CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

FOOD AND DRUG ADMINISTRATION/DHHS

Meeting of:

CELLULAR, TISSUE AND GENE THERAPIES ADVISORY COMMITTEE

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P R O C E E D I N G S (8:10 a.m.)

Agenda Item: Opening Remarks and Introduction of Committee Members.

DR. MULE: I would like to welcome all of you to the Cellular, Tissues and Gene Therapies Advisory Committee meeting.

The meeting will be held today, of course, as well as tomorrow. I would like to mention that we have a pretty full agenda in front of us.

A couple of announcements. The first is that I would like to recognize Dr. Rao, our previous chair, who has stepped down. We will have a recognition of him later in the program.

We also have several new members of the committee, those being Dr. .Calos, Dr. Chamberlain, Dr. Walter Urba, and Dr. Kurt Gunter. I would like to welcome the new members of the committee. Finally, we have several members who are cycling off, and we will recognize those members as well later on.

I think we will get started, and what I would like to do at this point is go around the table and have each member introduce himself or herself and affiliation. If we could start on the right?

DR. CORNETTA: Ken Cornetta from Indiana University. I am the chair of the department of medical

molecular genetics. My interest has been in gene therapy.

MR. TOMFORD: I am Bill Tomford at Massachusetts General Hospital. I am an orthopedic surgeon and professor of orthopedics at Harvard. I have an interest in cartilage transplantation.

MS. TERRY: I am Sharon Terry, president and CEO of the Genetic Alliance, which is a coalition of 600 patient advocacy organizations.

DR. GUNTER: My name is Kurt Gunter, and I am in charge of clinical affairs at ZymeQuest. I am a clinical pathologist, and I have had a long-standing interest in cell therapy and gene therapy. I am happy to be here at my first meeting.

DR. URBA: My name is Walter Urba, medical oncologist, interested in immunotherapy, from Portland, Oregon.

DR. CALOS: Michele Calos from the department of genetics at Stanford University School of Medicine. My lab works on gene therapy, developing site specific integration systems.

DR. HARLAN: My name is David Harlan. I am chief of the diabetes branch of the National Institute of Diabetes, Digestive and Kidney Diseases at the NIH, and I am interested in eyelet transplantation in immunotherapy for type I diabetes.

DR. TSIATIS: Hi, I am Butch Tsiatis from the department of statistics at North Carolina State University.

DR. ALLAN: I am Jon Allan from the Southwest Foundation for Biomedical Research, and my area if non-human primate models rights.

MS. DAPOLITO: Gail Dapolito, executive secretary for the committee.

DR. MULE: I am Jim Mule, associate center director of the H. Lee Moffitt Cancer Center in Tampa, Florida.

DR. PLANT: I am Anne Plant from the National Institute of Standards and Technology, which of course is responsible for measurements and standards in lots of aspects, including biotechnology, lots of different industry organizations.

One of the speakers here today is John Elliott, who works very closely with me in the cell and tissue measurement group.

DR. COUTURE: I am Larry Couture from the Beckman Research Institute at the City of Hope National Medical Center. I am the senior vice president for applied technology development, and the Center for Biomedicine Genetics, largely a biologics manufacturing facility on campus, and I have been in gene therapy for a long time.

DR. ROCKE: I am David Rocke. I am from the

division of biostatistics in the school of medicine at the University of California, Davis, and I work on statistical methods for assays, particularly these days gene expression arrays, proteomics and metabonomics.

DR. SNYDER: I am Richard Snyder from the University of Florida, where I am the director of biotherapeutic programs. My interests are in cell and gene therapies.

DR. MARINCOLA: I am Francesco Marincola. I am director of the immunogenetics laboratory at the clinical center of NIH. My main interest is developing strategies for monitoring clinical trials.

DR. GAVIN: My name is Denise Gavin. I am from the Office of Cellular, Tissue and Gene Therapy.

DR. SIMEK: Stephanie Simek, deputy director, division of cell and gene therapy, CBER.

DR. PURI: I am Raj Puri. I am the director of the division of cellular and gene therapies.

DR. WITTEN: Celia Witten, office director of the office of cell, tissue and gene therapy.

DR. MULE: We have just had Dr. Chamberlain join us. Jeff, if you could introduce yourself and your affiliation?

DR. CHAMBERLAIN: Jeff Chamberlain. I am at the University of Washington in the department of neurology.

DR. MULE: I would also ask the speakers today if you could use the nearest microphone and introduce yourselves as well and your affiliation.

DR. PROVOST: Nicole Provost, Dendrion Corporation.

DR. ELLIOTT: I am John Elliott from the National Institute of Standards and Technology.

MS. MANSON: Kelledy Manson from Therion Biologics.

DR. DANILKOVITCH: I am Alla Denilkovitch from Osiris Therapeutics.

DR. KASLOW: David Kaslow from Vical.

DR. BUTMAN: I am Bryan Butman. I am senior vice president of vector operations for GenVec.

DR. MULE: I would like to welcome the speakers and thank them for their time in sharing with us some information that will be very pertinent to the topic ahead of us.

At this time, I would like to introduce Gail Dapolito, who will talk about the conflict of interest statement.

Agenda Item: Conflict of Interest Statement.

MS. DAPOLITO: Thank you, Dr. Mule. Good morning.

The Food and Drug Administration convenes today's meeting of the Cellular, Tissue, and Gene Therapies Advisory

Committee under the authority of the Federal Advisory Committee Act of 1972.

With the exception of the industry representative, all members and consultants of the committee are special government employees or regular federal employees from other agencies, and are subject to the federal conflict of interest laws and regulations.

FDA provides to the meeting participants and the public the following information on the status of this advisory committee's compliance with federal ethics and conflict of interest laws including, but not limited to, 18 USC subsection 208, and 21 USC subsection 355(n)(4).

FDA determined that members of this advisory committee and consultants of the committee are in compliance with federal ethics and conflict of interest laws, including but not limited to 18 USC subsection 208, which is applicable to all government agencies, and 21 USC subsection 355(n)(4), which is applicable to certain FDA committees.

Congress authorized FDA to grant waivers to special government employees who have financial conflicts, when it is determined that the agency's need for a particular individual's services outweighs his or her potential financial conflict of interest -- that is subsection 208 -- and where participation is necessary to afford essential expertise, subsection 355.

Members and consultants of the committee who are special government employees at today's meeting, including special government employees appointed as temporary voting members, have been screened for potential conflicts of interest of their own, as well as those imputed to them, including those of their employer, spouse or minor child, related to the discussions of topic one, potency measurements for cellular and gene transfer products, topic two, the national toxicology program on retroviral insertional mutagenesis, and topic three, an overview of the research program of the Office of Cellular, Tissue and Gene Therapies.

These interests may include investments, consulting, expert witness testimony, contracts, grants, CRADAs, teaching, speaking, writing, patents and royalties, and primary employment.

In accordance with 18 USC subsection 208(b)(3), waivers were granted to the following special government employees. Please note that all interests are in firms that could potentially be affected by the committee's discussions:

For topic one: Dr. Jeffrey Chamberlain and Dr. Richard Snyder. Topic two: Dr. Jeffrey Chamberlain and Dr. Anastasios Tsiatis.

Dr. Richard Mulligan was granted a limited waiver

for topic two. He may participate in the committee discussions, but will not vote on this topic.

For topic three, waivers were granted to Drs. Jeffrey Chamberlain, James Mule, Anastasios Tsiatis, and Kenneth Cornetta.

Dr. Kurt Gunter serves as the industry representative acting on behalf of all related industry, and is employed by ZymeQuest, Incorporated.

Dr. Gunter is also a part-time employee of the University of Massachusetts Memorial Medical Center, and a scientific advisor for Viacell.

Industry representatives are not special government employees and do not vote. A copy of the written waivers may be obtained by submitting a written request to the agency's freedom of information office, Room 12-A-30 of the Parklawn Building, Rockville, Maryland.

With regard to FDA's guest speakers, the agency has determined that the information provided by these speakers is essential.

The following information is being made public to allow the audience to objectively evaluate any presentation and/or comments made by the speakers:

For topic one, Dr. John Elliott is employed by the National Institute of Standards and Technology.

In addition, there may be regulated industry and

other outside organization speakers making presentations. These speakers may have financial interests associated with their employer and with other regulated firms.

The FDA asks, in the interests of fairness, that they address any current or previous financial involvement with any firm whose product they may wish to comment upon.

These individuals were not screened by the FDA for conflicts of interest. The conflict of interest statement will be available for review at the registration table.

We would like to remind members and consultants that if the discussions involve any other products or firms not already on the agenda, for which an FDA participant has a personal or imputed financial interest, the participants need to exclude themselves from such involvement, and their exclusion will be noted for the record.

FDA encourages all other participants to advise the committee of any financial relationships that you may have with affected firms and/or products. Thank you, Dr. Mule.

DR. MULE: Thank you, Gail. So, we are going to get started with topic one, which is the potency measurements for cell and gene therapy products. I would ask each speaker to please keep to the allotted time.

At the end of each presentation will be a brief question and answer period. Then, later in the afternoon,

the committee will be asked to tackle questions which have been provided to the committee prior to the meeting.

So, the first speaker is Dr. Gavin from the FDA, and he will give the FDA introduction.

Agenda Item: TOPIC I: Potency Measurements for Cell and Gene Therapy Products. FDA Introduction.

DR. GAVIN: Good morning. My name is Denise Gavin. I am from the Office of Cellular, Tissue and Gene Therapy.

I would like to thank the committee members, as well as the guest speakers, for their participation in this potency assay discussion today.

Potency measurements are a critical part of product characterization, which is necessary for the approval of all biological products, and we look forward to the committee's input on this important topic. I would also like to thank the FDA participants who helped make this meeting possible.

We organized this advisory committee meeting to discuss the challenges related to the development of meaningful and relevant potency assay measurements for cell and gene therapy products, and to obtain perspective and advice from the committee members regarding the implementation of scientifically valid assays for measuring potency.

The Office of Cellular, Tissue and Gene Therapy regulates a number of complex products, as indicated by this list.

We recognize that many of these products will also present challenges for potency measurements. However, comprehensive discussion of potency for all of these products is beyond the scope of one meeting.

Therefore, today's discussion will focus solely on cellular and gene therapy products, and cell and gene therapy based tumor vaccines.

Cellular products have been derived from a number of different tissues, and include stem cells, differentiated cells, as well as immune cells and tumor cells from many different types of tissues.

The source material for cellular products may be obtained from autologous and allogeneic donors, as well as from established cell lines.

Gene therapy is the transfer of genetic material by means of a vector, either viral or non-viral. These vectors may be administered directly to subjects, or used to modify cells ex vivo for subsequent administration. Both the vectors and ex vivo modified cells are considered gene therapy products.

For today's discussion, we will only be considering tumor vaccines based on cell and gene therapy

products.

This could also include tumor vaccines that consist of cells and cell lysates that have been pulsed with peptides, proteins or vectors. Many tumor vaccines may also include the addition of an adjuvant.

As with all biologics, successful development and approval of cell and gene therapy products will require that the product be safe, pure and effective.

This requires full product characterization, and a demonstration of manufacturing and product consistency. This is necessary not only to comply with GNP regulations, but also to ensure continued production of a safe, pure and potent product.

Product characterization involves a number of tests in the final product as specified in the code of federal regulations.

Licensed biological products must be tested for all of these parameters, including potency, prior to vat release.

Potency is defined in the code of federal regulations as the specific ability or capacity of the product for tests of a given result.

Tests for potency shall consist of either in vitro or in vivo tests or both, which have been specifically designed for each product so as to indicate its potency.

These regulations provide considerable freedom when evaluating suitable potency assays.

While there is not specific guidance for determining potency of cell and gene therapy products, we generally follow the interpretations outlined in the ICH guidance for industry on specifications.

This guidance states that potency is the quantitative measure of biological activity based on the attribute of the product that is linked to the relevant biological properties. It also states that a relevant validated potency assay should be part of release specifications.

While the regulations require validation of assays used to characterize licensed products, there is no guidance on how to validate the biological assays used to measure potency.

The general guidance documents that are available that outline the process of validating analytical procedures are indicated here.

These state that the validation of a method is the process of demonstrating that the analytical procedures are suitable for their intended use. Does the assay measure what it is intended to measure.

The process of assay validation should demonstrate that the method meeds all of these parameters indicated

here.

Assay robustness if necessary to show that the assay is not affected by small but deliberate changes in methods, materials or operators.

The robustness of an assay should be determined during assay development. We would like the committee to keep these parameters in mind when considering assay design strategies discussed throughout the day.

While a validated potency assay is not required until licensure, there are a number of advantages to beginning potency assay measurements during early product development.

Potency measurements are critical to determine the activity, quality and consistency of products used during all phases of clinical development.

Demonstrating that a consistent product was administered is important when interpreting clinical data. Starting early also allows for the examination of multiple assays, as well as provides data to establish specifications for lot release, and provides data to establish product stability.

Finally, potency is an important measure in comparability studies. Since manufacturing changes are likely during product development, a good potency assay is necessary to determine the comparability of products

manufactured under different conditions.

So, what is a good or acceptable potency assay?? The Office of Cellular, Tissue and Gene Therapy has no particular preference for any type of assay.

The acceptability of all potency assays will be determined on a case by case basis. However, all assays will need to meet certain criteria as outlined here.

The assay will need to measure the biological activity of the product, will need to measure properties specific to the product.

Results will need to be available for lot release. Lots should be quantitative. The assay should demonstrate lot to lot consistency.

The assay should include a comparison to a reference standard or appropriate control, and the assay will need to be validated for licensure. That is a lot of requests.

Our speakers today will discuss a number of different potency assay methods. So, I will just give a brief overview of the type of assays that could potentially be used to measure potency.

Potency can be measured directly by means of a biological assay which is based on specific product characteristics, or it can be measured indirectly using analytical methods which are correlated with a specific

product activity.

For the purpose of discussion today, we, the Office of Cellular, Tissue and Gene Therapy, are using the term, analytical assay, to include any assay method that is not a bioassay.

Biological assay methods are meant to measure a product's activity within a living system. Bioassays may be performed in vivo using appropriate animal models, or in vitro using cell culture systems.

Assays based on immunochemical, molecular or biochemical characteristics of the product can also be used to demonstrate potency, provided that they directly reflect, or are correlated, to a biological activity.

In addition, the information obtained from these assays can be used to increase our understanding of complex cell and gene therapy products, and can also be used as tools for the development of relevant potency assays.

For example, many of these procedures could provide information about the functional status of, say for example, a cellular product, by distinguishing cells of different phenotypes and differentiation states, and also by measuring the level of expressed or secreted proteins.

Challenges. There are many. Many of the challenges related to potency measurements for cell and gene therapy products have to do with the products themselves.

Many of these products have complex mechanisms of action, making it difficult to determine what activity to test for.

These products can also obtain multiple active components with multiple activities that could all be necessary for the biological function of the product.

One should also consider the potential for interference or synergy between these components when designing your assay.

Product variability, due to variability in the starting material or source of cells may be unavoidable, and is also a challenge.

There may be a limited amount of material to perform all the lot release testing, and product stability should also be considered.

Many of the products need to be administered within hours of harvest, and storage and extended handling times may adversely affect many of these challenges.

Additional challenges relating to the assays are also possible. Biological assays used to measure potency can be highly variable.

Therefore, it is important to identify sources of variability, and design your assay to minimize these effects.

Some variability will be unavoidable due to the

source material, as described earlier. The high variability can complicate the ability to validate assays.

In addition, limited availability of reference standards and controls can further complicate the ability to evaluate consistency, as well as to evaluate these assays.

Many bioassays used to measure potency also can take a considerable amount of time to perform. This makes them impractical for lot release for many of these products, especially those with limited stability.

So, what are we, at the FDA, doing to help? We encourage the development of potency assays during early product development.

However, we are also very flexible when it comes to the implementation and validation of potency assays. We allow a phased in approach, which allows sponsors to begin clinical trials while they pursue product development.

We acknowledge there is no one assay that may be suitable for all products, and it may be necessary to develop more than one assay to demonstrate potency.

This may necessitate a matrix approach to testing. For the next few slides, I will explain what we mean by a matrix approach.

An ideal potency assay is a bioassay that meets all the criteria as demonstrated in this example. All the criteria is not really that important, just the idea behind

it.

It may not be possible or feasible to perform a bioassay that includes all this criteria for every product. For example, you may have a bioassay that may be quantitative and measures a biological activity, but it may not be available for lot release.

When a single bioassay does not meet all the criteria listed here, it may be necessary to develop one or more additional assays.

You could develop a single analytical assay that is available for lot release and meets all of these criteria. However, in the example shown, this assay does not demonstrate biological activity. So, both assays would be necessary to demonstrate potency.

If a correlation can be made between a surrogate analytical assay, or assay one, indicated here, and a relevant biological property of the product, then the bioassay would no longer be necessary, and assay one could be used for lot release.

Many of our products and many of these assays will have many challenges. The bioassay may be highly variable and difficult to quantitate, as well as the results not being available for lot release.

If one surrogate assay meets some, but not all, the necessary criteria to demonstrate potency, it may be

necessary to develop an additional assay.

In this example, the two analytical assays, combined with the bioassay, are necessary to demonstrate potency.

However, if the surrogate assays are correlated to biological activity, the bioassay would no longer be necessary for lot release.

In the example shown, neither assay one nor two meets all the necessary criteria. So, both assays are necessary to demonstrate potency.

It may be easier to demonstrate this using an example based on a cell therapy based tumor vaccine. The first is an example of a functional assay, such as measuring antigen specific T cell activation using a cytokine release assay, which is measured using ELISA or ELISpot.

The second approach might include correlating one analytical assay to the biological function, such as correlating antigen binding, as measured by flow cytometry, to T cell activation.

The third approach involves correlating multiple assays to a specific biological function, for example, correlating phenotypic cell marker and antigen presentation with T cell activation.

To summarize, I can't stress enough the importance of initiating potency assays during early product

development.

This allows sponsors to recognize challenges to meeting the requirements for potency, to evaluate more than one assay if necessary, and to collect sufficient correlation data to replace bioassays with an analytical assay if necessary.

It is really important not to forget that a well characterized product is important when evaluating clinical data.

I have included this slide for your reference. The CDER web site contains relevant links to guidance documents and regulatory information that are discussed today, and we will eventually have this slide as well.

I would like to end my session by thanking the speakers who have agreed to discuss their research and potency assay development experience with us today.

Their experience should provide additional insight not only into the complexity of cell and gene therapy products, but also into the challenges faced with developing assays for this complex class of biologics.

Their experience should be helpful to the committee in addressing the questions put forth for discussion.

I would like to clarify that the speakers have agreed to discuss their strategies for potency assay

development as a courtesy to the agency, and are not here to seek approval for their products or assays.

Therefore, the committee should keep in mind, they are not here to determine the acceptability of any of the assay development programs presented today.

We are here, however, to seek input from the committee regarding a number of questions, and we will discuss those later this afternoon. I would like to present them here today.

We are seeking input from the committee regarding assay design schemes that will be necessary to validate potency measurements, the types of data on studies that will be necessary to demonstrate valid correlation between analytical assays and biological properties of the products, what is necessary to adapt and implement state of the art technologies, such as micro assay, genomics, proteomics, to obtain the consistent results necessary for their use in potency measurements.

If time permits, the future research directions that may be necessary to demonstrate potency for cell and gene therapy products. I will stop here, and I welcome any questions or input from the committee. Thank you.

DR. MULE: Thank you, Dr. Gavin. Questions from the committee?

DR. CALOS: With the cell therapy products, you

mentioned them being pulsed with these different components. What do you mean by pulsed?

DR. GAVIN: Well, you can mix together, you can add these together.

DR. CALOS: So it is just mixing?

DR. GAVIN: Yes, I think so.

DR. MARINCOLA: A question more about terminology, so that I know during the day what we are talking about. If you could specify what you mean by cellular, the range of cellular products, and the same thing when you talk about adjuvants. Are cytokines included in a vaccine trial part of the discussion, or peptides versus cellular products, or is this a different part of the arena?

DR. GAVIN: We are only including the cellular based and the gene therapy based. So, there would be the cells and cell lysates from any source.

DR. MARINCOLA: So, no synthetic products.

DR. GAVIN: No. There will be some discussion -- you could include the tumor vaccines that have cells or cell lysates that are pulsed with or include antigens or peptides or proteins or vectors. Sometimes you will have a cellular that is transduced with a vector, a cell product transduced with a vector that is for a tumor vaccine, for instance.

We won't be talking about proteins themselves or the peptides themselves, only in the context of the cell and

gene therapy product. Does that answer your question?

DR. MARINCOLA: Yes, because actually it makes a big difference in the variants that we are going to be dealing with. If we talk about synthetic products, it is so much easier. Then we don't really have to worry about the tough stuff.

DR. GAVIN: For today's discussion, it will just be the ones that include cell and gene therapy products.

DR. GUNTER: You showed a slide that was entitled, acceptable potency assay. I was just curious, the criteria on that slide, is that in a regulation or a guidance? I am just curious about the source of those criteria, and are they negotiable.

DR. GAVIN: These are basically the criteria that would be necessary for approval of a bioassay that is for a licensed product.

The biological activity is from other guidances and the fact that these are biological product and the specific activity of a biologic product should have a biological activity.

The regulations state that it should be specific for the product. That it has to be available for lot release is also in the regulations.

Some of the guidances that are available, they also state that it should be a quantitative assay. The

demonstration of lot to lot consistency is one of our criteria, and the fact that it should compare to a reference standard is also in the regulations. The validation is also in the regulations.

DR. TSIATIS: In your matrix approach, I was confused by one of the things, and maybe you can clear it up. You showed a situation where the bioassay might not have a good lot to lot availability, but the other assay might, but it didn't necessarily correlate. I wasn't clear what good an analytic assay would be if it didn't correlate with the bioassay.

DR. GAVIN: This isn't a direct measure of biological activity. It may be a cell surface marker that is present, but is it showing biological activity, or do you need to correlate that marker expression to biological activity.

DR. MULE: Dr. Gavin, one of your slides is a recommendation, perhaps, for an advisement to begin potency assays early on. Maybe you can define for us what early on means, if you take a cell based vaccine, for example.

DR. GAVIN: Ideally, during your preclinical and early clinical studies, you are looking at the biological activities products.

You should be able to start getting information that will help you develop a potency assay based on the

functional activity of the product, or on basic characteristics of the product.

It is never too early. You can start as early as preclinical development. We don't require that you have an assay during early phases. We do ask for something that demonstrates some activity, but we don't necessarily ask for a quantitative validated assay until later, but it is never too early.

DR. COUTURE: I suppose it will be more in terms of how the discussion progresses later in the afternoon, but it occurs to me that biological activity may not even be an accurate definition or something to apply to all of these products, since in some cases some cellular, or patient-specific, autologous patient type product activity might simply just be survival, and just being a cell that exists, as a cell that is a protected from HIV, for example, just a T cell. It is just healthy.

I will be curious to see how biological activity gets layered on a cell like that. You haven't really made it do anything in a patient other than just be able to survive.

DR. GAVIN: Some of the part about biological activity, the specific activity is also defined by the product, and when you submit the information to us, you submit that information of how you are describing the biological activity.

A lot of the burden is on the sponsor to determine the biological activity on the product. That is also part of the reason we are having this committee meeting, is to get input from the scientific experts on what is considered biological activity for these products, or how you would correlate biological activity to an analytical assay or some other sort of assay measurement.

DR. MULE: Thanks, Dr. Gavin. Our next speaker is Dr. John Elliott, from the National Institute of Technology and Standards.

Agenda Item: Lessons Learned from Measuring Cell Response by Automated Quantitative Microscopy.

DR. ELLIOTT: I am John Elliott from the National Institute of Standards and Technology. I would like to thank everyone for coming this morning.

The National Institute of Standards and Technology, which is right up the road, is part of the Department of Commerce.

Our mission is to work on many of the measurement challenges that the nation faces. These include both measurement technologies and standards.

We have a variety of laboratories. As you see up here, we have chemical, a science laboratory, a physics laboratory, material science.

We have a lot of scientists who have significant

expertise in making measurements in the physical sciences and, more recently, they have been moving into the world of biological sciences.

As measurement scientists that really think about how do you control the conditions before you make measurements on cells, and how do you make very reliable measurements, cell biology presents us with a really interesting problem.

As I show here on a picture, one way that we can consider the cell is as a complex signalling machine that grabs a variety of input signals from extracellular sources and then, through complex signalling pathways, decides what the fate of the cell will be based on those input signals, and then begins to go into some cell substate, such as proliferation, or the cells begin to differentiate.

Then we measure that by looking at biomarkers that are representative of, or correlate -- a biomarker whose function correlates with the state the cell is attaining.

So, as scientists, the way we kind of can think about this problem is to bring it right down to that the cell is somewhat of a meter, and that it is measuring the input signals in the local environment, and then we are going to look at that cell and make a measurement of how that cell is behaving to those input signals.

So, with the complex input signals and all the

different methods that we can use to measure biomarkers, we have a lot of standards and reference issues and materials, both on the assay side, which would include a lot of input signals, and on the measurement side, that we can think about.

So, I am going to start talking about our program. We are the cell and tissue measurements group, and we have been working on what does it take to make highly quantitative bioassay measurements, a number of the issues that are important for potency assays or for typical bioassays.

Here is just a schematic of a simple cell assay that we can work off of. We have a cell in the center that we are going to measure our response in.

We are going to need some tools to measure the response at the bottom. We are going to need to known conditions that will be able to induce a known response in that cell, so that we have a validation that our tools can measure that.

Then we are going to need some statistical methods, to ensure that any differences we measure are statistically significant.

That is all before we even look at unknown materials. That is for validating the assay. Then we have, for test conditions for various changes that we make in the

system, we have to have a valid assay for that.

Every step of this process is going to require a number of issues, which I think we have outlined several of those issues, including physical standards for calibration the instrumentation, identification and validation of a biomarker that is going to be your cell response measurement, a variety of standard operating procedures dealing with how are you going to perform assays, known materials and reference conditions.

So, all of these things are issues that we are going to have to deal with in these bioassay measurements. So, to talk about a little bit of some of the measurements we have made, and some of the issues that we have come up with or learned about in our program in some of our studies, I am going to talk about a variety of the issues that come up during a bioassay.

These are some of the terms that were used: precision and robustness, kind of how we have identified these and the methods that we use; precision, this is reproducibility of replicates, mean standard deviation and CVs are typical metrics of that;

Robustness establishes long-term reproducibility, and that includes this variation of reagents or conditions have changed over time by unknown factors, sometimes. So, the robustness is something that we want to be able to

measure.

We can use something -- for example. I will show you a quality factor. I was calling that the Z factor, which is a nice assay, a nice metric for evaluating assay.

Then we would like to have accuracy, too. Now, accuracy is an interesting one, because we do need a standard to be able to compare our result to, to make sure that it is accurate, and that requires a reference material.

In some cases, in biological measurements, referencing materials are not readily available, but we can certainly think about -- there are certainly things such as dimensions, if you want to measure length scales on microscopes. Certainly scales are available for that.

Here is an example. This is a single cell clone that has been grown out. So, all the cells in these pictures have the same DNA.

These are NIH 3T3 fibroblasts that have been transfected with a green fluorescent protein driven by a promoter.

What you are seeing is, in the first red picture, you see cell shape. That has stained the whole surface of the cell, and it shows right off the bat that, even though the cells are genetically equivalent, we have a distribution of cell shapes.

Not every cell has the same shape. There is a

distribution of shapes. If we look in the gene activation panel where the green protein exists, that -- we see, again, that several cells in the red panel, which shows every cell, we don't see every cell expressing the same amount of green fluorescent protein.

So, even in the case of gene expression, we have a distribution of responses. That distribution of responses is something that is a true measurement of how these cells are behaving as a population.

So, our approach has been to really take advantage of that distribution data to really try to make some high quality measurements.

So, measuring the distribution of a cell response as opposed to a mean value in such a biochemical assay, in which all the cells are lysed, for example, could provide us with some more sensitivity in our measurements.

The choice, the methodology that I am going to talk about is automated fluorescence microscopy, automated quantitative fluorescence microscopy.

