay.

15 DR. NERENSTONE: Just a point of  
16 information, would you then go back for tamoxifen  
17 and re-write the PI to tell how the ERs and PRs  
18 were obtained for the pivotal data? Dr. Keegan?

19 DR. KEEGAN: Not actually directly  
20 reviewing those, I can't say. I don't know if that  
21 would be appropriate or not. I think that that is  
22 a decision that probably should be made based on  
23 looking at the data and the relative issues. In  
24 this particular instance, and having seen what we  
25 saw with the home brew assays and the variability

1 that exists, our concern was that if we don't say  
2 anything physicians would not even know to speak to  
3 their pathologist about what method was used.

4 DR. NERENSTONE: Ms. Mayer?

5 MS. MAYER: Patients don't normally have  
6 access to their pathologists and rely on their  
7 oncologists, of course, for all the relevant  
8 information. If the oncologist is not informed  
9 about the problems regarding a particular assay,  
10 and doesn't at least have a rudimentary sense of  
11 clinical outcomes for the assay, then he or she  
12 cannot communicate to patients in such a way that  
13 they can make, I think, the best treatment  
14 decisions. I think it is really crucial to get at  
15 least the sense of the reliability of this  
16 information into the oncologist's hands to  
17 communicate to patients.

18 DR. NERENSTONE: Dr. Blayney?

19 DR. BLAYNEY: I would like to say that I  
20 practice in several hospitals, one of which would  
21 be classified as a small volume IHC, and when I do  
22 talk to my pathologist she will tell me, you know,  
23 I know best. But hearing the lecture today on  
24 antigen retrieval and fixation I think is a very  
25 useful thing for me to discuss across the

1 microscope with my pathologist. So, I would  
2 encourage you to place at least a rudimentary  
3 amount of information in the package insert and --  
4 perhaps it doesn't rise to the level of a warning,  
5 but some caution that these are subject to  
6 interpretation when it is the case.

7 DR. NERENSTONE: Dr. Redman?

8 DR. REDMAN: I am not against informing in  
9 the package insert oncologists or medical  
10 practitioners of the inherent problems with  
11 different assays. I guess the question is next  
12 time a new assay comes out are we going to revise  
13 the package insert again, and again, and again?  
14 Or, are we just picking a target and saying  
15 HER2/neu positive and there are problems with the  
16 assays but we are not specifically recommending any  
17 particular assay?

18 DR. KEEGAN: I think that depends upon  
19 several things. One is, for instance, if we are  
20 approached, first of all, to put in additional  
21 information about the use of an assay selection  
22 method. The second would be if we are aware of  
23 information affecting the public health about the  
24 failure of a particular assay, in which case it  
25 would probably be appropriate.

1           Beyond that, there are probably some grey  
2 zones where we wouldn't have information in the  
3 package insert because it didn't fall into one of  
4 those categories. But I think for the other two we  
5 do need to make some information available, and the  
6 question was the extent. We do have the sense of  
7 the committee that you don't want to be over-  
8 burdened with data, but to put in relevant  
9 information on aspects that might impact your  
10 clinical decision-making and what precautions  
11 people should be aware of in samples, and the  
12 handling and interpretation.

13           DR. NERENSTONE: I certainly think in the  
14 pivotal trial, if either of these adjuvant trials  
15 are positive then a little bit of discussion about  
16 the assays and the patients who were treated is  
17 good clinical judgment, just the way we would write  
18 a toxicity write-up for a usual cytotoxic. Then  
19 you would not have to keep reviewing it as the  
20 assay changed because you are really referring to  
21 the pivotal trial upon which FDA approval was based  
22 for that indication.

23           Last question, again, I think we have  
24 touched on this a bit, the assessment of the  
25 clinical utility of an assay to accurately select

1 patients who will benefit from a therapeutic can be  
2 best be performed in the context of a prospective,  
3 randomized clinical trial.

4 Do you concur that this is the most  
5 appropriate method for assay utility assessment?

6 Once a trial has been completed and if we  
7 assume that samples from patients have been banked,  
8 is testing of the banked specimens using a new  
9 assay a reasonable alternative for assay utility  
10 assessment?

