UNITED STATES FOOD AND DRUG ADMINISTRATION

BIOLOGICAL RESPONSE MODIFIERS ADVISORY COMMITTEE

Bethesda, Maryland

Friday, 13 July, 2001

PARTICIPANTS:

DR. DANIEL SALOMON
Chair

MS. GAIL DAPOLITO
Executive Secretary

DR. STEVE BAUER
Center for Biologics Evaluation and Research

DR. ESTUARDO AGUILAR-CORDOVA
Harvard University Medical School

DR. BETH HUTCHINS
Canji

DR. RICHARD SUBLETT
Introgen Therapeutics

DR. PHYLLIS FLOMENBERG
Thomas Jefferson University Medical School

DR. ED SAUSVILLE
National Cancer Institute

DR. DAVID GAYLOR
Sciences International

DR. MAHRENDA RAO
National Institute on Aging

DR. BRUCE BLAZER
University of Minnesota

DR. RICHARD CHAMPLIN
M.D. Anderson Cancer Center

DR. JOANNE KUTZBERG
Duke University

PARTICIPANTS (CONT'D):

MS. ALISON LAWSON
Genezyme Corporation

DR. DAN SALOMON
Scripps Research Institute

DR. RICHARD MULLIGAN
Harvard Medical School

DR. GARY KETNER
Johns Hopkins University

DR. MARSHALL HOROWITZ
Albert Einstein College of Medicine

DR. GENE ROSENTHAL
National Institute of Health

DR. JOYCE FREY
Division of Cell and Gene Therapy

DR. PHIL NOGUCHI
Office of Therapeutics

DR. EUGENE ROSENTHAL
National Institute of Health

MS. ALICE WOLFSON
Consumer Representative

* * * * *

P R O C E E D I N G S

DR. SALOMON: Good morning everybody. We've fulfilled my criteria for beginning a meeting. Jay Siegel is seated. We're going to have to have a vote at the end of this meeting about what the criteria for the next meeting's going to be because Jay informed me that he wasn't going to be here, so --

Anyway, I'd like to welcome all of you to Meeting Number 30 of the Biological Response Modifiers Advisory Committees. So, 30, that means we've had 30 meetings, so when did that start? When did --

MR. SIEGEL: Probably ----.

DR. SALOMON: And we've had 30 meetings in or 12 years? I've been giving them a hard time for the last several meetings about not having more -- more titles, you know, good formal titles for these meetings. And, apparently, there was a title here. But it was taken off. Maybe

we'll get to see it later.

Anyway, one of my pleasures this morning is -- in addition to welcoming you here to this meeting is to introduce four official new members of the BRMAC. Dr. Katherine High, who I believe is not here yet, but will join us a little bit later; Dr. Mahendra Rao, seated over there; Dr. Bruce Blazer; and Alison Lawton, seated to my left. And, again, the dedication and the amount of time and energy of the members of the BRMAC to the sort of process is something I think all of us involved in it really respect.

I also see Abbey Meyers down there, which is a pleasure, welcome back. We're never going to let you go, is that what it is? Abbey's been a member of this committee off-and-on for as long as I've been a member and it's a real pleasure to see her back, as well.

So, I'd like to go ahead and get

started. We have a one-day meeting. The next meeting of the BRMAC will be three days-long, which, I guess, is proof that no good deed goes unpunished. So just when you thought you were safe to come back to Washington, maybe not, but anyway, I think it will end up balancing out.

This should be a very interesting meeting on adenovirus and return to my right-hand person, Gail.

MS. DAPOLITO: I'll read the Conflict of Interest Statement. This announcement is made part of the public record at this meeting of the Biological Response Modifiers Advisory Committee on July 13. Pursuant to the authority granted under the Committee charter, the director of FDA's Center for Biologics Evaluation and Research has appointed Ms. Abbey Meyers, Doctors Estuardo Aguilar-Cordova, David Gaylor, Marshall Horowitz, and Gary Ketner as temporary voting members. To determine

if any conflicts of interest existed, the agency reviewed the submitted agenda and all financial interests reported by the meeting participants.

As a result of this review, the following disclosures are being made. In accordance with 18 U.S.C. 208, doctors Katherine High, Estuardo Aguilar- Cordova, and Marshall Horowitz, have been granted a general matters waiver, which permits them to participate in the committee discussions.