Basically, it is a fluorescent microscope that is an off-the-shelf microscope with translation stage that can move the sample that is put on the stage in an XY coordinate plane.

It has got a focusing motor. It has got filter wheels that automatically can change so that we can image

multi-wave lengths in the cell.

What the automated microscope allows us to do is to unbiasedly sample the surface by simply scanning through 100 images, 100 positions, on the microscope slide.

So, we are not choosing areas. The microscope just takes pictures. So, we get an unbiased collection of data.

Just to tell you -- you see the three stains over there. We can use, for example, a red stain which identifies cell shape, and we can use image analysis software to outline the cells, and then we can use that to identify how much green force and protein is in the individual cell, or how many nuclei are in the individual cell.

So, this provides us with a lot of information so we can get information from several thousand cells. One of the great things about microscopy is that the image data is very right, in that there are a variety of information about the cells that can be in a set of data.

So, you can get multi-parameter information simultaneously with this technique. In some cases, fluorescence microscopy can be less labor intensive than, for example, another single cell method such as flow cytometry.

It can work with lower numbers of cells. So, this may address some of the issues of lot specifications and

have a limited amount of material. Fluorescent microscopy could be a future tool for measuring cell response.

So, using an instamed, we can look at some of the reference materials that we use. In the lab we need to really ensure that the lab intensity has been calibrated on the microscope, when we take measurements day to day that everything is working right, the lamp is working, the microscope is working correctly.

NIST has a history of doing standard reference materials, especially physical reference materials, for biological systems and measurements and instrumentation.

There are a couple of materials that are shown there, a fluorescein solution, flow cytometry beads that have been standardized, and the fluorometer, an SRM for fluorometry, florescent wave length SRMs.

These materials, in some cases NIST actually doesn't have them in production, but manufacturers now make these materials and they are traceable to the original NIST standard.

Over on the other panel -- in our group we have been working on a fluorescent glass reference material, and this is just a material that is very robust.

It provides us with a two point calibration. We have a piece of glass that doesn't fluoresce, a piece of glass that fluoresces, and we can use that in our day to day

calibration of the microscope.

So, when we take measurements, let's look at the kind of data that we get. What you see in the top panel is cell morphology.

We are going to look at distributions of cell shape on a surface. What you see, that distributional peak, is not the distribution due to measurement uncertainty. That is actually a single well. We are looking at 1,000 cells in a single well.

What you are seeing is, that is the true biological distribution of the different sized cells in that well.

The same is true underneath, with the GFP fluorescent measurements. We see that there is a distribution.

That is not a measurement uncertainty. What that is, that distribution is the distribution of the green fluorescent protein expression in individual cells.

On the lower panel, if you want to call it, it is a non-galcian response, in that it doesn't look at galcian, where actually the cell morphology looks a little bit more like a galcian, which will come up a little bit later in the talk.

So, we can measure a mean value of that distribution to look at what the cell response is in that

particular well, and then we can do the same kind of measurement for multiple wells, for doing replicates.

We see that the distribution of cell responses overlap in both cases, in these measurements anyway. So, that provides us with a mean and a standard deviation due to the replicates, and you can see on the side, that is an average mean cell area from four replicates with standard deviation and then we have an average mean intensity.

So, those are metrics that give us some idea of what our reproducibility is and our precision of our measurements.

Now, one of the things that is important to note is, as I said, those distributions that you saw are due to the natural variation in the cell response.

That mean value is used to characterize that distribution for our measurement, but that mean value is only a valid representation of that distribution if we had sampled enough cells.

For example, in this case here, if we had not sampled, that mean value is representative of this distribution, but if we do not sample enough cells, I will show you on the other panel, that mean value will no longer be representative of that distribution.

So, what you see here is that the mean sample of 1,500 cells, this is the mean value of that distribution. As

we get down to lower and lower numbers of cells, about to 250, that mean value that we measured in that distribution begins to call, no longer representing what that distribution is. We see the precision, the CV of the replicate measurements, also begins to rise when we get below 250 cells.

So, what is important is, because biological response from cells is a distribution of responses, there will be a minimum number of cells that are required to get a mean value that is representative of that response.

Now we can think about setting up a minimal assay. That was just some important information that we learned about by treating cell responses as a distribution. Cell number is important when you want to use a limited amount of material.

In setting up a minimal assay, here is how we can think about this. Here are control samples. What we need is a set of replicate plus and minus control samples that clearly indicate the biological response we are going to measure.

By assaying these wells, it will give us a metric that tells us our measurement of that biological activity is functioning correctly.

Then an example, this is an example that I am going to use from our laboratory. We have been working on

materials to grow cells on.

We can imagine that we have four different processing conditions to make these materials, and we are going to identify if these are similar to the controls.

One of the metrics we are going to use to evaluate that assay is what is called the Z factor. This is used routinely in the pharmaceutical industry to evaluate the assays which they use for high throughput screening.

It is a very elegant method to make a measure of assay quality. Basically, this is the mean and replicate standard deviation of your minus control and your plus control.

Basically, from the separation of means you can get the dynamic range of the assay. That is the denominator, and then you have the confidence intervals of each of the mean values, which are in the numerator.

What the Z factor gives you is, from your control experiments, we get a parameter that characterizes the assay range in the -- the dynamic range and how much of that dynamic range is taken up by the statistical uncertainty in each of the measurements.

So, this Z factor can be used to establish how good are our assays. We can now get a specification on our assays, and we can use that, then, on day to day evaluation of assay quality to the begin to address robustness.

One of the nice things about the Z factor is that it actually provides us some information about the ability to get the false positives and false negatives that we could expect from this assay.

When Z factors are high, above .5, the plus and minus control dynamic range is so large compared to the hoise, that we would rarely get false positives and false negatives. As the Z factor gets lower and lower, then we would begin to get to a place where a false negative and false positives would have to be part of the assay characterization.

So, here is an example from our laboratory, just a quick one, using this biomaterial. In our lab, we prepare a biomaterial that is a collagen film that induces a cell shape change.

These are smooth muscle cells. These are smooth muscle cells in polystyrene, these are smooth muscle cells on the films that we make. These are the control surface that we would compare our different processing conditions to.

Again, we measure the distribution of cell responses on each of these materials. This red is the distribution of cell response. That is five replicates on top of each other, and the blue is five replicates of the cells on the tissue culture polystyrene.

These would be our end points of our assay, our plus and minus controls, and we can plug them into the Z factor assays, these parameters that we measure, and we get a Z factor of .53, which from the page I showed you before, this means an assay that is going to have a high level of selectivity and specificity.

Then we can -- I am not going to show it here, but then I have taken data from years past, that we have done this for, and we can look at the Z factors of those.

What we can see is that the Z factors usually wind up -- because these are very robust measurements of cell morphology in these cell lines, and the conditions we use are very robust -- and we actually now have a specification of assay quality that we can use as a robustness measure.

One of the things I want to talk about just real quickly before I get off is that we have measured distribution information.

Even though I have only talked about mean so far, there is also -- you can use mean values to look for differences in the plus or minus control or different assay conditions.

You can also use what is called a D statistic, which is a part of the KS statistics test. Basically, what it does is, here are two cell distributions. You see the pink and the blue.

We form what is called a cumulative distribution, which is shown on the other panel. That is a cumulative distribution of the pink and the blue lines. Then we measure a D statistic, which is the maximum difference between those two curves on the vertical direction.

What that can provide us with is some sense -- we might be able to measure changes in cell response distributions that means do not pick up.

Here is an example. Here is the normal situation where we have two mean values that are separated. We can measure a difference in the means between a plus and minus control. We would also measure a D value, giving some reliable Z that we can calculate.

There is a possibility that your mean values don't change, because cell response may change the shape of the distribution and not necessarily the mean value, or the mean value doesn't change very much.

The D statistic will allow you to distinguish the difference between these two distribution where mean value wouldn't.

So, if we look at that Z factor using means, we would get zero, but using a D statistic, we would get a Z factor above zero.

Here is another condition as well. Mean values are zero, but you clearly change the shape of distribution. So,

an alternative method of measuring differences between cell responses can be valuable in many cases. This method takes advantage of the distribution data.

Finally, I just want to finish off with statistics, of course, will be extremely important. They are most powerful for evaluating small changes and determining whether that change is a significant change or due to random chance.

All statistics requires uncertainty values when you are comparing numbers. That means that replicates, of course, are important. I think everybody probably knows that and, as I said, statistical evaluation is going to be important when small differences -- when you want to conclude that small differences that you measure are significant.

In summary, we have dealt with the issue that cells exhibit a distribution of responses. So, by measuring that distribution, we can take advantage of that extra information that we get from the shape of that distribution.

Measurement of the distribution, though, does require that we sample an adequate number of cells, and that number of cells is going to be dependent upon what the shape of that distribution is.

So, we need to know what that response looks like before we can figure out what the minimum number of cells is

that we are going to need be sample.

Internal positive and negative controls can be used with that quality factor, that Z factor I talked about, so we can begin to measure assay robustness, and that can then be used to do day to day or long-term evaluation of robustness.

As I said, alternative methods to measure differences, you can take advantage of that distribution shape information, which can provide information that, for example, means would be less sensitive to.

Again, statistical analysis does need to be included as an important part of making these cell measurements. Thank you.

DR. MULE: Thank you, Dr. Elliott. Questions from the committee?

DR. MARINCOLA: I just have some questions. You really talked a lot about the Z factor which is very interesting. It was a great talk, thank you.

In some ways, I didn't catch what was the advantage of other parametric and non-parametric tests like a D test or why do you focus particularly on that. That is one question.

The other question, I was thinking hard when you were talking about the D factor, about some biological examples that would really be relevant.

I mean, it is going to be interesting. I am sure the distribution is important, so that the mean could be the same, but you have differences. I am trying to think, in what sense -- could you give us some examples of where that would be useful.

DR. ELLIOTT: Absolutely. So, where actually it has really come into being an advantage for us is the GFP expression that I showed is a highly asymmetric distribution, in that there are maybe 30 to 40 percent of the cells appear to not even express GFP, where the rest of them express a very wide, wide distribution of GFP.

When we put those cells onto other materials, that distribution changes, not a lot, but it changes. The mean values are not -- the difference between the mean values are not as -- the difference in the mean values is not -- the range of that difference, the D statistic would provide us a much better metric of showing the shape change than means do.

So, in the case of a highly asymmetric distribution where you have, for example, this peak and this long tail in the edge, alternate metrics other than means provide a good measurement. GFP expression in these clones, in that single cell clone, is a good example, where it is important to do that.

I imagine that there are a number of biological

responses that don't always have a significant -- that the distribution may be a big change and the means may not be changing that much, at least at the initial parts of the process of, for example, some biological response.

So, that is an answer to that second question. The GFP expression is one example. So, that is one thing. The Z factor is a metric that kind of takes into account the dynamic range and the noise of the positive and negative controls.

It is almost similar to signal to noise ratio, but it is taking into account both the positive and negative control and the dynamic range, which are the most important parameters of an assay, when that is a plus or minus control.

So, it is a metric that the pharmaceutical community does use it. For assays, it is a very -- it has a lot of great qualities to it. For example, it gives us a method to, here is your overall assay quality, which is a nice metric to have.

It goes all the way down to just about 100 million, 10^8 parts per ml. So, if we have a therapeutic preparation that is very low concentration, we can use the HPLC method.

As far as infectivity goes, we use what we call a focus-forming unit assay, FFU. Basically, it is an

immunofluorescence assay and it is based upon the expression of an adenoviral DNA binding protein that is expressed very early in the virus infection.

It shows up in the nucleus, and we have monoclonal antibody against that, and you simply infect and stain with a fluorescein conjugated monoclonal and count nuclei.

A positive control is run in each assay. We actually have a reference standard that we use as a control, and we run that every time, and that is how you are sure that assay calibration is not drifting.

DR. MULE: You mentioned in several cases robustness and long-term reproducibility. How would you invoke that concept in, say, a cell-based vaccine where you have a space in time of a few hours to an hour to inject this vaccine. In that scenario, what would you envision being robust and long-term reproducibility.

DR. ELLIOTT: Where are the two points that you are measuring? Are you measuring that there was not change in the -- are these cells loaded with the vaccine that you are talking about?

DR. MULE: The vaccine would actually be a patient cell.

DR. ELLIOTT: So, would you like to measure that the cells didn't change before and after a treatment, before you put it back into the person?

DR. MULE: Again, I think we are talking about a very short period of time. I am just trying to understand what you mean by robustness and long-term reproducibility. Is that taking multiple patient vaccines and looking retrospectively for reproducibility or --

DR. ELLIOTT: In my case, in the case that we are using here, yes, that is what it is. For example, cells have been frozen for a year or longer, and then we pull them out and thaw them and do an experiment with them. Reagents have changed.

So, that is the level of robustness that I was talking about here, and I think that is an important point of all of these assays, that that is particular to the ones that we were doing.

To talk about your particular assay, robustness probably does have a different meaning because it is not a very long process that it is outside, but there are other factors that robustness is probably related to.

DR. MULE: How transportable is this technology to the outside? It seems very technology intense, instrumentation intense. Is that really the case?

DR. ELLIOT: No. Certainly automated microscopy is moving into the laboratories pretty heavily right now. Besides the -- the measurements are relatively straightforward.

I mean, it is microscopy, but just done at a later scale. Imaging analysis tools are NIH -- I mean, they are all open source software.

It is very portable. There are some standards issues. How do I make a measurement on your microscope and my microscope and get the same answer on the same sample.

There are some standards issues there that need to get, really, I think from the microscopy community are going to really need some standards to really do interoperability comparisons or calibrations of instruments. Despite that, I think it is pretty straightforward to make those measurements.

DR. TSIATIS: I have a question on the Z value. You use Z value, in a way, to sort of determine what ranges you might get sensitivity and specificity for. Why not just use sensitivity and specificity as your measures, rather than this Z value which you would have to translate into sensitivity and specificity in their own mind. That is one question.

DR. ELLIOTT: The metrics that we get out of making multiple measurements are, for example, as I showed, a mean and a standard deviation.

Those plug directly into a Z factor equation. Just knowing my pharmaceutical colleagues, that is a directly plus in to the equation.

So, I don't know, formally you may be right, that it may be easier to do that, it might make more sense to do it the other way, but that is why I have done it that way.

DR. TSIATIS: The other question is that your Z value is a function of the mean and standard deviation which are, themselves, variable. Do you have a sense how large samples you have to take from the two groups to even have the Z statistic be reliable?

DR. ELLIOTT: Yes, and that is a great question. Again, because there is a distribution response, the Z factor is also dependent on the number of cells that you look at.

If your distributions are very wide or they are asymmetric, you need a lot more cells than if they are very narrow.

I guess if every cell turns on very bright and turns off in an assay, then you really only need to look at a couple of cells and that would be valid. With wide distributions, you need to look at a lot of samples to really get a reproducible Z value.

DR. CALOS: One thing I wanted to bring up that I think comes out of some of your assays is that a variable that is often important in biology is time.

For example, in the brightness of the GFP, I suspect that the variability is because you have

unsynchronized cells, and they degrade the GFP at a certain point in the cell cycle. If you had a synchronized population, you might get a much lower variability.

DR. ELLIOTT: Actually, yes, I agree that that was a question we asked actually and looked at. It ends up, in synchronized cells, most of the cells are in G-1 phase and you only move about 30 percent over to G-1 when you synchronize them.

Actually, we don't have a measurable change in GFP expression. The GFP expression appears to be a long-term process, and we haven't even characterized all the degradation and all that kind of pathway yet.

The measurement that we are measuring is a real measurement that we control for the synchronization, and not in those experiments that I showed. So, we have looked at that, and there is very little change in that data.

DR. MULE: Thank you, Dr. Elliott. Next up is Dr. Kaslow from Vical.

Agenda Item: Potency Assays for Plasmid-Based Vaccines and Therapies.

DR. KASLOW: Let me thank CTGTAC and OCTGT for inviting Vical to present our thinking on potency assay development for plasmid based vaccines and therapeutics.

Time doesn't really allow a discussion of all the details presented in the slides provided to the committee.

My goal is jus tot hit the highlights and to provoke discussion of assay development.

First, I just want to provide a context and introduce a term, pre-biologics, and then distinguish what we mean by potency and strength, and then go into the details of potency assay development, looking at both protein based potency assays as well as polynucleotide based potency assays.

So, for a plasmid DNA product to work, we rely on the central dogma of molecular biology, which is that we supply the gene sequence to the human host, and it is then the human host that has to transcribe, translate and, based on the cell type it goes to, post-translationally modify the protein.

So, our product is a pre-biologic, rather than strictly a biologic as defined by conventional products. As such, there is a real difference between strength and potency when applied to plasma and DNA products, shown on this next slide.

We use strength to determine how much of the product goes in the vial, and how much of that product gets injected into the humans. We use measures of DNA concentrations, such as AT-60, to determine the strength of the product.

Potency, on the other hand, is used to demonstrate

that we are manufacturing the product consistently, and that is the way that we have applied these assays.

If you go back to that central dogma, we use strength to define the dose of plasmid, and then use potency assays to look at product consistency as measured by downstream biologic effects, that being either mRNA or protein.

We use a variety of tools to do this. So, for strength we use AT60. I want to come to genetic stability and the role of genetic stability in determining the strength and potency of the product.

Then, for potency, we use tools such as RT PCR or protein based assays, such as immunoprecipitation, western blot, ELISA or FACS assays.

So, what are some of the key assumptions that we have made in developing potency assays for pre-biologics, and there are three of them.

First of all, we have focused on in vitro assays rather than in vivo assays for potency, and the reason for this is two-fold.

First of all, in our hand, in vitro assays, the responses in in vitro assays, are less variable and have a greater dynamic range than in in vivo assays, and I will show you data that support that assumption.

Secondly, the immediate biological activity of

pre-biologics is transcription. Therefore, the immediate biological result of our product is mRNA, not protein.

Finally, if a pre-biologic product is genetically stable, if we can demonstrate genetic stability, then there should be no lot to lot variability of the primary nucleotide sequence by definition and, if there is no lot to lot variation in the primary nucleotide sequence, there should be no lot to lot variability of primary, secondary or tertiary protein structure.

Really, the only potential lot to lot variability of a drug substance is its strength and its higher order DNA structure, be it super-coiled, open circular, or linear.

So, with these assumptions in mind, let's take a quick look at how potency assays have evolved, both in thinking -- in this case in the thinking that is used in vaccines -- but also how they evolved during product development.

I think this is a good way of thinking about how potency assays in general have evolved, and I rely on some vaccine regulations.

Here, vaccine potency in conventional vaccines, like live attenuated vaccines, as originally used, was a measure in the laboratory, and it was thought to be the most important characteristic to ensure human efficacy.

That thinking has evolved now and, for at least

vaccine potency, it is thought of as only one of many tools used to ensure that the manufacturing process yields and quality are consistent with the lots that were used to demonstrate efficacy in humans.

There are a variety of tools that are available to use for plasma DNA, and I just list them here, and I won't go through them in detail here, but will show you, on the next slide, how we think about the evolution of using these tools during product development.

So, originally, in preclinical, and even in the translational research part of it, we take plasmids and we look at IT western blot activity for developing, let's say, a cancer vaccine, or look a immunogenicity and efficacy in animals producing something such as a cytokine. We will look at proliferation effects in vitro.

Those are mainly assays that are used in preclinical and early phase I and phase II development. We then turn to more validate-able, more quantifiable, more reliable assays.

In protein-based assays, we used things such as ELISAs and FACS, and for polynucleotide things, such as IC PCR.

Originally, in preclinical and in phase I, where we don't have a good reference standard, necessarily, to look at relative potency, we used single point assays, and I

showed a couple of examples of that.

As the product develops and we have reference standards, we move to percent relative potency assays, and these are multi-point assays. I will go through the development of two of those assays.

So, for protein based potency assays, I want to use a case study, alavectin 7, and show how we used a FACS assay to develop a validated protein based potency assay.

Alavectin 7 is a product that we are developing for the treatment of metastatic melanoma It ia bi-cistronic plasmid. So, there are two proteins encoded on a single plasmid, human HLAB7 and beta 2 macroglobulin. That plasmid is formulated with a cationic lipid, demarine dope.

The key regulatory issues that we faced were to show that we could detect HLAB7 and that we could distinguish that HLA molecule from other HLA molecules that may have been on the cell substrate that we are using in the assay.

We were also asked to demonstrate that beta 2 macroglobulin actually complexed with HLA2B7, which is required to get the molecule to the surface, and to show that the HLAB7 and the beta 2 macroglobulin were the correct size.

So, the development strategy that we used was two-fold. One is to use the FACS assay as a quantitative assay

of that cellular surface expression, and then to use IT and western blot to show identity, particularly that we were making the correct sizes.

So, the evolution of this assay during product development was, we originally started off in preclinical, phase I and phase II studies using a single point FACS assay.

Working with the FDA, particularly with Suzanne Epstein, we developed validated assays for phase III, including an IT western blot and a percent relative potency assay using a dose response. I will go through both of those later assays.

So, for FACS percent relative potency assay qualification, we did an eight dose -- you can see the doses there -- transfection curves for both HLAB7 and beta 2 macroglobulin.

We generated 43 reference curves and did 32 pairwise analyses for HLAB7 and, for beta 2 macroglobulin, we did 21 reference curves of 24 pairwise analysis, and applied standard statistical analysis to the results.

What we found was that, for HLAB7, you can see it has a fairly large dynamic range, and the dose response model fits best with a slope ratio of untransformed data.

Interestingly, for beta 2 macroglobulin, you can see it has a narrower dynamic range, and actually the model

fits best with the log transformed data in a parallel line model.

With this in hand, we then undertook assay validation. These parameters that we have already talked about were used to characterize this FACS assay in validation, and we found that this assay met the pre-specified acceptance criteria for accuracy, precision, range linearity and specificity.

What I am going to show you are some examples of data using these assays. So, shown here are the results of five lots, phase II clinical trial material lot, retention samples that were subjected to this assay.

You can see that, in those five lots, the percent relative potency for HLAB7 was 85.2 percent, plus or minus 4.7, and for beta 2 macroglobulin it was 92.8 percent plus or minus 4.4.

Using the IT western blot to look at the correct size range, showing you an example here of this IT western blot, what we do is, we immunoprecipitate the HLAB7 beta 2 macroglobulin complex, and then separate that complex on SDS polyacrylamide, and then probe western blots either with antibody specific to HLAB7, shown in the left panel, and beta 2 macroglobulin in the right panel. You can see that we can identify specifically proteins recognized by those immunologic reagents.

Shown here are the results of, again, five phase II clinical trial material lots, identifying what the relative mobility was for HLAB7 beta 2, and they match up with the expected values for these two proteins.

So, in summary, with the help of the FDA, we were able to validate both a relative potency FACS assay and an IT western blot.

This assay was successfully validated using phase II clinical trial material retention, and that we intend to use phase III clinical trial material and validation lots to set the commercial specifications for these potency assays.

So, moving on to another type of potency assay, polynucleotide potency assay, first I want to talk about the genetic stability and the important role of genetic stability and then talk about the mRNA as the immediate given result of plasma DNA products, and the application of RT PCR in a case study of a CNB vaccine. I will also show you some data looking at in vitro in vivo correlation between the RT PCR assay and immunogenicity assays in mice.

So, genetic stability, we see genetic stability as a characterization, not a lot release assay, that will be determined once, based on manufacturing cell bank or a manufacturer's working cell bank.

We are using a stepwise approach to establishing genetic stability. Certainly at the IND stage we will have

sequenced plasma within the master cell bank.

We will use restriction fragment size pattern by gel electrophoresis on the drug substance to look at genetic stability.

During clinical development, we are developing some other assays to reduce the risk of doing a complete genetic stability analysis at the validation or commercial filing stage.

At the commercial filing stage, it is our intent to do a complete analysis of the plasma backbone at the end of production, at scale, and to look at a statistically significant number of individual colonies, and to complete sequence the expression cassette, again at the end of production at full scale.

In a sense, what we are doing here is taking a master cell bank, determining the plasmid DNA sequence from that master cell bank, and making sure it matches the predicted.

Then, using that master cell bank and the manufacturing working cell bank, we are producing a fermentation at scale to purify DNA at the end of production, transform and select individual plasmids -- so we are looking at individual plasmids, not a collection of plasmids inside a bacteria -- and then looking and sequencing those individual colonies, and looking at the

observed plasmid DNA sequence, and comparing it back to the predicted sequence.

We have done some statistical calculations, and we believe that looking at 30 individual colonies will be sufficient to establish genetic stability.

Basically, there is about a 95 percent probability of detecting one or more mutations in a sample of 30 independent clones, if the actual mutation prevalence is greater than or equal to 10 percent. We can discuss that, if you want, in the Q and A session.

Now moving on to mRNA and back to this slide, we believe that mRNA is the immediate biological result of injecting plasmid into human subject and, as such, we believe this is an appropriate assay of potency of the product.

We also believe that this is an easier assay to develop. In addition to measuring the immediate biological effect, it is less cell substrate dependent than the protein.

Cell substrate dependency, particularly post-translational modification, may depend on the cell type that you are in.

We also have found that it has a larger dynamic range and lower inherent variability than protein based assays, and that the reagents that you use in this assay

tend to be less variable, more stable, and more readily available than things like antibodies that are required for protein-based assays.

I am not going to go through this slide in detail, only to point out that we have set up this assay so that we only detect messenger RNA.

Essentially, what we have done is to develop a primer that spans the intron, and that primer will only amplify polynucleotide that lacks an intron. So, it has to be messenger RNA rather than the genomic copy that is in the plasmid. We use a tachman PCR to do that.

What I would like to do now is to go through a case study using CMV and to show you in vitro in vivo correlation between the RT PCR assay and immunogenicity in mice.

This product is a bivalent. So, there are two plasmids in it. One plasmid encodes a truncated glycoprotein B, secreted surface protein, and PP65 immutagenized tegument protein, PP65. It is in a similar plasmid backbone.

In this case, it is formulated with a non-ionic block co-polymer, a ploximer CL1005, and a cationic lipid, benzocolonium chloride.

What is shown here is the results of a multi-dose RT PCR assay in which we have made different concentrations,

20 micrograms per ml to five micrograms ml of the product, and then analyzed it in the RT PCR assay.

What you can see is that this RT PCR assay, the observed potency, matches fairly closely what the expected potency is, and you can see the dynamic range of this assay, which is very broad.

The goals, then, were to show how well this in vitro RT PCR assay correlated with immunogenicity. So, we ran some samples, and I will go through how we generated those samples in an RT PCR assay, and then use those same samples in a mouse immunogenicity study, characterizing immune response by antibodies to GB by ELISA, and T cell responses by gamma interferon ELISpot to PP65.

The method that we used to generate the material was that we found that subjecting plasmid DNA to 80 degree temperature for a prolonged period of time degraded that DNA and allowed us to generate material all the way from zero potency to 100 percent potency.

This gen electrophoresis shows what happens to plasmid DNA when subjected to 80 degree temperature over time, from zero to 87 hours. We selected samples at zero, 12, 24, 39 and 87 hours.

You can see where the supercoiled is, and that is the product of interest, and you can see what happens with that supercoiled with time, as it is subjected to high

temperature.

So, in the RT PCR assay, zero hour treatment, it had 100 percent activity, 87 had zero percent potency, and you can see that, at 12 hours it had 55, 24 is 22, and at 39 it had 11 percent potency in the RT PCR assay.

Then that material, 10 micrograms of each one of those time points was then injected into a group of animals, and then we characterized the immune response in those animals.

What we found was that, by GB ELISA, that there was a reasonably good correlation between the relative potency assay and antibody responses.

What was surprising was that the T cell responses were highly variable, they had very low responses and were basically inconclusive, and that to use animals, particularly mice, to look at potency for T cell responses is going to be very, very difficult.

So, our conclusions from this study are that you can use forced degradation of pDNA, and that degradation of DNA does correlate with a drop in relative potency by our in vitro assay, and that we can pick up that drop by looking at antibody responses, but not by ELISpot assay.

So, in summary, in terms of thinking about potency assays, particularly for plasmid DNA, and potentially for gene based vaccines in general, we believe that, if you can

demonstrate genetic stability and that you can then you total size restriction enzyme digest and HPLC analysis to look at the higher order structure of the DNA.