11 Maybe we will ask the pathologists to  
12 comment on that.

13 DR. O'LEARY: In terms of (a), I think it  
14 all depends on the exact design of that trial, but  
15 I think done in the context of a carefully designed  
16 clinical trial that really answered the relevant  
17 questions it is a very good thing to do.

18 I think that bank specimens are a pretty  
19 good alternative but that they have their strengths  
20 and their limitations. We invariably will learn  
21 more about assay performance after it gets out of  
22 the original few laboratories that started in  
23 clinical trial characteristics, and it will come  
24 out into the literature and that is why in this  
25 context, and in the context of the previous

1 questions, establishing that dialogue with your  
2 pathologist and encouraging them to be aware of  
3 what is going on is really, really important.

4 DR. NERENSTONE: Any other comments? Dr.  
5 Lippman?

6 DR. LIPPMAN: I think we discussed this  
7 when we discussed 1(e) really. I think ideally  
8 this would be the way to do it. The importance of  
9 banking is to first test it against clinical  
10 outcome and instead of, as Dr. Redman mentioned,  
11 doing a separate clinical trial, if you have tissue  
12 available for this you can answer that question  
13 within the context of a completed trial.

14 DR. NERENSTONE: Other comments? Dr.  
15 Keegan, do you have what you need from this  
16 discussion?

17 DR. KEEGAN: Yes.

18 DR. NERENSTONE: What I would like to do  
19 is adjourn the committee for lunch. We will give  
20 everybody an extra 15 minutes. So, if you can come  
21 back to the table by 2:00, we will start on time at  
22 2:00. Thank you.

23 [Whereupon, at 12:56 p.m., the proceedings  
24 were recessed, to reconvene at 2:08 p.m., this same  
25 day.]

AFTERNOON SESSION

1  
2 DR. NERENSTONE: What I would like to do  
3 is to go around the table again because there are  
4 people who were not here for the morning session,  
5 and just introduce yourself. Mr. Ohye, you did  
6 such a good job to start with.

7 MR. OHYE: George Ohye, industry rep.

8 DR. O'LEARY: Tim O'Leary, the Armed  
9 Forces Institute of Pathology.

10 DR. WATSON: Michael Watson, American  
11 College of Medical Genetics.

12 DR. BARKER: Peter Barker, the National  
13 Institute of Standards and Technology.

14 DR. CARPENTER: John Carpenter, medical  
15 oncologist, the University of Alabama at  
16 Birmingham.

17 MS. MAYER: Musa Mayer, patient  
18 representative.

19 DR. ALBAIN: Kathy Albain, medical  
20 oncologist, Loyola University, Chicago.

21 DR. GEORGE: Stephen George,  
22 biostatistics, Duke University Medical Center.

23 DR. KELSEN: David Kelsen, Sloan-  
24 Kettering.

25 DR. NERENSTONE: Stacy Nerenstone, medical

1 oncology, Hartford, Connecticut.

2 DR. TEMPLETON-SOMERS: Karen Somers,  
3 executive secretary to the committee, FDA.

4 DR. BRAWLEY: Otis Brawley, medical  
5 oncologist, Emory University.

6 DR. LIPPMAN: Scott Lippman, M.D. Anderson  
7 Cancer Center.

8 DR. BLAYNEY: Doug Blayney, medical  
9 oncologist, Wilshire Oncology Medical Group,  
10 Pasadena.

11 DR. TAYLOR: Sarah Taylor, medical  
12 oncology, University of Kansas Medical Center in  
13 Kansas City.

14 DR. REDMAN: Bruce Redman, medical  
15 oncologist, University of Michigan Cancer Center.

16 DR. GRIFFIN: Connie Griffin, Johns  
17 Hopkins University.

18 DR. GUTMAN: Steve Gutman, Division of  
19 Clinical Laboratory Devices in the Office of Device  
20 Evaluation, FDA.

21 DR. JERIAN: Susan Jerian, medical  
22 officer, Center for Biologics, Division of Clinical  
23 Trials.

24 DR. KEEGAN: Patricia Keegan, Division of  
25 Clinical Trials, Center for Biologics, FDA.

1                                   **Conflict of Interest Statement**

2                   DR. TEMPLETON-SOMERS: The following  
3 announcement addresses the issue of conflict of  
4 interest with respect to this meeting, and is made  
5 part of the record to preclude even the appearance  
6 of such at this meeting. Based on the submitted  
7 agenda and information provided by the  
8 participants, the agency has determined that all  
9 reported interests in firms regulated by the Center  
10 for Drug Evaluation and Research present no  
11 potential for a conflict of interest at this  
12 meeting, with the following exceptions:

