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IMMUNOLOGY DEVICES PANEL  
OF THE  
MEDICAL DEVICES ADVISORY  
COMMITTEE  
OPEN PUBLIC MEETING

Tuesday, November 9, 1998

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P R O C E E D I N G S

(10:30 a.m.)

**Open Public Meeting**

MS. MAGRUDER: Good morning. I want to welcome everyone to today's Panel meeting. I am Louise Magruder, Executive Secretary of the Immunology Devices Panel of the Medical Devices Advisory Committee.

Would the Panel members please introduce themselves, and give their affiliation, starting with our Chairperson.

DR. LADOULIS: Charles Ladoulis, State University of New York, the Health Science Center, Brooklyn.

DR. TAUBE: I'm Sheila Taube. I'm at the National Cancer Institute.

DR. HORTIN: I'm Glen Hortin. I'm at the Clinical Center at NIH.

DR. KEMENY: Margaret Kemeny, State University of New York at Stonybrook.

DR. MCCASKILL-STEVENSON: Wortia McCaskill-Stevens. The National Cancer Institute.

DR. HOMBURGER: I'm Henry Hamburger, Mayo Clinic.

DR. HACKETT: I'm Joe Hackett, one of the Associate Division Directors here in the Division of Clinical Laboratory Devices.

DR. JORDAN: Wilbert C. Jordan, University of Los Angeles.

DR. TODD: Mary Todd, Cancer Institute of New Jersey.

DR. REYNOSO: Gustavo Reynoso, Yale University.

DR. KAUL: Karen Kaul, Evanston Hospital, Northwestern University Med. School.

DR. CARPENTER: Betts Carpenter at King's Daughter's Medical Center at Ashland, Kentucky and Marshall University of Langton(?), West Virginia.

MS. AMMIRATI: Erika Ammirati, Industry Rep. and Independent Regulatory Consultant.

#### **Opening Remarks**

MS. MAGRUDER: I would like to ask all the Panel members to please pull their microphones closer to them and point it toward your mouth. There seems to be some difficulty recording our statements.

The Immunology Devices Panel last met on September 4th, 1998, in a joint meeting with the Hematology and Pathology Devices Panel.

The Panels discussed, made recommendations, and voted Approvable with Conditions, on a Premarket Approval Application for the Dako herceptest for immuno-enzymatic staining, indicated as an aid in the assessment of patients in whom herceptin treatment is being considered.

Future 1999 meeting dates for the Immunology Devices Panel are tentatively scheduled for January 15th,

April 9th, July 16th, and October 15th.

Now, I would like to ask Dr. Max Rabinowitz, Senior Medical Officer in the Division of Clinical Laboratory Devices to give a presentation on the Year 2000 Date Problem, and Computerized Medical Devices.

**Year 2000 Date Problem and Computerized Medical Devices**

DR. RABINOWITZ: Good morning, Panel members, ladies and gentlemen. This is to demonstrate the difficulty of using a computer.

This briefing is being presented to all the Advisory Panels of the Food and Drug Administration's Center for Devices and Radiologic Health, to alert Panel members of the concern of the Food and Drug Administration Health and Human Services in the Federal Government, about the Year 2000 date problem, and the possible adverse affects on medical devices.

The Year 2000 problem, or the Y2K problem, is the failure of computer systems to process or display dates properly, due to the representation of the year using only two digits in the early days of computers where there was a limit in the storage capability and memory, or other date-related problems, such as a failure to recognize a leap year.

An example would be, at the stroke of midnight,

instead of a computer reading 2000 on December 31st, 1999, it would read 00, and lead to a confusion between the Year 1900 and the Year 2000.

The FDA uses the following definition of Year 2000 compliance, for the purposes of the database that the FDA has compiled. Compliant means, with respect to medical devices and scientific laboratory equipment, that the product accurately processes and stores date-time data, including but not limited to calculating, comparing, displaying, recording, and sequencing operations involving date-time data, during, from, into, and between the twentieth and twenty-first centuries, and the years 1999 and 2000, including correct processing of leap year data.

Medical devices are subject to Year 2000 problems, and include the microprocessors, or PCs that control products, software applications, device interfaces to databases and record-keeping systems, including networking of computers. And the embedded chips for date display, or recording.

The FDA Biomedical Equipment Database can be found on the worldwide web site that FDA maintains, with the voluntary submission of data from manufacturers about certification.

It is searchable by the manufacturer, and is downloaded and freely in the public domain. Manufacturers

provide lists of products that are impacted, that are noncompliant or compliant, and certification of all products, both current and past production, that are not compliant; certification of products that do not use dates. This worldwide web link of the FDA is maintained with this data.

Many companies have not yet reported. We hope they are doing their assessments and these are in progress. So far, most noncompliant products involve date display or date recording; that is, date-stamping. But a limited number of products do have significant operational problems, and will fail unless these are corrected.

PC-based products in addition have PC-type problems, and the manufacturers are providing solutions, but there is a variety of approaches.

During 1998, in October, Congress passed and the President signed the Y2K, or the Year 2000 Information and Readiness Disclosure Act, otherwise known as the Good Samaritan Law, to promote free disclosure and exchange of information for Y2K readiness.

This will assist consumers, small businesses, and local governments in effectively and rapidly responding to Year 2000 problems, and hopefully, will lessen the burdens on interstate commerce by establishing uniform legal principles for disclosure and exchange of Y2K information.

We at the FDA hope this will increase the number of manufacturers who supply the information, so that everyone can benefit.

You can reach the product database at [www.fda.gov](http://www.fda.gov), and select the Year 2000 item. Also, there are links on this site to other government agencies and non-government agencies, particularly the National Institute of Standards and Technology, the Government Accounting Office, the Small Businesses Administration, and so forth.

It provides guidance to manufacturers, letters to manufacturers, databases of the product information, and the results of some of the monitoring and assessment activities to educate all of the stakeholders in medical devices.

What we would like the Panel to consider is how you can provide us advice regarding these problematic devices from your domain of expertise, to identify types of devices which, because of their use of dates, could present risks to patients, if not addressed, and suggest to CDRH what actions we might be able to undertake.

Please address your comments to our Panel Executive Secretary and they will be addressed to the appropriate sites at FDA.

The regulatory role of FDA in addition to the monitoring, does allow us to recall devices which may present significant risks to public health, and we hope that

by linking with the Department of Veterans Affairs, we will be able to expand the database, the outreach and communication with all stakeholders, and even increase our inspection capability, so that all healthcare facilities will benefit from this information, to develop contingency plans and plan and develop ways to respond to the Year 2000 problem.

Thank you for your attention.

MS. MAGRUDER: Thank you, Dr. Rabinowitz. And now I would like to ask Dr. Steve Gutmann, Director of the Division of Clinical Laboratory Devices, to come to the front. He will make a presentation to two of our Panel members.

#### **Presentations**

DR. GUTMANN: Thanks, Louise. Although as you noted from the schedule, there is a possibility that this Panel could meet in the early winter, we do not have a absolute product or policy to bring to the Panel, so there's a possibility it might not meet in the early winter, and we wanted to make sure we had an opportunity to thank two of our Panel members that have provided us with yeoman's service and helped us over the last couple of years move through a variety of exciting and complex deliberations.

Retiring from our Panel and going on to the Consulting Staff is Dr. Reynoso, and retiring from the Panel

and going back to his role as a private citizen is Dr. Jordan, and I want to present them each with a plaque and a letter of appreciation and thank them deeply and greatly for the contributions they have made.

MS. MAGRUDER: Thank you, Dr. Gutmann. The Panel is here today to discuss, make recommendations, and vote on the Premarket Application for a fluorescence in situ hybridization assay used in the detection of amplification of the HER-2/neu gene from subjects with node-positive, stage II breast cancer, to aid in the assessment of potential response to adjuvant therapy, leading to choice of therapy.

At this time, I will read the waivers for the Conflict of Interest Statement, and Temporary Voting Status into the record.

Conflict of Interest Statement for the Immunology Devices Panel Meeting, November 9th, 1998. The following announcement addresses Conflict of Interest issues associated with this meeting, and is made part of the record to preclude even the appearance of an impropriety.

To determine if any conflict existed, the Agency reviewed the submitted Agenda, and all financial interests reported by the Committee participants.

The Conflict of Interest Statutes prohibit Special Government Employees from participating in matters that

could affect their, or their employer's, financial interests, however, the Agency has determined that participation of certain members and consultants, the need for whose service outweighs the potential conflict of interest involved, is in the best interests of the Government.

Waivers have been granted for Drs. Karen Kaul and Charles Ladoulis, because of their interest in firms that could potentially be affected by the Panel's decisions.

Waivers are currently on file for Drs. Henry Homburger and Mary Kemeny.

The waivers allow these individuals to participate in today's deliberations. Copies of these waivers may be obtained from the Agency's Freedom of Information Office, Room 12A-25 of the Parklawn Building.

We would like to note for the record that the Agency took into consideration other matters regarding Drs. Karen Kaul, Wortia McCaskill-Stevens, Sheila Taube, and Mary Todd.

These Panelists reported past and current involvements with firms at issue, but on matters not related to the day's Agenda. The Agency has determined, therefore, that they may participate in the Committee's deliberations.

In the event that the discussions involve any other products or firms not already on the Agenda, for which

the FDA participant has a financial interest, the participant should excuse him- or herself from such involvement, and the exclusion will be noted for the record.

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

And now I would like to read the Appointment to Temporary Voting Status. Pursuant to the authority granted under the Medical Devices Advisory Committee Charter, dated October 27, 1990, Amended April 20, 1995, and October 1997, I appoint the following people as voting members of the Immunology Devices Panel for the duration of this Panel meeting on November 9th, 1998: Karen L. Kaul, Wortia McCaskill-Stevens, Mary B. Todd.

For the record, these people are Special Government Employees, and they're either a consultant to this Panel, or a consultant or voting member of another Panel, under the Medical Devices Advisory Committee.

They have undergone the customary Conflict of Interest review. They have reviewed the material to be considered at this meeting.

Signed, D. Bruce Burlington, M.D., Director,  
Center for Devices and Radiological Health, dated October

27, 1998.

And at this point, Dr. Ladoulis will convene the meeting.

**Open Public Session**

DR. LADOULIS: Okay. We will begin with the public meeting, correct?

MS. MAGRUDER: Yes.

DR. LADOULIS: This will convene the meeting, and we will begin with an open public meeting, after these comments from the Agency, and are there any individuals in the audience who wish to make any statement with regard to the issue before the Panel today?

There being none, then the time for public comment will be closed. Are there any other issues to be brought that is public?

MS. MAGRUDER: Yes. No one has contacted the Agency with a request to speak, but FDA did receive a letter to be entered into the record

The letter, which I will summarize, is from Philip Wyatt, MD, PhD, Chief of the Department of Genetics, North York General Hospital, North York, Ontario, Canada.

Dr. Wyatt states there has been confusion over the use of in situ hybridization in modern medical laboratory practice, and as a result, is writing a letter in support of the use of this technology.

He states that this technique has been available for several years, and with the availability of highly effective and well controlled kits, this technology is being used by more clinical laboratories.

As Chairman of the Laboratory Proficiency Testing Program in Ontario, which helps monitor quality assurance in licensed laboratories, Dr. Wyatt submitted a bulletin that was issued April 16, 1996.

The bulletin addressed the fact that in situ hybridization was becoming a part of routine laboratory medicine, and as a result, effective January 1, 1997, all laboratories involved in modern cytogenetic and other forms of genetic testing would use in situ hybridization techniques where they were pertinent.

This comment was put in place to ensure that all laboratories in practice in Ontario were aware that in situ hybridization is an expected laboratory practice that should be used in patient care, and as a result, the Cytogenetics Committee recognizes that the additional responsibility for the proficient and appropriate use of this technology requires a period of development, so that a formal assessment of the laboratories' performance will not begin until 1997.

A copy of this letter has been supplied to the transcribers.

DR. LADOULIS: Are there any other comments about this letter of submission from any of the Panel members, or any in attendance? There being none, then we can conclude this part of the presentation and begin now with the sponsor presentation, and Dr. Enns is Vice President of Regulatory Affairs of Vysis, and is going to make the introduction, correct?

**Sponsor Presentation with Panel Questions**

**Introduction**

DR. ENNS: Correct. Good morning, members of the Immunology Devices Panel, members of the FDA, and the public. Vysis today, has been announced, will be presenting our PMA Application for Premarket Approval of the PathVysion HER-2 DNA Probe Kit.

Today, I will be functioning as the moderator for our presentations. The speakers that we have today that will be presenting our information and data will be Dr. Steven Seelig, our Chief Medical Officer and Vice President of R&D at Vysis; Dr. Donald Berry, Professor of Biostatistics at Duke University; and Dr. Dennis Slamon, Professor in Medicine at UCLA.

I will give a brief introductory statement, followed by an overview presentation by Dr. Slamon on HER-2/neu status and its involvement in breast cancer.

This will be followed by Dr. Seelig making a

presentation on the general background of the FISH technology, and also presenting results from our 300 Clinical Trial for Reproducibility.

Following Dr. Seelig's presentation, we will have Dr. Donald Berry from Duke University present the results of the Vysis Pivotal Study, the 302 Final Report, and how it is associated with the CALGB 8869 Study.

Dr. Slamon will then come back to the podium and will give his experience as a practicing clinical oncologist and molecular biologist, and then I will have a few concluding statements and again read the Intended Use.

The slides that you have been given and overheads are in hard copy, xeroxed copies. The ones that we are presenting today have been checked with a checkmark. The other copies of slides that you have that do not have a checkmark are those that are going to be available for helping to answer the questions and answers session.

Also, in addition to the speakers, we have with us today, people who were significant in the performance of our 300 Reproducibility Study, Dr. Marilyn Bui and Dr. Shahla Masood from the University of Florida Health Sciences Center in Jacksonville, and also Dr. Diane Persons, who has done a lot of development work with this, from the University of Kansas Medical Center.

In addition, we have representing the people who

participated in the 302 Pivotal Trial, Ms. Lynn Dressler, who is at the University of North Carolina, who is an Assistant Professor, and is also the Principal Investigator for our 302 Study, and her assistant, Mr. David Cowan, who is the Lab Supervisor.

Also, from Duke University, is Gloria Broadwater, who is an assistant to Dr. Don Berry in Biostatistics.

To answer questions on specimen preparation for FISH test in breast cancer specimens, we have Dr. Mary Lowery from St. Francis-Penrose Hospital in Colorado Springs.

And then I also have some of my colleagues from Vysis, Mr. Scott McKenzie, our Director of Quality, answering questions that may come up on production and quality, and I also have our Manager of Clinical Affairs and our Biostatistician with us today.

Just briefly, on the Company background. We do have a number of FDA-cleared products with this same technology for different indications.

We have a broad line of 314 research DNA probes and ASR products. We have an installed base of 526 genetic workstations, in 28 different countries, and a worldwide distribution covering 42 countries, and we have 119 issued and allowed U.S. patents, with 56 pending in this technology arena.

The clinical products that have already been cleared for use are listed here. There are products for the use in leukemia that are listed. We also last year received clearance for an AneuVysion product for use in amniocentesis that involves five direct labelled probes. And we also have an image analyst system, which is an aid to standard G-band and karyotyping.

Pictures of our typical products that I have just explained to you are shown on this slide, and just from the standpoint of intellectual properties, Vysis has seven different patents that directly impact our ability to successfully market this product, if the Panel and the FDA approves it.

We have three specific patents, exclusive of Vysis, for direct fluorescence DNA label probes and their use. We have exclusive license of the ECF CEP patent by Gray and Pinkell, for unique genes by FISH.

We also have our own unique sequence FISH probe patent that was recently issued, and we also have non-exclusive licenses for access to using the HER-2/neu diagnostic marker that was invented and patented by Dr. Slamon at UCLA.

Just to show again, following up on the statement that was read into the Minutes by Dr. Wyatt, since its very first use of in situ hybridization in 1968 by Gaul(?) and

Purdue(?) there are over 20,000 publications now for in situ hybridization related to human chromosomes and disease.

Approximately 10,000 of these are for a combination in use in leukemia, in cancer, so this is not in situ hybridization, which originally used radioactive labels up until about 1980, and then switched to direct fluorescence.

It is a technology that is widely used, and is witnessed by the fact that the American College of Medical Genetics, in its Practice Guidelines, indicate this is a standard of care since 1993. The College of American Pathology does provide proficiency testing panels for this particular technology, however, not yet for HER-2/neu marker.

I am also the Chair of the NCCLS Subcommittee that has been convened to establish national standard guidelines for FISH technology.

And then just for our Intended Use, our original Intended Use that we submitted in June with our submission is that the PathVysion Kit is designed to detect amplification of the HER-2/neu via fluorescence in situ hybridization in paraffin-embedded specimens from subjects with node-positive, stage II breast cancer.

Results from the test are intended for use as a rapid assessment of the potential response to adjuvant

therapy, leading to choice of therapy. And for this, I will turn over the microphone to Dr. Slamon.

**HER-2 Background/Clinical Utility**

DR. SLAMON: Thank you. Thank you for the opportunity to present some of the information that we have developed using this approach in the FISH probe from Vysis.

Let me first state, as requested, I have no financial interest in Vysis. I have no stock or equity position there. I am neither a consultant for the Company. I have received this product under the usual Materials Transfer Agreement that we have with most of industry that we work with, and the only thing that has been reimbursed is the expenses for my travel to and from meetings where I have discussed this with the individuals at Vysis and of course for this Panel.

Now, I think most of you are aware that the problem with the HER-2/neu alteration is something that has become increasingly important of late. There are, as you know, 180,000 new cases of breast cancer per year.

\$6.0 billion is spent as direct medical cost annually for this disease. It has a 25 percent mortality rate, and there is clearly no question that improvement is needed for therapeutic selection and early detection in this disease, and there is an enormous effort going on, in large part, due to the advocacy movement among women in this

country, women and men, but primarily led by the women, to actually make this a national agenda item, which it has become.

Now, the gene we are talking about today is a gene called HER-2/neu, known as the Human Epidermal Growth Factor Receptor No. 2, also known as c-erb2, or neu.

It was first identified in the laboratory of Dr. Robert Weinberg in 1985 in a model system where pregnant female rats were being exposed to chemical carcinogens to determine if it would cause cancer -- the carcinogen would cause cancer -- and what genes might be impacted.

It was found that a disease developed in the rat pups delivered from these animals, and it was a neurogleoblastoma. When the DNA from this tumor was extracted and transfected into NI 3T3 cells, it was found to transform them, and ultimately that led to the identification of a dominant transforming oncogene in that DNA, known as neu.

When the gene was sequenced, it was found not to be a member of the RAS(?) gene family, which most of the genes that had been scored out of this kind of model had been seen, but rather was related to the erbB family. It was the first time that something that was not a rat homolog was found in this approach. And so, Dr. Weinberg called it neu, N-E-U, for the disease it induced, this neurogleoblastoma.

The gene has a homologue in the human genome, which is HER-2, also known as neu, or cerB2. It encodes a 185,000 molecular weight, transmembrane cell surface receptor, which is a receptor of tyrosine kinase.

It is a member of the receptor tyrosine kinase type I family. There are four members, EGF receptor being the prototype, and HER-3 and HER-4 have been identified. There has been an extensive search for other members from this family, as yet, nothing has turned up and many people believe that this family will be relatively restricted to just a few members. Currently, as I said, there are four, HER-1 through HER-4.

The HER-2/neu gene is clearly involved in the regulation of breast cancer cell growth. There is now substantial data in the literature that would I think support this statement.

It is also true that amplification and overexpression of this gene is clearly associated with an increase in many of the biologic factors of breast cancer cells, including their growth rate, their DNA synthetic rate, their ability to grow in soft agar, their ability to form tumors in nude mice, and lastly and most importantly perhaps in the model systems, their ability to form metastatic disease in those models.

The amplification/overexpression alteration occurs

in 25 to 30 percent of human breast cancers. Now, in terms of the mechanism of overexpression of this gene, it appears that gene amplification is the most common alteration that results in overexpression of this gene.

It results in an increase in the transcription of the messenger RNA, an increase in the protein expression, and of course the biologic endpoints that we have talked about.

Perhaps one of the most critical questions is -- and that's relevant I think to the discussions for the Panel today -- is how best to detect whether or not this alteration is present?

Now, much debate has been put forth, much of it generated by us at times, that there are different approaches that can be used to determine whether or not a tumor contains this alteration.

And then there has been an enormous amount of debate, subsequently, saying, what is really the pathologic entity that you are looking for? Is it amplification versus overexpression?

Carried to its logical extreme, even overexpression doesn't measure, because you are actually asking for the activity of the overexpressed protein, as measured by its kinase activity, or ultimately, endpoints such as increase in early transcription factors that would

result in DNA synthesis and cell division.

There are several ways that this can be looked at in terms of whether or not the alteration is present, and one of the questions I think before the Panel today is how strong is the product that is being proposed, how strong is it in its ability to detect this alteration when present?

Now, why is there such interest in the HER-2/neu gene status and amplification? Initially, it was all due to the association of this alteration with prognosis. I will tell you, as the laboratory that first published that there was an association here, we were not looking for a bigger or better prognostic factor when we started these studies.

What we were looking at, and what was our intent at the time, was to identify alterations that were playing a role in the pathogenesis of the disease, in hopes of developing more specific therapy, or specifically targeted therapy, for whatever alterations we might find.