Really, the only thing that you need for potency is the RT PCR relative potency assay, again in the context of genetic stability.

I want to point out that a large number of people have contributed to the work here. This takes a tremendous amount of work to develop these assays, qualify these assays and validate these assays, and I specifically want to recognize Lon Rolon, Keith Hall, Chris Carner, Beth Furr, and Rojay Majanian(?), in the work that I presented today, and I think I will end there.

DR. MULE: Thank you, Dr. Kaslow. Questions from the committee?

DR. CALOS: You kind of alluded to the different forms of plasmid DNA, the supercoiled, linked and linear. Do you find a different biological result with those, and do you just have a standard of a certain percent super coil that is your reference or something like that?

DR. KASLOW: We have not seen a tremendous amount of difference between the biological activity of different percentages of supercoiled.

The agency has provided some guidances on the percent supercoiled that they would like to see, and I would

actually probably ask them to try to respond to that.

There has been an expectation for the amount of percent supercoiled in the product, but that has really been driven by the agency.

DR. HARLAN: I wonder if you would expand a bit on the problem with the T cell assays. You said that they didn't correlate with your potency assay. Was it that even the potent stuff didn't generate a T cell response or vice versa?

DR. KASLOW: Great question. I think the difficulty is multi-fold. First of all, the dynamic range, the linearity in animals, is very, very narrow. So, we can see -- it is almost a binary type response. Either they make a T cell response or they don't.

We certainly have, at high doses, 100 percent of animals making T cell responses. The problem is trying to identify the dose that puts you in the dynamic range, that 50 percent of the animals give a response. That is extremely difficult to do, even in inbred mice.

Couple that with the inherent variability of the ELISpot assay itself, and this becomes a real intractable problem.

DR. MULE: For the alavectin 7, you mentioned the use of phase III trial material, I guess to lock in your potency or release criteria. Maybe you can expand on that.

DR. KASLOW: The idea is that at some point you have to run a statistically reasonable or appropriate number of lots to be able to set commercial specifications.

I don't think you want to set specifications on running the same lot over and over and over again. That is very different than running separate lots.

So, until we have run a sufficient number of lots using the commercial scale process, I think it is ill advised to set strict commercial specification.

Certainly, what we will do is, as we get more experience producing at commercial scale and have data, we will set specifications that are appropriate to show that we are making the material consistently. Until we have a sufficient number of lots made, it is hard to do that.

DR. TOMFORD: Did I understand from your talk that you don't feel you need to look at translation, you can stop at transcription?

DR. KASLOW: That is our thought.

DR. TOMFORD: Why is that?

DR. KASLOW: Because the immediate biological activity of the product is mRNA. It is not protein. To use cell substrates -- so, for example, we are using a mouse cell line to do our transfection experiments and using in vitro assays.

How the protein is made, how it is folded, how it

is modified in a mouse in vitro cell line can be very different than how that mRNA is transcribed and translated in an antigen presenting cell, a human antigen presenting cell.

So, to try to draw those conclusions, I think, is ill advised. What is know is that the gene sequence that we are providing is supposed to make an mRNA transfer, and different cells may be transfected by this, it may be the protein may be folded differently. I just think that it makes more sense to look at the immediate biological product, which is mRNA.

DR. CALOS: But that isn't actually the sort of biologically relevant thing. The biologically relevant thing is the protein in the tissue of interest.

You know, your particular assay, you know, you never mentioned in your presentation which cell line you were using, which cells at all, but that makes a big difference.

So, you could pick a cell where you get both of your proteins made, and that might not be the actual result in the animal. That would be the worry.

DR. KASLOW: The most important effect we have with our product is efficacy in humans, and we will use phase II for proof of concept and phase III for proof of efficacy.

Once we have established that, the most important thing to do is to make that material consistently and reproducibly, so we will be assured that we will get the same effect once you go into humans.

We need to establish that this product works in humans. That is a given. The next step, then, and the way to use the potency assay is not to continually prove that it is going to have efficacy in humans, but that you can reproducibly and consistently make the material that was shown to have efficacy in humans. I think that is the concept.

DR. PLANT: I have a question about when you do a FACS analysis looking for beta 2M production and HLA expression.

Is there any caveat associated with how you threshold what you are going to consider a positive or a negative response?

DR. KASLOW: Yes, there is a bunch of work that went into it. There was a lot of detailed analysis that went into setting up that assay, and it is quite important.

DR. PLANT: So, there is a statistical -- not that you are going to go into that now, but there is a statistical trick to doing -- to deciding how you did that?

DR. KASLOW: Yes, the gating and all of that, that was quite a bit of work.

DR. COUTURE: But the reality is that different cell lines -- I think cell lines are very important because different cell lines are more or less transfectable and, depending on the cell line you use in culture, you may mask a lot of difference that will actually manifest themselves in a clinical trial.

I suppose phase III studies would allow you to make the correlation, but you still need something to correlate to.

If it transfects as well in a 3T3 cell line, that may not give you any information in a clinical trial when you are seeing variable results in patients.

DR. KASLOW: Again, I think the concept here is, will that transfection of the cell line pick up subtle differences between lots that are important in efficacy.

If it does that, I don't think it makes a lot of difference if the cell line you are using has higher or lower efficacy than the transfectability in humans, as long as it can pick up the differences lot to lot.

DR. MULE: Thanks, Dr. Kaslow. Before we take a break, we are going to have one more speaker, and that is Dr. Butman from GenVec.

Agenda Item: Potency Assays for Adenovector-Based Therapies.

DR. BUTMAN: Good morning. What I would like to do

is take you through a discussion of how GenVec has approached potency assays.

Our platform is an adenovirus. Just as an overview, I just want to take a minute and provide context for the type of product that we are dealing with.

I think that is important to understand how we think about potency, and you have to think about the technology it is based on.

Then I will walk you through the matrix that we use for assessing potency. Then I am going to talk through some of the challenges that we have faced and tried to find solutions for to control variability of adenovector potency assays, what our thinking is, and our progress is, on validation of these assays, and then a summary.

So, our product platform is an adenovirus vector. Our concept is actually the drug is made in the patient. The gene is expressed, the transgene is expressed.

We are able to do this because we have made deletions in the adenovirus early genes to assure that we don't get replication of the virus in the patients.

We have made complete deletions in E1 and E4 regions, and a partial deletion in the E3 region, and that gives space to insert a transgene, and it also assures absence of viral replication.

Well, if you don't have viral replication, you

have to have a cell line to make it in. So, we have taken 293 cells and we have added in the open reading frame of E4 over reading frame 6, and we have created a cell line that complements for the missing viral sequences in the adenovector, that provides both E1 and E4 complementation.

This is just a picture of that. You can see that the E1 gene has been deleted and it has been replaced by an expression cassette driven by a motor.

E3 is partially deleted and E4 has been completely deleted, and we put a transcriptionally inert spacer where it lived.

The complementing cell line, 293 or F6, as we call it, results in no RCA generated in any of what we call a GenVec 11, GV-11 vector production, no E4 transforming gene expression.

It is a very stable, well characterized cell line, and it is non-oncogenic. We have completed our oncogenicity studies.

We are looking at both therapeutic and vaccine applications. Our lead product is called TNFerade, and it is being explored clinically in pancreative cancer, in rectal cancer, and in metastatic melanoma.

By the way, that former, the oncology product, uses TNF -- tumor necrosis factor -- alpha, as the transfene.

For severe coronary artery disease, we are exploring clinically the use of VEG-F 121 gene in our so-called biobypass platform, and we are looking at anti-angiogenesis in age related macular degeneration, where we have inserted the transgene for pigment epithelium derived factor.

We also are working with several government agencies to develop vaccine applications. We are working with the NIH to explore the development of a worldwide HIV vaccine in collaboration with NIAID.

We are also working with the U.S. navy to attempt to develop a worldwide malaria vaccine, and we are also working with MVI for other aspects of that malaria vaccine, and we are working with the U.S. Department of Agriculture to try to have a prevention of FMD as an economic threat.

The way that we think about potency is that we think of it as a matrix. IT is kind of like what the former speaker was alluding to, strength versus potency, but we take it all the way through to a biological effect.

So, any demonstration of potency has to start with an accurate quantitation of the virus particle concentration. So, that is the first question we asked.

The next question we asked is, can that vector infect cells. So, we measure viral infectivity. Then we actually look at the ratio of virus concentration to virus

infectivity, and the FDA has established a specification that that has to be less than 30.

Well, that is great, the virus has made it in. It is infected, and we detected a viral protein in the process, but the transgene is the payload.

So, we look for transgene expression. We have a quantitative method for that. That would be great to know that, but also we want to assure that it is biologically active. So, we have been working on bioactivity assays for that expressed therapeutic protein from the transgene.

I am just going to walk you through some slides to give you some specifics on how we conduct these assays. So, the viral particle concentration is very simple. It is based on absorption of UV light at 260.

We lyse the vector to liberate the DNA with detergent. There is a published extinction coefficient. We are able to then calculate the virus particle concentration.

In the adenovirus world, several years ago, there was a collaborative effort to develop a reference material. So, we are very fortunate to have that.

When we run that adenovirus reference material in our particle assay, we get very good correlation with the value that has been reported, by a very similar method.

This is really suitable for bulk drug, and it is also suitable for any therapeutic formulation, that we have

at least two times 10^{11} particle units per milliliter. IF you get lower than that, you get into uncertainty and you are kind of reaching the limitation of quantitation of the assay.

We then have an HPLC assay that uses a calibrator whose value was assigned by that particle method. So, you just continue that calibration all the way down to just about 100 million, around 10^{8} particle units per ml. So, if we have a therapeutic preparation that is very low concentration, we can use the HPLC method.

As far as infectivity goes, we use what we call a focus forming unit assay, FFU. Basically, it is an immunofluorescence assay and it is based upon the expression of an adenoviral DNA binding protein that is expressed very early in the virus expression, and it shows up in the nucleus.

We have monoclonal antibody against that, and you simply infect and stain with a fluorescein conjugated monoclonal and count nuclei.

A positive control is run in each assay. The range of attribution is sufficiently tight that you feel that you can get a pretty good prediction.

In order to do that, then the obvious experiment that you have to do is measure both the analytic assay and bioassay on the same product with a sufficiently large

sample size over a reasonable range of experimental conditions, in order to be able to assess the predictive value.

You run that every time and that is how you are sure that assay calibration is not drifting, because we have got a range.

If the assay does not fall into that range for the reference standard or the control, we know we don't have a valid assay.

So, that anchors the calibration drift. So, we know when we are counting in the test article, that we can believe the result.

The FFU assay correlates very well with the infectious unit titer that has been reported for the adenovirus reference material. As I said, it really enables us to express infectivity in terms of particle to FFU ratio.

Now, you might think, well, the agency has said that you can have a PU to FFU or a PU to infectivity ratio of 30. That must mean that you could tolerate as low as three percent infectivity in your preparation.

What you are running into there are the limits of an assay. Imagine this room is a culture well and we are the cells.

In suspension, all the way up to the ceiling, are viral particles diffusing around. Only a certain percentage

of those are actually going to be able to be assayed by us cells, coming into contact with us.

So, it is really not a true -- the ratio is not a true reflection of the infective titer, but it is a way of being able to express infectivity and show comparability, and we are certainly able to move that specification.

Next we say, great, the virus infected the cells. How about the transgene. So, depending upon the drug, we have target cells for TNFerade. We use A549 cells, and that is also what we use for our ad PEDF.

For our VEG-F 121 product, it was hard to find a cell line that did not have some level of expression of VEG-F by itself, and we found a cell line called RAT-2 cells.

We have a fixed ratio of the number of particles per cell in the assay. So, you know how many cells you started with. You know how many particles you put into that system.

Then the transgene -- the infection process occurs, and the transgene is expressed. Then we simply quantify the transgene protein concentration with an immunometric method for TNFerade.

There is a commercial TNF alpha ELISA that is available. Likewise, for the VEG-F 121 product, biobypass, there is a commercial VEG-F 121 -- I am sorry, it is the whole molecule -- yes -- of VEG-F.

There was not a system available for PEDF, because this is an emerging molecule. So, we developed a monoclonal antibody based immunometric assay in house.

One of the things we realized is, we really need to express the value, not in terms of picogram per ml or femptogram per ml or nanogram per ml, but to back calculate it down to the cell.

From well to well, from plate to plate, there is going to be variability in that cell number. So, we actually then calculate it in terms of femptograms per cell, or picograms per cell, depending on the promoter, and how productive that expression is.

We have a reference standard that we have created that is run in parallel every time. That is the ultimate comparator. That is how we control for inter-assay drift.

This was really a suggestion of the FDA, to look at the ratio of test article to reference standard when trying to assess.

This table -- I hope you can all see it -- in the first column, it shows the vector lot number. Actually, as you work your way down, the fourth one is a typo, it should be 0003, and the next one should be 0004. So, there are four lots there.

You can see that the particle units per ml are all

-- that depends on the manufacturing process and what concentration you want to freeze it down, but they are all around four to six times 10^{11} particle units per ml.

Then we have the FFU concentration and then the ratio, the PU to FFU, and you can see that it can vary from six to 12 or 13. It is well below the FDA specification.

So, you might think, wow, that is a lot of variability in your product. Then, when you look at the transgene expression, we know that cell based systems, there is variability, and I am going to be discussing how we control that variability.

You can see in, for instance, the TNF ELISA, you can see that we get concentrations -- well, we get expressions in the range of maybe one to two femtograms per cell all the way up to 10 femptograms per cell.

That is not due to an inherent difference in the lots, but rather assay to assay variability. By running a test article every time, you can prove that -- by running a reference standard every time, you can prove that.

So, we look at the ratio of test article to reference standard, and you see it is much tighter. That is how we control it. That is how we are moving toward a specification for transgene expression.

Also, in our PEDF system, these are stability data going out 48 months, and you can see that it is tight, plus

or minus 20 percent, over four years, as long as we are expressing it in terms of test article to reference standard.

If we showed the actual just concentration or the femtograms per cell, there would be a lot more bounce, but by using a reference standard as a comparator, it tightens it up and gives us assurance of reproducibility and comparability from lot to lot as the product ages.

Bioactivity. So, for TNFerade, we are looking at a cell killing assay because that is what tumor necrosis factor, that is one of its properties.

We looked at different cell lines and we found one cell line that is exquisitely sensitive to TNF. It is called EHI-13 variant.

We run this in a 96 well format. Samples and reference standards are run in triplicate. We actually generate a standard curve for each.

So, going back to the transgene expression assay, we know what the concentration is in that supernatant. So, we generate a standard curve for both the test article and the reference standard in the range of 100 to 1,200 femptogram per mil. That covers the full dynamic range of the dose response. Then we use a metabolic substrate as an indicator.

So, actively metabolizing cells take up the

substrate, and they enzymatically generate a formalin dye, which can then be read with a spectrophotometer. So, the absence of signal means cytotoxicity. It means TNFerade bioactivity.

Then we use a four parameter curve fit to apply, and we use the data from that four parameter fit to establish specifications for the maximum background and minimum signal and the slope and the delta between the top and bottom of the curve.

We use the midpoint on the standard curves, the so-called C value from the equation, to compare the test article to the reference standard.

We are trying to establish the acceptance criteria ultimately for the commercial product, but right now we are saying that the test article must be within 25 percent in terms of TNF concentration. The bioactivity must be within 25 percent of the reference standard. I will show you what those data look like.

So, these are two curves. So, you start with no killing at the top and, as you increase on the X axis the concentration of TNF, you see that the production of the formalin dye goes down, and you get a nice sigmoidal curve. This is what you want to see in these kinds of assays, and you can see that you have got a full titration of the reference standard compared to the test article, and we used

the mid-point, that C value, to show comparability between the reference standard and the test article.

We have specifications for the A value, a minimum signal for the A value, actually a minimum and a maximum signal for the A value. We also have a specification for the D value, which is completely lysis.

We have a specification for the slope, B, and we have a specification for the correlation coefficient on that four parameter fit.

If those specifications are met, now you have a valid assay, now you can look at the mid-range, the C value, and compare the reference standard to the test article.

For biobypass, this one has been tough. We have been using a cell migration assay and it uses the chemoattractant properties of functional VEG-F 121.

The target cells are a primary culture that you can purchase, human umbilical vein endothelial cells. We have looked at other cell systems, but those give us the best dose response.

So, you have one of these two chamber culture systems. In between the two chambers, the upper and lower, there is a membrane with pores in it that the cells can migrate through.

So, the cells are plated into the upper chamber of our trans-well plate, and recombinant human VEG-F 121 is our

positive control, or the expressed supernatant from the transgene expression assay for either the reference standard or the test article at a given concentration. Kind of a mid-point in the dose response are placed in the lower wells, and we typically use five nanograms per milliliter.

We also run an ad null vector, which is the same vector except there is no transgene, in case there are any cell effects from a virus creating cytokines. So, that is run. The test article and the reference standard are assays. I already went over that.

So, the cells migrate toward the VEG-F 121. They go through the membrane pores, they come up through the other side, and then we stain them with calcian AM florescent dye.

Then we simply visualize the underside of the membrane with an inverted fluorescence microscope, and we take a digital photograph of a low power field, and then we do computer based image analysis and we count pixels.

There you can see a dose response, the replicates of four with a control, very little cell migration, and we are already picking up cell migration at a tenth of a nanogram per milliliter.

It maxes out at 10 nanograms per milliliter. So, it is about a two log dose response, and actually a little hook effect, harkening back to my diagnostics days, even if

you go up to 100 nanograms.

So, you can see you get a nice dose response. So, if you simply now pick the mid-point in that dose response, you can compare test article to reference standard, and that is what we do.

Now, I would like to transition to the challenges and how we face them. The challenge is that adenovector potency assays are subject to significant inter and intra assay variability.

Number one, we have a very complex analite that we are trying to measure here. There are more than 12 distinct proteins in the virus, 32 KB of DNA.

It has all got to be packaged right. It has to have the fiber knob for binding the cells in place. The whole system, in order to show potency, requires cell infection, transgene expression, and proper biologic function. You can see all along the way you have got opportunities for variability.

Of course, there is going to be an inherent variability in cell based assays. So, just as you are passaging your QC cell bank, you might see changes over time. So, you have got to think about that variable.

The metabolic state of the cells is probably the greatest variable in these systems. You can't just say, well, when they are 70 percent confluent, let's use them.

You have got to think about what their parents were doing two days ago and what their metabolic state was. You need to think about the age of the culture after you set it up.

You need to think about the degree of confluency, and all of these, we have started learning, are important to control and to have specifications for if we are going to have a validate-able assay.

Then there is, of course, the reagent and the culture system, your collagen from lot to lot, your medium, your culture vessel. All of these things can be variable from suppliers, and you need to be able to qualify those.

Then there is the famous edge effect in a micro-well assay, due to evaporation or pH control. Back in my monoclonal days, we really worried about that. So, we just said, let's not put any cells in the outer wells, and that is really what we decided to do in the 96-well format.

So, to address the challenges, first of all, we have established QC cell banks for all of our assays. You can't really do that so well for the huvec, since it is a primary culture, but we qualified it. We pretest and make sure we have got a good responsive lot.

Then we have established criteria for passaging of the QC cell line. We don't let them languish. We keep them actively growing, actively metabolizing, but we also control

the maximum passage number.

Then, separate from the assay SOP, we have detailed full maintenance and passaging SOPs, so that we are controlling that aspect. We are trying to tighten that down.

Then we have detailed assay SOPs, with clearly defined system suitability and assay acceptance criteria. We have got to have a suitable system, or there is no sense looking at the data. So, that is the first question we asked.

Then, most important, we have product specific reference standards. So, we generate a lot of vector by the same process that our test article is going to be manufactured.

We know that our formulation is extremely stable. You can take an adenovirus, put it in our formulation, put it in a freezer for four years, and you are just not going to see movement. That is really helpful, because then you don't have to qualify a reference standard every six months.

So, we thoroughly characterized them, and then we monitor the stability of that reference standard, and we trend the data that it generates during the system suitability.

That provides a comparator to compare for assay drift. Then we even use that reference standard in our FFU

assay as a control with a specification to anchor calibration, because there are people looking in a microscope and counting nuclei, and you can imagine how much variability there could be there. So, we used the reference standard as a control there.

Also, on the quality system side, it is equally important, having qualified QC equipment is extremely important, having well trained QC analysts who have to meet certain specifications in their training assays. The consistency in the assay raw materials is one way to control variability.

We begin the process of assay validation, we kind of evaluate and get kind of a practice on this validation early. We do that during phase II.

You think you know your assays and you try to validate them and transfer them to another lab, and you are amazed at what you find out, the achilles heels on these assays, and they have been run by the in-house expert, and now we are asking the quality control laboratory to run them.

So, we learned that several years ago with our biobypass program in trying to transfer them to Europe and to validate them, and all kinds of things from that.

So, we have really learned from that experience and we said, let's validate in phase II because the last

thing I want to do is to find out that the assay has a fatal flaw and I am doing my formal validation ready for licensure.

Also, for transgene expression, we have learned to segregate the validation of cell infection -- that is one variable -- from the immunometric ELISA. That is another variable.

So, we nail down the ELISA first, and make sure we have got that under control, and then we are able to start looking at optimizing the cell side of it.

I will just say a few things about our assay qualification validation parameters. In terms of assay qualification, you have got to have a standard operating procedure, and you have got to have system suitability requirements, and that comes out during the assay development.

So, we make an initial assessment of sensitivity, precision, reproducibility, and specificity. Then we start thinking about validation parameters.

Well, in order to do that, we have to run it under QA authority with a protocol and a report with specifications determined ahead of time, for all the parameters which you find in the guidance documents and in the law.

We have well established acceptance criteria

before we start the validation. We figure, let's start that in phase II. We are going to have to repeat it as we move toward licensure, but it gives us a lot of experience in the behavior of our assays.

So, during phase I we develop our potency assays and we establish the qualification parameters. We establish an SOP before we do our first lot release testing, and we add what we understand to be the best system suitability requirements.

During phase II we actually begin our assay qualification and conduct the initial validation evaluation.

During phase III -- well, we are not in phase III yet, but what we will be doing, if everything continues to go well, we will then do our formal validation.

Then, you are not done. This is a product lifetime endeavor. We are even now evaluating assay performance. We trend our data. We ask, how many times do we get an out of specification result.

That tells you a lot about your assay, and we actually don't want to clog up our system with OOSs. It is not helpful.

Then we also, as we establish our commercial quality control laboratory, we might learn some new things along the way about assay performance. So, we will constantly be assessing assay performance and ask, is it

time to revalidate.

There is a lot more information here than you need, just to say that the spec PU, HPLC PU assays for particle concentration, infectivity, those have all been actually validated in kind of our phase II cycle.

The TNF expression assay, we validated the ELISA side and now we are nailing down the cell side. The bioactivity assay is qualified, and that is what we are going to do this year. We really do not want to wait. We want to validate that assay this year.

VEG-F expression was qualified at the contract laboratory and we are going to be doing validation at GenVec as well as the bioactivity assay.

We may need to change the format of the VEG-F bioactivity assay. We just don't want that to be a problem, and there are other systems available. So, we are taking a look at that right now.

PEDF expression is qualified, and we are going to be doing validation as that product moves clinically, and we are looking at PEDF bioactivity assay. It is pretty tough. It is an anti-angiogenic.

So, in summary, I hope that you can see that we have a comprehensive potency assay matrix. Controlling assay variability is essential to success.

Assay development, qualification and validation

program is in place, and it is designed to keep pace with drug development. Don't wait.

These efforts are conducted in concert with our FDA product reviewers, who have been extremely helpful and provided great guidance.

In summary, our therapeutic adenovector products have a well established, but maturing, program for measurement of potency. Thank you. I will take questions.

DR. MULE: Thank you, Dr. Butman. Questions from the committee?

DR. PLANT: I have a couple of questions. It is a very impressive program. It would be nice if you could elaborate, maybe by example, one or more of your reference standards. I would really like to hear exactly what constitutes a reference standard for you.

Then the other question I have is, clearly there are so many variables that need to be controlled. One thing that you mentioned, for example, is having humans count nuclei.

So, I am wondering to what extent you can envision improving that QA QC process by automating, say, your microscopic analysis and having more digital information handling, as opposed to relying on human.

DR. BUTMAN: As far as reference standards go, we actually -- the preference, and what we try to do, is not

even prepare those in the process development laboratory, but actually when we are establishing GNP manufacture, we do that at a GNP manufacturing site. So, it is really representative of what we are going to be testing.

Then we do the same degree of characterization, the complete profile -- purity, potency, impurities. We look at all of those, and we actually establish a certificate of analysis, and we do a kind of lot release on that reference standard.

Then that is vialed up and stuck in a freezer at minus 70 and we track its stability. So, we have a formal stability SOP to monitor that reference standard, and that is used every time.

A small aliquot is brought out, so you are avoiding freeze thaw variability, and it is treated as a very important reagent in the process.

DR. PLANT: Let me ask a clarification question. Is that then sort of a clone -- excuse the expression, but sort of a replicate of the product itself?

DR. BUTMAN: Yes. So, our product, you start with a working cell bank and a working virus bank. You combine them in a bioreactor, and then you purify the virus that is produced.

So, we actually manufacture the virus with the same transgene, exactly the same as we are going to in our

manufacturing process, because we want it to represent the process.

DR. PLANT: Then doesn't your reference standard, isn't it subject to the same variation and irreproducibility and non-robustness of your product itself?

DR. BUTMAN: Yes, you have got a chicken and the egg argument. Where do you start. When we are phase I, we actually use a research lot, because we have got to start somewhere.

Then, as we move through phase I, we use a phase I lot as we move into phase II. So, yes, there is variability, but one thing that doesn't vary is the potency.

So, it is staying frozen, and we can show that its PU to FFU ratio, we track that, and there is no ultimate standard.

We have the ARM, but it is a wild-type adenovirus. It doesn't have a transgene. So, there is no international standard for our product. So, we have to create one and monitor it and control it as well as we can.

So, really, the reference standard is used as a comparator, so that all the other variability can be controlled for.

Now, with regard to the FFU assay and counting nuclei, yes, there are other ways of doing it. You can certainly use FACS analysis, you can automate fluorescence

microscopy.

We certainly have thought about that. So, you have to work very hard on your training side to make sure that people have the same standard for recognizing and counting fluorescent nuclei.

We are able to show the PU to FFU ratio of a given lot is quite consistent within bounce from lot to lot, but yes, certainly automation, taking out the human variable is a good consideration.

DR. SNYDER: Bryan, I have a quick question for you. You showed a dose response for your cell migration assay, and actually the example before you showed a dose response, and both of those were based on nanogram per ml of protein. Why aren't those tied back to MOI, your actual product?

DR. BUTMAN: The expressed protein is based on a very defined particle to cell multiplicity of infection, if you will.

So, when we express it, we et that supernatant containing the transgene from an assay in which the ratio of particles to cells is fixed, 500 or 1,000. That is true for the test article, that is true for the reference standard.

So, once you measure the concentration, you now have, let's say, five nanograms per ml for the test article, and maybe four nanograms per ml for the reference standard.

We normalize those because what we want to know is, pound for pound, is the biological activity the same. We have already looked at is the quantitative expression controlled and comparable to the reference standard.

Now we want to know, is that biological molecule, based on mass, does it have the same biological activity. So, it is both sides of the equation.

DR. SNYDER: But like in the dose response curve for the cell migration assay, you were titrating nanograms per ml of your protein in that case.

Why wasn't that dose response tied to actual -- to the MOI. I know I am being redundant, but should that have been at MOI of one you get X number of nanograms and you get this response, an MOI of 10 more nanograms, more response, that relationship?

DR. BUTMAN: I understand your question. I guess the way we tie it back is the link between transgene expression and bioactivity. So, I think what you are suggesting is, can't you just take your supernatant and apply it in your assay.

We want it to be on the slippery slope of the titration curve, so that we know that it is a reliable result.

If we don't know what the concentration is, then we are not going to be able to do that. That is why we

quantitate first, make sure that is comparable and controlled. Then we ask, is the biological activity comparable.

In a sense it is linked to it, in an indirect fashion, because it is a two step process in demonstrating potency.