13                   In accordance with 18 USC, Section  
14 208(b)(3), Dr. Scott Lippman has been granted a  
15 full waiver. A copy of Dr. Lippman's waiver  
16 statement may be obtained by submitting a written  
17 request to the agency's Freedom of Information  
18 Office, Room 12A-30 of the Parklawn Building.

19                   In addition, Dr. Sarah Taylor's employer,  
20 the University of Kansas Medical Center, has  
21 interests which do not constitute financial  
22 interests in the particular matter within the  
23 meaning of 18 USC, Section 208 but which could  
24 create the appearance of a conflict. The agency  
25 has determined, notwithstanding these interests,

1 that the interest of the government in Dr. Taylor's  
2 participation outweighs the concern that the  
3 integrity of the agency's programs and operations  
4 may be questioned. Therefore, Dr. Taylor may  
5 participate fully in this morning's discussions and  
6 vote.

7 Further, Dr. George Sledge will be  
8 excluded from participating in the discussions and  
9 vote concerning the labeling supplement for  
10 Herceptin.

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

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

1 whose product they may wish to comment upon.

2           Again, I would also like to note for the  
3 record that Dr. Jody Pelusi, our usual and  
4 appointed consumer representative, had to cancel  
5 her participation in this meeting just yesterday  
6 and there was no time to get and prepare a  
7 replacement consumer rep. Again, we are fortunate  
8 to have Ms. Musa Mayer as a patient representative  
9 to provide that point of view. Thank you.

10                           **Open Public Hearing**

11           DR. NERENSTONE: We go now to the open  
12 public hearing portion of the meeting. Ms.  
13 Margaret Volpe, from Y-ME National Breast Cancer  
14 Organization.

15           MS. VOLPE: Good afternoon. My name is  
16 Margaret Volpe, and I am a breast cancer survivor  
17 and a volunteer representing Y-ME National Breast  
18 Cancer Organization. I would like to thank the  
19 committee for allowing me to speak this afternoon.  
20 I have no personal interest in Vysis, but Y-ME in  
21 2000 did get a small grant from Vysis.

22           Y-ME National Breast Cancer Organization  
23 would like to express its support of the use of  
24 fluorescence in situ hybridization testing using  
25 the PathVysion HER2 DNA probe kit, Vysis, Inc., as

1 a diagnostic method to select patients for  
2 Herceptin therapy.

3 Y-ME National Breast Cancer Organization  
4 is the premier resource for breast cancer  
5 information, education and support for those  
6 diagnosed with the disease, their family and  
7 friends, and those concerned about breast cancer  
8 and breast health. The mission of the organization  
9 is to decrease the impact of breast cancer, create  
10 an increased breast cancer awareness and ensure,  
11 through information, empowerment and peer support,  
12 that no one faces breast cancer alone.

13 The determination of thee level of  
14 HER2/neu expression for all newly diagnosed  
15 patients with invasive breast cancer is now  
16 recommended. HER2/neu level of expression is used  
17 to provide prognostic information to predict for  
18 the superiority of anthracycline-based adjuvant  
19 chemotherapy over CMF chemotherapy, and to predict  
20 for benefit from trastuzumab therapy in women with  
21 recurrent or metastatic breast cancer.

22 The new data presented at the ASCO 2001  
23 conference showed that the response to Herceptin  
24 occurs predominantly in patients whose tumors are  
25 positive by FISH, confirming that the FISH assay is

1 more reliable than IHC in identifying candidates  
2 for Herceptin. Mass et al. reported patient  
3 selection based on HER2/neu amplification by FISH  
4 may predict improved clinical benefit from the  
5 addition of H to C compared to selection by IHC.  
6 This includes a substantial survival benefit. This  
7 data supports FISH testing for selecting patients  
8 for Herceptin therapy.