What we found was that the HER-2 alteration was associated with prognosis, which led us to think that, perhaps since there was smoke here, perhaps there was fire, perhaps it was causing a role in driving the tumors. We have subsequently been able to prove that, and specific therapy has been developed for that, as this Panel is aware.

In addition, identification of the status is important with regards to, as some recent data has

generated, predicting therapeutic outcome in terms of response to therapy. So, not only is it a prognostic factor, but it is also a predictive factor.

With regards to its prognostic value, the initial publication in Science in 1987 showed that, in a node-positive cohort, this alteration was an independent prognostic factor, stronger than all of the traditional prognostic factors, with the exception of the number of positive nodes in predicting outcome.

There have been innumerable publications since this time, some of which did not agree, and many of which did agree with this, and we will talk about that in the course of the discussion today in terms of what has gone on.

In addition, this has been looked at in node negative cohorts, and it is found also, again, to be a prognostic factor in many of the studies that have been carefully done with large numbers and long term follow-up. And most importantly, with good reagents.

In terms of it being predictive for therapeutic outcome, there are two major areas that are being investigated quite actively now. One is, with regards to response to hormonal therapy.

There were small studies that indicated that this might be the case, but recently at ASCO(?) this year, a large study was presented from the Italian group, the so-

called GUN Study, in which a large number -- more than 1200 patients -- were looked at, and it was clear that those patients who had overexpression of the HER-2 protein, and amplification of this gene product, did not respond well to hormonal therapy.

In fact, there is some question that they may have responded even worse with Tamoxifen in this trial. And this is something that is a very, very active area of investigation, currently.

In terms of traditional chemotherapeutic approaches, there is the study that is going to take a lot of the discussion today, which is the well known CALGB Study, that showed that, with regards to what was considered dose-intensity, anthrocycline therapy, cytoxin, Adriamycin, based on anthrocycline-based therapy, CAF, that patients did better if they were HER-2 overexpressers, with higher doses of anthrocycline therapy.

Now, the higher doses of anthrocycline therapy that were alluded to in that study when it was first developed, are really what is standard dose anthrocycline therapy, currently. But clearly, the data were that patients that had this alteration were more likely to respond to this kind of therapy than those who did not, as can be seen by the graphs here.

This is particularly relevant, given the fact that

there had been previous large studies that indicated that there did not appear to be a benefit to CMF-based chemotherapy in the adjuvant setting, for patients who have had -- who have this alteration. So, this becomes a relevant question, I think, for some of the discussion we have later.

At this point, by way of background, I will stop. I don't know if this is the right place for addressing any of the questions and/or comments, or we're waiting.

MR. ENNS: We are waiting.

DR. SLAMON: Okay. So, Steve Seelig will go next?

**FISH Technology/Reproducibility**

DR. SEELIG: Good morning. I am presenting two aspects of the presentation today, What is FISH Technology, and Protocol 300, the Vysis Reproducibility Study.

For the FISH technology, I will provide a brief description of the technology, the design of the HER-2/neu PathVysion Probe, basic FISH assay procedures, a few microscopic images of FISH results, and our approach to PathVysion verification.

For reproducibility, I will present Protocol 300 that examined assay-to-assay, observer-to-observer, site-to-site, and lot-to-lot reproducibility.

Finally, it is important to remember that four other FISH-based products, three related to leukemias, and

one is an adjunct to standard prenatal karyotyping, are cleared by the FDA.

For each of these products, rigorous reproducibility studies were performed and all products showed high performance characteristics.

I believe the performance of the HER-2/neu PathVysion is comparable to those previously cleared products.

For instance, in situ hybridization consists of two basic elements. Biologic materials, affixed to a slide, to the surface of a microscope slide, and a DNA probe, to which fluorescent molecules have been attached.

The DNA probe in solution is applied to the surface of the slide, and through the highly specific process, of base pairing, the matching and binding of complementary DNA sequences, the fluor-labeled DNA probe becomes specifically attached to the appropriate genetic targets in the biologic materials.

After the reaction, and a simple wash procedure to remove unattached probe, inspection of the surface with a common fluorescent microscope allows for simple visualization of a DNA probe, attached to its molecular target.

In a few moments, I will show you FISH images so you can have an appreciation as to what the user will see

through the microscope.

There are many attractive characteristics of FISH technology. It is applicable to a wide variety of specimen types, from fresh-frozen to formalin-fixed paraffin-embedded tissues. Because this is an in situ-based technology, the important tissue morphology information is retained for the pathologist.

It is highly sensitive, in that it can allow an individual to visualize through a microscope a single genetic loci, and the Vysis direct label strategy provides for a high signal-to-noise ratio. The latter simplifies interpretation considerably.

As previously mentioned, the DNA probe binds to its complementary sequence, in a highly specific fashion. Essentially, each of the nucleotide bases must match, for the binding to occur.

DNA is a stable target, and appears considerably more resistant to degradation than either RNA or protein targets.

Lastly, FISH allows for the simultaneous assessment of multiple targets. This capability allows for quality and performance controls built in to the primary reaction.

The assay format is simple. Following location of the tumor on the slide, the interpretation is a visual read

through a microscope, and requires a low level of interpretive skill, basically counting. It is quantitative and highly reproducible. It is automatable, and finally, the equipment required for the work is generally available in most laboratories or hospital settings.

The design of the HER-2/neu PathVysion Probe is schematically shown in this slide. There are two genetic loci identified by PathVysion.

The first probe is the alpha satellite probe for the chromosome 17, and that hybridizes to the centromeric region of the chromosome, as shown here at 17p11.1 to q11.1.

This probe, labeled with green fluor, allows for determination of a number of copies of chromosome 17 in the individual cell.

The second probe is for the HER-2/neu gene loci, located at 17q11.2 to q12. The probe is 190 kilobases in length, with the HER-2/neu gene centrally positioned in this probe. And it is labeled in an orange-colored fluor.

In your packages, you have received a detailing of the PathVysion procedure, so I will only outline the process for you today. It consists of three fundamental steps. Specimen preparation. Hybridization. And microscope examination and enumeration.

There are a total of nine steps and eight individual reagents. The complexity of these steps are no

greater than other technologies, such as immunohistochemistry, a methodology currently practiced in most pathology laboratories today.

The kinds of images of the green chromosome 17 to the orange HER-2/neu, are schematically represented here. In normal cells -- in this case, the open circles are the CEP 17 and the dark, closed circles are the HER-2/neu -- in normal cells -- this is shown in A -- there will be two chromosome 17 signals and two HER-2/neu signals. The ratio of HER-2/neu signal to chromosome 17 signal, will be one.

I remind the Panel that this is not a manmade standard or reference point, but inherent in the genetics of man, and this well-understood reference greatly simplifies assay requirements.

When an observer sees four HER-2 signals, such as in D, and only two of chromosome 17 signals, the ratio is 2, and this is amplified.

Amplification can go considerably higher, as shown in Examples E and F.

Here is an example from an actual patient with breast cancer, and I think each of you can readily see two green signals in this cell here, which represents the CEP 17, and multiple copies of the HER-2/neu oncogene, and clearly, this woman has amplification in this, too. The signals are readily apparent.

The first step for formalin-fixed, paraffin-embedded specimens is to process the materials to allow for adequate hybridization of the PathVysion probes.

This general purpose laboratory product, the Paraffin Pretreatment Kit, is used for processing of these types of specimens, and includes four fundamental components: pretreatment solutions, wash buffer, protease buffer, and protease.

The purpose of this process is to free the DNA from entanglement from crosslinked proteins, which develop during formalin fixation, thus allowing greater reactivity of the target DNA, with the fluor-labeled probes.

These are the five components of the PathVysion IVD Kit. The core of the kit is the pre-denatured direct labeled probes, chromosome 17 and HER-2/neu, premixed in the solution required for hybridization.

Following hybridization, the SSC and NP-40 are used to wash the slide to remove all unhybridized probe.

A solution of DAPI counterstain is applied to the slide after the wash, to provide for a general nuclear stain, and to retard the loss of the fluorescent signal.

Included in the kit are ProbeChek Control Slides, which allow the user to verify that the assay is performing according to specification, and that they can achieve appropriate results.

One control slide in this set of slides has a normal ratio of CEP 17 and HER-2/neu. And the second control slide consists of a low level increase of HER-2/neu, of between 1.6 to 2.0, to test the sensitivity of the assay in the user's hands on a regular basis.

In this slide, I show examples of the results the user is likely to observe. This is a collage of four different images obtained at 1000 magnification. In the upper left corner, one can easily see two green signals and two orange signals, so this cell would have a normal ratio.

In the upper right panel, you can see three orange signals and two green signals, for a ratio of 1.5.

In the lower left panel, you can see two cells with three orange signals, and one green signal, for a ratio of 3, and this cell would be amplified.

Finally, in the lower right panel, you see two green signals, and multiple orange signals, indicating a high level of amplification.

As you can see, interpretation of these images is straightforward.

In addition to using molecular STS markers to verify the composition of the HER-2/neu locus in PathVysion, it is possible to obtain visual verification of specificity.

The approach is to perform sequential Wright-stained G-banding, followed by FISH staining. The two

images are paired, thus allowing for verification of location of the hybridization signal.

Fourteen to eighteen individual metaphases for each of the 13 IUO probe lots were examined, and between 6 and 12 metaphases were examined, for an additional 12 probe lots.

A total of 254 metaphases were examined. All showed correct localization of both the spectrum green CEP 17 probe and the spectrum orange LSI HER-2/neu probe.

In the next slide, in this slide, I show an example of those types of images. On the left side, you can see a standard G-band in metaphase, and you can easily see two chromosome 17s, and those are easily identified by an individual trained in classical cytogenetics.

Exactly the same metaphase has been sequentially hybridized to the PathVysion probe, and you can see the green CEP 17 probe, and right next to it on the Q-arm in the proper location, is a spectrum orange LSI HER-2/neu probe, thus providing visual verification that the probes are hybridizing to their correct locations.

Turning to the Reproducibility Study, the objectives were to assess assay-to-assay, observer-to-observer, site-to-site, and lot-to-lot reproducibility.

Protocol 300 was divided into two weeks. During Week 1 we specifically examined intra-assay reproducibility.

During Week 2, we examined the inter-assay, observer-to-observer, site-to-site, and lot-to-lot reproducibility.

The three participating sites are listed in this slide, Drs. Masood and Bui are here today to help us in the discussion of PathVysion later on, if needed.

For the Intra-Assay Reproducibility Studies, formalin-fixed paraffin-embedded tissue cell lines were used. The samples were blinded to the observers, and were wildcarded. The use of wildcard samples reduces the likelihood of entrainment of a user.

There were four levels of amplification, three different sites, and two observers per site, 100 nuclei per sample per observer were counted.

In this slide, I provide the mean of the two observers for each of the four replicate slides at each level of amplification at the three different sites. So, here are the three different sites, here are the four different levels of amplification.

There are a total of 96 observations represented in this table. I have not shown the statistical analysis for each of these points, to reduce the clutter on the slide. As one scans this data, one is struck by the close agreement among different within-assay results -- for example, 1.02, 1.03, 1.01, 1.04, 4.35, 4.41, 4.37, 4.26. And you can see that tight reproducibility in each one of

the values, not only across the different levels of amplification, but also across the three different sites. There is excellent agreement. Not a single mean value fell outside the expected range.

When one examines the precision of the estimates in this Study at the four different levels of amplification, we observe a coefficient of variation between 5.53 and 3.04 percent.

This precision approaches what one would normally expect within a standard chemical assay, rather than a biological assay.

In this slide, I show the correlation between the two observers for Week 1 of the Reproducibility Studies, for the different levels of amplification. And again, from a practical perspective, there is excellent correlation between the two observers, with a correlation coefficient of 0.98.

In addition, the two observers always placed the slide into the correct level of amplification. Rigorous statistical analysis that is included in your packet of information shows statistical differences between institutions, and observers.

These differences are observable because of the power of the statistical design, but have no practical or clinical effects on how PathVysion is practiced.

The second week of the Reproducibility used formalin-fixed, paraffin-embedded tissue cell lines, a Latin square design was employed. Again, the specimens were blinded and wildcarded. There were four reagent lots, four levels of amplification, four assay days, three sites, and two observers at each site. There were a total of 96 observations in this study.

In order to simplify the visual presentation of the data, I show only the various analyses by their mean values. Again, the details of the statistical analysis were contained within the submission.

As we examine different lots of PathVysion, Lots 1 through 4 here, we can see excellent agreement for the results across four different reagent lots, and this agreement was evident for each level of amplification that we tested these lots against. I think you can just scan through these and see very, very tight agreement for the results.

For site-to-site reproducibility, again, we see extremely good agreement among the three sites, across the four different levels of amplification.

For inter-assay reproducibility, that is, on four different days, again, we see extremely good agreement over the four different assay days, and this agreement is consistent across the four levels of amplification.

Finally, observer-to-observer reproducibility is shown in this slide. As previously noted, there is excellent agreement between the two observers.

This is further shown in the next slide, where the correlation was examined between Observer 1 and Observer 2, and again, you can see a correlation coefficient of 0.99, indicating excellent agreement.

As indicated in the detailed statistical analysis of this study, there are statistically significant variations among institutions, and between observers; however, these statistical differences are evident because of the power of the design of these studies, but have no practical or clinical effects on how PathVysion is practiced.

For the second week of the Reproducibility Study, I show the precision of the estimates of amplification, in this slide, and you can see that they range from 11.99 down to 3.28 for coefficients of variation.

Turning our attention briefly to the control slide, manufacturing reproducibility, the results of different ProbeChek slide lots are shown in this slide. For ProbeChek slides prepared to have a HER-2/neu to CEP 17 ratio of between 1.16 and 2, the average ratio was 1.92, and the range was between 1.77 and 2.0, with a coefficient of variation of only 4.7 percent.

For ProbeChek slides prepared to have a HER-2/neu to CEP 17 ratio of between 0.75 and 1.25, which would represent a normal, non-amplified situation, the average ratio was 1.02, with a range of 0.98 to 1.07, with a coefficient of variation of only 3.94 percent. These results clearly indicate reproducible manufacturing of the ProbeChek Control Slides.

In this slide, I summarize our total experience in the Reproducibility Study by specimen classification, according to the expected HER-2/neu to CEP 17 ratio, or the known ratio, to the observed ratio, as shown on this column.

Of the 192 specimens used in this Study, 190 were correctly placed into the expected ratio range, on the first attempt. So, you see a perfect correlation here.

One specimen was failed, due to immersion oil getting under the coverslip, and one specimen fell off the slide during the pretreatment steps. Repeats of these two slides were successful, and resulted in all 192 specimens being classified correctly.

To summarize, there were no significant differences in assay-to-assay, lot-to-lot, or day-to-day results. There was significant observer-to-observer variation and site-to-site variation; however, these variations are not of clinical significance to the practice of HER-2/neu PathVysion.

It is quite clear from these studies that HER-2/neu PathVysion assay is highly reproducible, robust, and quantitative, and will provide a reliable method for the measurement of HER-2/neu status. Thank you.

DR. LADOULIS: Question. The targets -- yes, this is Dr. Ladoulis, I had a question. The targets for the Study of Reproducibility were all of the cell lines --

DR. SEELIG: Correct.

DR. LADOULIS: -- that you used for the selection, right?

DR. SEELIG: That's correct.

DR. LADOULIS: And therefore, the statistically significant variation between sites and between observers were all on these cell line preparations, correct?

DR. SEELIG: That's correct.

DR. LADOULIS: What was the observer variability, the greatest inter-observer variability on the standard slides, approximately 4 or 5 percent?

DR. SEELIG: The maximum difference --

DR. LADOULIS: Yes. Yes. The maximum variability.

DR. SEELIG: What's the maximum variability observed, maximum range?

DR. LADOULIS: Inter-observer variability.

DR. SEELIG: Right. Maximum inter-observer

variability. That should be in the data file that you received.

DR. LADOULIS: Okay.

DR. SEELIG: Can I get back to you on the --

DR. LADOULIS: That will be fine. I'm asking you if you would restate the conclusion that you reached from these studies, with regard to the reproducibility of the PathVysion in practice. That applies -- you know, this inter-observer variability applies to these targets that are in -- that you use in the test kit, correct?

DR. SEELIG: That's correct.

DR. LADOULIS: Okay. You don't mean to infer -- or will infer differently -- how this applies to the patient population?

DR. YANG: May I answer the question? The observer-to-observer and the site-to-site variation is still 2, because the subjectivity introduced when you -- when the observer in the enumeration for the highly (?). Because we can see the site on (?), that's, if it is highly (?), then we will see across, the orange signals, and some of them, because in that, the (?) in the signal enumeration, sometimes you have to (?). And some of them will just try to (?) all and some entire, but in term of the classifications of the amplification status, there is no variation.

DR. LADOULIS: Maybe I am just anticipating, and you can answer this later, because I'm concerned about the inherent variability in the tumor of overexpression and as contrasted with the cell line variability for amplification that is used in each one of these three levels there. And therefore, the inter-observer variability has to be -- is additive or cumulative with the inherent variability biologically of that in the tumor cells. Are you going to address that?

DR. SEELIG: Let me -- we have performed what we would call a Portability Study, where we have actually used tumor specimens and examined that. The data is in the process of being prepared for submission. I will tell you that that looks as good as this data, in terms of reproducibility. But, that data is -- and we have the slides if we want to talk about that later.

DR. LADOULIS: Okay.

DR. ENNS: Russ Enns, again. Just to respond to the portability. We have put summary data into our 90-day response to the Agency, however the Agency has not had the opportunity to review the raw data that supports the conclusions that we drew on the portability data. We will be submitting that, probably within the next week or two.

DR. HORTIN: I have a question.

DR. SEELIG: I will now turn the discussion over

to Don Berry, our statistician --

DR. LADOULIS: I think there is another question from the Panel.

DR. HORTIN: Just one more question. Regarding the number of nuclei that were examined on some of these slides, it had a number of 100 nuclei, and I believe your final procedure calls for a scoring of 60 nuclei, and would you comment about selection of the number of nuclei that were examined and kind of the affect on variability?

DR. SEELIG: We have now conducted studies looking at 60 nuclei, which is the 302 Study, and we have actually gone down to 20 nuclei, and the mean value does not change as you go down in lower numbers of nuclei. The errors of the estimates expand a little bit, but it should not affect the reproducibility.

Again, part of the portability study is designed to look at going down to 20 nuclei and again, we're in the process of preparing that data. It doesn't make any difference, is what the data will show you.

DR. LADOULIS: Any other Panel? Dr. Reynoso?

DR. REYNOSO: Will there be an opportunity to discuss data reproducibility in actual paraffin-embedded tissue sections?

DR. SEELIG: As I indicated, our portability study is actually designed to look at reproducibility in paraffin-

embedded formalin-fixed actual tumor tissues.

DR. REYNOSO: Will we be able to discuss that today? I don't know if this is appropriate or not.

DR. ENNS: It is my understanding, we presented you that data in the 90-day Response to FDA questions, that does discuss the summary conclusions of our portability and different nuclei counting.

We also now have data from Dr. Don Berry and their analysis of our raw data in the 302 Pivotal Study that addresses the number of nuclei that can be reliably and reproducibly discussed, but we do have a slide number for that, and I'll have to look it up.

We have not been able to at this point submit the raw data as yet to the Agency for review, and I think that it would be their preference for us not to go into this in detailed discussion at this time, unless the FDA instructs me to do differently.

DR. LADOULIS: We can come back to this issue, perhaps at the conclusion of your presentation.

DR. SEELIG: I would like to turn the discussion over to Don Berry, our Statistician, CALGB, and Professor in the Institute of Statistics and Decision Science and Cancer Science, Biostatistics, at Duke University. He will discuss Protocol 302. Don?

**CALGB 8869/Protocol 302**

DR. BERRY: Thank you, Dr. Seelig. I should indicate my role in this Study. I am a statistician on the Breast Cancer Committee of the CALGB, and the CALGB is under contract with Vysis to carry out this Study.

I have no financial interest in Vysis. My consulting fee for Vysis was contributed by them to the CALGB Foundation, so I have no consulting fee from them, either.

Protocol 302 is a subset of a clinical study, which I will describe. The bigger study was CALGB 8541, the Study was designed to look at increasing dose, and increasing dose intensity in node-positive breast cancer.

The low dose was indicated here, Cyclophosphamide, Adriamycin, and 5-Fluorouracil. The high dose is just double that, both four cycles. The standard dose, no longer standard, the standard dose is six cycles, giving the same total dose as the intensified dose. So, these two are equivalent, except by intensity, and these are double the low dose.

In the overall study with 1549 patients, the intensive dose was shown to be statistically significantly better than the low dose, and the standard dose was shown to be statistically significantly better than the low dose.

CALGB 8869 was designed as a laboratory companion of CALGB 8541, designed to examine the role of various

markers, including those indicated here, including HER-2/neu, overexpression, and p53, and any other markers that would come along later.

Schematically, 8541 is shown on the right, 1549 patients, 8869 was really in two parts. It was published separately, a sample of about 400 patients, the initial sample, was published in the New England Journal of Medicine addressing the interaction that we will be talking about here, this morning.

The second part of that study, another approximately 600 patients were then -- blocks of these patients were collected to address the confirmation question, did the early results, the early observations that we made in the first 400 patients, did they continue in the second batch? We collected as many of the blocks as we could, totalling about 1000 from the 1500-odd.