Doctors Blazer, Champlin, Kurtzberg, Mulligan, Salomon, Sausville, and Ms. Meyers have associations with firms that could be affected by the committee discussions. However, in accordance with current statutes, it has been determined that none of these associations require the need for a waiver or an exclusion.

Ms. Alison Lawton is the non-voting industry representative for the committee. She is employed by Genzyme. Genzyme has

associations with various universities, investigators and research foundations that are involved in gene therapy. Ms. Lawton also has financial interests in several firms that could be affected by the committee discussions.

In regard to FDA's invited guests, the agency has determined that the services of these guests are essential. The following interests are being made public to allow meeting participants to objectively evaluate any presentation and/or comments made by the guests. Dr. Beth Hutchins is employed by Canji, Inc., and has a financial interest in a firm that could be affected by the discussions.

Dr. Eugene Rosenthal, is employed by the National Institutes of Health, Office of Biotechnology Activities. Dr. Rosenthal is substituting today for Dr. Amy Patterson, who is unable to attend.

Dr. Richard Sublett is employed by

Introgen Therapeutics, and has a financial interest in a firm that could be affected by the discussions.

In the event that the discussions involve other products or firms not already on the agenda, for which FDA's participants have a financial interest, the participants are aware of the need to exclude themselves from such involvement and their exclusion will be noted for the public record.

With respect to all other meeting participants, we ask, in the interest of fairness, that you state your name, affiliation and address any current or financial previous involvement with any firm whose product you wish to comment upon. A copy of the waiver's addressed in this announcement is available by written request under the Freedom of Information Act. Thank you, Dr. Salomon.

DR. SALOMON: Okay, going on, I'd like to -- I always try and start the

meeting with every body going around and just kind of giving a quick blurb on who they are. I think this is particularly important that today, as we have a couple new members and we're also joined at the table by several additional experts, Dr. Ketner, Dr. Aguilar, Dr. Horowitz. And, again, we're very glad that you guys are here with us. And so why don't we start with Abbey, just tell us a little bit about yourself and we'll just go around.

MS. MEYERS: Yes, I'm President of the National Organization for Rare Disorders, which is known as NORD and we're the orphan-drug folks. We helped to pass the law and we monitor the development of orphan drugs. And I served on the Recombinant DNA Advisory Committee for many years, and the Biological Response Modifiers has me since then.

DR. SAUSVILLE: My name is Ed Sausville, and I'm the Associate Director

for NCI's Developmental Therapeutics Program and we have an -- our major role is in the preclinincal evaluation and production of both drugs and biologics for use in clinical trials sponsored by NCI and by academic investigators with INDs.

DR. GAYLOR: I'm David Gaylor, I retired from the National Center for Toxicological Research of the FDA last year. I'm now working with a consulting firm, Sciences International. My area is biostatistics and risk assessment.

DR. RAO: My name is Mahendra Rao, I'm a Section Chief at the National Institute of Aging. My work is predominantly on stem cells, but I'm also interested in ----.

MR. BLAZER: My name is Bruce Blazer, in Bone-marrow Transplantation Division at the University of Minnesota and I'm involved in the immunobiology of bone-marrow transplantation and preclinincal

and clinical studies.

DR. CHAMPLIN: Richard Champlin, I'm the Chairman of the Department of Blood and Marrow Transplantation at M.D. Anderson Cancer Center.

DR. KURTZBERG: Joanne Kurtzberg, I direct the Pediatric ---- program at Duke University and work with umbilical cord blood transplantation.

MS. LAWTON: Alison Lawton, I'm the industry rep. I am senior Vice President of Regulatory Affairs for Genzyme Corporation and I chair the Cell and Gene Therapy Committee for the PhARMA Association.

DR. SALOMON: Dan Salomon, I'm in the Department of Molecular and Experimental Medicine at the Scripp's Research Institute, and my expertise and interest covers organ transplantation, gene therapy, tissue engineering and, more recently, angiogenesis. Thank you.

MS. DAPOLITO: Gail Dapolito, Center for Biologics, Executive Secretary for the Committee and Rosano Harvey, the Committee Management Specialist for the Committee.

DR. MULLIGAN: I'm Richard Mulligan from Harvard Medical School and we're involved in vector research and also stem cells.

DR. KETNER: I'm Gary Ketner, from the Department of Molecular Microbiology and Immunology at Johns Hopkins University School of Public Health -- excuse me, Johns Hopkins University, Bloomberg School of Public Health. I'm an adenovirus biologist.