9           Vogel et al. reported results corroborate  
10 earlier findings and suggest that FISH is a  
11 superior method for selection of patients for  
12 Herceptin therapy. A similar conclusion, that the  
13 determination of HER2 gene copy number by FISH may  
14 be a more accurate and reliable method for  
15 selecting patients eligible for trastuzumab therapy  
16 was reported by Tubbs et all.

17           The use of Herceptin for women with  
18 metastatic breast cancer or in clinical trials to  
19 determine its safety and effectiveness in the  
20 adjuvant setting carries great promise and serious  
21 potential side effects. Women need access to the  
22 most accurate form of testing available. Based on  
23 recent findings, Y-ME believes FISH testing using  
24 the PathVysion HER2 DNA probe kit, Vysis, Inc., as  
25 a diagnostic method to select patients for

1 Herceptin therapy should be approved. Thank you.

2 DR. NERENSTONE: Thank you very much. We  
3 now turn to Genentech to start the sponsor  
4 presentation for Herceptin indicated for the  
5 treatment of patients with metastatic breast cancer  
6 who have tumors which overexpression HER2, to  
7 include the use of fluorescence in situ  
8 hybridization testing using the PathVysion HER2 DNA  
9 probe kit as a diagnostic method to select the  
10 patients for Herceptin therapy. Dr. Armstrong?

11 **BLA 103792\5008, a Labeling Supplement for**

12 **Herceptin**

13 **(trastuzumab), Genentech, Inc.**

14 **Introduction**

15 DR. ARMSTRONG: Advisory committee  
16 members, FDA and guests, good afternoon. My name  
17 is Marianne Armstrong, and I am senior director of  
18 regulatory affairs at Genentech. On behalf of  
19 Genentech, I would like to thank you for this  
20 opportunity today to present our data to you  
21 regarding our s/BLA for Herceptin.

22 Our purpose in being here today is to seek  
23 approval of our s/BLA that requests inclusion of  
24 fluorescence in situ hybridization testing,  
25 commonly known as FISH, using the PathVysion HER2

1 DNA probe kit, manufactured by Vysis, in our  
2 current Herceptin label.

3 As many of you are well aware, Herceptin  
4 is a recombinant DNA-derived, humanized monoclonal  
5 antibody that targets HER2, the protein product of  
6 C ErbB2. It is important to note that more than  
7 60,000 women worldwide have received Herceptin  
8 since its market introduction.

9 In September of 1998, Herceptin was  
10 approved for two indications, the first being for  
11 first-line treatment in combination with paclitaxel  
12 in metastatic breast cancer patients whose tumors  
13 overexpress HER2.

14 Additionally, we were approved for second-  
15 or third-line single agent therapy in metastatic  
16 breast cancer patients whose tumors also  
17 overexpress HER2.

18 The only FDA approved diagnostic method to  
19 aid in the selection of patients for Herceptin  
20 therapy is immunohistochemistry, commonly referred  
21 to as IHC. The two FDA approved HER2 diagnostic  
22 kits include the HercepTest and Pathway. Only the  
23 HercepTest test is included in the Herceptin  
24 package insert.

25 In the presentations that you will hear

1 this afternoon, we will present data that  
2 demonstrate that PathVysion or HER2 FISH diagnostic  
3 kit is an appropriate method to aid in the  
4 selection of patients for Herceptin therapy.

5           The data that we will present to you will  
6 include an overview of HER2 biology and the  
7 scientific rationale. Concordance data from our  
8 Herceptin clinical trials will also be presented.  
9 At the request of FDA, we will present exploratory  
10 clinical outcomes analysis also based on our  
11 Herceptin clinical database.

12           Our agenda for this afternoon includes Dr.  
13 Michael Press, who is a pathologist and professor  
14 within the Department of Pathology at the Norris  
15 Comprehensive Cancer Center at USC. Dr. Press will  
16 speak to you regarding HER2 biology and methods of  
17 assessment.

18           Next will be Dr. Robert Mass, a medical  
19 oncologist and associate director within medical  
20 affairs at Genentech. Dr. Mass will present our  
21 concordance and clinical outcomes analyses, as well  
22 as our conclusions for the day.

23           In summary, our goal is to demonstrate to  
24 you that PathVysion is an appropriate method to aid  
25 in the selection of patients for Herceptin therapy.