DR. COUTURE: On the HUVEC experiment, we just heard a presentation that suggested that gene expression was all one should measure for potency, and you have gone beyond that to protein expression, and then you have gone beyond that to activity of VEG-F and the HUVEC cell migration assay.

I would like you to comment on that. The question, I suppose, would be have you has an assay in which you had detectable VEG-F and not correlate to HUVEC cell migration and, if you were to see that, would that be an assay failure or a failure of the product?

DR. BUTMAN: We haven't seen great variability. We haven't seen a lot of VEG-F that was adenovector producing VEG-F, where that VEG-F then was different from the reference standard.

I mean, it has just been made. We try to control a lot of the variables. So, actually we would be very surprised if we ended up having to reject a lot because the expressed protein level was comparable to a reference

standard but the bioactivity was not.

We haven't seen this, and I have been in this industry long enough to know that we could see it, but that hasn't been a variable.

I still think, in our discussions with the agency, this whole two-step process is very important and, frankly, we need more experience in producing lots and tracking them and showing lot to lot comparability, to really know how important that is, and whether we might be able to someday say, you know what, we have done 10 lots, every time we make it, the level of expression is comparable and the bioactivity is comparable.

I think we need a lot more -- as we validate our process and move toward commercialization, I think we need a lot more experience before we can make that assessment.

DR. MARINCOLA: You gave us a wonderful example and introduced an important concept of reference standard, which I do believe is really the key to assess potency in biologics where things can change with time.

Of course, your application in your case is very straightforward. You are looking at one protein at a time. I would like to see if you can give advice, since you have so much experience with the committee, about how would you use the same concept for cell products, things that were looking one gene at a time.

Maybe it could be very useful. You could use high throughput systems, for example, to see deactivated dendritic cells, or could you give us some thoughts on expanding that?

DR. BUTMAN: We have had to think about that for our vaccines. Ultimately, it is immunogenicity, which that is the ultimate biological activity.

I think it is a great question. I guess I don't have a good answer for you today, and we have got people at GenVec who think about this, too.

I am happy to follow up and give you a better answer than I am going to be able to do here, because my whole focus is the adenovirus.

I understand where you are going with it, because you are looking at the full context of what the agency is dealing with and you are trying to find continuity.

Ultimately, I think you do need a system where you can be sure that, when that product is administered, what you can expect in its biological activity. I think that is very important.

DR. HARLAN: I want to follow up on Dr. Snyder's question, and then expand on it a little bit. As far as the FDA is concerned, you have reported the amount of your gene product produced by the vector.

When you come to delivering the actual therapeutic

product, maybe you would define potency by how much that particular lot produced protein-wise, or I suspect you would have to base it on MOI, since MOI has an effect on how much of the product you can give to people. At least that is my understanding of adenoviral vectors.

Then the other little angles are that you have reported the potency assays and cell assays, but my understanding -- I am probably exposing my ignorance, which I do frequently -- is that adenoviral vectors in vivo tend to go predominantly to the liver. So, I wonder how you would target these things to the areas where you want them to go and avoid the immune response that occurs.

DR. .BUTMAN: We use a needle. We inject the -- I think it is a great question. Obviously, the whole field has been impacted by your statement.

First of all, we set our dose based on particle units. That is how we know. So, in our dose escalation studies in the clinic we start very low, and we work our way up until we get a maximum tolerated dose, and we have been able to see that in our programs.

In the TNFerade program, we inject directly into the tumor. For instance, in pancreatic cancer, with a percutaneous needle, or there is the concept of using a scope to deliver and inject.

In our VEG-F product, we use a catheter and push

it up just the same way that you are going to image ischemia in the heart, and we use an injection needle and put it right into the heart muscle.

The same thing for the PEDF product. We inject it directly into the eye. So, by using it locally, we avoid the systemic toxicity, and it is contained within the site of injection.

DR. HARLAN: Just following up, if you had shown MOI as opposed to protein, would you show the same dose response curves? How much variability did you remove by showing the gene product as opposed to MOI?

DR. BUTMAN: I am trying to think of how best -- it is a good question. I understand the basis. So, ultimately -- the only system that I can think of that comes closest to answer your question is where we use a transgene in an experimental system that is an enzyme.

So, we take alkaline phosphatase, and in order to develop our assays and understand whether our stability is good and to really understand our product, we used a model system.

The transgene was alkaline phosphatase. You can actually infect cells with a known MOI and simply put a colometric or luminescent substrate in the well, the same well, and you can measure biological activity of an expressed transgene protein in the same well.

Obviously, that is a model system but it is very instructive because it helps us know whether the assays in which we are not able to do that, whether we are missing a lot of variability.

We see that -- it follows, for instance, when you do a forced degradation or when you do a stability study, or when you are asking questions like how many times can we freeze thaw these products, you get the same profile. You get the same titration and dose response in a direct cellular system where you can do that. So, that is how we build that link.

DR. CHAMBERLAIN: I am curious how you monitor the genetic stability of your antiviral background.

DR. BUTMAN: When we create a new product, we do full length sequencing of the entire genome in our master virus bank.

Then we do run a PCR assay for every lot, where we are looking at key areas of the genome that we can monitor. We actually use it more for an identity assay.

We start off with sequencing, full length sequencing of the master virus bank. We have also done sequencing all the way back to when it was first created in research, multiple passages.

So, we know that the product is stable if you just compare what was the expected sequence back when it was a

plasmid, before you even had vector, and how you have expended and researched through vector seed stock, you have moved it over into development. You have now made a master virus bank. Is it the same.

So, you have got this historical context to say, actually, it is the same sequence as the original plasmid. That is how we know that, once we sequence it, it is representing the genetic sequence of the virus.

DR. SIMEK: I just want to throw this out based on everything that has been said. One problem with MOI that maybe we need to take into consideration, there are a lot of variables with MOI.

MOI depends on the volume that is being tested, the container the cells are in, the cells, the cell number. So, in itself, it is not the answer to everything. There are a lot of ingrained variabilities with MOI.

DR. BUTMAN: But when you go to give something to a patient, you are going to have to -- you can't know what the MOI is going to be in that patient; right?

DR. SIMEK: We administer based on virus particles. So, you know the infectious titer, but we have to base it on total virus particles.

DR. MULE: Some of these issues we will revisit in the afternoon session. What I would like to do is take a 15-minute break and have everyone back by 20 to 11:00, and we

will continue this topic.

[Brief recess.]

DR. MULE: Our next speaker is Alla Danilkovitch, who will give a presentation on potency assay development for a novel cell therapy product, prochymal adult mesenchymal stem cells.

Agenda Item: Potency Assay Development for a Novel Cell Therapy Product: Prochymal Adult Mesenchymal Stem Cells.

DR. DANILKOVITCH: Thank you very much for the invitation. I am very pleased to be here and show you our experience in development of a potency assay for cellular therapies.

I represent Osiris Therapeutics, a company developing cellular therapies based on the use of adult human mesenchymal stem cells derived from bone marrow.

Today I wanted to present potency assay for a particular product we have at our company now, this product called prochymal.

Prochymal is adult mesenchymal stem cells for treatment of graft versus host disease. At the present time, we have two ongoing clinical trials phase II and, based on the preliminary data in phase II, we started to design phase III.

So, we expect to have this product on the market

at the end of 2007 or beginning of 2008. Of course, we need to have potency assays to qualify each lot of our product.

Let me tell you, in the beginning, what is our product. Prochymal is human adult mesenchymal stem cells derived from bone marrow aspirated from healthy donor volunteers.

Because the frequency of mesenchymal stem cells, or MSC, is very low, you have to expand the cells in culture.

So, we isolate the cells and expand them in culture up to passage five. Passage five culture of human MSCs represent our product.

Then we freeze down the product, and here is how the product looks like. It is a plastic bag containing 100 million cells in 15 mls of plasma lipe 8 with human serum albumen and cryopreservative DSMO.

It is a homogeneous population based on several characteristics, and the storage at the present time is in liquid nitrogen, and we have stability data for the product, that you can store frozen cells for longer than two years and, at least after two years of storage, the viability of cells will be very similar to what you can see in the beginning. The product is supplied frozen in a metal cassette, to protect the bag inside.

As I mentioned, we use this product for treatment

of graft versus host disease. In several words, we can compare graft versus host disease to organ rejection.

If you transplant heart or liver from one individual to the patient, you know that the patient immune system can reject a transplanted organ.

Graft versus host disease is in some kind similar but in the opposite direction. When a kidney patient, after treatment, receives a bone marrow or blood transplant to reconstruct their hematopoietic system, they will receive healthy immune cells from donors.

These healthy immune cells can recognize host tissues as foreign tissues and attack them. So, the most common tissues that suffer from graft versus host disease is skin, liver and gastrointestinal system.

In severe cases, graft versus host disease in lethal in up to 80 percent of patients, who will die in a very short period of time.

When we started to think about potential application of mesenchymal stem cells, we had a very solid scientific evidence why this drug would be useful for treatment of graft versus host disease.

Potential underlying mechanisms are that when you infuse these cells systemically, they can hear signals and can migrate specifically to the sites of injury or inflammation.

Sitting at the side of inflammation, they can modulate immune response and, particularly if you have activated immune response, they can shut down this response.

With that, we observed very strong anti-inflammatory activity when these cells can down regulated the secretion of TNF alpha and interferon gamma.

In addition to that, cells will secrete a lot of growth factors, which will stimulate proliferation of epithelial cells and help to renew tissues.

What is also very interesting about this product is that we can use allogeneic cells and transplant cells from any donor to any patient without matching.

This is because of the unique properties of these cells. Briefly, these cells have very low immunogenic profiles. So, they will not trigger immune response. Number two, they can actually modulate immune response.

Instead of immune response, they rather can generate tolerance and can survive in the body for a long time and do their job.

When we started to think of potency assays for such a product, what we wanted to see, we wanted to link our potency assay to desirable effect of our cells, and desirable effect of our cells is suppression of immune response, inhibition of inflammation or healing of damaged tissues.

Among these three, probably the suppression of immune response is a very specific feature which is necessary to have for successful treatment of graft versus host disease. We started out potency assay development from this concept.

So, here is an illustration. If you take mesenchymal stem cells and mix them with peripheral blood mononuclear cells or isolated T lymphocytes, and you stimulate blood cells with some kind of stimuli -- in this particular case we used antibodies against CD3, CD28.

The lymphocytes will proliferate, and you can detect this proliferation by various methods. In this particular case, we used irradiated thymidine to catch DNA replication.

So, higher counts, the more proliferating cells you will have. You can see that, if you add MSCs, the more MSCs you add to lymphocytes, the stronger inhibition of proliferative response you can see.

This represents a potential bioassay you can use as a potency assay, but in reality, it is very difficult to validate such an assay because of high variability.

Moreover, what we notice, if you will check ability to inhibit immune response by the same lot of MSCs versus different blood cell donors, you can see great variability.

It happened because MSCs, they do not inhibit immune response just constituitively reproducing something. They respond to factors produced by activated immune cells, and then these factors will trigger a response production of different factors by MSCs, and finally you can detect inhibition of immune response.

So, what we observed was that some donors, blood donors, may have very low, for some reasons, very low activation levels. In this case you cannot detect very good inhibition.

So, again, this is a very difficult system to validate. Based on that, we wanted to replace this bioassay by a very reliable assay, if it is possible, using a bioanalytical assay, but we have to link our bioanalytical assays to the real biological effects we wanted to have for all cells.

We started with potential candidates that might mediate immunosuppressive activity of our mesenchymal stem cells.

Our strategy was to select candidates based on data in the literature and on our own experimental experiences, and then screen selected markers to find whether they correlate with MSC's immunosuppressive activity, and then validate potency assays and to do potency marker qualification experiments.

On the next slide you may see, on the left side, several potential candidates we screened. I would like to call your attention to the last one, human necrosis factor receptor particular type I.

Among all candidates we selected finally as a result of our screen, this is a prochymal potency marker, and there are several reasons why we selected this marker.

Here you can see the key result. The correlation between the level of expression of TNS receptor on MSC and MSC-mediated immunosuppression.

Bar graphs represent proliferation of lymphocytes, and bar number one, that is the lymphocytes alone. Bar number two, if you mix the lymphocytes with MSCs, you can see good inhibition of immune response.

Bars three, four and five, this is MSCs transfected with anti-TNF receptor type I oligonucleotides. You can see that the red line represents expression of TNF receptor.

If expression is dropped, MSCs cannot inhibit proliferation of lymphocytes effectively any more. The last bar is the control bar. It is very interesting. It is sense oligo.

We didn't expect that it would have any effect, but because we used pretty high concentrations of oligo, you can see here probably a non-specific slight inhibition of

TNF receptor expression, and it is very well correlated with proliferation.

We were very glad to see these results. It means that cells are very sensitive, and any change in expression of TNF receptor might be linked to desirable biological effects, such as inhibition of immune response.

So, this is a selected marker based on this experiment. What is also very good, to the 15F receptor expression, you can use a single bioanalytical assay like ELISA type assay, for our end point potency assay is a single ELISA.

There is a commercially available kit from R&D Systems, which allows you to measure solubility TNF receptor, but we validated the same kit and it can measure successfully full and receptor extracted from tissues or whole cells.

We did classical validation for bioanalytical methods, and I will not describe it, but we follow FDA guidance for that, and we did all possible testing, including stability of analite in our metrics, and we know all the limits, upper and low range, linearity, and what is possible interfering factors and cell lysates.

So, the assay looks like you take your product, which is frozen cells, five aliquot, and exactly like at the time when you use these cells and infuse this in the

patient, you lyse a portion of cells and you measure TNF receptor in cell lysate.

So, when we selected our marker and we validated the ELISA assay for measurement of TNF receptor, we moved to the very difficult part to qualify our potency marker.

In that study, we used two parts. Number one, when we selected our marker, we did several preliminary experiments, but we had no data, what we may see on cells derived from different donors.

Part one was to analyze expression of TNF receptor on MSCs from 30 different donors, and see whether their expression level will correlate with inhibition of lymphocyte proliferation in vitro.

Part number two was to establish a cut off point for what you consider bad cells, which will not inhibit proliferation of lymphocytes any more, and what you consider good cells, and this is part one.

Part two, very briefly, a schematic representation of the experiment. We took frozen cells, prepared as closely as possible to the manufacturing process, the same type of reagents, the same process, just very, very small scale.

We used cells at the P5, passage 5, exactly like we use cells in our real product. We prepared frozen aliquots, then the full cells, counted cells, and divided

into two parts.

One part was lysed, and we measured TNF receptor expression by ELISE-ing cell lysate. The second part was plated together with lymphocytes, and in five days we measured how cells can inhibit proliferation of lymphocytes.

Below, this scheme, you can see the summary of experimental results. What is very interesting is that, if you prepare your cells, you culture your cells, in the same conditions, variation between donors is not very high, at least the range of TNF receptor expression is the same if you look at this not as numbers, but as a biological mean. All the cells, they inhibit proliferation of lymphocytes at a pretty good level.

When we consider a good level of inhibition, we consider it potent cells if they inhibit lymphocyte proliferation at least 50 percent or higher. Less than 50 percent, that is not very powerful inhibition of proliferation.

Part two, we wanted -- we had several methods how to generate better MSCs. Finally, a very good method to use anti-sense oligonucleotides.

So, the design of experiments was that we started with our P5 cells, full cells, count cells, and plate cells in a petri dish.

We transfected cells with anti-sense oligos or

control oligos, and then, after one day, we collected these cells and divided them again into two parts.

One part we used for bioassay and the second part we used to detect TNF receptor expression. Here you can see the results.

Red dots represent proliferation, inhibition of proliferation by MSCs, and numbers showing percent of inhibition.

Blue bars represent expression of TNF receptor, and numbers show you a TNF receptor measure that is picograms per ml in cells.

So, you can see that, after 28 picogram per ml in cells receptor, you still have very good inhibition of proliferation. Then, when we developed expression, inhibition is not higher than 50 percent.

So, based on that type of experiment, we established our cut off point as a mean expression of TNF alpha receptor type I in cells, plus standard deviation.

It means that 15 picogram per ml in cells is our cut off point. Cells expressing low levels we will consider as non-potent cells, and cells expressing higher than 15 we will consider as potent cells.

At the present time we use this assay as a release criteria for manufacturing product for phase III, plus we are implementing this assay for all our other studies like

stability studies.

For example, we would like to switch storage of our product from liquid nitrogen to -80, and it will be much, much easier later to have our drug distributed into regular pharmacies. We need to know whether cells stored at -80 will be not only viable, but potent.

We are going to use the same potency if we will introduce any manufacturing changes, because it seems like this system is very sensitive to changes in culture conditions.

So, on the next slide, just one example. These are the so-called results of the so-called temperature tolerance study. When this particular study was done, it was to be able to ship our cells on dry ice instead of liquid nitrogen.

We wanted to know if, suddenly, the temperature will be shifted to higher than -70, what may happen with cells.

We stored cells for a short time at different temperatures. After that, we measured cell viability and we measured the expression of TNF receptor. In parallel, we measured potency of these cells in ability to inhibit the immune response.

What we found was that -80 and -78 is a good storage temperature. You can store cells at least for a

short period of time without losing cell viability and expression of receptor.

When temperature dropped and started to be higher than -60 or -60, you will lose cell viability, number one, and number two, these cells will be losing TNF receptor, and will be not potent in bioassay any more.

If you look on this graph you may say, okay, it looks like your viability correlates with TNF receptor expression. So, you have less cell receptor because you have more dead cells.

This is true, but only partially. When we played with the data and assumed that all dead cells still looked by cell analysis as a cell, they do not express TNF receptor.

We calculate expression of receptor on the living cells, and still you can see a huge difference between cells stored at -80 and -70 versus cells stored at -60 and -50.

As conclusions, we may say that TNF receptor seems the best marker for today, which we can link to MSC immunosuppressive activity, and this is a desirable biological activity for treatment of drug versus host disease.

So, an end point for the assay is a simple ELISA which is very robust, very quantitative, and meets all acceptance criteria.

So, at the present time, our experiments show that we can use this potency development assay approach for other drugs.

We have other drugs for cardiac and for muscular regeneration. We are going to developing a potency assay and select a marker for such potency assays based on product application. Thank you very much, and I will be pleased to answer your questions.

DR. MULE: Questions from the committee?

DR. CALOS: You identified this potency marker. Do you think that this TNF receptor, then, more or less completely accounts for the activity of the MSCs? If so, is that a better product than using the cells?

DR. DANILOVITCH: Yes, we think that this marker represents the best for today linked to cell biological activity.

DR. ROCKE: Did I miss something, or did you not tell us what the relationship was in those 30 individuals between the proliferation, suppression and the TNF receptor measurements?

You had means and standard deviations for them separately, but I didn't see a chart -- I would like to see a plot of the two.

DR. DANILOVITCH: Individually all donors. I will tell you that we saw some, in some cases, a little bit less

than 50 percent inhibition, and we have re-tested this sample several times.

What we observed, it was linked to biological variabilities. So, I might tell you that the range of TNF receptor expression that we found in this set of donors and the range of inhibition of proliferation were very good.

Let's say, everything that was tested was potent. That doesn't mean that all cells expressed 20 picogram receptors per million cells or 40 picogram receptor for million cells, they will be potent, so only if expression of receptor would be dropped lower than --

DR. ROCKE: You must have computed the correlation between those measurements.

DR. DANILOVITCH: Okay, I can provide such data. I understand your question. The correlation in that case, you cannot see that more receptor more inhibition.

In this case we saw that the range of receptor expression and very good inhibition. That is why we used anti-sense oligos, because we were not able to identify rat MSCs, the nature of rat MSCs.

DR. HARLAN: So, if I understand correctly, the TNF receptor level was a surrogate for anti-CD3, anti-CD28 stimulated proliferation.

Have you ever tested either of those assays in an animal model to see if they predict the ability of your

product to prevent graft versus host reaction?

DR. DANILOVITCH: Yes, we tried to do that. The problem is that you cannot use human MSCs in synergeneic stages.

DR. HARLAN: What about animal MSCs?

DR. DANILOVITCH: Yes, we were trying to use red MSCs in the rat graft versus host disease model, but unfortunately, our attempt failed, and failed for the reason that let's say the rat graft versus host disease is completely different from human.

DR. GUNTER: You worked really hard to find a relevant protein to serve as a surrogate for a functional assay. I think that constrained the universe of potential molecules to actually test.

One thing that I would just like the committee to consider this afternoon when we have a discussion is does the surrogate protein for a functional assay, does it really have to have any relevance at all to the function of the cells?

If you just show a very, very strong correlation with biological function of, say, an unknown protein, could that be an acceptable surrogate? That is kind of the principle behind using an array analysis, too. I would just like the committee to consider that question.

DR. MARINCOLA: I am just kind of putting together

two comments from Dr. Calos and Dr. Chamberlain. It seems to me a very important question Dr. Calos brought up.

One thing is biomarkers. Another concept is to look to identify what might be the single component of the therapy.

It is important because if you are considering, and you have to really prove it in one way or another -- I think this is just a biomarker, which might work very well for a very simple, straightforward in vitro assay, but it has nothing to do with the complexity of what the cells are doing in vivo. Then you have to find other biomarkers that may be relevant.

On the other hand, if this is the only biomarker, if this is the only component, you might as well give the TNF receptor one. It seems like an important concept to extrapolate.

What do you find, when you find a biomarker that is too relevant to the point that it is the only thing that really matters. I don't think I am convinced one way or the other by your presentation of what this represents.

DR. DANILOVITCH: Thank you very much. I Understand the limitation of the potency assay we developed, but for today this is the best that we can do.

At the present time we are building a data base to try to see what -- I kind of doubt, but anyway, we will try

to see whether the expression of TNF receptor on all cells would somehow correlate with the clinical outcome.

This is what finally we wanted to see. We wanted to link our potency assay, and we wanted to guarantee that each lot will do in patients what we want it to do. This is a very complex issue. I don't think just RNF receptor will cover this issue.

We will continue to work on it, and as soon as we generate more data, and it is very possible that this assay will be replaced by another assay, and we will continue to add more assays to have not just one assay but several, to have a more complete picture of the functionality of our process.

DR. ALLAN: I may have missed this, but it seems to me that you are into some human clinical trials, or you have put this product into humans; is that correct?

DR. DANILOVITCH: Yes.

DR. ALLAN: Have you been able to go back to some of the cells that you may have held back and then look to see what their expression levels are, if you were able to do that?

DR. DANILOVITCH: What I may tell you, that because our clinical trials are open label clinical trials, reaching dying patients with very severe forms of graft versus host disease, we can see the result how cells work in

patients before the completion of the clinical trial.

What I may tell you, we can see very good therapeutic effect of our cells, but when there was a bone marrow biopsy, and biopsies of some other tissues, we cannot find our cells in tissues.

It seems like at least in the body where there is a very high level of inflammation, cells will not survive for a long time. They will do the job and they are gone. This is the data that we have now.

In animal models, other animal models, we can see that cells can stay in the body as long as we followed the cells, and we followed the cells up to one year, and you can find cells in the body.

DR. TOMFORD: Do you do cell viability assays on every product?

DR. DANILOVITCH: Yes, this is our release criteria.

DR. TOMFORD: What is the viability? Is there any range?

DR. DANILOVITCH: Viability, the acceptance criteria that cells should be -- that viability should be higher than 70 percent, but what we see with our cells is that we have viability higher than 80 percent.

We check viability several times, before infusing the patient, at the sites where cell stems -- they will do

cell counts on each bag of the product. If viability is less than 70 percent, this bag will be rejected.

DR. CHAMBERLAIN: One thing I am a little confused about, it sounds like your criteria for the receptor expression is it has to be above 13 -- whatever, picograms per ml, but you have never encountered a batch of MSCs that is lower than that. At that point, what is the point of even doing that assay?

DR. DANILOVITCH: So, you know, it happened that all MSCs, when they are cultured, they look like good MSCs. Actually, bad MSCs are what are generated if you store the cells at like -60 and -50.

So, for some reasons, these cells will drop expression, and they will lose the ability to inhibit the immune response. At the present time, all lots tested, they all passed the acceptance criteria.

DR. MULE: Thank you. The next speaker is from Therion. It is Kelledy Manson.

Agenda Item: Potency Assays for Recombinant Viral Vaccines for Cancer Therapy.

MS. MANSON: I am going to talk to you about development of potency assays for our products, which are recombinant viral vaccines.

The agenda for today, I am going to give you some background of our products, the challenges we faced in

developing our potency assays and, working with the agencies, we have also adopted a matrix approach to potency validation, which I will describe to you.

I will talk to you a little bit about how we are starting to move into developing surrogate assays, and then answer any of your questions.

We use recombinant pox viruses as our vaccines to deliver genes for tumor associated antigens and immune enhancing proteins.

We have two. Our two lead products are currently in clinical trials, panvac VF in phase III for metastatic pancreatic cancer, and prosvac VF in phase II for metastatic prostate cancer.

There is a lot of information on this slide, and I am going to go through it in some detail for you. What I am going to talk to you about is give you a little overview of how we produce the vaccines, and then the series of events that occur from infection by subcutaneous infection through to our proposed mechanism of action, and this will help to describe why we are using a matrix approach to potency.

Let me start with how the vaccines are made. Our vaccines are made by in vitro recombination of the attenuated vaccine vector in the plasmids containing genes for the tumor associated antigens and immune enhancing proteins, and in a few minutes I will describe what those

are.

The recombination goes through a series of plaque titration purification cycles. The material is grown into a seed stock and then amplified in manufacturing to the drug product, which we test for lot release.

As I mentioned earlier, our vaccines are administered by subcutaneous immunization. Pox viruses are able to infect and replicate in just about any cell type.

Upon infection, we have the DNA is transcribed and translated into protein which is expressed on the cell surface either as whole protein or in the context of HMC peptide.

We also express a triad of co-stimulatory molecules, again which I will describe to you in just a minute, which are immune enhancing proteins. This triggers an immune response.

Our proposed mechanism of action for our vaccines is this immune response leads to tumor destruction by CTL activity.

Our vaccines consisted of two pox viruses which are administered in a heterologous prime boost, vaccinia followed by fowl pox.

Vaccinia is a very permissive virus that replicates in just about any cell type, and this replication leads to a rapid development of a neutralizing antibody

response by the host.

Fowl pox, on the other hand, which is given as a boost, is an avian virus. It infects avian cells. It can express the proteins -- excuse me, it infects mammalian cells and it does not replicate, but it expresses the proteins.

This protein expression, to date we have not seen any inhibition of it in the clinical trials, and we have been able to give fowl pox many, many times as a boosting agent.

Each of the vaccines -- vaccinia and fowl pox -- express tumor associated antigens and a triad of co-stimulatory molecules, which we refer to as tricon.

So, panvac V and panvac F each express CEA and MUC in the tricon molecules. Similarly, prosvac V and prosvac F express PSA in the triad of stimulatory molecules, B7, LFA-3 and ICAM.

You can see from this we have had to develop a number of different assays, not only to look at the different viral vectors, but also the different components that they contain.

A wish list for developing a biological potency assay is very much the same as we have seen earlier. We want to measure the biological activity as it relates to the product function. We want to ensure lot to lot consistency.

We would hope that these assays would be stability indicating, so we could see any changes that might happen to our product.

We, of course, want to minimize the variability that is inherent in all bioassays, and we want to include a suitable reference standard.

Specific to Therion, as I have already alluded to, we have a complex product with multiple components. We have the two viral vectors, we have one or two tumor associated antigens, and we have these co-stimulatory molecules. Then there is a series of events that lead to biological activity, which has led us to the matrix.

To help us reduce some of this variability and ensure lot to lot consistency, we have taken a statistical approach to our bioassay development, and this has been absolutely invaluable to us.

Following our feasibility studies, so we have an initial assay design, we actually engage a bioassay statistician.

This is someone who is familiar with what biological activities used for and becomes really a partner in our development, understanding our science and looking at our assays as a component for lot release.

Using this approach, this may lead to assay modifications and refinements along the way to produce a

more precise and accurate assay, but that is just the natural evolution of bioassay development.