Vysis 302 is a subset of these, of 8869, mostly a subset, and the numbers are shown here. The first set, we got 174 of these 397, in the second set, 349 of the 595. And the way we selected this is a complete random sample from the 992 patients. That was my preference, as I designed the collection, I designed the analysis, and everything that you see here, including that little 1 over there.

That patient was -- somehow we got a block in 8541

that was not included in 8869, and my attitude was all-encompassing; anything we looked at, we would include, even though it's a bit of a peccadillo. We don't have IHC or PCR on this patient, we do have FISH. And it is included as one of these 524.

What I will tell you about today's comparison of the clinical characteristics of the 302 subset, with the fuller subset, 8869, I will discuss the correlation between HER-2/neu status and clinical and tumor characteristics in both subgroups. Tell you the frequency comparisons, the relationships among FISH, the Vysis Probe, immunohistochemistry, and gene amplification using PCR. We have on about 900 patients, PCR amplification, in addition to the 992 patients that we have IHC.

I will indicate the proportional hazards models for disease-free survival, defined as being free of disease and also alive, a similar proportional hazards model for overall survival, and give you the Kaplan-Meier curves we need to split the IHC results.

The IHC was percent of cells staining positive, and we, in the original paper and continued to, when we draw a Kaplan-Meier, split into positive and negative, at the 50 percent point. So, 50 percent cells staining positive.

With respect to the Vysis FISH, we calculated, as has been indicated this morning, the ratio of HER-2/neu

amplification to CEP 17, and the cutoff that we established was two, for amplification versus not.

There is a bit of arbitrariness in this. If you go down to somewhat lower, the results don't change much. If you go up to somewhat higher, they don't change much, but two is the value that, consistent with previous studies, showed the best discrimination.

The comparison of patients. This is the overall total, broken out by low, moderate, and high dose. The overall total compared with the remainder of 8869.

As I indicated to you, this was a random sample, a pure random sample of the full 8869, and so there is not much difference between these. This is a random variation with those who received Tamoxifen. This is not a critical issue, but somewhat more patients in the Vysis Study received Tamoxifen than in the full study. And similarly, for S-phase. The S-phase was somewhat higher.

The comparisons -- and this is a critical issue in the Vysis submission -- is the relationship between the FISH assay and PCR, and especially IHC. The total number of patients -- there's the 523 that I indicated to you -- the total number of patients in the 302 Study is 524, but the fact that there is a relationship between FISH and IHC, and also FISH and PCR, means that in my view, we can borrow strength from the fuller study of approximately 1000

patients.

This gives the CAP a degree of agreement between the FISH and PCR. It gives a sensitivity, assuming that PCR is a gold standard, which of course, it is not; there is no gold standard. But sensitivity is quite high. Specificity is even higher. Similarly, for IHC, FISH to IHC, the sensitivity is high.

The overall concordance is 88 percent, and somewhat higher in PCR amplification, which is what one would expect.

This is a comparison. This is the third pair. This is comparing IHC and PCR, and we did that in the 524 patients in the 302 Study, but we also did it in the fuller Study, the 992, of which we had PCR on 894. And you see very comparable degrees of agreements, not as high between IHC and PCR, as between IHC and FISH, and PCR and FISH.

The -- there is a correlation, as you saw, in the previous slide, the FISH-positive, IHC-positive, of the 524 patients, there were 78 of those. The other concordance, FISH-negative, IHC-negative, 384 of those.

There were 12 that were FISH-positive and IHC-negative. Of those 12, PCR -- if you view this as being breaking the tie -- PCR was positive in 50 percent of them. Among the so-called false-negatives, FISH-negative, IHC-positive; PCR agreed with FISH in about 80 percent of those

cases.

This is the meat, if you like, of the Study. This is a proportional hazards model for disease-free survival, and I will tell you what it is for overall survival in a minute. This is a model which uses a dose of CAF, and other prognostic factors, well-known prognostic factors in node-positive breast cancer.

The number of positive nodes -- this is the square root -- a standard thing to recognize that doubling the number of positive lymph nodes does not double one's risk, but quadrupling the number of lymph nodes that are positive, approximately doubles risk.

These are well-established prognostic factors in node-positive breast cancer, the number of positive lymph nodes. Tumor size is -- this is tumor size greater than 2 cm. Premenopausal status. These variables were established by me in the original New England Journal publication, and so, we continued with these same variables. They are standard.

The number of positive lymph nodes is by far and away the most important. It is the biggest and well-recognized risk factor.

HER-2 is shown here for the FISH assay, for IHC, for PCR, and the contribution is not always the same. The important role here is the HER-2 by CAF. This is the

interaction between the two, and I will expand on that with survival curves.

The interaction between the two is significant in this 524 patients. And remember this number; this is the statistical significance for the comparisons that I will be showing you with Kaplan-Meier curves for disease-free survival. This is the significance for IHC, for the 523 patients that are part of this Study, and for PCR.

Similarly, the same thing really goes on, there is a strong correlation between overall survival and disease-free survival. The same sort of thing goes on here with these significance levels.

And this is to show you the interaction. There is the p-value, .033, for the comparison of HER-2-negative patients -- this is by FISH -- HER-2-negative patients, the dose effect. And the dose effect shows that there is not much difference between these doses; in fact, over here, moderate dose is creeping above high dose, in the HER-2-negative patients.

In the HER-2-positive patients, a rather different story. There is no difference between low dose and moderate dose, but high dose is a good bit better. This suggests that in the clinic, if a person is HER-2-negative, that person doesn't need the intensive dose -- and you will see in a minute that probably doesn't respond to Adriamycin --

whereas, if the person is HER-2-positive, that person does benefit greatly from an increasing dose of, in this case, CAF.

These are overall survival curves, and the same sort of thing going on here; for example, high and low, there is essentially no difference in the HER-2-negative, and there is a rather substantial difference in the HER-2-positive. The ten-year survival in the low dose is about 39 percent, and in the high dose is about 67 percent.

Now, in the -- this is not FISH, this is IHC, this is from the recently published Thor et al paper with the full 992 patients, and this is disease-free survival on this side, HER-2-negative, HER-2-positive, roughly the same picture that you saw in the subset, only stronger statistical significance because of the additional power, the additional sample size.

Now we have 272 patients in the overexpressing group. For overall survival, again, essentially no difference among the three doses, and again, not much difference in HER-2-positive, between low and medium, but a substantial benefit for high.

This is an important observation. It is not the case in my estimation, that this is a 524-patient study. This is a 992-patient study, because of the correlation between FISH and IHC, and it borrows also from other data,

other confirmatory data that I will mention from the NSABP.

Comparison to other studies. I have talked about the Muss and the Thor papers. Muss was the early subset, and Thor was the confirmatory analysis, confirming our original observations.

The Paik paper, which appears after the Thor paper in JNCI, is a report of an NSABP study, B-11, comparing PAF, L-pam, Adriamycin, 5-fluorouracil, with PF; that is, Adriamycin versus not Adriamycin, and they see exactly what you would predict from our studies. It confirms our study, and it identifies, in my opinion, Adriamycin as the player in this interaction between HER-2/neu and CAF.

Now, I don't have -- John, I wonder, can you give me -- before I go to conclusions. Can you give me the prognosis and core prognosis? Yes, those two, and that one. Because of the question regarding four or greater positive nodes, I put these slides on.

This is a paper I have written on the basis of the full study, the full 992 patients, and the motivation -- a motivation -- was to address the confirmation issue.

I broke the study into two pieces; good prognosis versus poor prognosis. And the reason I mention that is because of the positive nodes issue. In my opinion, breaking out into four or greater positive nodes, which is suggested by this question, is not appropriate.

This is a subset analysis; subset analyses are problematic; not impossible, but problematic. The number of positive lymph nodes in breast cancer; we know that breast cancer is a heterogeneous disease. HER-2/neu is one of the categories of heterogeneity. Estrogen receptor status is one of the categories of heterogeneity.

The number of positive lymph nodes probably is not. It is a level of disease, rather than identifying a different disease, and the worldwide overview makes that very clear. The number of -- if there's a chemotherapy that works in node-negative patients, it works in node-positive patients, and vice versa.

This study breaks out to poor prognosis and good prognosis, using as prognostic variables, number of positive lymph nodes, estrogen receptor status, tumor size; specifically not dose of CAF; specifically, not HER-2/neu.

I separate it into two pieces, equal pieces, based on the Cox proportional hazards model using those co-variates, and also premenopausal status. The number of the events in both cases were the same.

These poor prognosis patients are mostly those with a large number of positive lymph nodes, not exclusively. If somebody had four positive lymph nodes, a small tumor, ER-positive, that person would be in the good prognosis group.

If they, on the other hand, had two positive lymph nodes, ER-negative, and a 5 cm tumor, they would be in this poor prognosis group.

And what you see here, on the basis of the full study, is exactly the same as in the individual pieces; namely, no difference among the doses in the HER-2-negative group, and a very dramatic difference between low dose and high dose, with middle dose in between; for example, at five-year survival, only 15 percent in the low dose group, and about 60 percent in the high dose group. Really, a dramatic difference.

Now, maybe it is the case that the poor prognosis patients are carrying the day; maybe the good prognosis patients don't show any benefit. Not so.

In the good prognosis patients, the p-value is even more significant. Here we have two pieces of the study, both of which give a statistically significant correlation between HER-2 and CAF. No difference -- and this is where you want to pay attention is in here, the denominators out here are smaller for several reasons -- no difference between the doses in the good prognosis --

By the way, if you compare the previous picture with this picture, you will see that the curves are lower in both cases. That's because the former is a poor prognosis and these are good prognoses. But there is no difference

here and here, a statistically significant difference between high dose and low dose, especially.

Conclusions. FISH reliably detects HER-2/neu amplification. There is a significant interaction between CAF and amplified HER-2/neu. In particular, HER-2/neu amplified patients benefit from intensive CAF, ten-year overall survival; 39 percent in the low dose, 67 percent in the high dose.

In non-amplified patients who do not benefit, they do not benefit from intensive CAF, regardless of their number of positive lymph nodes. Ten-year overall survival, about the same in the two groups. And there is a similar interaction between HER-2 and CAF in disease-free survival.

The results are consistent with the corresponding results in the bigger study of immunohistochemistry and PCR. Thank you.

DR. ENNS: Again, if we could hold questions until the end of our presentation, we will have time I believe on the schedule to have questions and answers. We have obtained for you, the answers to your questions after Dr. Seelig's talk, so we will be able to answer those forthrightly for you, without going off limits on other data.

At this point, I would like to bring Dr. Slamon back to the microphone to give a clinical oncologist's and a

molecular biologist's perspective of HER-2.

**Clinical Oncologist's/Molecular Biologist's  
Perspective of HER-2**

DR. SLAMON: Well, I think now we can get into some of the issues that, for our own personal laboratory, have been issues that have been particularly perplexing and vexing to some degree with regards to the whole HER-2 story.

And hopefully, I will be able to give some perspective on this, in the sense that there are a number of different approaches one can take to determine whether or not a patient has this alteration, and they are listed here.

When we first started this study, we had no idea that the gene we were looking for would be HER-2/neu, we were just using a number of probes that we had obtained that were involved in the growth regulatory process, with the objective that I stated earlier; trying to identify an alteration that may play a role in the pathogenesis, with the objective, ultimately, of targeting that.

The best technique we had at the time, when we were using actual clinical specimens, were to extract the macromolecules from those specimens, DNA, RNA, and protein, and begin to look at comparisons of the DNA levels, or the expression levels in those tumor samples, relative to normal samples.

And the approaches are shown here, southern

blotting, northern blotting, western blot analysis, immunohistochemistry in frozen material as well as immunohistochemistry in paraffin-embedded formalin-fixed tissue.

After we did these studies, which shows you how long ago it was, PCR technology came online and that became another tool to be used, and even after that, FISH technology for these probes in solid tumors became available.

As each one of these technologies became available to us, we went back and reassessed, and asked its relative strength or weakness in telling us whether or not a patient had this alteration.

The work with regard to FISH, I will say right at the outset, has in large part been done in my laboratory by Dr. Giovanni Pauletti, who is here today if there are any questions some of the technical aspects of actually carrying out the technique with the research probes we were given from Vysis.

Basically, perhaps one of the best-traveled slides that we have was from this study that we did, where after we showed in 1987, there was an association between amplification and outcome, there were some studies by some outstanding laboratories that said, the amplification rate that we said was not at all correct, there was nowhere near

this level of amplification; it was more like 5 or 10 percent of the cases. And more importantly, there was no correlation between this alteration and patient outcome.

So at that point I decided we would go back to the drawing boards, and we spent an enormous amount of effort, rounding up some samples on which there was sufficient clinical material on which to perform what we called, a comprehensive analysis.

To be able to look at a tumor at the level of DNA by southern blotting, RNA by northern blotting, protein lysix(?) by western blot analysis, as this was one of the approaches that was then being used; and also have sufficient material to look at immunohistochemistry in frozen material, unfixed, unembedded, as well as those same exact samples that had been fixed, embedded, and also processed for immunohistochemistry. And this is the results from that study published in 1989 in Science.

Women who had a single copy of the gene as determined by southern blotting in their tumors, had this level of RNA expression, this level of protein as seen -- and these are representative examples of the cohort. This represents a 180-patient study.

When we stained for immunohistochemistry, in frozen material, it is very difficult to see here, but if you had this right under a 'scope in front of you, you would

see very faint membrane staining on the epithelial cells, specifically. The stromal elements were not staining to any significant degree. This is in the frozen setting.

If we took tumors that had a two- to five-fold amplification of the gene, as determined by southern blot, looked at the RNA, the protein and the immunohistochemistry, we saw this picture, where we began to see the membrane staining. I will show you this at a higher power in a moment.

Five to twenty copies. The RNA, the protein, and the immunohistochemistry in the frozen setting, and more than 20 copies, the RNA, the protein, and the immunohistochemistry.

There was a cohort in this study, and this becomes a critical cohort for the purposes of discussion, today, that appeared as if it had a single copy by southern blot analysis, and yet dramatically overexpressed the protein.

We called these in that paper single copy overexpressers, but thankfully in that paper, we added the caveat that these may not be really single copy cases, but rather amplified, and we had missed the amplification due to the inherent problem with looking, not at cell lines, but at solid tumor specimens. And if I can have the carousel, I will show you exactly what I am referring to.

This is something that I think will be obvious to

most of the Panel members, to all of the Panel members, but I've been consistently astounded at how it hasn't become apparent to many of the people publishing in the field.

If we are taking a tumor specimen, and extracting -- this is an invasive ductal carcinoma -- extracting the DNA, or the RNA, or the protein, essentially any macromolecule that we use for a solid matrix body. In extracting those macromolecules, we are getting the macromolecules, not only from these cells, but also from the surrounding normal cells, stromal elements, inflammatory cells, vascular cells, etcetera. Therefore, we are introducing right up front, a dilutional artifact that will, if anything, underestimate the frequency of this alteration; certainly, not overestimate it. That was the initial crux of what we were looking at.

I think the next slide shows, when we stain with HER-2/neu-specific antibodies, in the frozen setting, you can see the intense membrane staining that is restricted to the malignant cell population, with the normal cells not showing this level of expression.

I think -- go ahead. Yes, if we can go back to the computer. No, we are done with the carousel, just to make that point. And it's something that I know appears obvious, but it has been astounding, consistently, how that has not been looked at in the field, and I think that has

been part of what has confused the literature so significantly over the years.

Now, in addition to that problem, there is also a problem that when we go from frozen material to paraffin-embedded formalin-fixed material, there is another artifact when you are using paraffin-embedded material. And this is an artifact that, it was the first time we had seen it, and the first time I had become aware of it, but the people who work extensively in immunohistochemistry sort of laughed at us when we told them, specifically, we went to Hector Battefora(?) and said, you know, this loss is pretty dramatic and he said, but there is no antigen that he is aware of that doesn't lose something when you go into formalin fixation, and paraffin-embedding.

This is a tumor specimen that we knew to be amplified by the southern blot; we knew it overexpressed the RNA; we knew it overexpressed the protein by western blotting, and here is a frozen section showing the intense membrane staining on the cells. I apologize for -- maybe we may have to have a little more lights off to see this.

If you take this exact same specimen, embed it, fix it in formalin, and embed it in paraffin, you now have a negative signal. So, there is an inherent problem with using archival material, when you're using an immunohistochemical approach, that is difficult, if not

impossible, to overcome. And we will talk about that momentarily.

And I think that is best shown in this. We had done that with our own antibody, which is called the R60 antibody we developed in our laboratory. But in a study we did with Mike Press at USC -- he was at University of Chicago at the time and had come into the laboratory to do a sabbatical at UCLA, when we started to do these studies --

We asked the question, since there is so much confusion in the literature, with some people saying it is correlated, some people saying it isn't correlated. Let's take all of the antibodies that we can get, everything that was available at the time, and people were quite generous giving us their antibodies, as well as those that were commercially available. And take these molecularly characterized cases, the ones that we considered gold standard, platinum standard, indeed, in the sense that we had all of the data on them.

We had them in frozen and paraffin material, and look at all of the antibodies. And we did this blinded, so we coded the antibodies, so we didn't know which antibody was which, and then asked how often will we be able to detect amplification greater than five-fold; amplification in this moderate range, two- to five-fold -- single copy overexpressers, which really do exist, but in a much smaller

proportion, as I will show you in a moment -- as well as, how often will we have antibodies that are showing the alteration is present, when in fact, it isn't present?

This is a list of the antibodies we looked at, and you can see that there is considerable variability among the antibodies. This is for the samples that are more than five-fold amplified.

In general, for the antibodies that perform very well, you can take those samples, embed them, or fix them in gasoline and embed them in concrete, you will still detect it. Where you get a problem is when you get down to the lower levels of amplification. You start to lose sensitivity significantly.

The best available antibodies, the 9C2 antibody, which is a research reagent developed by AmGen for other reasons, as well as R60, only detect 80 percent of the cases that have the alteration when you go into formalin material. And then it falls off from there.

Astoundingly, however, many of the publications in the literature, some of the prominent ones saying that there is no association with outcome, are using antibodies like this antibody here, TA1, which has an overall sensitivity of detecting only 30 percent of the cases that actually have the alteration.

I can tell that when we did the studies that we

did, looking for targeting this, the antibody we picked to use for an entry criteria was an antibody that Genentech(?) developed called 4D5, which ultimately became herceptin, and we recognized that we were using an antibody that was far from perfect.

All of them are imperfect in paraffin, but this is far from the ideal, even in paraffin, but the reason we were using it was we reasoned that if this recognized it, we knew that at least every patient getting into the study had the alteration. That was the rationale. I was involved in those decisions when those were made. So, it wasn't because this was an ideal antibody, it was because of that single factor in my discussions with Mike Sheppard(?), who at the time was in charge of the program. And you can see the distribution of the other antibodies here.

This becomes one of the most critical pieces of data that I think we have, and it shows the considerable variability of all the antibodies, and I think explains quite clearly why there was so much controversy in the literature, because most of them were using antibodies in this range, that do not detect all of the cases that have the alteration.

Now, the issue of loss of sensitivity is seen here, again, from a paper we published with Mike Press, where we were looking at tumors that have more than five

copies of the gene, two to five copies of the gene, and do not have an increased number of the gene, and you can see that with the R60 antibody, we lose sensitivity, but we can still detect; with the 4D5 antibody, we have very significant loss when we get into this mid-range.

With the TA1 antibody, one of the antibodies in which a large clinical trial was published in Cancer Research that said there was no association, you can see that you do not detect it even in the higher amp, but occasionally you can see samples where you get this intense cytoplasmic staining, but not specific membrane staining.

So, I think the long and short of it all has been that, there has been a lot of controversy introduced in the literature and into the discussion in this area, that may not have a scientific basis, other than the fact that there are reagent variabilities in the artifacts of the techniques used.

Let's get back now to this group. The single copy, so-called single copy, overexpressers. As I said, because of the dilutional artifact problem, we allowed in that paper, that perhaps these were amplified, but we really couldn't detect it with the technology we had at the time.

Enter FISH technology. We were now able to go back and look at these exact samples. I should tell you, in this whole cohort, it was 11 percent of the cases appeared

to be single copy overexpression.

When we went back with FISH, we found exactly what we thought we would find, and that is that, these indeed were amplified, and that the true, single copy overexpression incidence in these specimens, was only 4 percent of the total cohort. So, it is a rare phenomenon.

In the submission, we have now in the new study that we will talk about briefly, looked at single copy overexpressers to determine if they have a worse survival, and the astounding factor is that the single copy overexpressers indeed have a survival curve that looks like non-overexpressers. So there is something unique to the amplification event, and the overexpression of the curves with amplification, that we think is critical in the pathogenesis of detecting this alteration and making decisions based on it.

So, basically, the benefits and risks of determining the HER-2/neu status, a false-positive result could lead to medical decision, causing the patient to undergo unnecessary therapy. I think you have heard from Don, as well as what is in the literature, about some decisions that are being made in terms of predictive strength of HER-2 in predicting what therapy.

Equally importantly, a false-negative result could lead to a medical decision depriving a patient of

potentially beneficial therapy, so I think these are very relevant points that I suspect are obvious to everybody on the Panel.

We undertook a study, looking at an Australian cohort of some 920-plus patients, to determine, finally in a large cohort that was consecutively accrued with sufficient follow-up time, to ask, what would things look like in a direct head-to-head comparison between FISH and IHC, in a large cohort, that included both node-negative and node-positive patients?