1 Thank you.

2 I now would like to introduce Dr. Michael  
3 Press, from the University of Southern California.

4 **HER2 Biology and Assessment**

5 DR. PRESS: Thank you. Before I begin the  
6 formal presentation I have been asked to address  
7 two issues. One has to do with my experience in  
8 this area, and the other one has to do with  
9 disclosure of my relationship to the sponsors.

10 I am a pathologist at the University of  
11 Southern California, with a long-standing interest  
12 in HER2/neu. My interest there really covers three  
13 different areas. One is a basic science research  
14 activity which has been grant supported. We have  
15 been active in this area continuously since 1987  
16 when we began a collaboration with Dr. Dennis  
17 Slamon.

18 The second area involves our laboratory as  
19 a reference laboratory for HER2/neu testing. We  
20 are a College of American Pathologists approved  
21 laboratory, and we analyze more than 2000 samples a  
22 year in this capacity.

23 In addition, earlier this year we were  
24 selected as the central laboratory for the breast  
25 cancer international research group, and we

1 anticipate analyzing approximately 7500 samples  
2 over the next two years in our laboratory.

3           In terms of my relationships with these  
4 two companies, I have a research contract with  
5 Genentech to analyze specimens for HER2/neu that  
6 are sent to our laboratory on a blinded basis. I  
7 have been invited to attend two pathology expert  
8 panels to discuss HER2 testing, by Genentech. I am  
9 also a member of the scientific advisory board of  
10 Vysis.

11           In terms of what we are going to discuss  
12 this afternoon, I will be talking briefly about  
13 HER2 biology, very briefly to outline it. I will  
14 discuss immunohisto-chemistry as an analytical  
15 technique in clinical samples. I will briefly  
16 describe fluorescence in situ hybridization, also  
17 as an analytical technique for analyzing clinical  
18 specimens. Finally, I will talk about the clinical  
19 significance of HER2/neu as an alteration in human  
20 breast cancers.

21           From a historical perspective, this  
22 alteration was first described by Dr. Dennis Slamon  
23 and his collaborators using Southern hybridization.  
24 They demonstrated that approximately 20-30 percent  
25 of human breast cancers showed an increased copy

1 number of HER2 in human breast cancer specimens.  
2 In the upper right-hand corner is an example of a  
3 Southern hybridization or Southern blot in which  
4 DNA has been extracted from pulverized tumors,  
5 separated by electrophoresis, and the amount of  
6 radioactive signal, illustrated here in black, is  
7 roughly proportional to the amount of gene copy  
8 number in the specimen.

9           Slamon and his collaborators demonstrated  
10 that the amount of HER2 copy number when it is  
11 increased, referred to as gene amplification, was  
12 associated with the worst clinical outcome in women  
13 that had this disease.

14           The gene is localized on the long arm of  
15 chromosome 17. On the right-hand side is an  
16 example of fluorescence in situ hybridization to  
17 demonstrate the site of this gene on chromosome 17.  
18 The gene is illustrated in red or in orange, as is  
19 shown here. They are unpaired sister chromatids,  
20 and chromosome 17 centromere is shown in green,  
21 immediately adjacent.

22           This gene codes for a membrane receptor  
23 protein that is in the epidermal growth factor  
24 receptor family. Members of this family have three  
25 primary domains, an extracellular domain, a

1 transmembrane domain and an intracellular tyrosine  
2 kinase domain.

3           The extracellular domain of three of these  
4 family members, HER1, HER3 and HER4, interact with  
5 known extracellular ligands or proteins hormones.  
6 The HER2, also known as C ErbB2 or neu, does not  
7 recognize any known extracellular hormone and is  
8 considered to be an orphan receptor. The  
9 intracellular domain of three of these has tyrosine  
10 kinase activity. That is HER1, HER2 and HER4.

11           In the biological activity of these  
12 membrane receptors, this slide very briefly  
13 summarizes and contrasts the role of normal  
14 activity of HER2 with those cases that have gene  
15 amplification and overexpression of HER2. As is  
16 shown on the left, other family members that bind  
17 to the extracellular hormone, either HER1, HER3 or  
18 HER4, subsequently interact and heterodimerize with  
19 HER2. As a result of this binding and interaction,  
20 these heterodimers are activated through  
21 phosphorylation and activate a signal transduction  
22 cascade within the cell.