Speaking of evolution, we have been working on potency assays at Therion for quite a long time. We started prior to phase I with a plaque titration assay. This looks at the number of infectious units, and that is how we base our dose or concentration of vaccine that is administered.

That assay has supported preclinical and phase I and phase II trials. During our phase I and phase II trials, we also started to develop our biological activity assay.

As Dr. Gavin mentioned earlier, you cannot start too early to develop your biological activity assays, because they can take quite a long time, especially for complex products.

As we moved into phase III, we began to implement the matrix approach, which we have already talked about a little bit this morning, and that is putting assays in place to look at the critical steps required that lead to biological function.

Moving forward in our product development, we are starting to look at quantitative surrogate assays that would help supplement or potentially replace the highly variable biological activity assays.

We have seen this slide before, but on this one I would like to just highlight the steps where we have

implemented our matrix assays.

The first thing that we need to look at is infection. The viruses must be able to infect the cells. So, we need to make sure that they can do that.

We need to be able to evaluate the genetic coating, to make sure that the appropriate genes are being delivered.

We then need to look at protein expression. Do we have the right proteins. Have we expressed a sufficient amount of protein in each of the virus particles. Then we look at the biological activity.

Our matrix, therefore, consists of a series of six assays. The first assay is the plaque titration assay, looking at the number of infectious particles in the drug product.

We ensured the genetic structure, encoding the tumor associated antigens and tricon molecules as intact by southern blot analysis.

We evaluate the expressed proteins by western blot, and also by an in situ immunoassay that tells us the percentage of virus particles that we have administered that express the proteins.

We have two biological activity assays. One is to evaluate the tumor associated antigens, and we do this in vivo by generating an immune response in mice.

We also have an in vitro assay to look at T cell activation due to the tricon molecules. I am going to discuss that assay to you in a little bit more detail, because that has been one of our more challenging bioassays.

Tricon is designed to work by turning just about any infected cell into an antigen presenting cell. So, T cell activation requires two signals.

The first signal is the recognition of peptide in the context of MHC. The second signal is the recognition of the co-stimulatory molecules expressed by the antigen presenting cell by the corresponding receptors on the T cell. This leads to T cell activation. In our case, we are looking at cytokine secretion.

To accomplish this, we have an in vitro assay where we activate human T cells using a non-specific signal one, which is con-A. Then signal two is provided by the pox virus infected cell line expressing the tricon molecules.

As I said earlier, we need to look at each of the components. So, in the case of this assay, we are only looking at the contribution of tricon, and we quantitate T cell activation by measuring cytokine secretion.

This assay is one where the design of experiment has been crucial. We have been able to identify all the sources of variability within this assay using single experimental designs, and this is where our biostatistician

has been very, very helpful.

In identifying the sources of variability we have been able to modify the assay so that we can increase the accuracy and precision.

We also have looked at a dose response curve by looking at different multiplicities of infection, and have been able to identify multiplicity of infection, which allows us to have increased sensitivity so that we can assess lot to lot consistency and the stability of our product.

We are currently in the process of evaluating surrogate assays for biological function, as we all want these to be quantitative and analytical and more reproducible.

What we are looking at right now is quantitative flow cytometry, which we would use to either supplement or replace our in vivo biological potency assay.

The assay in mice, while the precision is good, we have about a 30 percent variability -- it is not sufficiently sensitive to look at stability properties of the product. We have preliminary data showing us that quantitative flow cytometry is capable of doing that.

So, in summary, we feel that, for complex products, that more than one assay may be required to assess activity.

We use a matrix of both analytical and biological assays to evaluate critical steps. We feel that the statistical approach to assay design, bioassay design and validation is critical, and also that surrogate assays can be used to replace or supplement the very highly variable biological activity assays. I would be happy to answer any questions.

DR. MULE: Thank you. Questions from the committee?

DR. PLANT: I wonder, in your in vitro cell based assays, you have assigned as one of the variables the source of T cells, and I wonder if you don't have a sort of a standard T cell, or if you considered the idea of a standard cell that might remove that variability from day to day.

MS. MANSON: Actually, in going through the design of experiments, the source of T cells is not a source of variability.

The dose response curve from multiple donors are virtually identical. So, it was quite a happy surprise for us when we found that that was not a major source of variability. So, we are able to use multiple donor banks.

DR. PLANT: So, you have sort of tracked down what the largest source of variability is, and is it in the product itself then?

MS. MANSON: It is not in the product itself,

actually.

DR. PLANT: The cytokine release assay is an average assay, you are collecting material and analyzing it. I wonder if you have thought about developing an assay that would allow you to look on a cell by cell basis to perhaps look at the variation within that population, which might help you track down what the variabilities might be due to.

MS. MANSON: I certainly think that is a possibility, yes, for further assay development.

DR. MULE: If there are no other questions, thank you. Our final speaker is Dr. Provost from Dendrion.

Agenda Item: Potency Testing for Autologous Cellular Immunotherapy.

DR. PROVOST: I would like to thank the committee and the agency for giving us the opportunity to present the data today that we are going to show you.

As many of the speakers have before, I am just going to give you an overview of what I will show you, an introduction to the process and the product.

I am not going to go into exquisite detail in terms of the statistics, the validation protocols, all of that stuff. Suffice it to say that our release assays have been validated, and we have gone through several phase III trials.

We are in the process now of revalidating some of

those with an eye toward introducing better reference standards. So, I will talk a little bit about that.

I will introduce you to the product and the process. I will talk a little bit about a model system that we use to help model a product and predict assay results, look at the molecular assays and tools that we use, talk a little bit about correlating the antigen presentation activity with cell phenotype, kind of circle back to justifying some of the potency assays, especially with regard to clinical data, and then look at tracking potency over time, and comparing potency data with clinical outcomes, with efficacy data, and then leave a little time, I hope, for questions and answers.

This is an overview of the product. It is an autologous cellular therapy for prostate cancer. Leukocytes are collected from the patient. They are transported to a manufacturing center where the cell product is made. The cell product is then shipped back to the patient and reinfused.

Three of these courses of therapy constitute one full course of therapy. This is a live cell product. So, suffice it to say that it has a short shelf life.

So, we are talking about hours, not days or weeks, to test and approve the product. So, it puts quite a constraint on our quality control and GNP facilities.

This is a cartoon representation of the product and a bit of the process, how we make the product. We started with a mixture of leukocytes from a patient.

We add a recombinant prostatic acid phosphatase antigen, which we make and characterize, that combines with resting antigen presenting cells in culture.

The APCs take up the antigen, process them, and present peptides on their surface, the APCs also activate in culture. This is what we call our product, a mixture of activated APCs and other cells, leukocytes.

That product is infused in the patient, and we believe that T cells are then able to dock up with the activated antigen presenting cells, and that allows them to focus their attention on prostate tissue, in this case. So, the idea is that T cells can then proliferate and target the prostate cancer cells.

Now, this should look familiar, since you saw a different version in the last talk. In order to recognize and respond to peptides, T cells have to recognize the peptides in the presence of MHC.

They also have a whole variety of co-stimulatory molecules that they need to respond to. So, co-stimulatory molecules and cytokines help activate the T cells.

You would say that the ideal readout for a product like ours would be T cell specific peptides for prostatic

acid phosphatase peptides.

That would require MHC matched T cell reagents for every different type of MHC haplotype, which is a pretty daunting task when you are trying to standardize an assay.

Since we don't HLA haplotype the patients as they walk in the door, it is a double problem. I just want to go through some of our challenges.

We have a very heterogeneous starting material. It is a mixture of leukocytes. We have limited patient materials. We don't make these in large batches. They come in one blood bag at a time.

As I just mentioned, we have HLA restricted antigen presenting activity and we don't know the HLA haplotype.

As I said before, we have a short shelf life for the product. So, the speed and accuracy of the testing is important.

Bioassays, we all know, are difficult to validate. Some of the solutions we have come up with were to evaluate and incorporate healthy donor cells as a model mostly for product development, but also for looking at shelf life and stability studies.

We have used healthy donor cells and patient product cells to take a look at and characterize the product and process, so we know what to expect in terms of product

uniformity and control -- that is, what variables we can control and we can't, and we have also taken a statistical approach to that.

We have gone to great lengths to identify the target cells that are actually responsible for antigen presenting activity to T cells, so we know exactly what we should be measuring for potency.

We have tried to correlate the target cell phenotype and the antigen presentation activity, and develop assays that can be validated and related to clinical assays. I will show you some of that as we go along.

The tools that we use for the cell product characterization, as I mentioned, are donor cells obtained from aphoresis, and a variety of fluorescently labeled reagents, commercially available monoclonal antibodies, antigen that we label up with fitsi ourselves, and some T cell hybridomalines, which are HLA DR1 specific, which I will mention a little bit more in detail later.

Then patient cells that we have evaluated as part of product release, and then sort of gone back and done some correlations with manufacturing results and potency results.

This is an example of a product characterization data set. It shows you both the range of values that we see. In the blue we see patient values -- these are all lot release values.

In the red you see healthy donor values. In this particular case, we are just looking at correlations between CD54, which is an activation marker, and CD14, which is a monocyte marker.

We have made the cell product from either source, either patient cells or healthy donors, and then measured various lineage and activation markers.

We have used this type of analysis to characterize the product, the process -- that is, looking at step yields, looking at activity -- as we go along in the manufacturing process.

We have examined in process intermediates, and we have used all of this information to establish specifications from patient materials. So, we used the patient lot release data to establish all of our lot release criteria.

This is a really old slide, but it illustrates how we came about choosing the assays we did for potency release.

If you look at a mixture of cells, leukocytes, before and after the process -- that is, pre-culture and post-culture -- you see that certain activation markers, on their surface, upregulate, or increase, their expression.

So, fluorescence for C54 is plotted here, and other markers here. So, this toward the right indicates

higher expression, and you can see that after culture you get measurable and reproducible increase in the expression of these markers.

When we titrate out those cells and look at various measures of T cell activation -- in this case there is allogeneic and autologous T cell activation assays, you can see that you get better stimulation of T cells after culture than you did before, which is no big surprise.

It basically showed us that the upregulation of these various markers, we used this sort of analysis to home in on what markers we should follow to assay T cell activation, and CD54 turned out to be a particularly reliable marker of antigen presentation activation.

Another course we took was to look at where the antigen goes in that mixture of cells. Again, we labeled our antigen, which is named PA 2024, the PAF antigen, with fluorescein isothiocynate.

Then we did sorting experiments to show that that antigen goes into certain cell types and not into others, and here we have CD54 cells, CD54 positive cells, a marker of antigen presenting cells, and other lineage and non-lineage markers.

The take home message was that we are probably looking exclusively at a monocyte population, and not at T cells or B cells, which are denoted here by CD2 and CD19.

So, this told us that we were really looking at antigen presenting cells, monocytes, and told us where to home in on our activities.

So, from early clinical lots, we developed potency specs for the product, and we established a minimum number of CD54 positive cells, which are measured by flow cytometry and cell counts.

A CD54 upregulation or fold increase in expression is measured by flow cytometry before and after culture with the antigen.

The flow cytometry method utilizes partially available fluorescently labeled antibodies. We use fluorescently labeled calibration bead standards to standardize the instrumentation and acceptance criteria, and obviously we have standard operating procedures and qualified personnel training procedures, et cetera, so that we can do this reliably in a GNP environment.

So, we have established reproducibility and robustness criteria. We have done the initial validations to show that it is linear over the range of values that we see, and we are going back now and revalidating that assay with even more system suitability standards in it.

Now, one question we had was, how do we tie in the activation of antigen presenting cells with some tangible measure of T cell activation, and there has been a little

bit of discussion of that so far, but I will tell you our approach.

That was to establish a mouse T cell hybridoma cell lines which were from mice that were transgenic for human HLA DR1.

By immunizing the mice with our antigen, selecting for T cell hybridomas that respond to specific peptides, we could then assay the activation of the mouse T cell hybridomas by either proliferation of cells or secretion of IL2 in response to human antigen presenting cells.

I have shown it here without the co-stimulatory molecules because these T cell hybridomas don't require that second signal. So, we are really only focusing on the peptide presentation in the context of HLA.

So, I am going to show you a few representative experimental results where we confirmed with the T cell hybridomas that we were looking at the right population, and that the CD54 population and expression actually correlated with the T cell activation.

So, the two T cell hybridoma cell lines that we generated were nicknamed paparino and papillon. I don't really take responsibility for that, but that is how we keep track of them.

In this experiment, we took HLA DR1 typed healthy donor cells and produced cell product using the PA 2024 that

had been spiked with a fitsi labeled PA 2024.

So, the cell products were then sorted. The cells were sorted by flow cytometry into fitsi positive and fitsi negative, that is, those cells that had taken up the antigen and those that hadn't.

Each sorted cell mixture was incubated with one of the hybridomas and titrated to see what the response was. These data actually confirmed that only the cells that had taken up the PA 2024 could activate PAP specific T cells. So, it told us that we were barking up the right tree.

Another set of experiments focused on HLA DR1 cell product that was made with just the regular old variety of antigen, but then sorted into CD54, and CD54 positive and negative populations.

Again, we see that the only appreciable T cell activation comes with the CD54 positive cell population. So, this confirmed for us the link between CD54 positive cells and antigen presentation.

It still hasn't confirmed a dose response for CD54, but in other experiments in data I am not going to show you, we are seeing more of a threshold effect than an actual titration of the CD54 molecules over the surface.

Can these hybridomas be used for lot release? No, because of the HLA restriction and the long assay time, they are really not feasible for routine use in lot release.

We can still use them as tools for characterizing the product, and process characterization for any changes we make.

It also helps us to characterize the product in terms of stability and packaging and that sort of thing. So, I will show you a few examples of that.

This is an example of an experiment where we used the T cell hybridoma activity to demonstrate stability over time.

While we could show that the antigen presentation activity does decay with time and temperature, this assay is somewhat variable, and we are finding it is going to be really difficult to validate this.

We have qualified it so that we know it is reproducible and we have operator to operator qualifications, but we do see variability in the absolute amount of antigen presentation activity and, in this case, we use a reference standard, a standard cell product that is made and frozen away and aliquoted. So, we have a relative antigen presentation activity or RAP, it is called.

It at least showed us that we were on the right track in terms of, if we look under stress conditions and normally storage conditions, we can see the decay with time. So, we know we should expect something to show up.

The other potency assay that we use, which is CD54

mean cell fluorescence by flow, this shows the number of lots that are put on a stability study.

This is a development study looking at normalized mean fluorescence intensity over time, both in normal storage and stress conditions.

It shows us that we are able to detect changes in the product and relate them back to antigen presentation activity.

So, the next couple of slides I need to show you are a little key to show you what we are going to be talking about, which is going back to manufacturing data and quality control release data, and asking how does CD54 expression and upregulation vary from -- what is the range in value for various lots, and how does it vary over the course of treatment.

This is a box and whisker plot. What you will see are a number of values which are expressed mostly as the median -- the horizontal line here is the median, this is called the interquartile range, which goes from the 25th percentile to the 75th percentile. So, this is where the bulk of the data generally reside.

There are some lines here which indicate the 75th percentile plus 1.5 times the interquartile range. This gives you an idea of the scatter of the data, and then anything beyond that is really scattered.

So, we are trying to apply statistical analyses to a very heterogeneous product, but it is a way of actually conveying the information in a very succinct way.

So, these are clinical manufacturing release data expressed as box and whisker plots. This is CD54 cell numbers.

These are the four phase III clinical trials that we have gone through. Two of these are finished and the efficacy data are analyzed. 9902B is still in progress, P11 just finished enrolling. We have got a few stragglers left, but we are just about done.

What this shows is that generally the CD54 cell numbers are consistent across all the trials. There is a little bit of variability, but the range is pretty much the same.

Across all three treatments you see a little bit more scatter later on, but generally speaking we are seeing fairly consistent results across different patient populations and across different trials. So, that gave us a good warm feeling.

CD54 upregulation, where we look at what is the increase in expression -- so, this is the delta measurement from day zero to day two -- shows pretty much the same thing.

You see a pretty good consistency across all the

trials. The interesting thing for this data representation is that, for the second and third doses, we see a lot ore scatter and generally higher CD54 up-regulation, which suggests that some increased cell product activation is going on in the later treatments.

Now, one question would be, how does our measure of potency with the CD54 expression and CD54 upregulation relate to clinical outcome.

So, you may have heard, we have finished two phase III trials, and we found a statistically significant increase in potential survival for those patients that were treated with the prostate product.

So, we went back to those trial data and looked at what were the values of CD54 and CD54 upregulation that the patients had.

So, this is a Kaplan Meier survival curve, looking at the cumulative CD54 cell dose, that is, the cell dose over three treatments.

We split the patient data up into placebo patients, which are represented by this black line. Those patients received a cell product, but it didn't have an antigen in it.

Then the treated patients, the patients who are treated with probander spoolcell T(?), were then divided into patients whose cumulative CD54 cell dose was above the

median, and those are represented in red. Those patients who had a cumulative CD54 cell dose below the median, those are in blue.

What this represents is, you see a slight increase in potential survival for those that had the higher cell doses, but not much. There is not a big difference.

The other take home message is that all the patients that got treated with this product did better than the placebo, which is not unexpected, considering the overall clinical trial data.

Next we wanted to look at the CD54 upregulation. The key is the same here. The black is placebo, the blue is below the median, the cumulative upregulation, and the red here represents those patients that had cumulative CD54 upregulation above the median.

Here we see a pretty significant difference in the outcome of patients that had higher upregulation ratios. What this tells us is that, while it is really preliminary, it is pretty provocative.

It gives us some direction to go in and start to go back and reexamine our specs and see whether we can find either a threshold or some correlation with CD54 upregulation.

It also gives us a way to start probing the timing of the doses and whether we see some threshold effect after

one, two, or three doses.

So, in summary, I have shown you a little bit of data about donor cells. I am just going to assert that they mimic the clinical data for CD54 expression and upregulation.

I have described the generation of PAP specific, HLA DR1 restricted T cell hybridomas that demonstrate antigen presenting activity.

PAP specific antigen presentation activity resides with the CD54 cells. The CD54 expression and upregulation appear to be surrogates for PAP specific antigen presentation activity.

CD54 expression is stability indicating, and CD54 expression and upregulation appear to correlate with survival. We are still working on those numbers.

In summary, the CD54 cell counts and CD54 upregulation values appear to be biologically relevant potency measures, but they are used as part of a matrix, as most people have described here, including viability, total cell count, cell specific identity and other safety tests.

I would like to thank the crew that generated most of the data here. That is the immunotherapy development team, and the protein and analytical development teams, and I would also like to thank our Dendrion manufacturing, regulatory and clerical personnel that really contributed to

these data, and also our FDA product reviewers that have been so helpful along the way. I will answer any questions.

DR. MULE: Thank you. Questions from the committee? Maybe I can start. So, CD54 upregulation is provocative when you show a potential predictor of clinical benefit.

Is CD54 upregulation in the potency assay directly related to the presentation of the antigen? In other words, if you culture those cells in the absence of the recombinant protein, do you still see upregulation?

DR. PROVOST: If you culture the antigen presenting cells, you see a little bit, but not much, and we have a threshold acceptance criteria based on how much you see with and without the antigen.

DR. MULE: If you take that observation of upregulation of 54 expression, with the number of 54 expressing cells that are introduced into the patient, I guess it wasn't as clear that cell number --

DR. PROVOST: Correlates much at all.

DR. MULE: So, I am trying to --

DR. PROVOST: There is a large variability in the CD54 cell number. It doesn't appear to correlate strongly, anyway, with survival.

The other dilemma is that the way we make the product is that the patient undergoes an aphoresis and

essentially donates their own cells to the product.

We take everything we get and make product out of it and put it back. So, if we look strictly at cell dose, there is a bias that is introduced in terms of the weights, the health, the general immune status of the patient as well.

So, that is a really difficult thing to sort out in a product like that, whereas the upregulation is more independently derived.

DR. MULE: What is your plan for going forward with the T cell hybridomas? I guess it begs the question, DR1 is expressed on what proportion of all patients enrolled on trial?

DR. PROVOST: Less than 10 percent. We haven't typed all the patients. We are not planning on using that as either lot release or correlative. We are looking at it to establish criteria for stability studies and things like that.

DR. MULE: Is there a way of doing maybe off loading assays of antigen on --

DR. PROVOST: We are working on that. It is a mass spec based peptide assay. It is difficult. It is pretty dirty.

DR. URBA: Back to CD54, do you see the baseline expression change in the second product and the third

product?

Then you show the ratio is much greater in the second and the third, and does that differ between those that are pulsed or get the PAP and those dendritic cells that don't?

DR. PROVOST: So, the second question first, yes, you see that ratio is different, and we actually specify that the upregulation has to be above a certain level with patients that are treated. So, we reject product that doesn't upregulate.

Those acceptance criteria were based on with and without antigen, and also we circled back to the placebo patients that we have treated to make sure that that still holds.

The first question you asked had to do with, do we see the level rise over time. It is somewhat patient specific. In general, yes.

So, upregulation is basically day zero versus day two. On treatment two, you might see day zero come up, but we still require the upregulation.

So, the assay, strictly speaking, is based on day zero versus day two, but you do see a general rise, not in all patients, but in general.

DR. URBA: How often do you actually end up rejecting a sample because you don't get CD54?

DR. PROVOST: Well, total rejections are less than 10 percent. I would say maybe a couple of percent. It is not that large.

DR.HARLAN: The Kaplan Meier curves you showed were compelling. I just wonder if the studies are large enough for those curves to be statistically significantly different, first question.

The second question is, you said that if you looked at those where the median response was above the mean, you saw a particularly dramatic result, but can you translate that into what would be a number for a product release criteria?

DR. PROVOST: The first question, I will have to rely on our biostatistics, and we are putting that together, actually, for a submission.

For the second question, we intend to go back and reexamine our specs with regard to upregulation because that is directly applicable to this.

So, we have specs right now that are based on manufacturing and manufacturability and reproducibility, and now we are going to go back and start looking at thresholds that we see from clinical response.

DR. MULE: Maybe you showed this. Is there a direct correlation between the fitsi antigen uptake and the upregulation of CD54?

DR. PROVOST: Yes.

DR. COUTURE: I have two questions. Early on you showed that the CD54, CD14 measurements were kind of all over the place for patients, from almost nothing to almost off the chart. I wonder if that at all correlates with upregulation of those molecules when you treat.

The second part of it is maybe more of a commercial question, but what do you do, or what do you envision doing in the commercial setting with a patient who you fail a product for, and whether you fail the product or fail the patient in that context, or will you retreat, or quite frankly, will you charge the patient anyway?

DR. PROVOST: I can't speak to the charging part. Marketing and sales is not my thing. With regard to whether we fail a patient or not, what we have seen is sometimes cells come in. The age of the cells or the health of the patient may have some direct correlation.

This is completely anecdotal, but we have seen failing product where we have rejected a product. The patient comes back for another aphoresis, and they do just fine. So, we don't anticipate many problems on that score.

DR. COUTURE: The first part of the question was, is there a correlation between CD54 and 14 measurements in the patient when they come in, and upregulation, the ability of those cells to upregulate.

DR. PROVOST: There is a direct correlation between CD54 number and CD14 expression. That particular graph that I showed you was basically correlation studies to see whether we were following the right markers and see if we could drop any of them, see whether they were redundant.

So, yes, there is a direct correlation between CD54 cells and CD14 cells, but there is not a correlation between CD54 upregulation and CD14.

DR. HARLAN: I am confused about the protein uptake and CD54. Are there cells that take up the antigen that don't upregulate CD54, and do they have any effect? Do you have any knowledge whether that is an independent predictor of response?

DR. PROVOST: What I didn't show you is that the antigen is a fusion protein between GPCSF and PAP. The GPCSF actually activates the APCs, and that stimulates our uptake of antigen.

DR. MARINCOLA: I think you gave a good example after all what the ultimate potency marker is the one you bring from the bedside to the bench, is the only relevant one.

I was curious about the process. The CD54 association or prediction that you developed was based on a large broad set of markers, and this came out to be the one you used for predictors or is that now retrospective?

DR. PROVOST: We started out with a broad range of patient and donor products, to see what actually correlated with the product, if you will, because we didn't have clinical data, and CD54 came out to be the most robust and reproducible.

DR. MARINCOLA: Before or now?

DR. PROVOST: Before, early clinical, so phase I and II.

DR. MARINCOLA: So, it is a pretty striking example.

DR. PROVOST: I think we got lucky.

DR. MULE: How do you separate -- this gets back to Dr. Harlan's point. How do you separate GMCSF activating the cell population to upregulate CD54 as opposed to an actual antigen processing event.

DR. PROVOST: In the absence of specific T cell response data for each specific lot, we have to rely on this correlation between CD54 upregulation and T cell activation.

The other way to do it, which we are exploring, is to look at peptide presentation and just try to get at the peptide dilution sort of assay.

Again, the timing is pretty critical there, and you can get a cell mixture with a lot of stuff in it, and eluting those peptides is pretty difficult.

DR. HARLAN: I can't resist just one other

question, on that GMCSF thing. This is not related to potency release assays at all. It is just the science, but presumably you can give more of that peptide GMCSF fusion protein that you could just give to the patient. I mean, why stimulate these in vitro as opposed to just giving them the fusion protein.

DR. PROVOST: It targets the right population for one thing. We have a direct measurement of whether it is working in those cells, which are then going to go on and do their job in vivo. Yes, it would be a whole lot more convenient for doing the cell products.

DR. TOMFORD: What is the time line on the survival curves, and have you looked at something beyond the time -- you know, long time. What happens over a long period of time?

DR. PROVOST: The patients that are in these trials are metastatic, end stage patients. The time line for the survival trials is three years, which is a pretty decent time for measuring survival and doing follow up.

If you are asking, what is the prognosis long, long term, years and years, are we going to see any other untoward effects, we will have to see.

DR. TOMFORD: Not so much untoward effects, but in other words, how long is the effect, the robustness, as it was called earlier.

DR. PROVOST: All I can tell you is that three years later we are still seeing a significant difference. .We are following the patients out with immune monitoring to see when we get response from T cells via ELISpot and other assays. That is something that we will just have to address in future trials.

DR. MULE: I think these questions are important but, again, our mission here is to really work with the speakers and others as far as the potency release criteria of the product.

Again, the outcome data are crucial scientific questions, clinical questions, which may not relate specifically to our mission with respect to the potency of the actual product, or the validation end points.

DR. COUTURE: I think you mentioned that the mouse hybridoma HLA DR1 restricted model you weren't going to use it for correlative studies.

DR. PROVOST: We are using it for correlative studies for looking at stress conditions, looking at stability, looking at process changes.

DR. COUTURE: But you are not going to do a retrospective study or HLA typing of the patients you treated and then ask, at least in the context of those patients, whether you get some specific activity that correlated with CD54 expression. It seems like that is an

obvious thing.

DR. PROVOST: We have done it with those few patients that we have HLA typed and shown that it correlates, but it is awfully difficult to go back and do retrospective, especially with frozen cells.

DR. MULE: Okay, so I would like to close the morning session and remind everyone that the committee discussion and questions pertaining to this topic will be at 2:00 o'clock.

We are actually ahead of schedule. So, we are probably looking at 1:30. We will take a lunch break and we will reconvene here at 1:00 o'clock, and we will begin the open public hearing at 1:00 rather than at 1:30. Thank you.

[Whereupon, at 12:10 p.m., the meeting was recessed, to reconvene at 1:00 p.m., that same day.]

A F T E R N O O N S E S S I O N (1:05 p.m.)

Agenda Item: Open Public Hearing.

DR. MULE: We will go ahead and start the open public hearing. There are three individuals who have requested to speak, and we are allowing five to seven minutes for each.

Before I introduce those speakers, I just need to read the open public hearing announcement for general matters meeting.

Both the Food and Drug Administration and the public believe in a transparent process for information and decision making.

To ensure such transparency at the open public hearing session of the advisory committee meeting, FDA believes that it is important to understand the context of an individual's presentation.

For this reason, the FDA encourages you, the open public hearing speaker, at the beginning of your written or oral statement, to advise the committee of any financial relationship you might have with any company or any group that is likely to be impacted by the topic of this meeting.

For example, the financial information may include the company's or a group's payment of your travel, lodging or other expenses in connection with your attendance at the meeting.