The shortcomings associated with fixation-induced antigen changes I have shown you, that is clearly avoided by FISH. DNA by its nature is a more stable molecule with regards to fixation, and we do not have the losses we see that are fixation-induced, that are inherent with IHC.

The dilutional effects, I have already discussed, and FISH allows us, like IHC, to go on a cell-by-cell basis to determine if the alteration is there, and as I said, it was a large cohort chosen for statistical significance.

We also went and asked before doing this study, was this cohort representative of the breast cancer population in general? So we looked at traditional prognostic factors, and all the ones that are traditionally there are clearly, and indeed, statistically significant in this cohort. So, we were reassured that this was a

representative cohort of the population at large.

Here are the data. The samples, as determined by immunohistochemistry, graded as -- and it is difficult to see here, but 0, 1+, 2+, or 3+, and you can see here that the curves are essentially overlapping, until we get to the 3+ cohort, in which case we see a statistically significant survival difference.

If on the other hand, we use FISH technology to do this exact same cohort, there is very plain separation -- and it is significant separation -- between all of the groups, with regards to single copy, and 5 to 8, 8 to -- I believe that's 8 to 10, and greater than 10, in terms of signals.

Now, looking at the whole cohort with the corrected approach; that is, as you have heard from Steve Seelig, correcting for chromosome 17, it maintains exactly the statistical significance. And I will tell you, in this cohort, this is now being -- it has been presented publicly and is being prepared for publication as we speak -- this holds for the node-positive and node-negative components of this cohort, and the node-negative component of this cohort was in excess of 350 patients.

Basically, where the action appears to be, is in this area here. This is a graph depicting FISH signals per cell, and it is just grouped geometrically, in terms of the

progression.

This backwards would be considered normal, four signals or less. This forward would be considered amplified. And you can begin to get a sense of where you are seeing cases that are positive by immunohistochemistry, yet negative by FISH.

This represents what is that true, single copy, overexpressing group of patients, and in this study, it was 5.5 percent, in complete agreement with what we saw in the original study, and in agreement with other studies done by other groups -- Kelly and Nimi(?) from Pinkel Group -- that single copy overexpression is a very rare event.

The flip side of the coin is, the number of times you are seeing cases that are amplified, clearly amplified here, in terms of FISH signal, and yet in the green, light green, these are negative by immunohistochemistry, so the false-negative right here, is a very real phenomenon, and it is based on what we have talked about already, this fixation problem that you see. So, I think that that really does put it in perspective in terms of what we are looking at with regards to the various tests.

Steve has already talked about this data, I won't go over it. This is the inter-assay variability, and you have already heard from Don with regards to the analysis of the CALGB Study, so I think that, based on what we have seen

up to this point, this quote is dated -- there is an error in this slide -- this is dated 11/09/98. I've actually made this quote many times before this, once we did the first study.

There is no question in my mind, as someone who has been working in this field for some time, that the FISH technology is superior to all of the other technologies in detecting whether or not this alteration is present in a patient's tumor, due to the inherent issues that are in the biology of the tumor itself, as well as what we need to do to correctly, pathologically assess one of these tumors with regards to fixation and embedding. And I will stop at that point. Thank you.

DR. ENNS: In conclusion, one of the questions that was posed to us by the Agency in the original 90-day Letter, as well as in the questions to the Panel, is to discuss a proposed training program, and then I want to go back to the Recommended Intended Use, and we will be done here in just a couple of minutes.

Again, a proposed training program, the objectives are to provide training and specimen preparation, assay procedure, and interpretation of FISH testing of HER-2/neu amplification. And also, to validate performance of the PathVysion Kit on breast carcinoma specimens known to possess HER-2/neu amplification, or overexpression.

This is consistent with high complexity, clear requirements for introducing a new test into the laboratory. We believe it is essential that laboratories, for the benefit of the patients and the physicians being able to make intelligible choices and treatment decisions, that it is absolutely essential that laboratories validate the performance of even FDA-approved products in their own use.

We support the attitude and the idea of a training program, and we think it is important. It is negligent on the lab director's part if they introduce a new product without following the clear requirements to validate the performance of a new product.

Again, we just mentioned the benefits and risks of these statements for this product, and I think that that is very evident, that you need to have accurate reporting, and so training is part of the ability to do that.

You have also received, I guess I should just comment on one more thing. You have received a sample, I believe, of the sample training program that we provided for the Agency, what we think would be a suitable training program.

The PathVysion HER-2 DNA Probe Kit again is designed to detect amplification of the HER-2 gene by FISH and paraffin-embedded specimens from subjects with node-positive, stage II breast cancer.

This is what we are requesting of this Panel to review. You have heard comments about other aspects of breast cancer, but our specific indication before the Panel today is of the Pivotal 302 Study, and the indication is for node-positive stage II breast cancer.

The results from the PathVysion Kit are intended for use as a rapid assessment of the potential response to adjuvant therapy, leading to choice of therapy. And again, I point out that we are intending to market this product to people who are qualified to perform this test, and again, I will point out, that there are over 1200 board-certified American medical geneticists, from the American Medical Genetic -- American College of American Genetics.

A good number of these people that are board-certified in this field, are also pathologists, and there are people who have had experience, and we have a couple of those with us today, in the audience.

We have Dr. Shahla Masood, and we also have Dr. Diane Person. If you have questions about how a pathologist and a molecular geneticist can do this test, they would be more than happy to share their experience with you.

Thank you. That ends our presentation.

DR. LADOULIS: Thank you, Dr. Enns. I just want to make a comment and a question and then open it up for some questions before we take a lunch break. And I think

overall, I sense there is something missing here in the middle, in the performance of the kit. There is a submission of data about the performance of the FISH procedure on the target cells, as was described in detail, and with excellent description.

Then we have on the opposite end of the spectrum, the clinical relevance of an excellent presentation by Dr. Slamon, using the analysis of patient specimens. And what I would like to hear, and I hope that maybe after the lunch or during the presentation following the FDA, Agency's, presentation, some discussion about the actual examination procedure, using the reagents on patient specimens, and what the variability is, and what is the threshold levels -- from Dr. Pauletti and Dr. Masood, because obviously, it is an inhomogeneous population.

There is biological heterogeneity within the tumor, within the same patient. And so, in terms of performance characteristics, and that's relevant to training of pathologists and medical geneticists, you must have some principles of application, and then some pitfalls of the procedure in terms of the heterogeneity within the patient specimen, the numbers of nuclei, the number of signals per malpa(?) DNA, and those kinds of issues. Okay?

DR. ENNS: Yes. Thank you Dr. Ladoulis. We do have answers to your questions that we are doing, and right

after Dr. Seelig's presentation, we do have information from the Pivotal 302 Study. I believe it's -- I want to turn your attention to a couple of slides that are in your package, and you can look at those.

One is still in the -- mock cell lines, on -- it's in your orange book. It's in Volume Two, Tab 10. That's the green tab. And then, under the white tab, Tab 12, is page 19. And John, if you could call up again, Slide 103. And then Steve will come talk about it.

Okay, now this is an internal study done, again on cell lines, and I understand it doesn't answer your question yet on heterogeneity in specimens, but this was a study that was done internally, between and before we embarked on the 302 Pivotal Study, to justify counting of 60 nuclei, instead of the 100 nuclei that we did in the Reproducibility Study Protocol 300. And I think Steve can address that.

Then, we can go to -- we'll go to -- Donna, it will be Slide 107 when it comes to that, but let's hold this. It will be the actual analyses done by Duke, by Don Berry, on justifying the use of not only 60 nuclei, but 20 nuclei in the actual breast tumor specimens.

This was part of our response to the 90-day question, where they asked -- the FDA asked us the question, how did you go from 100 to 60 in the Pivotal Study?

This was based -- this is how we did it, based

upon our internal laboratory validation of mock specimens. Steve will address that. And then we will go to how going back and looking at where we did the actual 302 with 60 nuclei, and doing random analysis, you could actually show that you could get the same results doing 20. And we will come back to you and still answer your question about variation and variability.

Again, we do have a Portability Study that has been done. You have seen the Summary Response. It is in your packages, but again, the FDA has not had the opportunity to review the raw data.

Those were done on actual tumor specimens that were cut, they were sliced. They were randomized and blinded and they were sent to five different laboratory sites as part of our Portability Study, to show, again, that we had excellent performance in reproducibility and 100 percent correct classification of every amplification level from normal, nonmalignant cells, to very weakly amplified, to moderate, to strong amplification.

When I say, the weakly modified, the weakly modified is sitting right on our cutoff for clinical utility. So, we do have that information and I apologize to the FDA, because we have not yet had the opportunity to submit to them the raw data so that they can review the data for themselves for its integrity.

I can tell you, though, that we have been through a full GCP inspection, both at the 302 clinical trial site, and the University of North Carolina. All the case report forms for the 302 Study have been reviewed, and in addition, we have gone through a rigorous GCP inspection, at our facility. So, all of this data is there, it's available, and we will get it submitted to the Agency as quickly as possible.

Thank you. Steve, you want to come up and discuss this slide?

DR. SEELIG: This should go fairly quickly. This Study really looks at the effect of the number of nuclei that you count. Again, it's a synthetic system, three different cell lines, with different levels of amplification or HER-2/neu to CEP 17, counting 120 nuclei versus 60 nuclei, for those three different cell lines.

You can see, for example, MDA MB 231, 120 nuclei; five repeats. You have a mean value of 1.05, and you have your 95 percent confidence, and it's --

DR. LADOULIS: Excuse me. Are these cell lines?

DR. SEELIG: These are cell lines.

DR. LADOULIS: Are these paraffin sections or smears?

DR. SEELIG: These are formalin-fixed paraffin-embedded cell lines that have been placed down on the slide,

so it mimics the procedure that the specimen is going through. And again, it doesn't address the tissue variability that you have asked, but it addresses the other part of the question I think is, what's the effect of lowering the count? I'm only focusing on that piece. Don can address the tissue variability.

But I think you can see pretty clearly that, going from 120 to 60, the mean value is 1.05 versus 1.03 for this cell line. At a slightly higher level of HER-2/neu to CEP 17, 120, 1.85, down to 1.77, counting 60. Again, no real substantive difference.

Confidence limits here, again, are essentially the same range. And for a slightly higher level of amplification, the SKBR-3, going from 120 to 60, we have 3.52. Going to 60, you go to 3.55. And again, the confidence limits are slightly broader, the 95th -- are slightly broader than if you had counted 120 cells.

From an analytical point of view, you can reduce the cell count --

DR. LADOULIS: From the clinical studies, the statistical cutoff was two signals or greater?

DR. SEELIG: Two, right. So, we have spanned that statistical cutoff in this study. This is -- these are very low levels of changes.

DR. LADOULIS: What would be of interest is to see

a -- you know, at populations in which it is an average of approximately two, how much variability there is between 120 -- or 100 nuclei and 60 nuclei for those -- for particular cell lines that actually have two signals.

DR. SEELIG: Well, I have -- I have 1.85 and 1.77, and --

DR. LADOULIS: Well, I mean, since the threshold is two, the question is, what is the error, and it's introduced by categorizing a patient into one cohort or another, based on whether or not you count 60 or 120 nuclei, and actually, it's two signals, so it happens in that patient, you know. So, I mean, that's the threshold for the clinical studies.

That's why that actual biological variability and the methodological variation that either compounds it or un compounds it can be critical, because it then segregates patients, if that is what's being used, two or four, I don't know what --

We have maybe just a few minutes before we want to do a lunch break, and if you have some response here, or if there are any other brief questions from the Panel before we break.

DR. ENNS: I have two very insignificant, brief comments to be made. One first by Dr. Don Berry, using the actual 302 Pivotal data. John, the hidden slide 107. And

this is again from the actual patient data. And so you will hear from the biostatistician how he used random analysis to qualify going from not only 60, but down to 20 for significance, using the raw data.

This was a part of our 90-day response. We went a little beyond, why did you go from 100 to 60? We went from -- 60 was too much, but you could do 20. And then for many of you that are really concerned as oncologists and practitioners, what do you do with this data? What does it mean? You asked the question about heterogeneity.

I'm going to have Dr. Shahla Masood, who is a practicing pathologist, who is familiar with both immunohistochemistry methods as well as FISH hands-on experience, will talk to you about the practicalities based on her experience, in actually measuring breast cancer specimens. Thank you.

DR. BERRY: This slide is much too busy. Let me just explain the process that we undertook. We selected randomly from these 60 cells, 20 cells, in each of the 524 cases, and compared the proportion of positives with the 20 cells, on the basis of -- with 60 cells. And you see the concordance that we get, that the correlation between the two in multiple iterations of this, was over 99 percent.

This is selecting cells randomly. You might think that the reader would pick different cells for the first 20,

then random cells. And so, we also did an experiment based on a selection of 100 of these 524, looking at the first 20 of the 60 cells.

Now, I don't want to break any protocol here. This was not presented to the FDA as yet. We've just recently finished it, and so I won't tell you about it, unless you want me to, Dr. Ladoulis. No? Okay.

DR. LADOULIS: No, I just -- the mean is irrelevant. It's the cyrtosis and the skewedness around a threshold value that's chosen to segregate cohorts of patients is what's important.

And so, if you go from 60 to -- 100 to 60 to 20, and the actual population of that patient happens to be two, so the question is, how does the skewedness that you introduced by the smaller sample, even though the mean might be close to two, how does it affect the selection of the patients that might be in that borderline?

It may be irrelevant if you're choosing in clinical use, actually, for practical purposes -- and maybe Dr. Masood or Dr. Pauletti might say that, you know, when you are looking at four signals or more -- I mean, I --

DR. BERRY: Let me give you just a little bit of evidence.

DR. LADOULIS: But I mean, maybe it's one of these peccadillos that you'll say --

DR. BERRY: Yes. Well, there is no difference. When we did these selections, it didn't -- we had the lowest correlation among 100 simulations was 99 percent, and so there is very little variability here.

With respect to what you were just saying, we have a little bit of evidence from this study. We did a duplicate analysis with different readers on 22 of the patients; not a big sample size, but some evidence in the actual clinical setting.

And in those 22, there were no discrepancies between the two. There were only two that were positive, but both readers got the positives, and in the other 20 cases, they were all negative. So that's some evidence in that direction.

DR. LADOULIS: Are there any other questions or --

DR. ENNS: Dr. Masood would like to just, from her experience as a practicing pathologist, answer your question on heterogeneity and the use of FISH.

DR. MASOOD: I'm really here to just make you aware of the problem of heterogeneity, and how we as practicing pathologists are dealing with that.

The concern that you have about the presence of heterogeneity, intra-tumoral and inter-tumoral heterogeneity exists, even between this cell block of tissue.

There is significant heterogeneity between the --

not only morphology, but also the expression of variety by marker. And that has been already published in (?). (?) morphology, morphology as well as hormone receptors and to others.

And there is practically no way that you can overcome that heterogeneity except to be very, very aware of that concept, and also from the beginning, try to section the tumor appropriately, and try, when you look at the selection of appropriate technology for assessment of a biomarker or anything else, try to make sure that a pathologist is aware of that heterogeneity and then select a block of tissue that is most representative of that given tumor.

Occasionally, we have come to that understanding that, for some of this testing, the best thing is to have two blocks of tissue, so that there has been a little bit more representation of the entire tumor, rather than a selective process that naturally, any one of us would become biased when you see more of the more aggressive nature of a lesion.

Therefore, heterogeneity is something that has to be dealt with in selection of the tissue block that is most representative, and FISH naturally, being modified for a paraffin-embedded, formalin-embedded, you know, paraffin tissue, is more suitable, simply because the pathologists

have that chance to really go through the entire tumor, see what is the most representative, and then if the tumor is the same everywhere, therefore one block can be chosen.

If there is significant heterogeneity, then the choice of having two blocks in the same setting of specimen and testing is not going to be a major problem. Any questions?

DR. LADOULIS: Yes, Dr. Kemeny?

DR. KEMENY: Has anyone looked at that heterogeneity for HER-2/neu, within the tumors and like to the lymph nodes?

DR. MASOOD: I'm not aware that has happened, but I guess I'm not the best person to respond to that.

DR. SLAMON: It has been extensively looked at in the research studies that we did, and that is not part of this submission, and Dr. Pauletti is here. He can address briefly what the issues are. But I have been struck significantly by how relatively homogeneous in the population we see this within the tumor.

There are clearly section artifacts you can't get around. You're cutting a 4-micron section through a 12-micron or greater nucleus, so you're only getting a representation, but if you count --

In our own studies, when we are looking at the low end amplified cases, we are counting 100 nuclei, but in the

high end amplified, we can get by with 20, but as we started to go down and look at the relative distribution frequencies of the signals, you can get down to 30 nuclei and still be very consistent that this is not something that you are going to get fooled by.

The issue is, this alteration occurs, which we have done the studies now that show us, the alterations occur somewhere in the biologic life of the tumor, between atypical ductal hyperplasia and DCIS.

So, it's relatively clonal, in the clinical life of the specimen. When you are actually biopsying and looking at a tumor tissue, malignant tumor tissue, the cells are very homogeneous cell to cell, unlike p53, hormone receptors, RAS protein products, as well as HER-2 overexpression as determined by immunohistochemistry. The FISH is very reliable, and very nondistributed all over the spectrum with regards to --

Do you want anyone to add anything in terms of the numbers? Basically, that's it? What he says is, he agrees.

DR. LADOULIS: Yes, a question?

DR. BERRY: Actually, can I make one comment about that? We did not FISH, but we did an IHC, Invasive Interductal Components, in the same 302 Study, the CALGB 8869, and found extremely high correlation.

In other related protocols, we have looked at the

HER-2/neu in the positive lymph nodes, and again, we find a very high correlation between the tumor component and the nodal component.

DR. MCCASKILL-STEVENSON: Wort McCaskill-Stevens. Actually, this is for Dr. Berry. Regarding the positive lymph nodes, I just want to be clear that your statement was inclusive of the ten positive nodes, as well. In terms of amplification and being positive and --

DR. BERRY: Yes. There are only about 5 percent of this patient population that has ten or more positive lymph nodes, but exactly the same thing is true regardless of number of positive nodes.

DR. LADOULIS: Are there any other brief questions? If not, I think it is time for us to take a lunch break, in which we will reconvene in exactly 60 minutes, if we can do that, at 1:45. Thank you.

MS. MAGRUDER: I would like to make an announcement about the lunches. The ones that were preordered are here, and the Panel will be eating in Room 20H, and the Sponsor will be eating in Room 20C. Thank you.

(Whereupon, at 12:41 p.m., a recess was taken until 1:45 p.m. that same day.)

P R O C E E D I N G S

(1:46 p.m.)

DR. LADOULIS: I think most of the Panel members have returned, and the Sponsor members are here. Is that all the Sponsor's representatives? Let us reconvene, then, for the afternoon.

We are scheduled now for a presentation from Agency staff. Dr. Maxim, Dr. Weng and Geretta Wood. So, who will be making a presentation first, Geretta Wood? Okay. Thank you.

And I guess I should also announce, also, that Dr. Liu, Director of the Division of Clinical Sciences of the NCI, will be available to answer Panel members' questions regarding the Cancer And Leukemia Group B Study, Protocol 8869. And Dr. Liu will sit at the presenters' table during the Open Committee Discussion. Without further ado, then, Geretta Wood.

**FDA Presentation****Geretta Wood, Scientific Reviewer, Immunology**

MS. WOOD: Good afternoon. As you all know, today's subject is the PathVysion HER-2 DNA Probe Kit, manufactured by Vysis, Incorporated of Illinois.

The Intended Use of the HER-2 DNA Probe Kit is to detect amplification of the HER-2/neu gene using FISH. The Kit is intended for patients with node-positive stage II breast cancer. The assay is indicated to assess the

potential response to adjuvant therapy, leading to choice of therapy.

The two main components of this device are the two DNA probes, the LSI HER-w/neu probe contains sequences specific for the HER-2/neu human gene locus. The probe is labeled with spectrum orange.

The chromosome enumeration probe, or CEP 17 probe, contains alpha satellite DNA, and serves as an internal control, to determine copy number for chromosome 17. This probe is labeled with spectrum green. The DAPI counterstain for nuclear DNA stains blue.

Hybridization efficiency was tested on the Sponsor's control slides. The average percent of cells with no hybridization signal for either probe, was 0-2 percent. Therefore, the hybridization efficiency of the assay is expected to be around 98 percent.

Stringency studies were also performed to determine the optimum denaturation time, and temperature. Hybridization was significantly affected by both the hybridization temperature and time, with the hybridization at 37 degrees Centigrade for 18 hours, showing the highest overall quality.

The sensitivity was tested in the Clinical Reproducibility Study, which I will describe later.

The limit of detection in interface cells was

estimated to be a ratio of 1.5.

To test the analytical specificity, the Sponsor performed locus specificity studies, with 254 metaphase spreads, examined by G-banding to identify the chromosome 17 and the HER-2/neu locus, followed by the FISH assay.

No cross-hybridization to other loci was observed. The stability of three similar devices manufactured by the Sponsor is two years. Stability studies for this product are ongoing and are expected to have about the same stability.

Repeatability was tested on ten consecutive sections of normal, and ten sections of amplified breast tissue. These studies indicate that the assay is reproducible in different sections of the same tissue block.