23           In tumor cells that have amplification and  
24 overexpression there is an increased concentration  
25 of HER2 on the membrane, which we will discuss

1 later, and through this increased concentration  
2 HER2 homodimerizes and is activated, and also sets  
3 up a signal transduction cascade within the cell.  
4 As a result of this activation, biological  
5 activities within the target cell are changed.  
6 They include such things as cell cycle progression,  
7 transcription and a change in cell death.

8           This morning you heard discussions about  
9 the relationship between HER2/neu gene  
10 amplification and overexpression. One of the  
11 discussions that was raised was what kind of sample  
12 would one like to work with if you have the  
13 opportunity, and it was mentioned that the most  
14 desirable to work with would be frozen specimens.  
15 In 1989 we published a paper, in collaboration with  
16 Dr. Dennis Slamon and his group, in which we  
17 analyzed a series of 187 frozen breast cancer  
18 specimens looking at this issue.

19           I would like to briefly summarize our  
20 findings. We analyzed the DNA using Southern  
21 hybridization to determine whether the gene was  
22 amplified. The messenger RNA that was coded by  
23 this gene was analyzed by Northern hybridization.  
24 The protein product coded for by the gene was  
25 analyzed by Western immunoblot analysis or frozen

1 section immunohistochemistry.

2 In this cohort of cases there was 63  
3 percent of the samples, illustrated by this outside  
4 lane, that did not show gene amplification. The  
5 number of copies of the HER2/neu gene was not  
6 increased. Nevertheless, the gene was expressed at  
7 the messenger RNA level and at the protein level,  
8 and this level of expression was considered the  
9 basal level of expression.

10 Among 27 percent of the sample that were  
11 analyzed there was gene amplification. Two- to  
12 five-fold are greater amplification of the gene,  
13 and it was found that the level of amplification  
14 roughly correlated with progressively increasing  
15 amounts of messenger RNA by Northern hybridization,  
16 protein by Western immunoblot or by frozen section  
17 immunohistochemistry.

18 Together, this represented 90 percent of  
19 the samples in the study, and that left 10 percent  
20 of the samples that didn't fall into one of these  
21 two categories. Those cases -- 18 of the 187 were  
22 cases that did not show gene amplification by  
23 Southern hybridization but, nevertheless, had  
24 increased amounts of RNA and increased amounts of  
25 protein by analysis of expression. This

1 represented ten percent of the samples in our  
2 cohort.

3           Before I leave this slide, I would just  
4 like to address three issues briefly. One of them  
5 has to do with immunohistochemistry. You will  
6 notice as you look here at the immunohistochemical  
7 staining at the bottom of this slide that it is not  
8 heterogeneous. It is not a percentage of cells, 10  
9 percent or 30 percent. If you look, the amount of  
10 expression in the cells that are shown here in  
11 frozen samples tends to be relatively even in the  
12 vast majority of the cells. In addition, you can  
13 also note that this represents a subjective  
14 judgment in terms of the amount of immunostaining  
15 that is present.

16           Secondly, I would like to point out that  
17 among these cases that we looked at every case that  
18 had gene amplification in this frozen cohort also  
19 had overexpression, pathologic overexpression in  
20 these tumor samples. There was no case that had  
21 amplification without having overexpression. There  
22 were 10 percent of the samples that we refer to as  
23 single-copy overexpressers that did not show gene  
24 amplification by Southern hybridization. I would  
25 like to address those cases now.

1           There were 18 such cases. In the original  
2 paper that we published we suggested that single-  
3 copy overexpression could be related to one of two  
4 factors, either it was an artifact of the way the  
5 specimen was produced in Southern hybridization so  
6 that we missed recognizing gene amplification, or  
7 there was possibly a change in promoter enhancer  
8 elements in the gene so that a single copy could  
9 get overexpression.