Likewise, FDA encourages you, at the beginning of your statement, to advise the committee if you do not have any such financial relationships.

If you choose not to address this issue of financial relationship at the beginning of your statement, it will not preclude you from speaking.

So, our first speaker is Dr. Peshwa from MaxCyte, Incorporated.

Agenda Item: Statement of Madhusudan Peshwa, MaxCyte.

DR. PESHWA: I would like to thank the organizers for the opportunity to present here today. What I want to bring to the attention of the committee is the need to understand control manufacturing process variability as being a key facet in consideration of characterization and potency requirement assessments.

If you look typically at these complex biological products, the variability in the potency of the product can be categorized in addition to the inherent biological variability of the assay on two variabilities overall, the biological variability of the cells themselves, and the manufacturing process variability.

The biological variability is typically more difficult to control. The manufacturing process variability is typically less difficult to control.

Hence, when one talks about characterizing these products, one needs to ensure that there is sufficient attention given to developing manufacturing processes very early on in the process, that optimize for biological function of the intended cell product, as well as ensuring that the process is engineered to be robust and scalable.

If one looks at any generic cell based product manufacturing approach, there is a source of cells from where one identifies and isolates a tell type or population of interest that is subsequently manipulated in ex vivo culture conditions using a variety of supplements, cytokines, growth factors, leading to an expanded cell population that is delivered and intended for therapy.

When one looks at this process, the major portion of variability in this process comes about in the ex vivo manipulation phase.

Our approach at MaxCyte is to control this ex vivo manipulation phase variability by developing a technology solution which allows one to intervene by delivering plasmids, RNA, SR RNA, macromolecules, pharmaceutical agents, directly inside the cell using aseptic closed processes, hence bypassing the variability encompassed by using cytokines, growth factors and supplements in the media.

I will share with you two examples to illustrate

this point, and then talk about the impact from a product and process characterization perspective.

The first example is for a cell therapy product, and it is a model system that I want to present over here, where we are looking at delivery GFP as a model plasmid into cells, looking at assessment of transgene expression in the cells, as well as viability of the cells post-loading.

The technology platform that I talked about is robust. It is scalable from developmental lots as low as a few million cells to a couple hundred billion cells going forward.

Typically, in preclinical research and development, most of the experiments are carried on in small scale.

When one scales up the process to clinical scale, here in this particular example, representing a scale up from 40 million cells being processed to 10 billion cells being processed, one sees that there is comparability in terms of transgene expression as well as cell viability.

Not only is that the case, it is a flow based cell loading process. So, if one takes fractions during the unit operation of cell loading and evaluates the different fractions, one sees that there is consistency in terms of cell loading, viability, and transgene expression.

How does this translate itself into improving

opportunities for characterizing products. What I want to do is walk through a dendritic cell vaccine example, where one loads up tumor lysates into dendritic cells to use them as a vaccine.

The normal process of doing this is co-incubating dendritic cells with tumor lysates. So, we will compare co-incubating dendritic cells with tumor lysates with force feeding the tumor lysate antigen into dendritic cells.

Just to exemplify the situation, what I have over here is not dendritic cells, but it is a different cell type, loading fixated, high molecular weight dextran as an example to exemplify this effect.

When one looks at co-incubation processes, typically only about five percent of the cells pick out the macromolecule, and the intensity of the expression of fitsi, which is representative of the amount of loading per cell, is much lower than if you can force feed the cells. That is the key difference in terms of how, in this particular example, we are controlling the variability in the manufacturing process.

Looking at functional assays, evaluating interferon gamma production from naive T cells, comparing the co-incubation process to this forced loading process, one sees significant improvement in interferon gamma secretion indicative of TH1 type response being generated by

these dendritic cells.

That is further evaluated in an in vitro chromium release assay, indicating that the T cells generated with this vaccine have a higher avidity in recognizing and killing tumor cells.

That translates in terms being suggestive that, in an in vivo tumor model, this dendritic cell vaccine does lead to inhibition in the rate of growth of the tumor cell, as well as the same results appear to be replicated in an in vivo model of therapeutic vaccination. The preliminary results from here were published in the Journal of Immunotherapy a couple of months ago.

The point I want to make over here is identifying and establishing aspects that lead to variability in the manufacturing process up front allow one to essentially close the dots and look at various facets and give one an opportunity very early on in development to look at multiple variabilities in the potency and characterization metrics for subsequent evaluation through the clinical development process.

The second example is an example of viral vector production, where what one is trying to do is load up component plasmids for viral vector into a cell line, and getting the cell line to transiently express and produce the viral vector.

What one can see is, subsequent to optimization, here on the right panel, showing eight different experiments done at small scale, this is the coefficient of variability plotted in terms of viral titers, as measured in an infectivity assay.

Again, the tightness of the control is indicative of the robustness and the consistency of the manufacturing process.

When one scales up from a T150 scale to a cell factor scale, which is typical of a phase I type clinical lot, one sees that the titers are maintained and so is the consistency.

So, I would like to summarize by stating that, when one considers requirements for characterizing the potency of cellular products, one needs to pay attention to manufacturing consistency in all phases of product development.

What I have shown you is one particular example of a technology solution that we are utilizing at MaxCyte. There are other such similar technology solutions also available.

I would like to end by saying, if one can understand and control this processing variability, one would hopefully be able to lead to more effective product characterizations. Thank you for your attention.

DR. MULE: Thank you, Dr. Peshwa. The next speaker is Dr. Leslie Wolfe from Genzyme.

Agenda Item: Statement of Leslie Wolfe, Genzyme.

DR. WOLFE: Good afternoon. Thank you for the introduction. My name is Leslie Wolfe. I am an employee of Genzyme. I am here today to give you a quick overview of our lessons learned on potency assays in terms of potency assays for cellular therapies.

As a little way of background, we have been working on cell therapies for a while at Genzyme, and we have come up with some understandings that I have heard a lot this morning.

A lot of these products are autologous. They have very small batch sizes or lot sizes. They are variable from patient to patient, and the final product itself has a very short shelf life.

For example, carticell, which was the first product on my Genzyme experience list, has a three-day shelf life. It is autologous cultured chondracytes for implants in cartilage defects of the femoral condyle.

At Genzyme, we have been marketing this product since 1997, and over 11,000 patients have been treated with it.

We also have an epicell product which is cultured carotinocyte autographs for extensive burn victims, greater

than 30 percent total body surface area, and we have treated over 1,000 patients there.

We have two major development programs in the cell therapy area, a second generation carticell, which is called MACY, or matrix assisted chondrocyte implantation, which is really chondracytes embedded on a collagen membrane, and a cardiac cell therapy program, which is the culturing of skeletal myoblasts for use for myocardial infarctions.

As an example of one of these products, I am going to focus today on carticell. However, a lot of the things that I am going to go through will apply to all of our products.

Carticell starts with a defect in the femoral condyle of the knee. The surgeon takes a healthy piece of cartilage and sends the biopsy to our cell processing facility, which is in Cambridge, Massachusetts.

This is a GNP facility with well controlled systems, raw material acceptance, quality control assays, and validations, et cetera.

Four to five weeks later, of culturing of the chondracytes, we produce a cell suspension, which is shipped back to the orthopedic surgeon, who then implants it in the defect with a periosteal path, and the recovery is the production of hyalin-like cartilage in that area, and specifically can be marked by the expression of type II

collagen in aggrecan, which are markets for the production of cartilage.

When we take the biopsy and release it from the matrix and culture it in our facility, we are culturing it in a monolayer condition, and then producing the product for release.

However, at that state, the cells undergo what is known as a de-differentiation stage. What we mean by that, in this particular case, is you no longer see production of type II collagen from these cells. You now see expression of type I collagen, and then a small amount of aggrecan, and aggrecan shuts off.

Now, this would be in the cell culture portion. We are able to mimic what would happen for the re-differentiation stage by a couple of assays that we have produced.

However, these assays that I am going to describe actually take some significant time in terms of actually producing them. So, they are not done at lot release.

The first lot, on the left-hand side, is our in vitro assays, which they are three dimensional systems using agarose cell cultures or alginate cell culture.

We also can re-implant the cells, obviously, back into an animal model, where we have found much difficulty in defining these animal models and getting them to work for

us, and of course, the patient could be a model of the re-differentiation.

Ultimately, after this lag time in these conditions, the cells will re-differentiate, and you can then pick up the markers that you were looking for, which is the expression of type II collagen and aggrecan.

Here is an example of the agarose system. Here is the monolayer culture of the cell that is in our cell culture facility, where you move those cells into the agarose system.

Three to six weeks later, you see that these cells form these great chondrocytic clusters, and the orange stain is acridian orange, which picks up the expression of aggrecan in the clusters.

Do the same type of assay. This time we put it in an alginate system, and we put an antibody in for type II collagen.

Here it is two weeks in the system, here it is six weeks later, and you start seeing, after six weeks, you are seeing the expression of type II collagen.

Now, those are examples of how you do it on the protein side, but take a look at it on the gene expression side.

We would put the cells into our alginate model, which is shown here. We then do an RNA extraction and run

RNAse protection assay, looking for the expression of type II collagen and aggrecan and other proteins.

This is a time course experiment, where we have set this up, shown up here on top. If you take a look, it really takes almost 15 days to start seeing the re-expression of type II collagen from the cultured chondracytes.

So, we have gone about approaching this cell product, this is the carticell approach. What we can do is characterize these in the cell culture facility.

We monitor quite closely the cell yields from the cultures and viability and the growth metrics. Obviously, if growth metrics aren't met, we know there is something wrong with the culture. Obviously, we are also monitoring the cell morphology prior to lot release.

What we can do is monitor the potency using our agarose and alginate systems, which have been developed and validated, but these take three to four weeks to complete, and cannot be done at time of lot release.

For the aspects to the potency of carticell, though, it is important to point out that we really feel that the control of the process is key, the cell expansion process is controlled first by quality and GNP systems. The process is the products. We want to make sure we are following all of that very consistently.

The process validation has been shown by using both the agarose and the RNA assays, and we continually monitor this ability for ourselves to de-differentiate and re-differentiate by running a whole bank of characterized reference strain cells of chondracytes in our facility on a quarterly basis through the manufacturing plant to confirm differentiation.

Then, of course, if any process changes are brought about, those process changes would be validated using the reference strain cultures, and redemonstrating the ability to re-differentiate.

So, a number of discussion points just to kind of finalize. We appreciate the opportunity to present today. These are the kinds of things we are kicking around at Genzyme, but we are taking a look, is it realistic to expect expression of potency markers from de-differentiated cells at time of product release, or obviously that you need to go after some kind of validated surrogate if you can find it.

Can we reliably connect the surrogate potency measure and de-differentiated cells at product release edit to an eventual clinical outcome. That is not known.

Then the third bullet is, obviously, depending on the assay, if we did have a potency assay that could be done, either in an ELISA or a PCR type of technology, would it be quantitative, or is qualitative measurement

sufficient, depending on the assay technology.

So, just to wrap this, we feel it is a really clearly challenging subject the panel is addressing today. We appreciate the opportunity to present, and we encourage further dialogue. Thank you.

DR. MULE: Thank you, Dr. Wolfe. Our next speaker is Dr. George Ashkar.

Agenda Item: Statement of George Ashkar.

MR. ASHKAR: Good afternoon, everybody. My name is George Ashkar, retired physicist. I have no relationship with any company except my retired wife.

Since I was five years old, I was determined to find the cure for cancer. I was involved with finding a cure for cancer. All my life I was involved with finding a cure for cancer.

Finally, I have the full developmental mechanism of cancer, and I developed a particular method. I can treat cancer 100 percent.

I won't disappoint you. Cancer is not a medical problem, period. Cancer is physics. So, physics I could do. I could find a treatment.

So, I want to start from the beginning. So, what is disease. When bacteria, virus or bacteria, microorganisms invade the human body, they start to destroy cells.

These destroyed cells give you symptoms of the

disease. So, to cure disease, we have to kill bacteria that cause disease, or eliminate bacteria. That way, we can cure disease.

So, when I finished my doctorate degree, I decided to be fully involved with cancer research and find a solution for it.

Before starting involvement in cancer research, I wanted professional advice from medical doctors to study what is cancer, and then how to cure it.

So, I asked a doctor, do you know, what is cancer? He said no. I said, do you have a cure for cancer. He said, no.

I thought maybe I am asking the wrong person. So, I asked what specialist you are, and he said, expert oncologist.

I was surprised. How can you be an expert oncologist when you don't know what is cancer. So, what exactly expert oncologists are doing.

When they say that the disease can be cured by medication, by eliminating the cause, so they said, this is the chief way, and people doing that, they don't have any medical education or medical background.

So, they decided to make different ways. The different way is this. Instead of eliminating carcinogens, which cause cancer, they started to eliminate cancer cells,

which is the victim of the carcinogen.

So, they destroy cancer, and they find that, in the body, there is no cancer, and they declare like a hero, you are cancer free, we have cured your cancer, but they never say your body is full of carcinogens, because they didn't treat carcinogens.

So, after a few months or years, the cancer comes back. Then they blame the victim. They said, you did something wrong, so the cancer comes back, but they don't say that carcinogens were inside the body and came back.

So, what is my treatment method? It is to take our carcinogens from the body. Since carcinogen is not viral or bacterial -- it is chemical -- the only way to cure cancer is to take out from the body this carcinogen, chemical. So, I am doing that.

I find out that 100 years from now, back 100 years, everybody was trying to destroy cancer cells, which is not the problem. The problem is carcinogens. That is the reason there are no cures.

Now I would say the biggest medical fraud for the 20th century is gene therapy, because gene therapy absolutely has nothing to do with disease.

Genetics is only the physical construction of the body. So, this is not enough. Now going to the next step, super medical fraud, stem therapy, which has absolutely

nothing to do with any diseases.

So, if you are going to cure cancer, you have to take out the carcinogens. If you are interested to know more about cancer mechanisms and treatment, I have my web site. I give you my web site. You can get more information from there.

I would say -- I would recommend -- the advisory committee to shut down all research centers, all institutions involved in cancer research.

Cancer is a very simple disease. It is much simpler than flu, because cancer is not spread from one person to another person. So, it is easy to cure.

I have cured already about 200 people and it has never come back, and myself, finally myself, in September 2003 I had pancreatic cancer.

So, after a five-and-a-half hour surgery, they have not been able to save my life. So, they tell my wife to prepare a funeral for me.

So, the five doctors decided what to do with me. So, they say the best research to cure you is chemotherapy if you want to do that. I said, thank you, but traditional therapy and chemotherapy can only cause cancer. It cannot cure it.

So, I said, thank you, I will go home. So, I treated myself and I am here. So, if you have any questions?

DR. MULE: Thank you, Dr. Ashkar. We have a few minutes if there are any additional comments from the area. We have a couple of minutes. Otherwise, we will move ahead.

Agenda Item: Statement of Vladimir Slepushkin, VIRXSYS.

MR. SLEPUSHKIN: Thank you, Dr. Mule. I am Vladimir Slepushkin from Virxsys Corporation. I would like to address one problem that unfortunately was not addressed during this hearing.

That is the kind of products that our company is making. It is an ex vivo transduction cell product that is transduced with lentiviral vectors.

I think that also the same problem concerns the retroviral vectors, and the problem is that we have kind of a two part product. One is vector and another one is autologous cells from the patient.

I would like the committee to address the question in this aspect, the aspect of potency. Would it be good enough just to have a quantitative biological potency assay for the vector product, and then have an analytical assay for the final cell product. Thanks.

DR. MULE: Thank you.

Agenda Item: Statement by Robert Bard.

MR. BARD: Robert Bard, Astrom Biosciences. Just a comment for the committee as they review this. We have

discussed autologous cells. We have discussed the limited time frame that we have for our products for testing.

We have not discussed complex cell mixtures, where we are taking bone marrow asferants and growing them up and taking the whole mixture.

I would like you to at least think about that there are products that are not homologous products. They are heterogeneous and they have a lot of differences from what has been discussed today. So, I would hope you would include that in some of your thoughts. Thank you.

DR. MULE: Okay, we are going to move ahead now and tackle the questions.

Agenda Item: Committee Discussion of Questions.

DR. MULE: What I would like to do now is have the committee have ample time to comment and offer advice and so forth on the questions. If time permits -- and I think it will -- then we can have a couple of comments from the audience.

What I will do is raise the comment and follow up with the question and then ask the committee to provide feedback.

So, the first is assay design and validation. In this case, assay validation is the characterization of assay performance that allows the significance of the values obtained in an assay to be evaluated.

Biological assays are particularly prone to assay variability, and therefore it is necessary to design and execute the assay, to reduce variability as much as possible, while providing a valid measure of the reproducibility of potency estimates.

So, the question here is, please discuss assay design schemes that would be necessary to successfully validate biological assay, and allow accurate quantification and interpretation of the results obtained. Please consider in the context of cell and gene therapy products.

DR. HARLAN: I think the silence is that we heard such a wide variability, even the last question, such a wide variability in these products that it is hard to generalize testing schemes for a validation assay. The products are so different, and it would have to be individualized, it seems to me.

DR. WITTEN: I think we recognize that they have a to be individualized, and that is what we face every day in our review.

So, perhaps if you can't give us something that would cover the waterfront, which I can imagine you could perhaps just speak to your experience in a more narrow area, and what you would suggest.

DR. ROCKE: I do think there are some general things we can say, though. One is, we saw one example of

what some people call ruggedness testing.

That is looking at the performance of assays under deliberately perturbed conditions to see what factors would change accidentally or randomly in the course of performing the assay are important sources of variability.

I think that is just a completely critical part of the development of any assay that you can trust, is you have to understand what the sources of variability are.

We saw one very good example of that. I don't think we saw the data, but we saw the design, which was very nice in terms of source of material, day to day variation, operator to operator variation. So, I think that is a general principle.

I assume that we are supposed to leave out of the discussion of question one is whether what you are trying to measure is actually of any consequence, or is that part of this also?

DR. .MULE: Actually, we will come back to that, I think, as part of question two.

DR. ROCKE: So, mainly this is technical. There is a lot of experience in developing assays, a lot of it coming out of NIST from the days when it was the National Bureau of Standards, anyway.

Anybody wanting to deal with this could probably read Jack Yuden's(?) from a long time ago on the development

of assays.

Basically, you need to make sure that it works under a variety of conditions and you need to make sure it works in a variety of labs if it is going to be something that is generally useful.

Perhaps if it is just manufacturing QC, you don't need to worry about if it is going to work everywhere. Otherwise, you have to think about interlaboratory studies and robustness studies, and this is old, old stuff.

This is, I am thinking, 1956 for the little book of Jack Yuden's. This is stuff that we all knew once and then a lot of people have forgotten this.

We know how to do this. We know how to develop assays and validate assays. It is just that not everybody takes the trouble to do what they need to do, because it is a lot of work and it can be quite expensive.

DR. PLANT: Thank you for invoking NIST or the old NBS for all of us. That publication predates me by some number of years, thank goodness.

I think you are right, that the general rules haven't changed, and there is sort of a philosophy for assay development that invokes those rules, like how do you -- just identifying what the sources of variability are.

Of course, biology, quantifying biology is sort of a new concept anyway, and it is certainly new for NIST.

Being part of NIST right now, it is a challenge to figure out how to take those physical principles about assay development and reproducibility that everybody knows so well at NIST and sort of move them into the biology realm, and what is relevant to biology.

I have a couple of things that I would like to say with respect to that, one being that a very important source of variability in biology that we have to recognize, and I think as biologists sometimes it is easy to forget because it is so close to you, is just that natural variability in cell response from cell to cell.

Of course, that is going to be compounded from patient to patient, but it is also going to be compounded from minute to minute, perhaps, and also from environmental condition to environmental condition.

So, one of the things we have stressed in our work is looking at cell to cell variability and finding assays that actually allow you to examine that variability.

What we don't have right now are easy assays that allow you to examine variability in time. So, I think there are some really important new measurement technologies that need to be developed and made accessible to everybody, that will allow us to define and measure some of the more complicated aspects of biology that might be sources of variability.

So, one of the things that I would also like to say is that NIST is working on this problem. One of the ways that we can most effectively work on this problem is with input from the user and needer communities, which can really help guide our programs and provide advice about what are the most important needs, and what kinds of technologies and other measurement tools might be necessary for addressing them.

That includes standards development, but also reference protocols, new measurement technologies, and various other things.

So, any suggestions that come out of this, I will certainly keep my eyes open for those kinds of things, but if you think of things specifically that would involve advanced measurement methods or standards, please bring those up, because those are things that NIST can address and should address.

DR. COUTURE: I am not an expert at designing experts and I tend to agree that I think investigators and companies have a pretty good feel for how one goes about controlling and establishing an assay and reducing variables.

There are technologies that are yet to be developed. We didn't see any micro array data, micro RNA data or any such, or proteomics today, and that is all

emerging technology, and I am not sure how that would apply to the technologies I am developing, but I know sooner or later that technology will emerge and prove itself useful in the field.

I am actually more concerned about how we apply these assays and what it is we are actually testing, and what it is that we define as I think -- I think what it is supposed to be is to effect a given result, which is what potency is all about, and I think that is probably what needs to be discussed.

We heard today a spectrum of how people are interpreting that definition, and similar genetic therapy type technologies, where the expected result is the nucleic acid ability to express an mRNA, to the effect of a protein product on a target cell.

Both companies could, quite frankly, have taken exactly the same approach and limited it to mRNA. Both of them could have gone all the way to asking, does this protein do something in particular.

I am more concerned about establishing precedent and having lots of companies go down a pipeline or a pathway of developing assays that may or may not be the assays we want people to perform to release a product. It is just an opinion. I think it is worth discussing.

I tend to agree with the first speaker, that a

nucleic acid technology is expected to produce a particular mRNA. That is what you want your nucleic acid to do.

What happens in the cell after that may or may not change from patient to patient. That would be figured out during clinical trials, whether that expression correlates with activity, so you can go backwards and do that correlative study maybe retrospectively, but certainly during your clinical trials.

I think coming up with almost random markers to trace on a cell or to ask for biological activity of a molecule that is well characterized, well studied, and is not actually what the company is actually producing doesn't make as much sense to me.

DR. MARINCOLA: I think that is a very important point, because of course if you look only at RNA, it is always a much easier and simpler way of comparing products, lots of products, with each other.

The other important concept is that if you start looking at micro arrays or situations where you are really going to be looking at ways to validate the products, not based on one gene but in more complex systems, the only way I can imagine is only using RNA, because I don't think there are proteomics tools that allow now days to really look at the big picture and be as reproducible as micro arrays or something.

So, it is a really major choice, and I think it is tough to really say which one is the best approach, but there is kind of a nice concept, just to look at RNA, or at least the direct byproduct of what the product is supposed to do, rather than just doing all this testing, and then eventually the study, I mean, the bedside to bench, tells you whether it was important or not.

DR. MULE: It seems to me that one of the hurdles ahead in validation and potency assays really lies in the area of cell based vaccines where it is patient specific.

In other words, we heard several speakers talk about vectors to deliver proteins. It seems to me in that case -- and we saw some, in my mind, really compelling validation assays with respect to locking in a cell line or clone that one can use to transduce transfect, and then follow that up with a commercialized or in house created assay measure that is very quantitative, in this case ELISA, for the release of protein.

To me, that is the holy grail, if you will, of where we need to go with personalized types of cell based therapies.

Now, the hurdle, of course, is a scenario -- and we heard a bit of this from Dendrion -- is a scenario where it is patient specific.

One needs reliable surrogates, in that case, then,

to measure activity, and we heard that it is very difficult. One is not able to have a cell line that can span all patient haplotypes, for example.

One is not able to have an effector cell or a cell for the readout system that is a clone, that can recognize whether it is an antigen or what you have.

So, in my mind, that particular scenario is the most difficult in validation and potency assays and maybe, as we go forward, we can tackle some recommendations of how we might take what we know from the gene therapy transduction field and maybe move that into areas of personalized cell based therapies, and maybe we can get some comments from the committee about that.

DR. PLANT: I wonder, when you talk about certainly the variability in the patient derived cells, how important it is to also have touchstones on the assay itself, so that you can validate that you have run the assay correctly today with this patient's cells, and you can compare that with an assay that you run tomorrow with another patient's cells, for example.

In that process, if we thought about measurement tools that might help to standardize or provide reference for that process, if the use of, say, standardized cell lines might be useful, such that one could take out of the freezer cells that have -- maybe several cell lines that

have different ranges of phenotypes that are of interest, and run those in the assay in parallel with the patient cells, in order just to validate that assay is running correctly, if that is necessary, is my question.

DR. MARINCOLA: We have been trying to deal with the scientific level, not at the practical level, by doing exactly what you are talking about for micro arrays.

Every 25 slides we repeat the same combination of reference systems, which I really liked a lot of these concepts, which is always the same unchanged.

Then a cell line, and I guess you could use different kinds of cell lines to see how much co-reference concordance you have in your experience.

So, in spite of all the changes that are in different lots, you can quantitate how reproducible your experiments are, even yours, in this.

So, I think it is something that can be done, and it really allows you to have a perpetual -- also, for example, in our case, as a reference system, we use peripheral blood lymphocytes from people.

There is a nice paper from Pat Brown where he showed that if you took peripheral lymphocytes from five donors, put them together at any time, as long as they were healthy donors, you are going to find always the same results.

So, if you use that, you can use that until it is finishing, but then you can go back and make more and test against the other. So, you can perpetuate your reference.

The third way in which it can be done pretty well, and you can quantitate exactly your reproducibility, even in high throughput systems so that you can then compare different cell lines with different products, whatever the variance here -- and we might not be able to tell which of the variants are important, but at least you can document. You can go back gene by gene and say, well, you know, these guys did better, what happened, you can do some sort of supervised retrospective analysis.

DR. PLANT: So, one question I would have, coming from NIST is, is it feasible or is it really a large burden for each individual laboratory or company to devise their own cell lines, or would it be helpful to have a repository of validated reference cell lines. If such a thing existed, would that be a useful thing to the industrial community?

DR. MARINCOLA: That depends. In my experience, if you use one cell line, it doesn't really matter what it is. Then what you are going to find -- the only thing that really matters is how much you can reproduce the data compared with your reference system.

Then, even when you use your test samples, whatever the reference is, it really doesn't matter what the

reference is, as long as it is always the same. So, instead of using housekeeping genes, you have a reference for a single gene.

The only disadvantage of that is, if some genes are not expressed in your reference system, you really don't have a good control for that.

So, it is nice to have some extra controls for different kinds of cell lines, but I don't agree that maybe you need that many.

I guess, for example, if somebody is making some particular product that involves dendritic cells, maybe you want to add every 25 or whatever the cyclic control, to be more relevant than just having something random.

Otherwise, really what you are getting is just some way that you can reproduce the same experiment over and over, like you use beads and a FACS, so you know the diameter is never going to change.

So, the principle may be simpler than really having a large panel of cell lines. What we found is that, if you do that, you increase the variance anyway, because it depends on how much you culture the cell lines, in what conditions, what percentage. Then you change their data anyway.

That is why we ended up not doing or using the Stanford system with many cell lines, because actually it

was more variable, and even Pat Brown actually mentioned that.

DR. CORNETTA: I think what I am hearing, too, is that I think the cell lines are interesting. To have a series of them may be useful, but it is still, cell lines are usually not necessarily a predictor of the biologic activity that we are expecting for many of these products.

For many of these products, the real biologic activity may only be really assessed in the patients or in some animal model, and I think what we are hearing today is, how do we move to understand the actual activity in the patients that are being treated with some surrogate model, and where do we broach that, and what is enough information to make that.

I think, while we heard, for example, mRNA might be a nice way to assess a plasmid vector in terms of its activity, I think one of the things that would run through my mind is, are there formulations from time to time that may have additional DNA forms, or there may be something about a lipid component or some other process that almost gets into the purity of the material that might, for example, get a signal transduction activation in those cells or something else.

So, in the primary cells, their ability to engraft in an ex vivo situation may change their activity in the in

vivo situation, and a cell line or a QPCR assay may really not be able to detect that.

I think the real challenge is how do we go from understanding -- I think we saw a nice example today where they are trying to correlate their phase III data with those assays.