Due to the difficulty of consistently slicing 4-micron sections, the Sponsor chose to test different thicknesses of tissue to determine the effect on amplification. Tissues were tested, ranging from 2 to 8 microns, with no statistically significant difference.

The Sponsor performed a reproducibility study, identified as Protocol 300, and a clinical utility study, identified as Protocol 302.

Protocol 300 examined reproducibility in the following areas. Between-day, between-site, between-lot, between-assay, and between-observer.

Three study sites tested a total of 120 paraffin-embedded tissue sections from four cell lines with known ratios of HER-2/neu to CEP 17. One hundred nuclei were counted for each specimen. Each sample was tested by two technicians.

We will be evaluating the data presented for the feasibility of counting 60 nuclei, as well as 20 nuclei, however, this may necessitate new reproducibility studies at that level.

There was no significant day-to-day or probe lot-to-lot variation, however, there were statistically significant observer-to-observer, and site-to-site variation. These differences did not result in misclassification of amplification.

The Sponsor performed their clinical study utilizing archived samples from the Cancer and Leukemia Group B Study.

I would like to briefly describe the CALGB 8541 Study. This Study included 1572 women with node-positive, stage II breast cancer.

The study was a prospective, randomized trial of CAF administered in three doses and intensities.

The patients were enrolled between 1985 and 1991. The three doses of CAF administered in 8541 are listed here. As shown on the slide, the cumulative doses of CAF were

identical in Groups 1 and 2, and 50 percent lower in Group 3.

The CALGB 8869 Study is a companion study to the 8541. 8869 included a subset of 442 retrospective archived samples, randomly selected from the 8541 trial.

Of the 442 patients randomly selected, 397 specimens were technically satisfactory for analysis.

This Study investigated HER-2/neu expression by immunohistochemistry, DNA index, S-phase fraction, and p53 accumulation. The IHC used in this study utilized a research-use antibody.

The Study indicated there was a significant dose-response effect to adjuvant chemotherapy with CAF in patients with overexpression of HER-2/neu, but not in patients with no HER-2/neu overexpression.

Vysis initiated their clinical study in May of 1997, and completed it in May of 1998.

Retrospective tissue specimens used for this protocol were drawn from the CALGB 8869 Study. The objective of the Sponsor's study was to determine whether the amplification of HER-2/neu, as assessed by FISH with DNA probe, provides statistically significant and independent prognostic information pertaining to recurrence rate, disease free survival, and overall survival in stage II, node-positive patients receiving adjuvant therapy.

All FISH assays were performed at the University of North Carolina, Leinberger(?) Comprehensive Cancer Center, and the signal enumeration was performed at both UNC and University of Kansas Medical Center.

The following criteria were utilized for subject selection and exclusion. Patients with node-positive, stage II breast cancer receiving adjuvant therapy in CALGB Protocol 8869. Sufficient archival, paraffin-embedded tissue available for the FISH assay, and complete information available on relapse, survival, as well as other relevant clinical data.

Vysis selected 711 specimens from the CALGB 8869 Study. 139 were excluded due to failure to meet the Study inclusion criteria. FISH was performed on the remaining 572 specimens, however, 45 were excluded from analysis because of FISH assay failure, and three were duplicate assays. This left a total of 524 specimens for statistical analysis.

The goal was to select 160 specimens from patients in each treatment arm of the 8869 Study. 179 specimens were selected in the high dose treatment group, 167 were selected in the moderate dose group, and 178 specimens were selected in the low dose group.

Protocol 302 consisted of a total of 524 specimens. Of this 524, 433 were HER-2/neu-negative; 91 were HER-2/neu-positive, and were equally distributed among

the three treatment arms of the 8869 Study.

Cox proportional hazards analysis demonstrated a statistically significant dose response effect of adjuvant chemotherapy with CAF in patients with amplified HER-2/neu, for both disease-free and overall survival.

At seven years post-randomization, the estimated disease-free survival probabilities for patients with HER-2/neu-negative tumors, are 55 percent for the low dose, 63 percent for the moderate dose, and 61 percent for the high dose group.

For patients with HER-2/neu-positive tumors, the probabilities are 36 percent for the low dose, 44 percent for the moderate dose, and 66 percent for the high dose group.

Overall survival probabilities showed similar trends. These results are consistent with those observed with HER-2/neu protein expression by immunohistochemistry in the 8869 Study.

FDA performed subpopulation analyses on the data we had available, the 524 patients in the Protocol 302 Study submitted in support of this PMA.

When separated by lymph node status, 296 patients had less than or equal to three positive lymph nodes; 228 patients had greater than or equal to four positive lymph nodes.

These patients are further categorized by HER-2/neu status. Our statistician will further discuss the statistical analyses performed on these groups.

DR. LADOULIS: We have Dr. Weng?

DR. WENG: Yes.

DR. LADOULIS: Okay.

**Teng S. Weng, Ph.D., Statistician**

DR. WENG: Good afternoon. I am Teng Weng, a statistician at FDA. This is the first slide, which is a continuation of Ms. Wood's presentation. This is about the Study 302, which used 524 evaluable patients, who were all node-positive with stage II breast cancer.

These patients could be divided into two subgroups; 296 of them had less than four positive nodes, while the remainder had at least four positive nodes. I will call this the node-negative group, although they are all positive, but just for simplicity, for the sake of simplicity. And this group will be referred to as the node-positive group.

Each of these groups can be further divided, dichotomously, into two subgroups. The first group has 242 patients. They were all HER-2 gene implication negative. The others are positive. And similarly, for these two groups.

For simplicity, I may just refer to this group as

the double negative group. This is the positive node-negative group. And this is the negative and the positive. And this is the double positive group.

I will start with this group. This group is the double negative group, having 242 patients. They are almost evenly distributed among the three dose groups. When we isolate this group and plot their survival curve using the Kaplan-Meier method, the curve looks like this. Here, 1 is the code for the highest dose; 2, the lowest dose; 3 is the standard dose.

See in this case, all three seem to benefit from the dose treatments, even the worst group; that is, the group who received the standard dose, had about 70 percent of survival probability at the study cutoff, while the group who received the highest dose, had about 85 percent survival probability. The lock(?) rank(?) test shows these differences to be significant at 5 percent error, similarly for the recoxin(?) test.

The recoxin test lays more weight to the beginning phase of the survival experiment, and the lock rank lay more emphasis on the final part.

Now, I am going to single out the positive and negative groups, to see how they respond to the different dose treatments.

Again, in these 54 patients are evenly, almost

evenly distributed among the three dose regimens. In this case, these three curves didn't show statistical significance, because the tests are not significant, but just notice that the highest dose, 1, and the low dose had about 60 percent of survival probability at the Study cutoff, while the standard group had about 55 percent. They are moderately high, all of them.

Now, let us look at the negative and the positive subgroup. This group is amplification-negative, but node-positive. The highest dose group had about 60 percent of survival probability at the Study cutoff, about the same as in the previous group, but the low dose and the standard dose group didn't show statistical significance, but they are significantly lower, compared to the high dose group. They had about over 40 percent of survival probability at the Study cutoff.

Now, let us look at the double positive group, which is amplification-positive and also, node-positive. The difference in the three dosage groups is remarkable. While the low dose and the standard dose are about the same, they have only about 15 percent survival at the Study cutoff, but the highest dose group, which had about 75 percent survival probability at the Study cutoff, and the difference is highly significant statistically.

Now, so far we have been isolating the subset of

patients and looking at how they respond to the dosage regimen treatment, or dosage regimen. These are the so-called local(?) analyses(?). They were performed against the backdrop of the more comprehensive model, which also includes other contributing factors, like the tumor size, menopause status, or some other important factors. This is a simplified form of Professor Berry's results. I just reproduced it here, but he has already made an interpretation for this, so I will stop here.

If you have questions, I am ready to entertain them. Thank you.

DR. LADOULIS: Are there any questions from members of the Panel? Yes, Ms. Wood?

MS. WOOD: We request the Panel's input on the following issues. Are you satisfied the data support the proposed Intended Use to Detect Amplification of the HER-2 Gene accurately?

FISH assays are technically complex. The Sponsor has proposed two different training programs. One plan, designed for experienced users, offers product users a booklet of color photomicrographs, representative of breast cancer tissue sections, with varying levels of amplification.

The second plan, for less experienced users, is a hands-on training program conducted at Vysis. Do you feel

these programs are adequate?

Subpopulation analysis of HER-2/neu-negative patients, with four or greater positive nodes, indicated that these patients benefit from higher dose CAF therapy. Will knowledge of the HER-2/neu-negative status offer any benefit in the management of these patients?

Does HER-2/neu status offer an independent benefit, in addition to node status in considering the use of high dose therapy?

And finally, in the Panel's opinion, are there any additional issues concerning the Vysis HER-2 DNA Probe Kit? I would like to take this opportunity to express my thanks to my review team for their help.

DR. LADOULIS: Thank you. Any other comments from the Branch? Dr. Maxim? Okay. All right.

Dr. Liu, you may sit at the presenters' table. I understand that Dr. Liu will have to leave at 3:30, is that right? So you will be available to answer any questions about the CALGB Study Protocol 8869.

You have heard the presentation from the Agency, and from the Sponsors. We are open for Committee discussion at this time. So, I would like to hear from any of the Panel members, if there are some questions. Dr. Kemeny?

DR. KEMENY: I have a few questions, but one of the things that I wonder about is, about the statistics on -

- okay, let me start out with my first question, then.

This may be a stupid question, but I want it in my own mind to be clarified as to, under the Intended Use for this, why are you combining the Intended Use specifically for node-positive, stage II breast cancer cases, when we have already heard from Dr. Slamon that they are already looking at people with node-negative breast cancer.

I just wondered why Intended Use is --

DR. ENNS: The Intended Use is consistent with the Pivotal Trial that we did. The Pivotal Trial was accessing the comparative 8869 Study to the CALGB 8541, and the inclusion criteria of that study was for node-positive, stage II patients. That is the Pivotal Study that we did.

Dr. Slamon presented data to you today that was basically from the literature and his own experience, and so that is not what we -- not what we have in our Pivotal Study. So, the Intended Use is consistent with the Pivotal Study that was presented. Did that answer your questions?

DR. KEMENY: Yes.

DR. ENNS: Thank you.

DR. KEMENY: Can I just ask another question? I found it now. It's on page 41, it was Slide 82. It talked about the comparisons of the patients in the trials, and it's -- Dr. Berry, actually, this is actually to Dr. Berry.

You talked about -- he said he selected a group of

patients to look at, and then he showed the comparison of the patients to the remainder of CALGB 8869. And it showed that the S-phase fraction was significantly different than the remainder, to the tune of a P of .001, where the S-phase fraction was much higher in the selected patients than in the remainder.

Now, first of all, I want to know why you think that happened. I mean, that there should be such a significant difference between selection and the regular group, and then, has anyone looked at the S-phase, whether S-phase had the same results on these patients as far as high, low, you know, levels of chemotherapy?

In other words, was that also significant, when you just looked at S-phase?

DR. BERRY: With respect to the differences in the two groups, all I can say is, S happens. It's a random phenomenon that I can't explain. As to whether or not it is important, that's the second part of your question. It was P .001, but this was, I don't know how many comparisons we looked at, and while that is still small, is the most extreme of 16 comparisons, it is -- it's not too unexpected.

As to whether it matters, indeed, the 8869, the first measure that we looked at was S-phase fraction, and we hypothesized a prognostic -- originally, a prognostic value to it. And my inclination was to look at therapeutic

interaction, and I did, for S-phase, and found none.

S-phase is correlated with p53. The story is a little bit different for p53, but there is some interaction, but for S-phase, it is absolutely none. So, this discrepancy between the two, would not have an effect. Did I answer your question?

DR. KEMENY: Yes.

DR. LADOULIS: Do you have another question?

DR. KEMENY: Probably.

DR. LADOULIS: Yes. Dr. McCaskill-Stevens.

DR. MCCASKILL-STEVENSON: My question relates to how this -- the Pivotal CALGB Study was interpreted, and this morning I have heard it referred to, and I think that is quite appropriately, that it was interpreted in the medical oncology community as being a study that helped us with the threshold, below which we didn't have efficacy, and I guess my question specifically is, will there be limitations or comments made about the dose of Adriamycin for which this is applicable?

Because it was 60 mg, during a time that it was not thought to be standard, but which is now 60 mg at a standard dose.

DR. BERRY: Yes, and that dose is now standard. One of the FDA questions, by the way, said high dose. High dose in my lingo is bone marrow transplant, so let's just

say, the current standard dose of Adriamycin.

There is a -- there has been a conjecture all along, and maybe Ed Liu can speak to this as well, about 8869, 8541, that the low dose was in fact no dose. The fact that it was an inefficacious dose, and that the comparison was with the chemotherapy versus not.

That is, I think, probably true, on the basis of the NSABP, on the basis of other data which we have not shown, that I have looked at in databases, that the issue is Adriamycin at a sufficiently high dose, as opposed to Adriamycin at a lower dose.

So, the issue is not high dose Adriamycin, but Adriamycin at 60 mg per meter squared, as opposed to not.

DR. MCCASKILL-STEVENSON: Okay, so that would be your answer to -- because as you may well be aware, some of the things that are coming down the pipeline are using higher doses of Adriamycin that have not quite reached the bone marrow transplant doses, which is why this is one question I am concerned about.

DR. BERRY: Yes. Adriamycin is not used as part of transplant, because of non --

DR. MCCASKILL-STEVENSON: Right. Right.

DR. BERRY: But in the follow-on study, the 8541, CALGB 9344, we accrued 3,000-plus women, looking at 3 x 2 factorial. One of the factors was Adriamycin dose.

Specifically, Adriamycin. Cyclophosphamide at 600, in all cases. Adriamycin at 60, 75, and 90.

And on the basis of the early analyses of that study, we see no benefit for increasing above 60. And so the next generation of studies in the inter-group, will consider 60 mg of Adriamycin as standard.

DR. LADOULIS: Yes. Another question, Dr. Kemeny.

DR. KEMENY: Just a follow-up to that question, and then back to, kind of my original question is, then again, in the Intended Use it says, response to adjuvant therapy, but actually, what we are talking about is response to Adriamycin at a dose of 60 mg.

So, I mean, then if you are going to say in Intended Use and refer to that study, then shouldn't we be a little more specific than that?

DR. BERRY: I think you should be. In fact, in earlier studies in node-negative and also node-positive patients, a node-positive study carried out by the inter-group, the formerly Ludwig Group, considered CMF versus -- the standard CMF versus CMF perioperatively, and did not find the interaction that we are looking at here, and in fact, found almost the opposite interaction.

So, that, too, suggests that it is Adriamycin-based, but clearly, it should be at least a CAF. My take on it is, it's Adriamycin versus not.

DR. HORTIN: I have a question, also. Maybe Dr. Berry might want to address this. For the data comparing the FISH versus immunohistochemistry, looking at -- the immunohistochemistry picked up about an additional 30 percent of patients.

And if you looked at the response to chemotherapy, the high dose or low dose chemotherapy, in terms of the statistical significance, it actually turned out to be higher for the immunohistochemistry. And it looked like the relationship was as strong if not stronger for the immunohistochemistry, suggesting that for the additional 30 percent of patients picked up, that they were actually biologically relevant.

Maybe you would comment in terms of that relationship, and whether the immunohistochemistry looked at, statistically appeared to have a stronger correlation than the FISH?

DR. BERRY: Yes, it did, and you are quite right in seeing that on the slide. As to whether or not that level of statistical significance is statistically significant from the level of significance in the FISH, is another question.

The gray areas, the discordant results, I spent inordinate time looking at that set. The set that are FISH-negative, IHC-positive. The set that are PCR-positive and

FISH-negative, and all combinations of those, to try to answer the question, is there a best assay? And is there a best combination of assays?

Should we do two? Should we do FISH and IHC, and you are positive if you are positive on either; or you are positive if you are positive on both. And I stand here, admitting my failure in that regard.

I cannot tell you that one assay resolves discrepancies among the other assays.

DR. TAUBE: Wouldn't you have to do that study using adjacent sections, so that you would eliminate the potential effect of heterogeneity within the tumor, so if you wanted to take a set of specimens, whether it's these or any other set, and do the side-by-side assays on adjacent or nearby sections, it seems to me that that would be the only way that you could really address the issue of comparison.

DR. BERRY: Well, you could, but that still doesn't come to grips with the natural heterogeneity in the tumor.

DR. TAUBE: True, but it's a better --

DR. BERRY: That would be the -- that would be the best --

DR. TAUBE: -- it's a better test --

DR. BERRY: That -- that would be the best --

DR. TAUBE: -- of --

DR. BERRY: -- to do, I agree.

DR. TAUBE: -- variation.

DR. LADOULIS: Yes.

MS. DRESSLER: Hi. My name is Lynn Dressler. I was the PI at the UNC site. And let me just make a further comment to Dr. Taube's question.

As someone who performs a variety of marker studies on tissue blocks, that kind of a question is a concern to all of us, and especially, for me, who performs both immunohistochemistry and FISH analysis.

And I can tell you that, in terms of the HER-2 marker, the HER-2 marker is a homogeneous marker, much more homogeneous than most of our other markers. And in studies that we have done, non-CALGB, within our Institution in breast cancer, I often have to go back to a block that I have originally cut for an assay, and recut it later on, after maybe 100 or 200 microns have been obtained.

And I can tell you for the HER-2/neu assay, that that is very consistent, whether we look at an adjacent section, Section No. 1 that was cut off, or whether we look at Section No. 10, or 20, that was cut off.

DR. TAUBE: Is that true for IHC, as well?

MS. DRESSLER: For IHC is what I can talk to you on. For the FISH studies that we had done, looking at different levels, we don't -- in the non-CALGB setting -- we

don't see any evidence that the signal amplification changes as you go into a different depth in stage II breast cancer specimens.

And so, I think for this marker, and for some other markers, too, that is not as much of a concern in this disease.

DR. SLAMON: Just a follow-on to the response to Dr. Taube. Your point is well-taken and we did exactly that in the 900-patient cohort study. We did a serial section. The very next section was compared from the IHC to the FISH.

And there, the numbers still hold true. The FISH was more sensitive in detecting the alteration. We had what we perceived was a larger number of false-negatives, using the IHC by the approaches we were using, compared to the FISH, but that was a serial section study.

DR. LADOULIS: Dr. Taube?

DR. TAUBE: Yes, I have an unrelated question. You have chosen two, a ratio of two as the cutoff, and I understand -- I mean, I read all the material and I understand that two was a neat cutoff.

My question is, what data set determined that? Did you use the current data set to establish your cutoff?

DR. ENNS: The cutoff for clinical utility was established, based on the Pivotal 302 Study, the CALGB 8869. Don can come up and give more details, but basically, I

think we looked at from about 1.3 to about 2.8, somewhere in that range, looking for the best fit. And two, two looked like the best fit.

DR. TAUBE: Yes, but isn't there --

DR. ENNS: I'll let him answer the --

DR. TAUBE: -- isn't there a certain danger --

DR. BERRY: Yes, there is.

DR. TAUBE: -- in using --

DR. BERRY: Yes, there is. I am not sure what the clinical -- the other clinical studies were that addressed the issue, but when I was approached by Vysis, they told me, two. And I -- I used two, but I also looked at other things. And if you -- and I am keenly aware that if you do searching --

DR. TAUBE: So, two, within your study design, from the up-front --

DR. BERRY: -- two is in -- I'm not sure it's stated --

DR. TAUBE: -- to validate.

DR. BERRY: I'm not sure it is stated in the protocol, but when Vysis came to me, they said, two, before we had looked at any data.

When I looked at other cutoffs, indeed -- if you remember the slide I showed, if you increase it above two, there aren't many cases there. If you are positive, these

tumor specimens are reasonably homogeneous, with respect to HER-2.

So that if you are positive, if you are above two, you are really above two. So, if you said, three, there would be very few patients that would be in the two to three range, and the results would be similar.

I looked at decreasing it from two down to -- you know, down to one, even, and find a more or less monotone relationship between the clinical significance, the interaction. The p-value goes up as you drop it below two.

So, I was reasonably satisfied that what they told me initially was the right value, and I think Dr. Slamon might have views on two, as well.

DR. ENNS: Just before we try to add some more to it, again, Dr. Seelig presented this morning, nature -- that we do have a normal level of two chromosome 17s, and one gene on each chromosome, in a normal diploid, nonmalignant cell.

Our cutoff maybe or maybe not is corroborated by other information in the literature. Dr. Slamon presented some. I think he has also found, and reported this morning, that about the same thing is true. And this Panel has also reviewed, and the Agency has reviewed, another sponsor's product, where they didn't use a ratio, but they used actual copy number. And that copy number was a copy of, I think,

four.

Copy number of HER-2, that seemed to have the best biological -- biological fit. So, I think there's some consistency, then, with what we have presented today with other -- with others.

MS. MAGRUDER: I would just like to remind the Sponsor to please confine their issues to data that was submitted in the PMA.

DR. ENNS: Okay. Dr. Taube, have we answered your questions satisfactorily?

DR. TAUBE: Yes.

DR. ENNS: Thank you.

DR. TAUBE: I mean, my concern is that once you -- that if you set a cutoff, based on the data set that you are analyzing, then you are not doing a validation of that cutoff.

And so, if you are putting this into your -- into your protocol for people, and for the interpretation in your package insert of this, you haven't really validated that cutoff in independent experiments.