10           Fortunately, neither Dennis' lab nor our  
11 lab put an excessive amount of effort in analyzing  
12 the promoter enhancer elements because this didn't  
13 prove to be the case in these samples. What we did  
14 do was return to these 18 samples, and more than  
15 two-thirds of these samples turned to be stromal-  
16 rich breast cancers in which there was a relatively  
17 small number of tumor cells in the sample relative  
18 to the other cells that were present. So, when one  
19 homogenizes the tissue sample the amount of tumor  
20 DNA was probably diluted out by the normal DNA  
21 present in the sample, and we underestimated the  
22 amount of copy number by Southern hybridization.

23           In the lower right-hand corner is shown an  
24 example of the fluorescence in situ hybridization  
25 that we conducted in these cases. More than two-

1 thirds of these turned out to be gene amplified by  
2 fluorescence in situ hybridization. Those of you  
3 that are near the front, perhaps you can appreciate  
4 that within these nuclei there are multiple red  
5 signals. In the upper right-hand corner is shown  
6 an example of the immunohistochemical staining of  
7 one of these populations of tumor cells to  
8 demonstrate the overexpression in this particular  
9 case.

10           Let me then summarize, we had a population  
11 of 10 percent of the samples that appeared to be  
12 single-copy overexpressers but, nevertheless, when  
13 we analyzed gene amplification by FISH the majority  
14 of these samples, the predominance of them were  
15 actually amplified overexpressers that we had  
16 missed by Southern hybridization. In the total  
17 cohort, it left a total of 2.8 percent of the  
18 samples in which there was not a direct link  
19 between the gene copy number and the level of  
20 expression. We feel that 2.8 percent is within the  
21 range of experimental error. So, our working idea  
22 is that this is an experimental error rather than a  
23 change in promoter enhancer elements in 2 percent  
24 of the samples.

25           To summarize then in a schematic fashion,

1 HER2/neu gene amplification is considered to be a  
2 genetic alteration that is responsible for  
3 overexpression of the protein product in these  
4 tumor cells. As is illustrated schematically here,  
5 there is an increased number of copies of the gene  
6 within the tumor cell nucleus, increased amount of  
7 messenger RNA and an increased amount of the  
8 membrane receptor protein on the cell membrane,  
9 referred to as pathologic overexpression.

10 This morning I think you also heard about  
11 the issue in which there appears to be gene  
12 amplification but there is not overexpression. I  
13 would like to briefly address this issue which we  
14 also tried to address in our initial publication.

15 In this group of frozen breast cancer  
16 specimens that had gene amplification there were  
17 cases that were immunostained on frozen section.  
18 However, when we recovered the paraffin-embedded  
19 blocks from the surgical pathology laboratory, an  
20 immunostain by the same antibody by  
21 immunohistochemical staining, we could no longer  
22 demonstrate the membrane staining, as you see here,  
23 on the right.

24 My personal view on this would be that in  
25 situations where there is gene amplification that

1 is identified but a lack of overexpression in  
2 paraffin-embedded tissue samples, the highest  
3 suspect has to be an artifact related to the way in  
4 which the material is analyzed, or the way in which  
5 the tissue is processed so that it prevents  
6 identification of the overexpression.

7 I would like to briefly talk about  
8 immunohisto-chemistry as an analytical method using  
9 the clinical trials assay that was used by  
10 Genentech, prior to my collaborating with the  
11 company, as a way of screening patients for entry  
12 into their clinical trials. The clinical trials  
13 assay involved a primary mouse monoclonal antibody.  
14 One of two different monoclonal antibodies was  
15 used, either the 4D5 monoclonal antibody or CB11.  
16 An indirect avidin-biotin technique was used to  
17 identify each of these antibodies bound to  
18 HER2/neu, as is shown schematically on the right  
19 for 4D5 and CB11. However, the antibodies were not  
20 reacted in the same tissue section but in serial  
21 tissue sections, one tissue section for 4D5, the  
22 next one for CB11. Antigen retrieval was used with  
23 both of these antibodies. With 4D5 protease  
24 digestion was used. With CB11 microwave heating  
25 was used for the tissue specimen.