That may be an area where we need to continue monitoring or some planning in that process so that, as folks are moving into the phase III trial, that it is not just a matter of trying to compare what is happening with their formulation and that specific assay, but that specific assay in a cell line may still not be very representative with it. I think the problem is that animal models, as we are hearing, are usually very difficult to standardize.

DR. ROCKE: On the cell lines issue, I think one of the problems with that is that there is so much variability that is caused by the culture conditions, the particular history within the growth situation, that it may not be possible, really, to standardize it the way you can some things.

Maybe the important thing is to use internal controls all the time, meaning, if you are trying to detect something in an altered cell type that you have, an unaltered cell type to compare it with, so that you can look for differences, that seems to be the most stable thing.

This especially applies in areas like gene expression arrays. In fact, in a certain sense, these are never quantitative in the complete sense. That is, nobody can take the spot intensities on an Affametrix gene chip and turn that into picomole concentrations of the species of messenger RNA.

The relationship between the concentration and the intensity is different for every probe. So, you would have to have, on the latest affametrix gene chip 1.3 million standards curves in order to be able to actually produce purely quantitative numbers.

What you can produce is changes, and those are best changes that are internal to the lab, to the project. It is hard to just compare that to something from the outside.

What you would have to do, if you had these standard cell lines -- and of course we do have standard cell lines -- is to take that cell line and do something to it and not do something to it, or do three things to it and compare those.

So, it is tied in with the design issues. As for the quantitation, I wonder exactly, in terms of validating an assay, what does it mean that it is quantitative.

I think gene expression arrays are quantitative in a certain sense. You have more of a particular transcript,

you have a brighter signal in general, and you get a number out of it, but it is not quantitative in the sense that you can tie it to an absolute concentration.

DR. HARLAN: I just want to echo Dr. Cornetta's point, that the danger in establishing a surrogate potency assay is that we start focusing on that surrogate potency assay and it is not what we are after. We are after a clinical benefit.

We don't know that these surrogate markers are one to one aligned with all that we want to achieve. In fact, the literature is filled with examples where we think we are shooting toward one target, and we are wrong.

That is why I think we need to keep an open mind. Gene array studies, all of these things are nice and quantifiable, but they may not be the direction that we are shooting for.

DR. CALOS: This is sort of obvious underlying I think what we are struggling with, but kind of the harder it is to formulate the assay, kind of what it means is, you don't understand the science of what you are doing.

If you really understand everything going on in your product, and what you are doing, then it is not hard to come up with an assay.

So, we are talking about products or approaches nowhere there are an awful lot of unknowns when you are

doing some of these cell therapies.

You don't know what the active component is. Therefore, it is very difficult to come up with an assay. You don't know what to assay.

It is sort of symptomatic in a way that these are not the final products one would like to see, because these are very crude products where we don't understand what the active component is. That is why it is hard to figure out the assay.

So, I think the assays are always going to be unsatisfactory as long as you really don't know what your active component is, and how your product is actually working.

We are in this terrain now, and it is, I guess a little dangerous in a way, because we are throwing around things, very complicated biological therapies, with only a very partial understanding of what they are doing and why they are doing it. So, how can you -- it is hard to make a simple assay for it.

DR. URBA: I think at some level we are trying to determine the mechanism of action of the treatments at the same time we are asked to do potency, and they are different.

We use drugs all the time we don't know how they work. There are patients who get treatments with all kinds

of things that we don't know how they work.

We think we know what their target is but we don't know, we don't ask them to figure out what the target is, and have you affected the target it. We ask them, does it work and can you make it the same way every time and does it have that potency.

Clearly, the Dendrion story will make the water a little murky because you don't have a well defined product, but perhaps you have something that works.

Are we going to say you can't use it until you figure out how it works and you know how to measure exactly what it is that tells you it works, or are we going to let patients get it when we know it works and there is a surrogate marker that you can -- CD54, I don't know, is it something else.

I don't know, but I would love to know the mechanism of disease, but we are not going to get there any time soon. so, we are going to have to somehow be comfortable with unsatisfying answers to those questions of potency and correlation, and some of this is going to be a little leap of faith that that mRNA put in the right place is going to do what it is supposed to.

If we are going to rely on having an assay that measures whether Tricon all three co-stimulatory molecules and the antigens are produced in the appropriate antigen

presenting cell in every patient that gets it, we are not going to have a product that anyone can use.

DR. COUTURE: That is why I would certainly agree with what the FDA has been saying all day, is why you should start developing these assays early on in your clinical studies, because we are really talking about correlation with clinical outcome.

Regardless of whether it is CD54 or mRNA expression, if you can't correlate that with clinical outcome as a company, you are going to have to go back and address that and come up with something different to test perhaps, or perhaps a multiple thing.

I agree. I guess my perspective is to keep it simple. So, there is no reason to come up with very complex downstream biological assays that are three or four steps removed from what your product is actually supposed to do, which again in a gene therapy context is expressed in mRNA. It is not supposed to make VEG-F, make cells migrate.

That is something that VEG-F does in a patient. That is not what a piece of DNA is supposed to do. If you can't correlate that expression of that mRNA to activity in a clinic, then you have got a problem.

DR. HARLAN: It sounds like we are coming to some consensus here to me. I think the whole issue is that we can't define potency, that what we are really talking about

is product characterization and then testing it.

The potency is what happens to the patient, and we don't know what the potency assay are yet. We don't know the science well enough.

I am suggesting that we are all saying, let's characterize the product as best we can, make sure it is the same every time, and then test its potency.

DR. CALOS: I think it indicates that, in some ways, we have to depart -- we have a complex biological product and before it was a simple product with a small molecule drug.

In both cases, what counts is the clinical potency and, in the case of the small molecule drug, they had no problem doing the lot to lot consistency and all of that, because it is a small molecule.

Now I think what we are facing is, do we have to have a different standard. You are not going to be able to get that kind -- you know, if you don't know the mechanism with the biological, you can't characterize the consistency and all that, because there are just too many things to measure. You don't know what you should be measuring.

So, are we saying that we have to depart from that standard, that we are going to let people use those drugs, some mixtures where it is really not definable right now what the potency is, because we don't know how it works, but

it does work.

I mean, it is problematic because, in terms of consistency and all that, how do you get it if you can't define how it works. How do you get that.

I think it is really -- you know, one is talking about the possibility of a different standard for biologicals than you have for chemicals.

DR. MULE: Again, I think it is important that surrogates be used in situations where we have an undefined cell based product that is individualized.

The reality is you are almost held hostage by the amount of tissue that you have available, the quality of that tissue based on a patient's treatment course prior to the acquisition of that tumor or tissue.

A lot of variability goes in before cells are even presented to an individual, to prepare a product. In that case, the luxury is not there where you have defined clones of readout cells or cells that can consistently answer the mechanistic question, how is this vaccine presenting an antigen to T cells or to B cells to produce antibody.

It is not potency. It is actually validating the concept of, if you are going to put a crude vaccine, cell based vaccine, into a patient, how do you characterize that vaccine unless you have surrogates. Are there other ways to do that in the absence of surrogates.

DR. HARLAN: Larry, you asked the question earlier about the Dendrion product. If it doesn't achieve a CD54 standard, do you tell the patient, sorry, we can't give you the cells back.

If we say that, how do we know we are not throwing out the baby with the bath water. Maybe that patient that didn't respond with CD54 will have a very nice response.

I mean, we have to do these studies before we say we have the answer with our surrogate end point, it seems to me.

DR. URBA: I guess I would just like to address the idea of a different standard. I don't think I am talking about a different standard.

I am an oncologist. We give people drugs. Those drugs are in a vial. People don't ask if the adriamycin inhibits topolysomerase and binds to DNA and alkylates. We don't ask that. We just give it because people did studies and we know it works, and because the company knows how to put the same amount of the same drug in the vial every time, so that when we open it and give it to a patient, it helps.

So, I don't think I am asking for a different standard. As complicated as that mixture is, if the process of doing A,B and C, whether it is with whole cell vaccines, with transfected cells or whatever, if that process leads to a product that provides patient benefit and you can control

the process, and you can find a marker that is present on those cells or something that they do all the time, I think we are talking about the same thing.

DR. PURI: I would just make a comment about the comment Dr. Harlan made, that what happens in the patient is not efficacy, that is efficacy.

The potency, as Dr. Urba is saying, potency is a measurement of characteristics of a product that you can measure again and again, lot to lot consistency, some characteristics that may or may not relate to what happens in the clinical outcome. That is a different issue.

DR. SIMEK: I would like to take that even a step further. You are all saying the same thing. I mean, the difference with small molecule chemotherapeutic drugs is that you do manufacture it consistently.

You have a structure. As long as it is not degraded, it has that structure, and you say that it will work.

For the example that is the most difficult example, which is the autologous cell product, number one, that product isn't pure, number two, that product isn't consistent from subject to subject because each subject is different.

That product isn't even consistent when you make lot to lot from each subject. So, how will we know. Again,

efficacy is the end.

What we want is some parameter, which is characterization, because we need to know, in order to assess clinical data, are we giving as consistent a product as possible.

If the product is frozen, even if it is a cell therapy and it is frozen, if it is frozen and a clinician wants to use that and we know that the stability is for two weeks and they want to give it at 15 days or 13 days, is it as consistent and stable as it was on day one.

I mean, we may not have something that is a direct correlation to what we think is happening because we don't know what is happening, but potency is essential, at least for these products, and much more complicated than a drug.

We are relying on it to answer a lot of questions. I think what you are saying is really useful to us, and what I hear you saying is that it doesn't matter if it directly correlates as long as -- I think I am hearing you say that it is a marker of some sort that you can at least measure consistently. So, it is almost like a process validation, in a sense, but it is a characteristic trait. Is that right?

DR. MARINCOLA: Actually, I would like to expand on that. I think that is the beauty of the concept of potency, is this basically is a measure of viability which, you are right, may not be necessarily correlated with the

clinical outcomes, but definitely allows a better interpretation of the results.

IF you know that something is always the same, it is much easier to compare the outcome than if something is very variable. It is a measurement of variability.

That becomes really important in complex systems, where you really don't know what you are measuring. So, going back to what Dr. Calos was saying, you really have no idea.

In that way, I have to disagree with Dr. Rocke in saying that micro arrays are not a competitive system. They are very competitive.

Affimetrix is going to have a problem because they don't have a co-hybridization system. So, they don't have a reference that is applicable to every single system.

There are ways in which you can actually very accurately, in a semi-competitive way definitely come up with a measurement of what is different and what is changing and you can keep a record of what you give that eventually you can use and go back if you want to discover a different approach, come back from the bedside to the bench and say, well, you know, maybe it was not CD54, because in that case it was a lucky break, an impressive prediction marker it worked, but in many cases you might be able to find it the other way around. So, potency is a very useful concept as a

measure of the variability, and that is what I think it should be.

DR. MULE: With respect to genomics and proteomics, we will be coming back to that with question three. So, save your thoughts on that as we move ahead.

DR. ROCKE: I think the situation here is very much ont analogous to a vial full of adriamycin. It is like saying there are 15 ccs of fluid in this, I don't know what is in it, but there are 50 ccs of fluid in it, and I am going to give it to the patient.

You wouldn't do it. With a small molecule, you can say how much of the active principal is in that. In some of these complex therapies, we don't know what the active principal is.

If you just say there are a bunch of cells here and we can count the number of cells and it is the same every time, that is like saying there are 50 ccs of fluid that you are giving to the patient to treat their cancer.

Then that means is, if you have absolutely no idea how it works, there is no way of deriving a potency assay. It has to be based on some at least plausible biological theory of what is going on.

If it is a gene and if it is going to make an mRNA species, then we can assay the mRNA because we know at least what the path of causality is that we are trying to impose.

If all we are doing is handing cells back to the patient and we don't have any idea what it does, then we are helpless.

The other thing this means is, somebody earlier asked, what about surrogates when you don't know what they are. So, you run it through CELDI(?) and you have a mass peak at 1,642 daltons, and you say, wow, that is a biomarker for something, or that is a measure of potency.

Not on my watch, if I have anything to do with it. If you don't know what it is, if you don't know what the therapy is, really, and you don't know what the marker is, there is no biology in it. It is just -- there are just too many ways to go wrong, and we all know stories of how that can happen.

I think that we have to define measures of potency based on a biological theory that has some plausibility. It may be wrong and it may be that CD54 is only correlated with the potency and not really measuring the potency, but you have to do the best you can.

That means acknowledge that it is not a small molecule, that it is complicated, that we don't understand all the biology, but do the best we can to establish a plausible mechanism and measure components of that mechanism.

DR. PLANT: Also, I think to add onto that,

because of that ambiguity, something that Denise mentioned early on was the idea of having maybe more than one marker that you measure simultaneously, because one could be wrong.

DR. CHAMBERLAIN: I think what ties in with that is, in a lot of these cases, particularly with early stage clinical trials, you are still testing a hypothesis.

An example might be the hypothesis is that monocytes are going to be a very useful cell to elicit immunity, in which case your measure of potency is to say, how do we define monocytes these days. It is usually just a collection of cell surface markers.

So, you isolate those cells, you see, do they have the standard array of cell surface markers, and to me that is the end of the story.

You can then go on and test those cells, are they working. If not, then you have to go back and change your hypothesis and start looking for a different cell type.

You want to make sure you have isolated these blood cells and you don't miraculously end up with a collection of myoblasts rather than monocytes or something like that, and that should be a fairly straightforward assay to do.

If you can then see efficacy in the clinic, then I don't know how much more potency you would really need in a case like that.

DR. MULE: Let's move on to the next question. In fact, it is relevant to our discussion here. The second question is the correlation studies, what data and study considerations are necessary to demonstrate valid correlations.

So, assay design, statistical analysis, controls, limitations, and so forth, it is really continuation of our current discussion. We have it up there on the screen.

DR. ALLAN: I get confused easily. When I think of potency, I think it must be doing something good or it wouldn't be called potency.

What I am hearing is, you have these assays that have no real relationship with potency, other than the fact that you think they might.

In other words, just hearing about monocytes, well, we think that monocytes are probably the way to go. Therefore, it is a potency assay, when I don't see it as a potency assay. I see it as a marker.

So, to me, it is like you get closer and closer to the efficacy with your assay systems, and it gets dirtier and dirtier and dirtier. It gets muddier and muddier and more expensive, and you are not really sure what you have got.

It seems to me that the closer you get to that end, the better off you are. You know, what people want to

do is, they want to go to the other end, because it is cleaner, it is simpler and it is cheaper.

So, you see that happening with some of the companies because it is like, well, I just want to do messenger RNA because it is clean, it is simple, I can look on the thing and you have got a nice curve and you are done.

I am not sure that is potency or not, or even reflects potency, because things can be so complex, especially when you get into cellular systems.

So, I get really confused when I go through all these different assay systems. To my mind, it seems to me that the closer you get to the efficacy end of it, the better off you are.

That can be very difficult in a cellular based assay, so we are into this what correlate, correlations with running a messenger RNA system or something else, and I find that very difficult.

Again, it seems to me that the hypothesis or the science driving the process is the following question. We have a cell which we believe is going to present an antigen that is going to trigger the immune response, then I think the validation should show that.

Maybe not necessarily have a surrogate that you think is a good marker for a biologic event. Again, I get back to what is easy.

What is easy is if the question is, if I infect a cell, get gene expression and the gene is encoding a protein that I can measure by an ELISA, you are done.

If you are asking a biologic question which is, am I presenting an antigen that is going to trigger the immune response, then the easy way is to say, well, we can show it in the patient later, once that vaccine goes in, or we can validate that assay in a way that proves the biologic mechanism.

DR. MULE: I think that is where the hurdle again is with respect to cell based, individualized types of products.

The cop out is, we can use a surrogate, we can get a marker which we think measures that biologic event. I would argue, why not really roll up your sleeves and really design a validation assay that shows the biology.

DR. HARLAN: I couple of quick comments. One is, I think Dr. Allan and I have served on this too long because we think alike.

I am hung up on this potency term, too. The other general comment I want to make is, i any individual product that we have talked about, I could see how we could settle on a surrogate end point.

We have to do something like the framers of the constitution did, and come up with general guidelines that

would guide all of these types of therapies.

Remember, the last question in the open session was, there are some cell products that are heterologous mixtures of cell types. How are we going to characterize those.

I think we have to be careful about identifying -- I like the word characterizes the product rather than establishing a potency assay.

DR. ROCKE: I think in terms of double standards, I think we have to be careful that we don't establish a standard that is actually more difficult for these products than for small molecules.

You have to show efficacy, of course, to get a drug license, but you don't have to show efficacy every time you make a batch. You just show there is the right amount of drug in there.

The issue of potency assays is the issue of saying, is there the right amount of stuff in that vial. Now, when we don't know what the stuff is that is the actual active principal, this makes it more difficult, and it would be nice if we always knew that.

Supposing we don't. You don't have to measure the ultimate downstream outcome in order to say that this vial of stuff can be sent to the patients. That is your comment, I think several other people have said the same thing.

We have to distinguish what you need to do to show the drug is effective, the whatever it is, the therapy is effective, from what you need to do to show that you got the right amount in the vial, that the process has been controlled, so that you are doing the best you can to deliver the same thing every time.

There I think surrogate markers make a lot of sense when we don't know enough about the biology. Even when we do know the biology, your point again, if it is a gene that is supposed to produce messenger RNA, that is the potency.

The efficacy is whether that messenger RNA does what you want it to do. That has to be answered, but it is not the question of potency assays. It is a completely different thing.

DR. URBA: I agree with that, and I think some of the examples we heard from Vical and maybe even Therion, they are like drugs, because they are well defined, you can characterize them and the potency can be the stuff.

There is a spectrum to the most complex that she mentioned and the mixtures, but you want the holy grail, it sounds like, Jim.

I guess my question is, if your hypothesis is that you have got a cell that immunizes against an important antigen and you have clinical data that shows that that

general approach is beneficial, you have shown that you can make these cells reproducibly, and granted we have heard why, for lots of reasons, you can't measure the immune response in every patient, you also can't show that every dendritic cell preparation induces an immune response, but if every preparation of dendritic cells that has X on the surface does, when you have tested it 50 times, does -- when X is above a certain level, those dendritic cells are capable of producing an immune response, isn't that a potency marker, and wouldn't we accept that or not?

DR. MULE: I think that is a very valid point, Walter.

DR.HARLAN: What do you do if the potency assay is absolutely consistent every time you do it, and all of a sudden the product stops working. You have got a very potent product that is not working, or may be harming the patient.

That is why I don't like the term potency. I like characterizing it, and I guarantee you it is going to happen. You know it happens in drugs where we think it is working a certain way and then we learn it is not working that way at all. This is a more complex system. It is, Dr. Rocke. These are much more complicated than small molecules.

DR. URBA: You don't stop studying it. You keep working on it. If we held everything to that standard, we wouldn't do anything, I don't think.

DR. CHAMBERLAIN: I think you might also want to think about the concept of different levels of potency or different degrees of surrogate markers depending on where you are in your clinical trials.

If you are at the very beginning and you really know nothing about the cell type, I think finding a marker that seems to be consistently there is a reasonable way to go.

As the system advances and you find out more and more that it is working in some patients and not in others, that perhaps you need to go back to the idea that you are dealing with a heterogeneous mixture of cells and they all share that marker, but what else is there.

Can you start finding correlations by exploring more and more. For example, if your surrogate marker was that the cell has ATP, that is clearly -- any living cell is going to have that, so that is not really telling you a lot in the long run, but there may be other more subtle, more important things.

You can't necessarily do that all at the very beginning, but if you find something that is working, and the more you get into your trials and you find out more of the system, then that gives you more time to go back and refine your system and develop better and better surrogate markers over time.

DR. COUTURE: I think that is a very good point, but I would like to go back to the point of making sure that we don't set the bar a whole lot higher for biologics than we do for small molecules.

I think it is fair to say there are a lot of small molecules that go into patients that work in some patients and don't work in other patients, yet there are not massive amounts of requirements to go back and really sort that out, although pharmacogenomics is really becoming a better thing and that ultimately hits us, too.

I think doing the correlation studies, whether it is CD54 or ATP or whatever it may be, if you can show, through up to marketing, that there is a correlation between the two, you are good to go, and I think there is going to be an increased onus on manufacturers to continue to understand that.

We are biologics. We are mechanism of action oriented drugs. We are not small molecules that just go in and block pathways or whatever.

I think there is going to be an increased impetus to study that afterwards, but I want to make really sure that we try up front not to automatically set the bar higher than you would for a small molecule.

DR. ALLAN: I think you have to set the bar higher because of what you are working with. If it is just this

mish of cells, it is not a small molecule that you can just throw it in an assay and you get a readout.

The cells, it can be very difficult to come up with something that actually correlates with a biological effect at the end. So, I find it hard to not inherently have the bar raised over a drug or a small molecule.

DR. COUTURE: I am not sure we are saying anything different. I am not suggesting we don't characterize the product.

I mean, just like small molecules, they have to be well characterized biological products, very well characterized, but we are all kind of saying the same thing. In some of these cases, potency just doesn't apply. There isn't going to be an actual potency assay.

The Genzyme product has no potency assay. It is cell viability. So, there are products that just won't have it. That is especially going to be true in the autologous cell products.

The plasma DNA -- I produce both. We do both plasma DNA and we do engineered CD34s that are in culture for just a very short period of time, engineered, and live on PBMCs, that we use pools of five to six because we found the same thing, that five to six will give you the same effect.

I tell you, you get cells out of a patient, and

those five or six PBMCs that were used for feeder layers are all over a chart.

What you get out of a patient from different patients can be radically different. We saw that in the CD14 analysis.

I mean, these cells, patient cells from one patient to the next can be completely different. So, it is very difficult for me to understand how you then layer on a fixed standard for what they all have to do look like when they go back into a patient.

That means we perhaps come back to this notion that the process is the product itself, and I realize that is kind of just a hand waving way of getting around saying we don't have a potency assay, but that is the reality.

That doesn't mean that we lower the bar. I think it has to be just as well characterized, but I am suggesting we don't increase the bar and go into these post-marketing studies a priori and force companies to figure out, if it is not CD54, what is it, just to narrow down that range of patients who don't have an effect.

DR. TSIATIS: For question two, are we supposed to think of it as sort of a theoretical question in the sense that, if there really was a bioassay that we agreed on -- and based on this conversation we are still struggling with that -- how might we do a study to see if another assay can

be substituted for that. Is that what question two is getting after?

DR. MULE: I am not going to answer for the FDA. Dr. Gavin, during her talk this morning, listed some criteria that validation assays are recommended to follow.

In some cases, one assay itself may not be enough, and one might have to evoke a second assay or even a third assay.

So, from the committee perspective, if that a daunting task. If the FDA would like to clarify this particular question for Dr. Tsiatis, that might be helpful.

DR. WITTEN: I think what you said is accurate, which is that we are asking, assuming we understood what the biological assay would be, and we want to -- but we don't have any one measure which will meet all of those criteria, then what.

DR. TSIATIS: There is something where you can talk about predictive distribution, and the notion there is, if you do the right analysis, then you can talk about, given that I got an analytic assay, then what do I expect the bioassay -- what I expect the distribution of that bioassay to be, given the value that I got for the analytic assay.

Once you get that, you can decide whether that is sufficiently accurate, that the range of that distribution is sufficiently tight that you feel you can get a pretty

good prediction.

In order to do that, you need the conditions to assess that predictive distribution with some degree of accuracy.

That is why I was asking, if that is the theoretical question and you have sort of something that you want to be your goal, then you should be able to do such a correlative experiment.

DR. ROCKE: Let me add one thing to that. When you can, it is always a good idea to actually be able to manipulate the surrogate, as we saw in a couple of cases in the presentations, and demonstrate that that causes a change in the biological activity.

Then you have actually got not just a correlation, but you have got more evidence that there is actually causality going on.

I have to say that I am not sure that the developers of these products would like the predictive distributions that come out of this suggested analysis, because even when the correlation is fairly substantial, certainly significant, those distributions can be pretty wide. It is information that would be very useful to the FDA, but it is a little discouraging to the investigators.

DR. PLANT: I think you would have to know those distributions, because if you don't know those distributions

well, then you don't know your reproducibility either, and I think this is one of the primary things that has to be known, is what is the reproducibility of the assay.

So, if something is different, you can recognize that, and you cannot evaluate whether some result is different from another result unless you have the statistics for reproducibility within those two kinds of results.

DR. MARINCOLA: Actually, going back to -- I really like the concept that was brought up about this dynamic interactive development of seeing the patients and the product, in the sense that maybe we should refocus, in such a way that the standard is not too high for the potency assay.

The requirement would be that whoever is doing the study is going to organize the sample collection in a way that is going to be helpful to come back maybe to the second phase study in which you can improve your potency assay and so forth, like this.

So, basically do the potency assay in the patients. That is the really relevant part. As long as it is safe, I think it is a reasonable thing to have a leap of faith that it is going to do what it is supposed to after the basic standards are met, and continue that way.

I don't think that is done as much in clinical trials, really, besides looking for the end point, really,

to collect materials and try to appropriate see if, in fact, the potency of that is there.

DR. MULE: Getting back to Walter's point, Franco, would that be setting a standard over and above what is generally acceptable, if you will, in providing drugs where you think you know what the mechanism is and yet, lo and behold, you have a patient population that is responding to a drug where theoretically you never would have predicted that.

I am just wondering, it is a valid point to have maybe secondary end points in the trial where you are collecting cells as a follow up to give you proof of concept, and then back that toward your validation assay, or potency assay. I think it is valid.

The concern, I think, is raised, that are we now layering on top a whole series of requests or recommendations that take it to a different level.

DR. CORNETTA: I wonder if it is almost a matter of sort of surrogate clinical markers that we are really talking about.

I think, from prior comments, I don't think it is necessarily efficacy we are talking about, in what the eventual outcome is with the drug.

For example, if you had a cellular product -- and just for example take core blood that you were going to

manipulate -- we would want to know if that actually engrafted.

So, we don't know if the patient might be cured of leukemia. If we are going to give some type of vaccine where we would expect that there may be an increase in cytokine levels in the peripheral blood at some point, that might be a helpful marker to know that whatever it was in the manufacturing or somewhere along the line, that product did not get changed.

If we, just for example, look at a CD4 or CD54 marker or some other value on those cells, we still don't really know the biologic activity, because something else may have happened, that they still express that marker, but their ability to have some effect in vivo has changed.

I think that is the challenge that we are talking here. It is not necessarily efficacy at ultimate outcome, but how do we know that that product that went in really was similar from batch to batch.

DR. MULE: We have a few minutes, and I think it would be worthwhile to ask if any of the speakers have comments with respect to the first two questions, if you could identify yourself and use the microphone.

DR. PATEL: My name is Suman Patel. I am from Plantarian(?) Biologics. The perspective that I would like to bring to this group is the characterization is the theme

that I am hearing.

Let me link that to a scenario where we can put more emphasis on it and understand it a little bit better. Let's say, for example, we have a gene therapy product, and you have it in the clinic, and you are using a placebo in the clinic.

Placebo is the same vector, found to be safe, a tolerable safety profile during toxicology or early clinical studies.

Then you have the same vector carrying those genes in that vector. Now you evaluate in your clinical setting, and you find that a vector containing the genes that you have designed into it.

We take a lot of pain and a lot of effort to design that vector, recombinant technology, to get certain things in there.

Now, if it is active, if it is efficacious, in clinical settings, I think as manufacturers and folks who control this kind of quality, it is our obligation to make sure what we design into the vector is there.

Make sure that what it is designed to do, it is doing. I think if you are doing that, you would have a lot of value in going with this well-characterized product profiling, and also applying multiple assays that relate to the characteristics of what you design in.

Now, I am not trying to say here that biological potency is irrelevant. Certainly that is a goal we should continue to work toward, but if there are other assays that are more precise, performance characteristics wise, that better able to allow you to put control on the product, I think that should be out goal. Thank you.

DR. PROVOST: Nichole Provost from Dendrion. I think there are two major themes coming out in the need for potency assays.

One is to initially characterize and figure out what is going on, but the other is the need to have some standardized, reproducible, consistent thread through your manufacturing process and through your clinical trials, so that you can tie any changes in your product, whether they be from source material or from manufacturing process.