DR. ENNS: I think in our actual protocol, we left the door open for evaluating the best cutoff. I don't think that we had a specific cutoff. I think we might have mentioned one, based on experience and pilot studies, but I think we said that the right thing to do is to kind of take

an approach of receiver-operator curves, and make sure that you have the best fit of the data.

DR. TAUBE: But, how are they -- well, that raises a further question. I mean, how -- are you saying that that is the instruction to the individual laboratory?

DR. ENNS: No, no, no, no, no. I think what we have to do is we say, this is what we found. I think if you go in the light of CLIA complexity, and again this is not what I'm suggesting, based on the data that we have, and we presented this as what we are recommending.

If somebody else runs a study, or does something, and in their hands, they find something else, I think CLIA requirements would say, you validate your own thing. And it might not be exactly the same, but that's a different population, then.

DR. TAUBE: Yes, because -- but it is difficult to validate this type of intended use, not just the detection, but the clinical decision, unless you have a data set that includes the outcome under these particular circumstances.

You know, I mean, I think probably that two is a reasonable cutoff. What I am indicating is that it's not clear that that's been proven.

DR. BERRY: I agree with the concern that you raised. If you -- if you go into a data set and you are looking for various cutoffs to see whether or not you can

find a cutoff that is more highly predictive of whatever you are looking for, you will find one. There will be a best one out there.

If, indeed, this -- if I were to view this study as hypothesis-generating, then it would take another study to verify what we have seen. And I am not sure what Dr. Enns is saying about the other studies, but my understanding is, and my clear understanding at the beginning of the study was, that two had been identified in different studies.

Now, that said, it doesn't matter much. Suppose we had picked three? You would get essentially the same results, because the patients who are greater than three are essentially the same patients who are greater two.

DR. TAUBE: But you don't know whether that's true for the general population. That's the problem. And this data set, from 8869, has been looked at over and over and over again, and been reused, and multiple looks, essentially, have been done, so there is always the question about whether the significance test should have been modified, and so on.

DR. BERRY: Yes, that's correct. I mean, these are not different -- they have been reused, but these are different sections of the block that we are looking at, and this is the first FISH study.

DR. TAUBE: They're not different cases, they're

not different outcomes.

DR. LADOULIS: Yes, please.

DR. SLAMON: That's why I had said things about the other cohort, which was the esterating. That is a separate cohort, and in that, it is also two. We selected that cutoff so that we would not be adjusting the cutoff, arbitrarily, to see what was the best fit.

Our rationale, valid or invalid, was, this is a cutoff set by biology. You should only see four spots at the most, when a cell's dividing, or somewhere between zero and four, depending on whether or not you have truncated the nucleus and gotten everything in.

If you do a sizeable enough sample -- and ours was 30 nuclei -- you would get a true read of what was going on in that tumor, as long as you had a control, the control being, meaning, if you got extra signals, it wasn't due to ployee(?), but it was due to true increase, due to amplification.

So we used a biologic cutoff that nature had given us, and two was the best fit for that data set, which was much bigger than the CALGB data set, which was also two.

DR. LADOULIS: I think just to maintain our focus, the questions we are getting are around that first question, whether or not the Panel is satisfied the data support the proposed Intended Use to detect amplification of the HER-2

gene accurately? And I would qualify that, saying that, amplification as defined in the proposal, as two, or four copy numbers per diploid cell, or two. Is that a fair statement?

Any other concerns or questions about that?

DR. HORTIN: In terms of Intended Use, is this product going to be specifically identified as not suitable for identifying patients for herceptin therapy?

DR. ENNS: The Sponsor is silent on that subject.

DR. HORTIN: Maybe Drs. Slamon or Liu would want to comment about, in terms of expected clinical use. If this assay was approved, would it be likely to be used for selection for herceptin therapy?

DR. MAXIM: The FDA has not silent on that. It will not be part of their Indications for Use.

DR. HORTIN: Pardon me?

DR. MAXIM: It will not be part of their Indications for Use.

DR. SLAMON: Cannot be -- right. It's clearly been my understanding that that won't be at all a consideration for what the label indications would be. If you are asking the question, whether -- some of us as oncologists, then I have to review it as a very biased oncologist in this particular area -- if we will use that test as the definitive test to treat patients?

My answer as an oncologist -- not as a representative of the Company. I am not in this venue -- is absolutely, unequivocally, yes, based on the data.

DR. LIU: If I could comment on that. I think you have to understand the tolerances of the tests as well as the toxicities of the therapy that one would give.

At one point, Adriamycin-containing regimens was considered relatively toxic. At this point in our practice, Adriamycin-containing regimens are actually less toxic than CMF, as far as I am concerned. And the same holds true for the herceptin, save the cardio-toxicity issues.

And so, at what tolerance do you accept how robust is the test? And I would argue that, any test of HER-2, whether it's a validated, immunohistochemistry, or a FISH analysis, is very robust, for all the things that you have mentioned here, and they tend to be cross-correlated.

The second thing is that most of the therapies that would be used for the HER-2-positive populations, tend not to be dire therapies, and therefore, one could miss a few individuals, accept a few false-positives, and still not hurt the population, in general.

So, in the context of practice, I think that there is a considerable range of flux, no matter what cutoff you use, as long as they're within a certain range.

DR. LADOULIS: Are there any other questions or

concerns about this -- the Proposed Intended Use, and this detection of amplification?

DR. HORTIN: I have one other comment, or question. What is the sample stability? Have you done studies -- in some of these studies, it looked like you were going back to blocks that were probably about ten years old.

In terms of evaluating how old a sample is acceptable, for performing this treatment, maybe you could address that issue, so people can know whether they can go back to a sample ten years old, or 15 years old.

DR. ENNS: Lynn Dressler is coming, as the Principal Investigator, that did the 302 Study. Again, I will remind you that the 8541, the first two numbers in that study means it started in 1985, and the samples, I think, probably were distributed across the range of the study that was done, that was enrolled from 1985 to 1991. So, Lynn, would you like to comment, please?

MS. DRESSLER: I think that's a concern that people may share, and I was pleased to find that it didn't matter if we looked at the cases that were accrued early on, versus the cases that were accrued later on in the trial, in terms of whether or not we could perform the FISH analysis, so that, ten-year old blocks versus earlier blocks did not seem to be a problem, within this Study.

I can also tell you that I have also gone back on

non-CALGB studies, to answer questions of degradation, just from protein, and wondered if tissue sections, not just blocks, but tissue sections sitting around for five years, would still be able to allow us to look at the signal. And I can tell you that, both from immunohistochemistry and from a FISH standpoint, we were able to see signal in five-year old sections, stored at four degrees, or in one-year old sections.

I was actually pleased to see that the length of time or duration of how long a tissue was stored either in a block, or as a cut section under appropriate storage conditions, still maintained the signal or the expression.

Did that help to answer --

DR. HORTIN: Yes, does --

MS. DRESSLER: Does that help to answer your question?

DR. HORTIN: And would there be any tissue preparations that would not be suitable, that need to be identified? Say, if they were infected with glutaraldehyde, or some other sample preparation, that would not be suitable?

MS. DRESSLER: In the studies that I have performed, I can tell you that we have focused on formalin-fixed, paraffin-embedded specimens, so I really can't speak to that. Perhaps some of the other people in the Vysis Q/A

can.

Our study specimens have all been screened for formalin fixation, as far as we know. That would be my recommended condition of fixative for these assays.

DR. HORTIN: -- follow-up to that --

MS. MAGRUDER: The transcribers have asked that you give your name, each time you come to the microphone.

DR. SLAMON: In follow-up to the question, we have looked at different fixation preparations and it appears that Boeing(?) or Picker-Casset(?) fixatives do not do as well as formalin-based fixatives in the non-CALGB cohort that we looked at. I don't know what the data are, or if the CALGB group looked at that, but we did find that finding.

DR. LADOULIS: That has been my experience, also, but I think Dr. Masood is in the audience, also, still, and I wonder if you had any comments about, in your experience, both the -- whether the cutoff of two or four copy numbers is conservative, or not conservative? And this issue about fixatives, too.

DR. MASOOD: I endorse what has been already said regarding the fixation, and also storage. If the sections are kept under appropriate temperature, then they usually should signal very, very easily.

We also have the experience of looking at archived

material, almost about 20 years of age, and we have been very pleased and often surprised that we still can really look at this, again, in a condition that they have been really kept properly.

We don't have that much of experience of looking at FISH in different fixatives. What we had done in formalin-fixed tissue, it appears that it's really appropriate and it can show us the signal, as appropriate.

Occasionally, we will receive a specimen in Boeing, or even in mercury-based, other mercury-based fixatives, and we have been able to see the signal. It's not as clear as it usually is in formalin fixation. Did that answer your question?

DR. LADOULIS: Yes, thank you. Are there any other comments from the Panel with regard to this question? Maybe we can bring this question to closure, and move to the other questions that have been posed here. And the second -

DR. TODD: I have a question.

DR. LADOULIS: Oh, yes, sorry. Dr. Todd.

DR. TODD: It's not necessarily related to this specific question, but I just had a question of Dr. Liu, since he has to leave.

In your article in September in the Journal of NCI. Is it not your caution that we really needed more

clinical validation, that there is a correlation between expression, co-expression and --

DR. LIU: Yes. And you know, one is always conservative when you have one study no matter of the magnitude of its numbers or what have you, that it could still be a statistical aberration, and so the most conservative thing is to ask for another group with another cohort, to do exactly the same thing, and again, within the same addition of the GNCI, the NSABP with a B11, used immunohistochemical assay, and found that there was an interaction with HER-2, overexpression and the need for Adriamycin, or Doxyrubicin-based adjuvant chemotherapy to achieve the maximum outcome.

This was not the case for HER-2-negative individuals, where regardless of whether treated with a non-Adriamycin-containing regimen, which was palpa(?) - fluorouracil, versus PF and Adriamycin, there was no difference in overall survival.

This was a study with 600-odd more cases and much longer follow-up, so we felt that in the totality, that there was validation of the concept of HER-2 being an important predictor for the need for an anthrocycline-containing regimen.

Just, if I could -- I just recalled a very early study that we did, and it has to do with the two copies, Dr.

Taube.

DR. TAUBE: It's the ratio of two, it's not two.

DR. LIU: The ratio of two, yes. We, as you recall, before the FISH analysis, we actually did a molecular validation, using microdissection and differential PCR, and found remarkable correlation. But the interesting thing was when we plotted immunohistochemical staining versus the number of copies, the break-off was at two.

In other words, anything below two, there was -- there was, you know, just sort of a random scattering of a percent staining cells, and then once two copies and exceeded, it was at the 85 percent range of cells within that tumor that stained positive for HER-2.

So, there is a biological, you know, shelf that appears to occur. And I believe that was corroborated by others, using western blot and southern blot hybridization, which was, you know, using larger tumor sizes.

I hope I answered your question. Thank you.

DR. LADOULIS: Before we perhaps go to the question about the proposal for training programs, I think the other questions that the Agency has asked that the Panel address are related to this, and that has to do with the subpopulation analysis of HER-2-negative patients with four or more nodes, indicated patients benefit from higher dose CAF. Will knowledge of the HER-2/neu-negative status offer

any benefit in the management of these patients?

I wonder if any comments -- Dr. Liu, you want to maybe amplify on that, or if any Panel members want to raise that question, again?

DR. LIU: My only concern about the -- actually, the four or more nodes positive, I have no problems with it, in fact, it doesn't surprise me, actually. I was a little surprised at the data presented just today, an hour ago, of the less than three nodes positive, and showing that the higher dose had an effect.

I think that Dr. Berry should actually comment on it, because I think he looked at that in a very different -- and came to some slightly different conclusions. Though, I have to caution the Panel that, unless some adjustments are made, because of the imbalances in sometimes the perceived -- of the prognostic variables in the various arms --

It is on occasion hazardous to look at each individual subset analysis, so again, so I think that Don should comment on that piece.

DR. BERRY: Yes, I do subset analyses all the time. You can't help but do it. But you have to do it with a filter on your glasses. You can't be too taken by unusual things.

Richard Pieto(?), who did a worldwide overview about seven years ago of breast cancer, concluded on the

basis of a subgroup analysis, that premenopausal women who are estrogen-receptor-positive, don't benefit from Tamoxifen. It was a --

You know, you split it every which way. You do premenopausal, you do ER-positive, -negative. He now feels that he might have contributed to the early deaths of many women, because he did not -- because he did that subgroup analysis.

In fact, the latest overview shows quite convincingly, that estrogen-receptor-positive patients that are premenopausal, benefit just as much as postmenopausal.

I regard the -- you will notice that Dr. Weng indicated a number of very small subgroups. I regard the entire set of 524 patients as a subgroup. If you do the full set of 1,000 patients, you find that in the four-plus node group, there is essentially no benefit for increasing dose, if you are HER-2-negative.

And the transparencies, the slides that I showed you this morning, showed that in fact, that's true for the good prognosis patients, as well as the poor prognosis patients. So, I think it's an artifact.

DR. KAUL: I've got a further question here. Looking ahead a little bit, how is the growing practice of doing sentinel lymph nodes, if we are using a cutoff of multiple lymph nodes, going to impact upon this potential

use of this product in years to come, and do we need to be addressing that now?

DR. SLAMON: I think that that is a very real concern. I think it's very appropriate, because the morbidity associated with a fairly extensive axillary node dissection, versus a sentinel node procedure that the surgeons have pushed, I think is really, there is a benefit there, that can be gained.

And so, when we have less information in terms of the number of positive nodes, these other data are going to become even more important in informing the medical oncologist how best to approach that patient. So, I think that your point is right on target.

I also think that, relevant to the CALGB Study, with the four or more nodes positive subgroup, assuming for a moment that that subset analysis were correct, making that assumption, that a standard dose anthrocycline therapy versus other forms of therapy. There are other forms of therapy that can be used for these patients, if you don't think they have to have the anthrocycline.

So, I think determining the HER-2 status in that subgroup, or in the group that are going to be considered for adjuvant therapy, node-positive, it is important, because there is still CMF, that is useful in the HER-2-negative population. That has clearly been shown by the

large cooperative group trials, and by Dr. Wright in Great Britain.

So, I think that that approach is important, independent of whether or not you are talking about the different levels within the CALGB Study. CMF is still a regimen that is used, used frequently throughout the world.

You can argue that it's better or the same, but in HER-2-negative cases, I think there is still a lot of room, and it's an important issue to be resolved.

DR. BERRY: I point out -- a very good question. I point out that if you are positive for sentinel node biopsy, you are positive. If you then do not do a complete dissection to find out whether you have four or more positive nodes, then a statement -- any such statement in a product such as this, would not be usable.

And so your question implies, I think, that you can't indicate -- because sentinel node biopsy is clearly coming, you cannot indicate a plus/minus, depending on number of positive nodes.

DR. LADOULIS: My question I guess is, if the patient is stage II, whether one or more, what is, you know, the proposal then in terms of the knowledge of the HER-2/neu-negative status, in a patient who is staged as stage II? And based on your statistical studies, now, including all of those patients, I guess Dr. Liu and Dr. Berry, can

you maybe address this?

Now, patient is going to be staged somewhere, stage II, is going to have one or more nodes positive. What is the significance of the HER-2/neu-negative status?

DR. LIU: Well, I think that that is the bigger challenge. I think that at that point, the data, both -- and I have to pull in the composite data with studies of CALGB and NSABP, would suggest that HER-2-negative individuals, with a sentinel node-positive, not dissecting any further out, would have the choice of an Adriamycin-containing regimen, or not an Adriamycin-containing regimen.

I do think that that is an important choice, because if you are a 36 year old woman who we still don't know what the 30-year effect on the heart is going to be, given Adriamycin, I do think that that is a serious consideration, and I certainly make that consideration in the patients that I treat.

And if they are equivalent, then I would argue that a non-Adriamycin-containing regimen, potentially for the really long term side effects of premature cardiotoxicity, may be an important consideration in selecting a non-Adriamycin-containing adjuvant regimen.

Perhaps Dr. Slamon could comment on that in terms of the practice in California.

DR. LADOULIS: Any other comments on that question

from the Panel?

Another question that is near the bottom of the list here is, does the HER-2/neu status offer independent benefit in addition to node status, in considering the use of high dose chemotherapy?

Have we answered that question to your satisfaction, Panel, in the process of the discussion around this? Are there additional issues concerning this Probe Kit, coming back to the top of the list of questions, the second question. And that has to do with the Sponsor's proposal to offer training programs for the performance of the assay.

DR. KEMENY: I want to go back to the question you just asked.

DR. LADOULIS: Okay.

DR. KEMENY: Again, I think, as many people have said, we have to be much more specific in how we ask that question, because first of all, high dose chemotherapy to most of us now means bone marrow transplant.

DR. LADOULIS: Okay.

DR. KEMENY: And that is a whole different question. And also, Adriamycin needs to be put into the mix here, because it's -- it's what they were calling -- it's standard dose Adriamycin now. I mean, so, it needs to be specified a little bit more clearly.

DR. LADOULIS: Well, the Intended Use of this in the proposal is, you know, is a device for determination of the HER-2/neu status of a patient. And the additional, you know, justification for the performance of this test and prescribing it, is as a rapid assessment, as stated, for the potential response to adjuvant therapy, leading to a choice of therapy.

Does there need to be some modification to the label, to reflect these concerns that you have?

DR. KEMENY: I think so. Yes.

DR. LADOULIS: What modifications ought to be made?

DR. KEMENY: Well, that's, we're saying --

DR. LADOULIS: Delete any specificity?

DR. KEMENY: I think they should delineate if they want to go with this Intended Use, then they need to delineate that we are talking about Adriamycin, and the dose of Adriamycin that you are talking about. Either that, or drop that altogether.

DR. TAUBE: The data support what is now standard dose Adriamycin therapy, so I think that the Intended Use Statement has to include --

DR. ENNS: Excuse me. This is Russ Enns. I would like to ask for further clarification from Dr. Kemeny, what -- where -- what you meant by your question about being more

specific on the Intended Use? I'm not clear. I'd like to make sure we are clear on that before we try to answer it.

DR. KEMENY: My original point was that, the way you have written up the Intended Use now is limiting it to node-positive, stage II breast cancer, and then saying it's for the potential response to adjuvant therapy, leading to a choice of therapy.

Well, if you want to make an Intended Use like that, then it seems to me, sticking to the material that you have, which is what you were saying you want to do, that you have to be more specific about -- because it's not really telling you anything about adjuvant therapy, because it's not going to tell you anything about CMF therapy. It doesn't tell you anything about that, as a matter of fact.

So, it really doesn't -- it doesn't help you in the case of node-positive people, as far as giving them CMF. No, it doesn't help you with that.

The only thing it helps you with is for people -- whether or not you should give Adriamycin in the doses that are now considered standard. Or, actually, what it helps you with more is whether you shouldn't give Adriamycin, for people who are negative.

DR. LIU: Can I make a comment on that? I think your point about the Adriamycin, I certainly can agree on, however, the dose may be problematic, because -- I will cite

two examples. One is the 8082 Study, which was published with a first author, Dr. Berry, in actually a statistical journal, where we looked at a randomized study from 1980 CALGB.

It was a limited study, granted, but the study was CMF VP, four cycles, node-positive, randomized then to CMF VP, another four cycles versus VATH chemotherapy, Valdine(?), Adriamycin, Thyotepin(?), Hylotestin(?).

It was not a perfect study for Adriamycin versus no Adriamycin study, but we found that with that regimen alone, with the VATH, that it gave the same effect, that use was subsequently published in our -- in the CALGB Study, as well as in the NSABP B-11 follow-up study with the HER-2.

That is, the addition of Adriamycin normalized the differential of survival, between the HER-2-positive and HER-2-negative groups. Those people who were -- were not given Adriamycin, the HER-2-positive individuals did more poorly. The dose there was something like 45 per meter squared.

In the PAF versus PF Study, which is B-11 -- and I brought this along just for clarification, because I figured that perhaps the dose issue was going to come up. The Adriamycin was at 30 per meter squared every three weeks. It was the low dose arm of a CALGB.

I don't think that the dose threshold has been

defined yet, so I would only argue that, given that the dose threshold has not been defined, that perhaps that shouldn't be stipulated.

DR. MCCASKILL-STEVENSON: But, are we not clear that, I thought that the lowest dose in the CALGB Study was 30 mg --

DR. LIU: Yes.

DR. MCCASKILL-STEVENSON: -- and that was the one for which we had the lowest efficacy.

DR. LIU: Yes, but the B-11 had a much longer follow-up, and it is not clear to me -- you see, you have to understand that the CALGB Study was not a comparison between an Adriamycin-containing regimen versus no Adriamycin. It's just doses of an Adriamycin-containing regimen.

For all we know, and if we had four arms, one without an Adriamycin-containing regimen, that it would do considerably worse than the worst dose arm of the CAF. We don't know that for a fact.

The only reason why I bring this up is that the dose threshold has not been determined, as of yet.

DR. LADOULIS: Okay, I think that we need to get now, in the last 25 minutes, until 3:30, go down the list and reaffirm what we have, I think as a consensus, and going from the top of the list. And the first is, is the Committee satisfied the data support the Proposed Intended

Use to detect amplification of a HER-2 gene? And it seems that the, with very little qualification, that is affirmative.