1           The samples were scored in a subjective  
2 way, and it is illustrated briefly here to the  
3 extent that we can. Those cases that showed a lack  
4 of immunostaining were referred to as negative or  
5 0. With weak, discontinuous membrane staining in  
6 the sample, it was referred to as 1+. With  
7 continuous membrane staining of moderate intensity,  
8 it was referred to as 2+. With complete membrane  
9 staining circumferentially around the cells, it was  
10 referred to as 3+. Once again, this is done  
11 subjectively by an infection looking through a  
12 microscope at the tissue sections and evaluating  
13 the degree of brownness, brown, browner and brownest  
14 because, remember, in frozen tissue samples even  
15 non-amplified cases show a basal level of  
16 expression that can be identified by  
17 immunohistochemical staining.

18           Like any analytical technique that is  
19 being used in a clinical arena, there are both  
20 advantages and disadvantages to this method. I  
21 will briefly summarize them as they are shown on  
22 this slide. Some of the advantages include that  
23 immunohistochemistry is widely available. It is a  
24 relatively rapid procedure. It is light microscope  
25 based. Importantly, there are two

1 immunohistochemical assays that have been approved  
2 by the FDA for Herceptin eligibility selection.

3           Disadvantages of immunohistochemistry in a  
4 clinical setting would include the following?  
5 Importantly, there is variable antibody sensitivity  
6 and specificity in fixed paraffin-embedded samples.  
7 This is highly impacted by what fixative was used  
8 and how long that fixation took place. The  
9 sensitivity of the antibodies can be quite variable  
10 in this setting and we, in our laboratory, have  
11 used our molecularly characterized cases as  
12 standards to evaluate 30 of these different  
13 antibodies to establish their relative levels of  
14 sensitivity. The amount of immunostaining is also  
15 affected by antigen retrieval and reagent  
16 variabilities.

17           Next, there are a number of non-FDA  
18 approved assays that are in routine use across the  
19 country, and their performance characteristics are  
20 often not well described. Finally, there is  
21 subjective scoring criteria that are used to  
22 evaluate the immunostaining by the observer looking  
23 through a microscope. There tends to be relatively  
24 lower pathologist concordance in this type of a  
25 setting and inter-laboratory variability for this

1 type of assessment, as was described I think  
2 earlier this morning in the session.

3 I would like to briefly summarize  
4 fluorescence in situ hybridization as another  
5 method of evaluating clinical samples that have  
6 been fixed and paraffin embedded. The key features  
7 of this are shown schematically on this slide. It  
8 involves cutting tissue sections and putting them  
9 on glass microscope slides. These tissue sections  
10 are then digested with a proteinase to remove the  
11 protein so that the DNA is more accessible to a DNA  
12 probe.

13 The DNA, shown schematically by this oval  
14 area of the nucleus, is heated so that the DNA  
15 within these tumor cell nuclei in the tissue  
16 section is denatured and unwinds. A directly  
17 labeled fluorescent probe that corresponds to the  
18 DNA sequence of HER2/neu is incubated with the  
19 tissue section so that when it is prepared under  
20 high stringency conditions the location of these  
21 fluorescent probes corresponds to the location of  
22 the HER2/neu gene.

23 In summary, the probes are directly  
24 labeled. They correspond to the HER2/neu sequence.  
25 In addition, there is a chromosome 17 centromere

1 and an alpha satellite DNA probe that is also used  
2 as a second internal control for this procedure.  
3 The procedure is interpreted by signal enumeration.  
4 Nuclei are scored in terms of their counts for each  
5 one of these probe signals in 16 nuclei in a tumor  
6 specimen. The ratio of the HER2/neu gene copies to  
7 the HER2/neu chromosome 17 centromere ratio is  
8 determined as showing gene amplification when that  
9 ratio is greater than 2.

10 This shows an example of such fluorescence  
11 in situ hybridization. On the left is an example  
12 of a breast cancer that does not have gene  
13 amplification. For example, here is a tumor cell  
14 nucleus, in blue, with two red signals  
15 corresponding to HER2/neu; two green signals  
16 corresponding to chromosome 17 centromere. The  
17 ratio of this is approximately 1.

18 On the right-hand side, for example, is  
19 another breast cancer. Here, in the center, is a  
20 blue tumor cell nucleus and there are multiple red  
21 signals that correspond to HER2/neu, and three  
22 green signals that correspond to chromosome 17  
23 centromere. The ratio is greater than 2.0 and this  
24 is an amplified breast cancer.

25 In terms of looking at the HER2/neu gene