You have to be able to have something that is consistent and reproducible, some assay that will, in every case, allow you to compare product to product, trial to trial, and some biological assays aren't going to do that.

There has to be some thrust to be able to have something consistent there. Otherwise, when you get to the end of a clinical trial and try and compare all of your manufacturing lots, it is going to be very difficult.

If you want to make changes, and think about not just the active, but any other formulation or excipients

that might have an effect, you need to be able to measure that and tie it back to some consistent assay, and that might be a surrogate or some other analytical assay.

DR. BARD: I am from Astrom Biosciences. I just wanted to make a comment of how this discussion is going. We are using a legacy term of potency, which seems to go back to drugs, and there are a lot of biologics that fall directly into the concept of potency.

We are talking about cellular products, things that don't go down to the level of individual chemicals. They have a degree of complexity that we are missing here.

I think Dr. Couture said that you can't just throw cells into a body and say, that is okay. If you do cardiovascular repair, that is exactly what people are doing today.

They are throwing mesenchymal -- they are throwing skeletal myoblasts, they are throwing bone marrow cells just back into the heart.

That is giving it a little bit simplistic, but we do it for fractures, we do it for a host of things and just saying what the cells are isn't good enough.

Maybe that is good enough. Maybe that is the potency. We tell you that there are CD9s, CD34s, AC133s. Somebody has to come back and tell me why that isn't sufficient.

If we do that time and time again, have we not given you what we think is the active ingredient in our product, and we can show you that we have a manufacturing scheme that handles that time and time again.

Now, there are other products that gene therapy, maybe that is not the right way to go, but maybe the FDA should have a little flexibility in a term of art that doesn't seem appropriate for all of our products that fit within this room today.

DR. GUNTER: I would like to react to that. I think that the briefing document that we got in preparation for this meeting made it pretty clear that an analytical test could serve as a surrogate potency for a functional test.

I don't see why you couldn't do some kind of phenotypic characterization, flow cytometry, perhaps, on a population of hematopoietic or other cell types and eventually correlate that with a functional effect, and use that flow phenotype as the potency assay. I don't see that as any kind of a problem inconsistent with the technology we have today.

DR. MARINCOLA: I want to make a comment on that. Going back to what I was mentioning before, I think that the point is not the lower the bar.

Of course, there is going to be some logic why the

products are developed in some kind of logical potency, where it could be simple monitors or more sophisticated as we understand the process better.

I think there are many ways in which you can, and I think the Dendrion example is a good example. For example, they were able to show some kind of a very simple biological relationship in the mouse model.

Then, for them, it would have been really impossible for them to have a potency assay in humans because of the genetic background issues and so forth.

So, you could save a lot of money not doing a lot of experiments that are impossible, but collecting phoresis in the patients, so eventually you can test and say, well, now we learned what is really important with the patients who are really responding. They have this particular HLA type or they have these particular T cells or expressor cells or whatever.

Then come up with a second generation of potency assay based on the patient. That is what I meant by this kind of interacting thing, which fine tunes what you are really doing in the patient. So, you can have a development of your potency assay as your product is developed.

DR. MULE: First of all, Dr. Gavin, this morning, told us that an assay should be accurate, precise, sensitive -- I am just reading those things -- and I said that I

thought it was difficult to come up with hard guidelines.

I actually still think that. I think that we should ask each person developing a product to speak to those criteria, what is your assay for these things.

In some cases, it may be impossible, and a jury of their peers will say, yes, it is impossible, but this is still logical to proceed.

Let's go ahead and move to the third question, which is related to incorporating state of the art technologies.

We heard one example this morning from Dr. Elliott of a flow based morphologic type of assay. Issues came up of transferability and so forth.

I think that raises issues about state of the art technologies and how one might be able to benefit from those, and what the transferability might be, as a start to the discussion.

I know, Franco, you touched on some of this early on with a discussion about micro arrays. Maybe you would want to comment first.

DR. MARINCOLA: I do believe that, as we get into more complex systems, the potency is going to fade, because you really don't know exactly what you are measuring.

I am not saying there is not a logic in why you are doing whatever treatment you are doing, but the fact

that you don't know what the most relevant markers are.

So, you have to go into a discovery driven approach, where you try to document what you are doing, and then eventually be able to sort out what it is.

To me, there are lots of state of the art technologies, but if RNA -- I don't think studying RNA in the cells really tells you what the cell is doing. It is really telling you what the cell is trying to do.

Still, it documents the thinking process of the cell when you are giving it to the patient or whatever, and maybe be able to give you some hint retrospectively of what your effectiveness was, and why some patient was responsive or not.

I do think that there are very good ways now to quantitative that, to really come up with ways in which you can say, well, you know, among these cells that we have given to different patients in different situations, while some were more close to what we were thinking we were giving compared with other ones, for whatever processing problems that may have happened, maybe the genetic background of individuals, and there are a lot of things that can be learned about that.

So, I think a simpler way, a relatively simple way to analyze and come up with some kind of standard in a complex system is to apply micro array technologies.

Other technologies are a little bit more complex like proteomics, and many times you really -- you see only the surface when you do proteomics of what is really happening, but you have no idea, particularly if you look in cytokines, and you are not even going to see them with mass spec.

So, I don't think -- it would be very nice to look at the proteins, but I don't think that the technology is now at that point where you can actually do it in an efficient way, but a minimal decrementation, it is a very interesting way of applying that and trying to do that.

So, I think that can answer two questions. One is the variability and, second, you can go retrospectively and maybe try to find relevant markers.

DR. MULE: In your view, what would be reference samples that one might envision if one were to use, say, micro arrays as part of the analysis for validation of a product.

This holds true for flow cytometry based assays. What are valid reference materials that one might need to use those assays.

DR. MARINCOLA: Well, I have my own bias. The bias is that it doesn't really matter as long as it is always the same, that it is consistent, and whatever reference you are going to use, you are never going to change that. So, it

really gives you an exact measurement.

For micro arrays, really, it doesn't matter. Just about any reference would work. That is a point that I think is very important to keep in mind.

When you do reference systems on a gene by gene basis, like, for example, having a co-hybridization, you are really doing a different kind of validation than when you use an external system, like housekeeping genes or things.

There you are assuming that the genes that you are comparing is let's see how many of this is over that, assuming that there is a constant expression of those genes. That, frankly, doesn't happen. We have tested that in so many systems, and it doesn't work.

I mean, there are genes that are more consistently expressed than other ones, but if you have a reference system that is always the same and is gene by gene, because basically you are co-hybridizing the same thing, it really doesn't matter that reference you are using.

I mean, there could be -- it would be nice to have a standardized reference so you could compare things among different laboratories directly, rather than based on kind of independent results.

One way to compare it, we compare all the time Affametrix results our data because eventually, look at biology, what is upregulated versus -- as Rocky was

suggesting, you can compare twos situations and then see what happens dynamically.

Otherwise, it would be nice to see a standardized reference system, but then you get into another problem which is who is going to want to do that. Are we going to force someone to use your reference system, or another. I think that consistency is by far the most important thing, through time.

DR. MULE: So, in your case, would the reference be individualized for each patient, or would it be a standardized reference, say, of a housekeeping gene that all patient product validations would be compared against.

DR. MARINCOLA: It should be always the same thing. For example, we use peripheral blood lymphocytes simply because truly they don't change.

If we take five donor -- five normal healthy donors, and then we take another five from any other donor, we will see more than 99 percent concordance all the time.

So, there is a way you can have a constant reference that is independent of cell culture and other conditions.

Then, for each gene, you have your own reference system, because you compare, actually co-hybridizing the two. So, it doesn't matter about the lot.

One problem with the Affimetrix system we find is

that, when you change the lot, you change a lot of results, a lot of the reagents you are using. When you use a co-hybridizing system, it doesn't matter, because the lots are going to always be the same. So, you eventually compare.

So, there are a lot of techniques to really look at this reference concordance concept, but the important thing is really just to identify a reference system that is stable and then you can perpetuate that, because you are comparing one to the other, and eventually forever you can keep going, and then you can develop huge data bases.

DR. ROCKE: You said earlier we disagreed on the quantitation of micro arrays, but I don't think we do.

DR. MARINCOLA: I said partially.

DR. ROCKE: I don't think we disagree on that part at all. I don't know that reference materials for micro arrays, standard reference materials, are going to be very useful.

I am a little disillusioned myself with two color arrays. In many cases, there is a gene specific, that is a probe specific, dye bias, which is very large.

It is often the largest effect on the array. That is, if you normalize the heck out of everything, this co-hybridization which is supposed to take care of a lot of the variability actually introduces another factor, which is that SI-5 is much more sensitive to degradation than SI-3,

and that for whatever reason, it depends on the species.

Either it is because of the probe or because of the labeling environment, I don't know. In any case, if you want to do two-color arrays, whatever reference you have, just what Dr. Marincola said. Whatever reference you have is fine. You don't really need a standardized reference that comes from somewhere.

The important principle, here, I think is you are doing experiments in the internal controls. So, that is where the information comes from.

So, if you want to see if a certain transformation has been effective in introducing a change in the cells, well, you have got the before and you have got the after, to see if there is some effect that you can detect.

DR. HARLAN: I just think we are on very thin ice with this whole line of discussion. We are talking about a specific assay, these gene arrays, and it is just a slippery slope.

Then there are going to be proteomics and FACS analysis of all these products. I think we would best stand back from recommending specific ways of characterizing things and let the field develop.

There will become an industry or product related standard for each new thing, and we had best not specify what those things are.

DR. MULE: That said, however, I think we are tasked with at least some discussion on future assays, and what value they may have in the field.

So, keeping in mind the discussion of micro arrays, are there instances that one can come up, where this assay may, in the future, be valuable, either in the characterization of a cell product as one example.

Are we there yet? Probably not, but I think it would be valuable to give some examples, if we can, of where micro arrays fit in the general scheme of product validation, and maybe we can come up with some examples.

DR. MULE: I would have thought that if micro arrays were on the cutting edge, that we would have seen something from one of the companies or some preliminary data or at least something from somebody that gave us a little data.

Since we didn't see that, I sort of wonder whether it is just a crystal ball approach, a guess as to what the future is going to be. I don't see it either.

DR. MARINCOLA: Novartis is a good example, in San Diego. They are doing huge studies in micro arrays, and then base a lot of their -- I think it was the biological therapy meeting they gave a good example of how you can standardize what you are doing based on it. So, there are large companies that are using micro arrays.

I think the question is, looking at the future. I mean, I don't think there are good examples now because people are not doing it. It is expensive, it is not well clarified how you do it. There is a learning curve. There is disagreement what platforms to use.

There is a lot that can be done to document what you are doing. Basically it is like repeating -- if somebody was doing northern blots or whatever, it is like doing 20,000 northern blots instead of one. So, there are advantages to document what is happening.

DR. MULE: As one example, we heard today about upregulation of CD54. In my mind, if one had a micro array system -- again, looking into the future -- you are not measuring one event, but you are measuring several events that you can maybe put together a picture of what is happening in the validation of a cell product. Then that may be valuable.

It may, in fact, help you to determine what surrogates you may want to measure with respect to flow cytometry.

DR. GUNTER: I am glad you mentioned flow cytometry. To me, it is interesting that flow is mentioned in the same paragraph as genomics and proteomics, because they are probably at least one or probably two decades apart in development.

We saw companies this morning that are actively using flow. So, my point here is that flow is coming, and it is going to be used as a potency assay, I believe.

I know there are companies out there working on hematopoietic cell therapies that want to use flow. Again, we saw some this morning.

So, it would be useful, I think, to the industry, speaking for the industry, for this committee to give some guidance on how to validate, to correlate, analytical flow with a functional assay.

I think that would be very important general guidance, if we could get it out of the committee either now or in the future.

DR. COUTURE: Just to go back to the micro array data, the main thing that concerns me about it is that I think it is a technology that is yet to come.

I think it is a great technology and has the potential to offer a lot when we understand how it works, when we understand what it means.

We heard 99 percent similarity from half a dozen patient samples, and yet we had two groups show today two different genes they are monitoring, and the cell surface expression is all over the place. So, maybe they just hit the one percent that there is a lot of variability from patient to patient.

We have tried micro array data a little bit, and what we find is that it is good for gross differences from gene to gene.

You can find fold differences, and you can probably tell me I am wrong, you guys have it down much better for that.

For an assay to meet all the criteria, it has to be very sensitive, precise and accurate, and we are looking at differences of levels of expression of a cell surface marker of 20 percent, and I am not sure that is something that we are going to be able to readily analyze by micro array data in the very near future.

I do think it is something that is going to come down the road. I think we should be very cautious, again, that it is a slippery slope, trying to push things like micro array data onto investigators.

DR. PURI: First of all, I appreciate all this wonderful discussion that is pertinent to the complexity of the products that we deal with on a daily basis.

Use of micro array, I just want to say that micro array provides an opportunity to do a global view of the product, particularly complex cell therapy products that we deal with.

Perhaps using this technology, given the discussion that we had about Affimetrix versus two color

hybridizing, and there are efforts ongoing where the large consortiums are trying to address the built in control standards and types of standards that would be in there, or doing the hybridization this should be put in, spiking the controls to control the variability or have external RNA control in two color hybridizations versus Affimetrix, not able to see the image after you hybridize, are you making the correct assessment of the data.

I just want to say that there is a tremendous amount of research that is ongoing, that perhaps a lot of people are aware of, where they are trying to address a number of these issues.

So, the forward looking statement is that this technology is here, and is going to be applied, and it is being applied.

There are a number of efforts. I heard talk at the cancer vaccine meeting earlier this week where the sponsor -- one of the investigators is looking at the characteristics of dendritic cells right after the pulsing, and non-pulsed dendritic cells, looking to identify the gene expression pattern.

Perhaps you identify the marker that can be useful in looking at the characteristics, or perhaps can help you with an idea for developing a potency assay.

Similarly, we encourage here the field that, while

we are trying to identify the complex products, such as cellular therapy products, it may be useful to apply the cutting edge technologies such as genomics, proteomics, that are still ahead.

Maybe it gives you an idea. You may come up with a cluster of genes, or maybe come up with five genes or 10 genes, and you might have a resource, that antibodies might be available, that primers might be available.

You can use that to characterize this product in an early stage of the product development so that it can help perhaps later, two years down the road or three years down the road, to move this field forward.

DR. ROCKE: So, in my opinion, the micro arrays are the best developed of these newly highly parallel technologies, which doesn't mean to say that we understand everything.

The repeatability on good micro arrays is on the order of 10 percent. So, you can detect 20 percent differences, if you knew what genes to look for. The big problem is there is the needle in the haystack deal.

A big advantage of micro arrays compared to most proteomics and metabolomics technologies is you have some idea of what you are measuring because you know the sequence of the probe and you know the putative gene anyway.

I think that the next best developed one is

actually not proteomics which, as one of my colleagues like to say, proteomics is easy to say and hard to do and metabolomics is hard to say and easy to do.

Particularly, focused metabolomics in which people are looking at, let's say, whole classes of oxilipids or glicans, focusing on very specific questions that are relevant to the disease or the therapeutic situation.

I think there is a lot of potential here. I think we didn't see people presenting it possibly because, when you are talking to FDA you tend to be a little risk averse, and because even though micro arrays have been around for seven, eight years anyway, they are still not completely developed.

In particular, a large fraction of the papers I read that use micro arrays are basically wrong, because of the way in which they analyzed the data.

This is the reason why biomarkers for cancer X at Massachusetts General don't seem to be the same as the biomarkers for cancer in Nashville.

I think there are human beings in both places. So, we have problems with the technology that are relating to how people use it, not so much to the technology itself, which actually I think is pretty good stable stuff, two color arrays, affimetrix, there are many other platforms that are really quite good in terms of producing sample to

sample, similar values and looking at changes that are similar between when you go validate it with PCR.

It is just there is a lot of misuse and not a widespread understanding of how to deal with it. It is coming, though, and there is going to be a lot of work presented, I think, to FDA as validation, as surrogate markers, as, for heaven's sake, diagnostic tests, although I guess we are not dealing with that one. We have to deal with it.

DR. CHAMBERLAIN: I think it is important to keep in mind the concept of simplicity also, that probably what you want as your potency standards are the simplest possible tests that you can come up with.

The more complex your assays become, the more inherent chance there is for variability and errors. As you layer on more and more assays, you have a greater and greater likelihood of making a mistake or introducing more and more variable.

I think it is important to ask, what is the simplest assay that you can come up with. If there is a simple one, you should use it.

As an example, the comment we heard earlier about transplantation of skeletal myoblasts, if you can isolate some cells and ask, do they express PAC7 or do they express MYOD, and that is all you need to know you have a myoblast,

that is great.

There is no need to do an extensive proteomics or micro expression array to let you know that is a myoblast. Certainly it has to be done on a case by case situation.

In terms of -- I think you asked earlier, is there an example where micro arrays might be a needed validation assay, to me it is hard to think of an example of that, although one possibly might be where you are dealing with a very complex mixture of cells or a very unstable cell type.

Maybe you have got a particular stem cell that you are trying to isolate from the blood, and it is present at only one in a million.

In the process of isolating that, if something goes slightly off, suddenly the telomerase gene is shut off, then you may need to go in there and really look at a lot of things to make sure you have got that really rare cell type that you are looking for, and that you haven't messed up anything.

At the same time, though, I would agree that once you go through and figure out, what are the important markers, what is it that you go through and figure out what are the important markers, what is it that you learn from the micro arrays, then maybe you are back to the simple case where there are only two or three proteins that you need to assay, and that is going to be your validation for potency.

DR. MARINCOLA: Actually, there are impoverished examples of how micro arrays can tell you about a product. I can give you an example in my lab.

We were doing a study where we were seeing what the effect was of giving IL2 to different subsets of lymphocytes in different ethnic groups.

At one point, there was a group of arrays that were totally separate from everything else. Well, it turns out that the person who was processing suddenly decided that he would treat the PBMCs, leave them overnight in the refrigerator and do the separation the day after.

Just to tell you an example, how you can in general sort out what happens, just look in the micro array and tell if there is something wrong with this product.

I think in some ways you can look at tools like this. So, when you look at your myoblast, probably all the CD8s we are looking at the CD8 marker, but we have totally different cells.

So, it is in some ways good that you -- as long as you have a marker for some of the various therapies, it is a good way to use your marker to assure that at least you will get myoblasts versus something else.

On the other hand, it might not be also a very good way to look at the variability of what you are really giving based on all the other -- so, that was just the

comment I had.

DR. CORNETTA: I think I, as often in life, have a schizophrenic view of this. In terms of the micro arrays, I think especially you have products which are not characterized, or they are coming from multiple sources, different patients.

How do we try to go from one single marker to tell us of activity which I think, from batch to batch, worries me about those batches that fail and trying to detect those, and having something that really has a very broad view is very attractive. The micro array gives you a lot of data.

I think the other side of my opinion there is having to sit in front of an FDA inspector and explain, yes, the ten ones that we were looking for look good, but these vary -- why these other ones which aren't really genes we were looking for are varied from product to product to product.

So, the problem with the micro arrays is they give you a lot of information. It is one thing to have that discussion in your lab group with your students, and it is another one to know how the FDA would view those variabilities.

I don't mean -- I think that is just a consideration that companies and everyone who will do this has. How are those variations that occur with this assay,

how are they going to be viewed with all of the data there. That is just a comment to the FDA.

DR. MULE: I think Raj wanted to respond to that, and then we will have Dr. Snyder.

DR. PURI: I just want to clarify that the FDA has released a guidance document earlier last year, March of last year.

It is a voluntary genomic data submission on the pharmacogenomics guidance document that we have, addressing exactly the point that you are mentioning.

There are mechanisms where some of the preliminary data that you obtain from your studies and you can submit to the FDA and the FDA can meet with you and advise you how to, and what kind of algorithms apply.

If you are not going to use it in regulatory decision making, or is it just only exploratory. So, we had thought about that, and those concerns have come up a number of times and, because of that, the guidance document is out there.

I encourage you, if you are considering applying a genomics type of analysis looking over this, finding out that this marker has this identity or purity or potency of your product, that would be a very useful part to look at.

DR. SNYDER: I guess I want to just get back to Dr. Harlan's comment about slippery slope. When Dr.

Chamberlain that PAC7 is a suitable marker for myoblasts, isn't that identity testing versus potency testing, if you are just looking at a marker to verify that you are administering the proper cell type. In my mind, that is an ID test, that is not a measure of potency.

DR. GUNTER: I would agree that, if you are just using it in that way, it would be an identity test. If you actually go the next step and correlate it with a functional biological assay, then you would characterize it as both an identity test and a surrogate potency test. So, that is how I would approach it.

Then one more comment, if I may, just a throw away, but I always liked the idea of array testing as a nice way for a company making, say, several different heterogeneous cell products to have an identity test.

It may lend itself to automation and high throughput to distinguish one kind of cell product from another product rather easily.

DR. PLANT: One of the things that seems to important about array kinds of technologies is that the complexity of biology is very poorly served by looking for one marker at a time.

I think it is really critical to think about array technologies as a way of developing a matrix of markers that will actually be identifiable fingerprints of what the

phenotype is.

A phenotype may not ever be adequately expressed by a single biomarker. So, I think that is one thing to really consider.

Along that path, some of the work that NIST is doing in trying to help standardize array technologies, for example, just the capability of the readers to be linear, et cetera, and then of course the incompatibility between platforms is just this huge thing, and one of the reasons why taking data from one lab and comparing it to another lab is so difficult to do.

I think it is important for the community to be involved in these kinds of standardization and platform -- what is the word I am looking for -- to standardize all of these kinds of assays in order to make them work is very important.

The other thing I just wanted to mention is, when we talk about advancing technology, sometimes it doesn't really take advanced technology to get really good data.

So, some of the data that were shown this morning from Dr. Elliott was just fluorescence microscopy. Probably everybody's lab has a fluorescence microscope in it. The only difference is that it has a motorized stage on it.

Really, it is how you take the data, how you set up the experiment, and how you analyze it that can give you

a lot of very important results.

DR. HARLAN: I like the rule, be careful when you make a rule because then you have to follow it. It is true that these various micro array techniques have nice inter-assay variability, but let's keep in mind that there are three commercial products out there.

When you compare the results from one commercial product with another, there are very big differences, and I just think that we could actually put a damper on the field if we say we want people to characterize all these things because they are expensive.

In the Dendrion experiment that we heard about this morning, there were 180 patients in there. That is 180 preps that we are going to analyze, and we don't even know what we are looking for.

Companies are going to do this, no question, and as trends start to become evident, then that will be the standard in the field, that you have to do it, but I think we are on dangerous ground when we suggest that it is necessary.

DR. MULE: I haven't heard the word NIST.

DR. HARLAN: I think companies know that they want to characterize their product, and they will do it. I think we can let them do it, and the standard will evolve as the field does.

DR. MULE: We have some time for comments from the floor.

DR. PROVOST: I just want to comment on the micro arrays because we pursued that approach. We presented a poster last year at ASCO detailing the work with micro arrays and the patterns and upregulation, if you will, that we found both in the process and for immune monitoring for patients over time.

The patterns that we found were nearly identical to what we were seeing with flow, same genes, same gene products coming up, same co-stimulatory molecules.

The conclusion for us was that it wasn't worth it. It was too expensive, very difficult to apply reproducibly. It was not industrialize-able for us.

So, it was nice that it confirmed what we had already had a hunch on, but from the standpoint of standardizing assays and actually making them industrial grade, it wasn't going to work. So, until it gets cheaper, easier, faster, we will stick with the flow.

DR. MARINCOLA: What platform are you using to do it?

DR. PROVOST: We looked at a couple.

DR. MARINCOLA: It depends. You can make arrays -- if you make your own, you can pay $8 per chip. It is not that expensive. It depends on whether somebody is going to

charge you $1,000 per chip, then it is going to be very expensive. The technology is very, very cheap.

DR. PROVOST: Until you get some feedback and home in on what you are looking for, it is still expensive.

DR. MARINCOLA: That is an external thing. The technology is so cheap, we make them very, very cheaply. Eventually, if there is an extra charge, that is what you are referring to, but in fact, the technology, I cannot think of anything cheaper than micro arrays, compared with the information that you get. Per gene, it is costing you nothing, basically.

DR. PATEL: Again, this is a great idea. As we are more and more successful, as we understand our products and their characteristics more and more, I think that there is a great deal of incentive, not only just from the point of your controlling the quality of the product data, but also what makes them data.

If you understand the products, how they work, with advanced technology, think about 1984, 1986, the interferons, that time. We were not asked. We were required to quality control interferons by CP assay, biological assay.

Those assays are still being done, but those assays are not driving the quality of those products. The quality of those products is being driven by advanced

technologies.

We have got mass spec, we have got NMR, all kinds of technologies that we developed to better characterize those products.

Now, that is an incentive. You don't have to do a multi-million-dollar clinical trial to show that this product, everywhere you look around the product, is very comparable to the product that you had, or what you change is designed in a certain way and this is how we characterize those changes.

This is great, and I think industry has a lot of incentive in developing advanced technologies, but I think it might take time.

I go with the comment that it shouldn't be a rule. It should be an opportunity for us to explore. That is what I would like to say.

MS. DE NAGEL: My name is Diane DeNagel. I am in assay development at Cell Genesis, and I have two comments. I am a protein biochemist by training. I have worked in assay development a long time.

One comment you had touched on earlier was the dynamic range of expression of proteins which can vary from just a few per cell to over millions per cell.

For flow cytometry, as much as I like that platform, one of the challenges is that sometimes a product

would lead you to develop an assay based on some of the over-arching comments here about potency or efficacy.

The commercial products may not, in fact, be in the dynamic range that you need. So, as an assay developer, you are struggling with how to stabilize this assay. So, that is one comment.

How do we maybe get our standards from commercial vendors for what I would call boutique users in a range that is going to span what we need.

Then the second comment that I just want to touch on is that sometimes the best markers are covered by patents.

In companies, that is actually a very big deal, and so that is the other layer that is not covered in here, is how do we keep doing what we need to do, and work in a time line and also do the right thing for the product. That is all I want to say.

DR. MULE: Let's move on to the final question which, again, I think flows with the discussion we are currently having, which is future research needs.

Please discuss what new research is necessary to bridge the gaps in our scientific knowledge of how to assess potency for cell and gene therapy products, in order to facilitate product development. Thoughts there?

We have had quite a bit of discussion on the pros

and cons of micro arrays. Are there gaps in our scientific knowledge of how to assess potency and can we sort of visualize what new research might be valuable for moving ahead? Any comments there?

DR. ROCKE: Let me throw in one comment. We can all take turns making self serving comments. So, I will make one, which is that one of the big holes in a lot of these highly parallel technologies is, we don't know how to deal with the data.

There are other holes, like we don't know enough about the biology, we don't have good enough methods for identifying proteins in 2D gels or mass spec, but a big hole is we don't know a lot about doing the data analysis even of micro arrays, which is the best developed of these.

By the way, I don't disagree with Dr. Harlan at all about the issue of, I don't think anyone should be encouraged to play with explosives or use micro arrays unless they know what they are doing.

So, it will come, but there is no reason to push it. It will come when the science and the technology are there.

Research issues, there are a lot of research issues. We don't know very much about how to do proteomics for sure, and even metabolomics, which is newer but in some ways better developed, is also very difficult, especially

broad-spectrum metabolomics.

About 10 years from now, we are going to see a lot of that, and we are going to see a lot of expression array type stuff, we are going to see a lot of mass spec data, but it will come when the technology is there, and the technology can be pushed by funding research that gets that stuff done.

I think there is a clear appreciation of that, for example, at many agencies and many institutes at NIH, that these are technologies that need to be developed for future use, and probably at FDA also.

DR. MULE: Well said. Any other comments? If not, before we close, I would like to ask representatives of the FDA as to whether there are any lingering issues with respect to the questions that we may help you with.

DR. WITTEN: We would like to thank you for your attention to these questions, and I think they are addressed as well as they can be, given our state of knowledge right now.

DR. MULE: I will close by thanking the speakers as well as those who were able to come to the microphone during the open session with their comments, and I think we are done at least for this part of the agenda for today.

[Whereupon, at 3:30 p.m., the meeting was adjourned.]