That is the consensus I get from the Panel, that given this, as defined for amplification, in the Sponsors, okay? Again, I would like to skip Bullet 2 for the moment and go to Bullets 3, 4, and 5 in this question, and will knowledge of the HER-2/neu-negative status offer any benefit in the management of these patients? And this may be a sticky one.

DR. KEMENY: These patients are the four positive, you skipped what they are, those patients.

DR. LADOULIS: Oh, yes, of these -- subpopulation analysis of the HER-2/neu-negative patients with four or more nodes, indicated patients benefit from higher doses CAF therapy.

Will knowledge of the HER-2/neu-negative status offer any benefit in the management of these patients? That is, HER-2/neu-negative with positive nodes. Any consensus on --

DR. TAUBE: I think that because that analysis was based on a subset analysis, that the original study does not and did not include that we can't -- that we don't -- we can't really use that information. And I think if we just took the question, will knowledge of the HER-2/neu-negative

status offer any benefit in management of the original set of patients, which are node-positive, any node-positive --

DR. LADOULIS: We don't know --

DR. TAUBE: -- stage II. Then we can say, yes, I think that the data do support that. But not -- I'll take the subset. In other words, eliminate the first part of --

DR. LADOULIS: You mean, that would be any stage II patients?

DR. TAUBE: Any stage II, node-positive patients.

DR. LADOULIS: Any node-positive, HER-2/neu-negative patients will benefit from this test?

DR. TAUBE: From knowledge of the results.

DR. LADOULIS: From the knowledge of this result?

DR. TAUBE: Well, probably. Possibly.

DR. LADOULIS: Would you benefit by knowing that you had a stage II patient who is HER-2/neu-negative?

DR. KEMENY: I think it will benefit the clinician to know what -- if we look at the data, yes. But, I totally agree with Sheila that this is a subset of a subset.

DR. LADOULIS: Right.

DR. KEMENY: I mean, basically, this whole group is a subset, I mean, to a certain extent. Because you are taking a big study and looking at these HER-2/neu --

DR. LADOULIS: So in response to the Agency's question, it is not a -- the Panel might affirm that the

knowledge of the test will be of benefit in the management of stage II patients, regardless of -- and without any reference to the numbers of nodes positive, because that's - - that's not --

DR. KEMENY: Any node-positive.

DR. LADOULIS: -- just a state.

DR. REYNOSO: Yes, I guess -- just if I -- in my own mind, whatever we say about these questions, with knowledge, etcetera, will be answered on the basis of the entire set, with regard to this subset --

DR. LADOULIS: Yes, with a node-positive --

DR. REYNOSO: -- subset of --

DR. LADOULIS: These are node-positive patients.

DR. REYNOSO: Right.

DR. LADOULIS: Okay. Bullet No. 4, the question posed for the Panel was, does the HER-2/neu status offer an independent benefit in addition to node status, in considering use of high dose therapy? And I thought --

DR. KEMENY: This is where we wanted to be more specific.

DR. LADOULIS: That we couldn't get a consensus as to what is high dose therapy, or the definition of the dosage, and whether or not that is even pertinent.

DR. TAUBE: Yes. It needs to be modified to replace high dose with Adriamycin --

DR. LADOULIS: Dr. Reynoso?

DR. REYNOSO: If -- are we modifying that question to say, considering the use of Adriamycin-containing adjuvant therapy?

DR. KEMENY: Yes.

DR. TAUBE: Yes.

DR. REYNOSO: That is what we are doing.

DR. TAUBE: Yes.

DR. REYNOSO: And also, in the Intended Use, in the second part --

DR. LADOULIS: Is that part of the submission here?

DR. REYNOSO: No, but that's what we are discussing here.

DR. TAUBE: But we can request that.

DR. REYNOSO: That is what's coming out. That is what's coming out. That is what's coming out from the data.

DR. LADOULIS: Okay. Would that clarification be sufficient, Dr. Maxim? Are you getting a sense of the Panel?

DR. MAXIM: Oh, yes.

DR. LADOULIS: You're getting a --

DR. MAXIM: I think what you need to do is, group those three questions and what we were looking there -- what we were looking for is additional clarification, on your

feelings as far as the Indications for Use on the test.

The Intended Use, as stated, and being able to detect amplification of HER-2 in the tissue specimens, I agree, and concur with the Panel that that has been established.

We need now to look in the current venue of what is going on as far as therapy of these patients and how these patients are being managed; precisely how this test could be indicated.

I will also ask you to remember that, in the sense of getting an overall view of what is going on in the field, we have allowed an awful lot of information to cross the table this morning, with regards to various cohorts and various studies, that amplify on what was presented in the PMA.

You are looking at, as Dr. Taube has pointed out, a subset analysis of 524 patients. The breakdown in even smaller numbers, when you start looking at the interplay between HER-2 and other risk factors, which was one of the things we were trying to do.

Now, granted, you lose power there, and granted it may not be a powerful indication of exactly what is going on, but what we need to know from the Panel as part of your deliberations is, how -- or what is your best recommendation as to how this product can be used in the management of the

breast cancer patients that you see?

And you can't go past stage I to other stages. I'm going to question whether you can go past Adriamycin therapy to adjuvant therapy completely, because that just was not a part of the PMA.

You are still limited to the Study the Sponsor did, and the data they presented here today, as part of that Study.

DR. LADOULIS: And these are stage II patients?

DR. MAXIM: Pardon me?

DR. LADOULIS: These are stage II --

DR. MAXIM: Stage II, node-positive patients.

DR. LADOULIS: Right. Right.

DR. MAXIM: Treated with CAF, right.

DR. TAUBE: And we are not allowed to deal with information that they presented in response to your 90-day Letter, as --

DR. MAXIM: It's additional literature information, it's clarification of some points. It's not part of their Study.

DR. TAUBE: Okay.

DR. BERRY: May I have permission to --

DR. LADOULIS: Yes.

DR. BERRY: I have to leave, and there is one thing I want to correct, the possible Panel interpretation.

The NSABP B-11 did look at 30 mg per meter squared, but they did it every three weeks, as opposed to every four weeks, and the total dose was up to 300 mg, so that's ten cycles of 300 every three weeks, as opposed to our low dose, 300 every four weeks, which is -- at times, four -- which is only 120.

It's not clear how you put those two things together, but indeed, the NSABP B-11 had a good deal more Adriamycin than the low dose.

DR. LADOULIS: Okay. So, where are we left? We're still with stage II patients, node-positive patients. Knowledge of the HER-2-negative status is important in the management of these patients.

DR. KEMENY: I didn't --

DR. LADOULIS: Without any further qualification? Okay.

DR. KEMENY: No, that's not where we're left.

DR. TODD: I'm not sure that I would be comfortable in saying that -- well, I guess if we're saying in addition to negative status. Did you want everyone to take into account -- I mean, I agree with Sheila, it's a subpopulation -- that it is a subpopulation that we are looking at, you know, it's a subgroup of a subgroup to begin with. So, I'm not sure that you want to --

DR. TAUBE: It's not independent.

DR. TODD: -- to leave it as it is the way it is

written.

DR. KEMENY: I think we're all saying pretty much the same thing, which is on Bullet 4, where it says, does -- that whole bullet is problematic because it's not independent, since we're only talking about node-positive. And it's not the use of high dose therapy, since we're talking about Adriamycin therapy. So, that bullet seems flawed.

DR. REYNOSO: Yes, we are talking altogether, node-positive patients. So then the way the Intended Use statement reads, we don't want to be more specific?

DR. LADOULIS: So we were -- the most relevant consensus is on Bullet 3, and that is, the knowledge of the HER-2-negative status offers benefit in the management of these node-positive patients?

DR. TAUBE: Node-positive, stage II.

DR. REYNOSO: I guess what I'm saying is that, that question is in a way, irrelevant, because the whole Study is based on node-positive patients.

DR. LADOULIS: Well, but that's all it's Intended Use for, and so we have to confine ourselves to that, correct?

DR. TAUBE: That's right. That's right.

DR. REYNOSO: Yes, that's why I'm saying that this statement from the FDA is maybe out of line, whatever the

word is. We don't have to consider it.

DR. LADOULIS: We don't have to tell them any more than they already know, which is to say that they know that they have a proposal for a node that is examined, and is the application for node-positive patients.

DR. TAUBE: Can we go back to the Intended Use Statement itself, and talk about what possible modifications we would want in the Intended Use Statement?

DR. LADOULIS: Let's do that.

DR. REYNOSO: But this is what we suggested. We may add there, for the potential response to Adriamycin-containing adjuvant therapy. And that will account for some of the data we have, and this would be in regards to that.

DR. KEMENY: You can even leave out adjuvant, can't we?

DR. LADOULIS: I don't know if you need it in there.

DR. KEMENY: You don't need adjuvant in there.

DR. KEMENY: But can I ask a question of Vysis that, when you say, node-positive, Stage II breast cancer, I mean, anybody who is node-positive is automatically stage II, then there are people who are stage II who are not node-positive. Is that -- are you saying that, or are you -- and that means you're not considering the node -- the stage III patients, right?

DR. ENNS: I'm not a clinical oncologist. I'm going to let -- oh, excuse me. Dr. Russell Enns, Vice President, Regulatory Affairs at Vysis.

I would like one of our clinical oncologists -- and we have two here representing today, the knowledge, that can speak to you. Dr. Slamon or Dr. Liu.

DR. LIU: This is Ed Liu, and I would say that we are talking about node-positive individuals.

DR. SLAMON: I would concur with Dr. Liu. We're just talking about node-positive individuals --

DR. LADOULIS: Yes, then let's just recommend that modification to the Intended Use Statement, that limits it to that which is -- for which it was intended, that the results from this PathVysion Kit are intended for the use as a rapid assessment, for potential response of node-positive patients, and --

DR. KEMENY: Where are you reading?

DR. TAUBE: The Intended Use --

DR. LADOULIS: And, now, how else do you want to word it?

DR. TAUBE: The Intended Use. Well, I'm only --

DR. KEMENY: -- rapid assessment, yes. Oh, sorry.

DR. TAUBE: I'm only a little bit concerned that we have no data on node-positive, stage III patients, and how they respond, whether they get any benefit -- you know,

I mean, I think node-positive, stage II patients is -- I mean, I -- Ed?

DR. LIU: Well, it's up to 5 cm, so it's -- I think we're cutting a large swath of humanity by covering that.

DR. TAUBE: Yes. Yes. Right.

DR. LADOULIS: So, would you be comfortable with stage II, node-positive patients?

DR. LIU: I think your concern would be, actually not node-positive, stage II, but actually, the interpretation of node-negative, stage II. I think that -- Dr. Kemeny, don't -- that was your concern. So, however you want to word it, to make sure that --

DR. LADOULIS: But the submission on the -- the submission has no data with regard to node-negative patients.

DR. LIU: Well, you know, the Study itself, the studies that are on the books, is for node-positive breast cancer --

DR. LADOULIS: Right, and that's --

DR. LIU: -- and it specifically excludes stage III.

DR. LADOULIS: Right.

DR. LIU: And so --

DR. LADOULIS: So, we have to address what it is

the Sponsor has submitted, and that is for the Agency and the Panel to make recommendations for, and that is, for stage II, node-positive patients.

DR. KEMENY: Yes, or you could just say, it's node-positive and stage II. I mean, just put an and in there.

DR. LADOULIS: Fine. Node-positive and stage II.

DR. TAUBE: Not stage II --

DR. KEMENY: Stage II -- sorry, let's go back. Stage II, lymph node-positive. We need to say it that way, first.

DR. LADOULIS: Stage II, lymph node-positive. So, the Intended Use might read, results from the PathVysion Kit are intended for use as a rapid assessment of the potential response of stage II, lymph node-positive patients, to -- if you want to make it to therapy, or and --

DR. KEMENY: Adriamycin --

DR. LADOULIS: Do you want to be specific about including something to it?

DR. KEMENY: Yes.

DR. LADOULIS: To Adriamycin-containing therapy, and that's a recommendation you would like to make?

DR. KEMENY: Yes.

DR. LADOULIS: Okay. So, that language modification, will be -- is that understood? Okay. Now,

the final statement of Intended Use is that the testing would be performed in a CLIA high complexity laboratory, and that brings us to Bullet Item No. 2, in which the Sponsor has proposed different training programs, two different training programs, one for experienced users, and one for inexperienced users.

I don't know that you want to make any recommendations with regard to that.

DR. HORTIN: I have one question, and part of it is a comment, too. This test, although presented as a relatively simple, kind of count-the-dot assay, looks to me relatively complex, and if you read through the details of the procedure, actually the critical element in terms of performing the assay well, is probably not being able to count the dots, but it may turn out to be in terms of reliably identifying the cells that you can count them on, because you don't want to perform your analysis in stromal cells, and you want to make sure that you are performing analysis to tumor cells.

In general laboratory practice, this would not be considered usually a test that would be suitable for performance by technologists. It is basically analogous to a histochemical assay that would usually be interpreted by pathologists, who basically --

The reason for that is, because you have to

interpret reliably, which cells are the tumor cells, and which section within the tissue that you are looking at, and it says specifically in the directions, that there is supposed to be selection of the tissue sites by analysis of an H and E section that is performed in parallel.

I think as a -- I would raise the question about, as presented by the manufacturer, that this is really not a suitable test for performance by technologists, and in terms of general laboratory practice, I don't know, maybe other people would have comments about some histochemical assays that are performed routinely by technologists.

I think, if you look at the assays, say the cytogenetic assays, that people are used to having performed by cytotechs, basically, you do not have to make the same selection in terms of a tissue section, to identify the regions of tumor infiltration, which are tumor cells, and in terms of PAP smears, in terms of cytotechnologists, every positive smear basically requires confirmation by a pathologist's review. So, I raise that as kind of an initial point and kind of a question for --

DR. KAUL: Actually, I can -- to clarify a little bit. I think we need to be careful about what we say, performed, and I would like to ask the Corporation.

I think being performed could be the staining of the slides by a tech, but who is their intended interpreter

of these?

DR. HORTIN: Right. I'm referring to the interpretation, not in terms of the physical performance of the --

DR. ENNS: Dr. Ladoulis and Panel, this is Dr. Russell Enns, VP of Regulatory again, for Vysis. If you would permit me to bring another level of experts who participated in our clinical trials, both the 300 Reproducibility and the 302, we have representatives.

We have a Ph.D., a laboratory expert in molecular genetics, who is the Lab Director at St. Francis-Penrose, is Dr. Mary Lowery.

Following her, we have Dr. Diane Persons, who is a pathologist, and I believe has also -- I think is board-certified in medical genetics. I would like her to come and comment.

And then Lynn Dressler, who is a Master's Associate, is Assistant Professor at UNC, who was our Principal Investigator, and she has a medical technologist who is her lab supervisor who is here today, who ran many of these assays, after a qualified pathologist, who is not able to be here today with us, Dr. Navatny(?) at UNC, who reviewed the slides and scored the appropriate areas on the slide as instructed in the procedure of the protocol.

And I think that Mr. Cowan is a technologist and

can also say what his experience was in reading and reviewing, so --

DR. LADOULIS: Before you do that, could you clarify one question first?

DR. ENNS: Yes.

DR. LADOULIS: What is it that you are proposing?

DR. ENNS: What I am proposing, if you understand CLIA high complexity; CLIA high complexity is way too much for us to go into in this meeting, the CLIA 88, but it has all of the requirements for qualified physicians, laboratory directions for the appropriate staffing of laboratory directors, what their qualifications are and also technologists.

We believe that any laboratory that meets accreditation and certification for CLIA 88 High Complexity, is qualified to run this test. And again, it is my understanding that the FDA and the Panel does not mandate specialties in the performance of products when they are cleared and approved.

DR. LADOULIS: Dr. Reynoso, you had a comment?

DR. REYNOSO: Just following the comment, and perhaps it is important that I say something before these experts answer. And that, many of us have experience in performing and interpreting similar tests, fluorescence, tumor markers, immunopathology and so on.

What we're talking about here is two key steps, and that is the identification of tumor cells to be counted versus stromal cells, necrotic cells, and so on. And also, the selection -- and the protocol calls for the selection of a field, a specific field, to be counted.

I think there is a role here for a pathologist or a similar person who has the experience, the knowledge, the skill, to identify the area to be counted. And once the proper area has been identified, then the actual counting can be done by a number of other competent people.

I wonder if they considered an approach this, which is the approach I use in my laboratory for many other markers, and that many other pathologists in immunopathology and so forth is used. So, I think that we are talking two different things.

I think that, as has been said before, interpretation of the proper area of the slide is an important step, and interpretation that the right nuclei are being counted. And I think that these are the variables that we haven't discussed sufficiently today.

DR. LADOULIS: But I think you are about to address that. I think with the comments from the Sponsor --

DR. REYNOSO: Yes, that's why I just wanted to clarify my thinking before we hear the answers.

DR. LADOULIS: -- because I -- I understand,

that's what you are going to address, correct?

DR. LOWERY: Thank you. I am Mary Lowery. I'm the Clinical Laboratory Director of Clinical Cytogenetics and Immunopathology at Penrose Hospital in Colorado Springs, Colorado. We're a large not-for-profit hospital system.

Actually, my lab is designed as a High Complexity Specialty Lab to support the main clinical pathology laboratory, and I am a clinical cytogeneticist, not a pathologist.

We happen to do a lot of FISH in association with all the uses that FISH is used for in clinical cytogenetics, but we also do a lot of solid tumor FISH.

We work closely with our pathology group, who does exactly as you have described. The H and Es are selected. Tumors are selected based on a positive H and E evaluation of tumor, and it is confirmed, marked on the slide. The specific slides are submitted for FISH, to be tested.

My technologists are highly experienced in FISH. As I mentioned, we do it on a routine basis, and so we have to meet all the regulations and the policies and the criteria that are related to the private lab in a clinical laboratory; meaning that we have to have competency validation and annual review of competency, for every test that we do clinically.

So, my technologists do the test. They are

comfortable performing the test. Two technologists evaluate it and it's all backed up by histopathologic confirmation of tumor within the specimen.

DR. REYNOSO: Thank you. That obviously answers the question. My point is that nowhere in the protocol does it say this is going to be done.

DR. ENNS: I think in our protocol we did mention that when the tumor selection, when the data center did their random access to the CALGB 8869, required the repository to ship the sample that was received at UNC, and a board-certified pathologist with expertise in reviewing breast cancer tissues, reviewed the slides by H and E, and scored them for their appropriate area for FISH assay results to be reviewed.

And then the people in Lynn Dressler's laboratory, under her supervision, performed the FISH assays. And maybe, Lynn, do you want to add anything to this?

MS. DRESSLER: I think that the discussion that was proposed by Dr. Reynoso is basically what we consider good clinical practice, in that in my experience in running clinical laboratories and research laboratories, that's a given. That in order to identify the appropriate area of a tissue specimen, no matter what test you are doing, you need to have that confirmed, pathologically, so that you know first the area that should be scored.

In the 302 protocol, that is just what had happened. A pathologist first reviewed the adjacent H and E section -- a section was taken, H and E stained, and the adjacent sections were assayed for FISH.

Now, before FISH assay could take place, the pathologist not only had to indicate that it was representative, invasive cancer for this study, which was stage II, node-positive study, you needed representative invasive cancer. Our pathologist also demarcated on the H and E section, in Sharpee(?), that area that the technologist was to score, and we were able to superimpose that on the unstained section, actually trace that area and luckily, too, what happens is, you can put that slide under the fluorescent scope, and actually see that Sharpee line, so that our technologist can move right within that Sharpee-lined area, and say, I'm just counting and scoring within that area.

So, yes, we consider that first step in good clinical practice of any clinical assay, and I'm sure this is much more detailed in CLIA 88, is that the pathologic area, the representative area, is identified first, and then the technologist can score within that area, just as you had indicated.

DR. REYNOSO: Yes, but just -- thank you. I just wanted to make sure what you said, and that was very

important. After the pathologist is supposed to go back and identify the area again, not just previous, in the H and E slide.

MS. DRESSLER: It actually doesn't really necessitate that occurring afterwards. As you know, many sites have pathologists off-site and are not right there next to where a laboratory assay may be taking place.

The H and E slide is prepared, and usually is available in any surgical pathology area any time we have a block, so that you know where the area of tumor-rich cells are. And that that can be demarcated, and that area can be superimposed on your unstained section, prior to doing a FISH analysis, after doing your FISH analysis, wherever you want to do that, but it doesn't necessitate a pathologist coming back and then re-reviewing to make sure that was the case, unless a lab, we did our own QA and QC in the 302 protocol, that our pathologist did do it at both ends, but that's not something that needs to be done in a normal clinical setting.

DR. LADOULIS: Well, I think, you know, we probably can come to a consensus about this, that in fact, if there is a program sponsored, that it really needs to be that for a high complexity assay, such as for other, you know, such assays, FISH assays I think of in hemen(?) eipathology(?), where it is designed for a professional

staff, that is, pathologists and medical geneticists, as well as for technologists.

And I think that's the kind of training programs for FISH and immunohistochemistry, that traditionally has been taking place, rather than strictly a technical -- there has to be some training for physicians on how the sampling has to be done in conjunction with the technologists, to make sure that the sampling is appropriate. And that the appropriate areas are subjected to analysis. Ms. Ammirati?

MS. AMMIRATI: I should make a comment, I think that just sort of builds on this as what we're going to see, I think are subtle differences from laboratory-to-laboratory, pathologist-to-pathologist, as to how the practice of the laboratory you know, just goes on and progresses.

That is what CLIA 88 is supposed to do, so saying that this is high complexity, it puts the responsibility on the laboratory director, at some point, to make sure that each person is trained to some level; there is proficiency testing, quality control and all these other safeguards. And it's really, I believe, beyond the scope of the Sponsor to have to dictate as to, you know, what actually has to happen on the level of the pathologist or the technician.

DR. LADOULIS: What is the staff asking for the Panel's advice on here, the Sponsor has proposed two

different training programs and you are asking if we feel these programs are adequate?

I mean, clearly, the consensus of the Panel is that there needs to be attention to the detail of the interpretation, as well as the technological performance of the assays, certainly, in a qualified laboratory staff, including professionals and technicians, technologists.

MS. WOOD: This Geretta Wood, FDA. Yes, that's -- what exactly we were looking for there is, if you felt that their programs were going to be sufficient, or if you had other recommendations, such as the ones that you are making now, as to specific areas that the training program should concentrate on, or specific qualifications of individuals who would, in fact, locate the proper areas of that slide to be analyzed.

We, basically, wanted to see if you were satisfied with what was proposed, or if you wanted more information?

DR. KEMENY: I'd like a point of --

DR. LADOULIS: Go ahead.

DR. KEMENY: From the FDA. I mean, do we usually specify this, or -- I mean, do you usually have to specify that a pathologist has to be involved, or do you not have to specify that?

DR. LADOULIS: To my knowledge, that's not what has been an integral part of this initiative, as far as I

understand, but you're asking for some guidance, rather than a specific recommendation in labeling?

DR. ENNS: Yes, basically.

DR. KEMENY: I didn't understand. Do we usually, or do we not, usually?

DR. LADOULIS: Not. That's not part of our usual request.

DR. CARPENTER: Could I bring up an issue, just related in general, it relates to the training program, that we really haven't discussed yet. Is the test interpretation -- I know this is an issue, a borderline test interpretation is an issue in every laboratory test we do, and I just wonder what the Company's recommendations were, about when you get a 1.9, you know, I see you have our -- I see you have a little blurb about how to deal with that, but I wondered if you could just clarify it a little bit, if you repeated what you say is one possibility, and you --

Say, you get a 1.8 the first time, and the second time you get a 1.9, or you get a 1.7 and a 1.9. Or, alternatively, if you repeat it and you get a 2.1, do you -- you know, how do you recommend what you report out on the report form, and how you reconcile the treatment the patient should receive?

DR. ENNS: Dr. Carpenter, your question is a very good question, and it's a question that every laboratory

test that's introduced into the laboratory medicine, the lab director has to question, you know, how to use the interpretation of results.

In this case, we are recommending a discrete cutoff, and I think we showed the data that the ratio greater than or equal to two, is a good and sufficient cutoff. Yes, you are going to, on a small number of cases, you will find samples that lie near the cutoff, and this is true of every diagnostic test I've been involved in developing, maybe over 25 different tests with DNA probes that the Agency has reviewed.

Basically, what you have to do, I think in the practice of good laboratory medicine, is there needs to be communication between the laboratory reporting results, and the physician who is the attending physician with that patient.

If the lab results are not consistent with what the physician believes he is seeing from the rest of the clinical presentation and history, then I think there should be a discussion between the lab director, or somebody in lab management, with the physician, as to whether the results are meaningful in this particular case.

If there is not a consensus on that, then I think the logical thing to do -- and I think NCCLS has more broad-lined guidelines that have probably been written more for

clinical chemistry, but it tells you what to do, and it suggests that you can do reflexive testing, repeat testing. In this case, you are certainly probably not going to go back to the patient who has breast cancer and ask for another biopsy, but you could go back to the tissue block, or you could go back to the slide and reread it.

And so there -- or you could use maybe another technology, to try to assess, you know, the same type of an answer, so I think you have to use good judgment, but the task as the sponsor will say, our technical service staff will be trained to say if it's greater than or equal to two, it's positive for HER-2. If it is less than two, it's negative.

And we're not in the business of practicing medicine. We're manufacturers. We are looking at this Panel and the Agency to establish the final labeling that will be approved, and then it will be up to us to tell the people who will be using the test, follow the package inserts.

I always tell people, don't go and try to recreate a different product. Follow the package insert if you want it to perform correctly. And then if you go off of that line, then I think CLIA tells you, if you go off the line of the recommendations on the approved labeling, then it is your obligation and responsibility to validate that, and I

know that that is what I put into my MM3A(?), which is a performance standard recognized by the FDA, as of February of this year. And that was for DNA probes for infectious diseases, including both amplification and regular DNA probes.

So, it's really important that if a laboratory goes off-label, they have got to revalidate --

DR. LADOULIS: Well, since you have been heavily involved in the standard-setting with NCCLS and you are the Sponsor, and you have already recognized that the package insert itself should contain sufficient information, I think it's the sense of this Panel, from the comments from Dr. Reynoso, Dr. Hortin and others here, that perhaps this package insert ought to contain specific information that actually recommends the participation of the pathology or medical genetic staff in the assessment of the slide adequacy and all the quality assurance and selection areas. Can you do that?

Can you make some insertion of recommendations in the package insert, that would --

DR. ENNS: I think, we certainly, certainly are comfortable in making recommendations who are the appropriately trained and qualified people to do this, and I don't have any compunction against that.

As a manufacturer, it is to our advantage to make

sure that the appropriately trained people are using the product, so that we don't have people who are unqualified, use the product.

The last thing I want coming back to my company is complaints, and complaint management, saying the product doesn't work. We don't want to use it. When it's somebody who is not appropriately trained or qualified to be using it.

DR. LADOULIS: Well, I think for your benefit, and the benefit of the Agency staff, and that's what seems to be the consensus of the Panel members around here, that there ought to be some recommendations in the package insert, if nowhere else, that there needs to be professional and qualified staff, pathologists, immunopathologists, medical geneticists, who oversee the quality assurance in the supervision of the personnel who provide the testing --

DR. ENNS: Again, I also -- I also have been nominated, haven't been approved yet, for helping to develop genetic testing guidelines at the CDC, and the issue I guess that I'm concerned about is, again, working with CLIAC(?) Committee, is the CLIA 88 requirements do very specifically spell out requirements for meeting, and the CLIAC Subcommittee, that I believe Dr. Gutmann is a part of, representing the FDA, is working through, saying we are not going to make a lot of different changes for medical genetic

testing than what is currently existing with the rest of the areas of medical laboratory diagnostics.

So, my recommendation is, I think I've tried to capture the essence of this in the last sentence of the Intended Use Statement saying that this product is intended for high complexity CLIA laboratories.

DR. TAUBE: It's on page 18 of the package insert document that you gave out today, where you have the section on signal enumeration and the recognition of the target signals and the selection of the optimum viewing area, and the valuable nuclei.

If you just had a simple sentence in there, reiterating that appropriately-qualified individuals should be involved in doing that.

DR. SEELIG: On, I guess it's Volume 11, page 3463, on Tab Insert 3 in Volume I, which is our draft package insert information.

It specifically states, selection of tissue for PathVysion assay should be performed by the pathologist. And it's specifically referenced there, and that's the way the clinic --

DR. LADOULIS: That's an insert -- that's an insert?

DR. SEELIG: It's in Tab 3.

DR. TAUBE: Page what?

DR. SEELIG: It's Volume 11, 3463. Under Specimen Collection and Processing. The second paragraph. Next-to-the-last sentence.

DR. TAUBE: Yes. Yes. Yes.

DR. SEELIG: So, it's contained within our --

DR. TAUBE: Right.

DR. LADOULIS: Maybe you ought to put that on the top of the sentence, in the beginning of the paragraph, instead of buried in the middle. I think that's probably the implication that you get from the sense of the Panel. That the process needs to be led by a team member.

I think we have given you the sense that -- I think we have given the Agency the sense of this question. I think we have probably come to the end of the concerns to debate. Dr. Hortin?

DR. HORTIN: This is moving on to a slightly different topic, but in your Study it was noted that about 10 percent of the specimens were not considered technically acceptable for analysis. That may have related to incomplete clinical data, or whatever, but do your package inserts include information that would allow the user to decide on what is a technically acceptable block, or -- would you comment on whether there are some specimens that are not suitable?

DR. ENNS: I'm asking Lynn Dressler, our Principal

Investigator for 302 to come answer that, since they were qualifying the inclusion criteria of specimens.

MS. DRESSLER: I think that's a good question that you asked, and I think for the purposes of this study, looking at stage II node-positive cases, that one has to have a representative invasive cancer nuclei on the tissue block. And that is something that needs to be evaluated by an appropriate person to do that.

Some of the cases that were excluded prior to even getting FISH assays performed on them, were excluded on the basis of pathologic evaluation, indicating that it was such a small, tiny specimen, there were just a few like that, that had so few tumor nuclei on that, that the pathologist working on the study didn't feel that that was representative.

There were some cases that just showed DCIS on the section that we looked at, because these blocks had been cut for many other assays.

So, the bottom line would be that you need, like in any other assessment of tissue specimens, you need to ensure that you have a representative cancer, and in this case, it's representative invasive cancer, with sufficient cancer nuclei, and that's not just two nuclei.

We didn't quantitate that. I think, any professional person in the area looking at representation of

cells, will be able to make that assessment.

DR. HORTIN: You need to specify a minimum number of nuclei or cells, or just enough to count, or --

MS. DRESSLER: The -- you could say that it's a somewhat subjective analysis that falls into the hands of the appropriately trained person.

Certainly, when we only have one or two tiny cells, you can't even make the assessment that you have an invasive cancer. So, you need to show that there is representative invasive cancer nuclei on the specimen, and I wouldn't give it a number. If the appropriate person indicates that it's representative, then that is sufficient. Does that answer your question?

DR. LADOULIS: If there are no other burning questions, comments, I think we have come to the point where we are going to take a 10- to 15-minute recess. At 4:00, we will have opened the meeting, that will be open public session, and we will vote in the concluding part of this Panel. Okay?

(Whereupon, at 3:45 p.m., a recess was taken, after which the Panel reconvened at 4:00 p.m.)

#### **Open Public Session**

DR. LADOULIS: We are now reconvened and we have now the obligation to have our open public session, and any interested persons who want to address the Panel and present

any information relative to the Agenda of this meeting are invited to do so now, and state whether they have any financial involvement with the manufacturers or of any products being discussed, or with their competitors.

Would anyone like to address the Panel? There seeming to be none, then we can close the public session. The Chair recognizes no one who wants to speak, so now we convene in closed session again, for our final deliberations, and I will turn to our Executive Secretary here, Louise Magruder will make some comments in preparations for our deliberations.

#### **Closed Session**

MS. MAGRUDER: At this time, Dr. Ladoulis will be calling for a motion, and he will be asking the voting and temporary voting members of the Panel to make a recommendation on this PMA.

For today's Panel, voting members present are Drs. Carpenter, Homburger, Hortin, Kemeny, Reynoso, and Taube.

Appointed as temporary voting members for today are Drs. Kaul, McCaskill-Stevens, and Todd.

The Panel recommendations may take one of three forms. You may recommend Approved, with no conditions attached to the approval. The second recommendation is Approvable, subject to specified conditions, such as resolution of clearly-defined deficiencies that have been

identified by you, or the FDA staff.

Examples of deficiencies could include the resolution of questions concerning some of the data, or changes you would like to see in the draft labeling.

Or you may conclude that post-approval requirements should be imposed as a condition of approval. If you believe that post-approval requirements are necessary, then your recommendation must address the following points:

The reason or purpose for that requirement.

The number of patients to be evaluated.

And the reports required to be submitted.

The third recommendation is you may recommend, Not Approvable. Section 515(d)2(a-e) of the Food, Drug and Cosmetics Act, states that a PMA can be denied approvable for any of five reasons, three of which are applicable to your deliberations and decision. These three reasons are:

There is a lack of showing of reasonable assurance that the device is safe, under the Conditions of Use prescribed, recommended, or suggestion in the proposed labeling.

The second reason, there is lack of showing of reasonable assurance that the device is effective, under the Conditions of Use prescribed, recommended, or suggested in the proposed labeling.

Or, thirdly, based on a fair evaluation of all material facts, the proposed labeling is false or misleading.

Safety is defined as reasonable assurance that the device is safe, when it can be determined based on valid scientific evidence, that the probable benefits to health from use of the device, outweigh any possible risks, and that there is absence of unreasonable risk associated with the device under its Conditions of Use.

An effectiveness determination must be based on valid scientific evidence, that in a significant portion of the target population, the use of the device, its Intended Use, and Conditions of Use, when accompanied by adequate directions and warnings against unsafe use, will provide clinically significant results.

If you make a Non-approvable recommendation for any of these stated reasons, we request that you identify the measures that you believe are necessary, or the steps that should be undertaken, to place the PMA in Approvable form.

**Panel Vote and Recommendations to FDA**

DR. LADOULIS: Okay. I think that we are ready to poll the voting members of the Panel for their sense as to whether or not you would find the application Approvable, Approvable with Conditions, or Not Approvable. If you would

have conditions, if you could so state them.

I can go around, I would like to go around the room from my left, across the way, and who is the first voting --

MS. AMMIRATI: I'm not a voting member.

DR. CARPENTER: Yes, I would approve with the conditions that we have discussed, in terms of the changes in the Intended Use, to mention Adriamycin. And -- do you want me to go through all of these that we have already discussed in the Committee, or --

DR. LADOULIS: You can refer to what we have discussed, if you would like.

DR. CARPENTER: And --

DR. LADOULIS: Could you identify yourself, please?

DR. CARPENTER: Dr. Carpenter. The changes with Adriamycin and maintaining the stage II, node-positive patients as the group of patients which are targeted.

One other, which this is kind of a minor change, which we alluded to, was in the package insert, where it does mention that selection of tissue for PathVysion assay should be performed by a pathologist, but we only alluded to this.

I think that maybe the paragraph should just be changed around so that it's either bolded, or it's at the

beginning of the paragraph, because I think it is real easy for people to just read over that, and not see it, and I think that is a very important concept, and one that, since it is that a pathologist is required, the lab may not necessarily know that, so I would include that.

Those were the two main --

DR. LADOULIS: Okay. Thank you, Dr. Carpenter.

DR. KAUL: Karen Kaul. I vote Approvable, subject to the conditions that we have already discussed as a Committee.

DR. LADOULIS: Thank you.

DR. REYNOSO: Reynoso. I will vote Approvable, subject to the conditions specified.

DR. LADOULIS: Next.

DR. TODD: Mary Todd, and I would vote Approvable, subject to the conditions we have already specified.

DR. LADOULIS: Next. Who is next voting? Dr. Jordan, I'm voting. Dr. Homburger.

DR. HOMBURGER: I'm not sure my voice works, I haven't said anything all day. I wish I could say more, but I vote Approval, subject to the same conditions relating to specification of Adriamycin in the treatment regimen, bolder, more easily identifiable indication that a pathologist be involved in selection of appropriate tissues for examination and testing.

DR. MCCASKILL-STEVENSON: Wortia McCaskill-Stevens. I vote Approval with the conditions as previously discussed by my colleagues.

DR. KEMENY: Margaret Kemeny. I vote Approval with the conditions as specified.

DR. HORTIN: Glen Hortin. I would recommend Approval with the conditions we have specified. I do have a little concern about the assays relating to the data that we presented.

It would suggest that, the assay may be 20 to 30 percent less sensitive than immunohistochemistry. Given that we have no absolute gold standard, we don't know necessarily whether that represents false-positive from immunohistochemistry. We have no way to resolve that conclusively.

It does raise a little bit of a concern, and I think the assay performs well in the sense of having an internal standard. It's quantitative, gives relatively precise results, so it does have some favorable characteristics.

One thing that I would recommend the Company develop in terms -- this relates to the training program, somewhat. I wouldn't consider this necessarily condition for approval, but they should work in developing some sort of proficiency testing program.

I think that with their key element in terms of making sure that their training has been successful, and that the proficiency program should actually include real tissue sections and not simply the cell lines that they -- I know that requires quite a bit of work in terms of finding the resources, but I would think that that is one recommendation I would make, both at the initiation of testing, people should test in proficiency samples to make sure that they are able to perform them reliably, and also have a periodic proficiency testing.

I'm not aware of any materials that would be generally available to do this, so I think the Company could serve a great service if they could provide those.

DR. TAUBE: Sheila Taube. I also vote Approvable, subject to the conditions that have already been mentioned. I would like to just emphasize again that the pathologist has to be involved in identifying the area to look at, and also in the interpretation of the results.

DR. LADOULIS: Charles Ladoulis, I'm the Chair and I'll make a last comment. I would like to recommend that the one condition be that the -- any statement of the variability in the enumeration or assay, be subject to the final FDA staff review of submitted data, which we had not received until this meeting, which had to do with tissue sections. And therefore, no claims should be allowed, with

regard to coefficient of variation, based on just the target samples of cell culture lines, since this does not pertain to the actual patient environment in which this assay is to be used in the marketplace. And that is my final comment. Louise?

Now, we can summarize, therefore, I think the conditions and then we will have a show of hands for the vote. Therefore, this --

DR. HOMBURGER: With respect to the coefficients of variation --

DR. LADOULIS: I'm sorry. Elaborate?

DR. HOMBURGER: My question had to do with whether or not the individual lab users would not find it useful to keep track of the coefficients of variations in their own results, on the various control slides, to indicate that they were in fact -- that's another indication of their own proficiency in performing the tests, so I think that guideline in the package insert is actually somewhat useful to people.

If they are three logs removed from what the test sites were able to get, then they should consider going for more training.

DR. LADOULIS: I have no objections to that. My objection was to an inference that that guideline for control slides applies to variability in the patient

specimens. That's all I was concerned about, having a more realistic assessment, which I think will be derivable from the data which has been submitted, but is not completely analyzed yet by the Agency, and so I think the staff can take that under advisement, and make the judgment as to what the variability is. Is that fair? Okay.

Then I think the final consensus of the Panel, for which we would vote is that this application would be recommended for approval, subject to the conditions set forth. That the -- if I can just specify it for Dr. Reynoso -- that -- do you want to specify the condition that you had mentioned, or I guess it was Dr. Carpenter.

DR. CARPENTER: Just that the statement concerning the requirement for a pathologist to review the slide was in a more prominent location in the package insert.

DR. LADOULIS: A, that a prominent --

DR. CARPENTER: The beginning of the paragraph, or in bold, or --

DR. LADOULIS: -- change of the package insert to highlight, specify, or otherwise qualify that a pathologist professional be involved in the process of specimen and field selection. And B, Dr. Kemeny?

DR. KEMENY: I think B would be to change the wording on the Intended Use to say, subjects with stage II, node-positive breast cancer, so change that around, and on

the next sentence to say, potential response to Adriamycin-contained adjuvant therapy, or Adriamycin-contained therapy.

DR. LADOULIS: That's B and C, okay. Any other condition? We have those three conditions.

DR. KEMENY: And then, what about --

DR. LADOULIS: And D is the condition that the variability in the performance of the assay be subject to the final review with the Agency staff, based on actual slide data, which has been submitted.

DR. HOMBURGER: This is Dr. Homburger. I think Dr. Hortin did make an excellent point earlier with respect to -- and I don't recall whether this is specifically listed. Specimens that are clearly not suitable for analysis.

DR. LADOULIS: Okay.

DR. HOMBURGER: And he enumerated several, those that have insufficient tissue for -- I don't want to paraphrase what you said, but I thought it was a point well-taken, because running a reference lab, I can see myself getting all kinds of samples sent to me, and --

DR. LADOULIS: So, therefore, E, another condition, that clear requirements be spelled out in the package insert as to what specimens are disqualified for assay, based on properties such as insufficient fields of tumor --

DR. HOMBURGER: And necrotic tissue --

DR. LADOULIS: Improper fixation.

DR. HOMBURGER: Improper fixation.

DR. LADOULIS: Or improper handling of the blocks, excessive exposure to heat. Can that be handled by -- will that recommendation be sufficient for the staff? All right. I think those are recorded, duly, and understood by the members of the Panel. Then I think if there is no further questions, that we can have a show of hands of the voting members of the Panel. For those who would vote for this recommendation of Approval with those conditions.

Dr. Carpenter, Dr. Kaul, Dr. Reynoso, Dr. Todd, Dr. Homburger, Dr. McCaskill-Stevens and Dr. Kemeny, Dr. Hortin, and Dr. Taube -- it's unanimous.

(Whereupon, the Panel voted unanimously by a show of hands for Approval with conditions described previously.)

So, that recommendation is for Approval with those conditions is passed.

Any other business to come before the Panel? Move to adjourn.

MS. MAGRUDER: I would like to just say a few words.

DR. LADOULIS: Oh, please.

MS. MAGRUDER: On behalf of the Center for Devices and Radiological Health, I want to thank this Panel for

their participation in the Center's activities. I want to congratulate the Sponsor, Vysis, on their well-prepared presentation.

FDA also thanks Dr. Edison Liu for making himself available for Panel questions. And I especially want to thank all of the FDA staff, for their thorough and effective presentations, especially Joan McGlenn-Bennett, who was doing the overheads under adverse conditions.

As a final reminder, this is a housekeeping reminder. Would you please put all of your disposable items in the wastebasket as you exit? And thank you very much.

DR. LADOULIS: Thank you.

(Whereupon, at 4:20 p.m., the Panel was adjourned.)