DR. AGUILAR-CORDOVA: I'm Estuardo Aguilar- Cordova, I'm with the Harvard Gene Therapy Initiative, I'm involved in vector research and clinical trial applications of gene therapy.

DR. HOROWITZ: I'm Marshall Horowitz, I'm the Chairman of Microbiology

and Immunology at the Albert Einstein College of Medicine and a member of their clinical and the pediatric infectious disease group. I work on adenoviruses, I work on their immunoregulatory genes. In a sense, I'm coming home, in a way, I served four years on the FDA Vaccines and Related Products. And at the time, which was about ten years ago, related products started to include gene therapy. It's nice to see the expertise in the room this morning. It was very hard to find it ten years ago, and I look forward to the discussion today.

DR. ROSENTHAL: I'm Gene Rosenthal, I'm a biotechnology program advisor in the Office Biotechnology Activities at NIH. I'm substituting today for Amy Patterson. One of the committees that we act as the support staff for is Recombinant DNA Advisory Committee.

DR. BAUER: My name is Steve Bauer, I'm a product reviewer in the

Division of Cellular and Gene Therapies.

DR. FREY: Joyce Frey, Deputy Director of the Division of Cell and Gene Therapy.

DR. NOGUCHI: Phil Noguchi, Director of Cell and Gene Therapies in the Office of Therapeutics.

MR. SIEGEL: Jay Siegel, Director of Office of Therapeutics, CIBER (?), FDA.

DR. SALOMON: Well, please?

MS. WOLFSON: Alice Wolfson, I apologize for being late, I was driven to the wrong Holiday Inn. I'm the consumer representative.

DR. SALOMON: Been there, done that. And Katherine High hasn't joined us yet, so, that's okay.

Well, I want to say it's a pleasure to be with real professionals. All of you knew to push your button to speak, and then to turn it off, I'm impressed. That bodes well for the rest of the morning.

Okay, Gail, are we ready to go? Okay, thank you all very much, and I think we're ready to go.

The first speaker today is Steve Bauer, our own Steve Bauer, Office of Therapeutics Research and Review and he's going to talk about Adenovirus Vector Titer Measurements and RCA Levels. Steve and Joyce Frey-Concells, who are there were sort of the lead FDA people on putting together today and the questions involved, so they'll be very much involved in the rest of the discussions this morning with Phil and Jay.

DR. BAUER: It's a pleasure to talk to you folks this morning and I'd like to express my gratitude to all of you for being here and being willing to participate in this deliberation this morning about adenovirus gene therapy products.

My purpose is three-fold: I want to talk, first of all, just to update everybody on an initiative from the gene

therapy community to develop a wild type adenovirus reference material that we can use to better or improve our ability to measure the amount of adenovirus product, in terms of viral particles and infectious particles that we administer to people.

And the second purpose is to update the Committee and the gene therapy community and members of the public on recent changes in recommendations about some of the measurements of adenovirus products.

And the third is to introduce some of the questions related to those measurements and how they impact the clinical trials.

To put that in perspective, I have here a chart that shows current active gene transfer INDs and I wanted to point out a few things about adenovirus. This is our second most common vector class, about 58 current active INDs in this area. The majority of them, shown in green are direct

in vivo injection of the vector by a variety of routes. And then there are some, as well, that rely on ex vivo transduction.

And also, there's a variety of patients that are treated with these different vectors, so in order to put in perspective the kinds of patients who would be exposed to RCA and would be administered these products, I put this chart together to show you that the majority of patients are cancer patients. There are some coronary/vascular applications, some genetics and even some normals and then another thing that's relevant to some of the discussions we'll have later is how are these products actually administered, what is the rout of administration. And I've broken those out. So they go from injection directly into the lesion or the tumors. I mentioned ex vivo transduction of cells, but they're also intravascular, intraperitoneal, oral, directly into muscle and down the

respiratory tract.

So, there's a variety of routes of administration; a variety of kinds of patients. We'll be discussing later and I think it's an important issue: What is the status of the patients in terms of their ability to respond to either infectious virus or replication-defective adenovirus? A total of 58 current INDs, so that give you a perspective on what are the activity in this area.

So, the first thing that I'm going to do is tell you about this development of an adenovirus reference material. When it comes to looking at an adenovirus preparation final product, we measure several important parameters of what is actually in that product. And I diagram this here. There are a non-infectious particles and, actually, this is a challenge to measure infectivity and Dr. Aguilar-Cordova will talk about some of the

technical challenges that accompany that later.

And one of the important questions is what is our ability to measure infectious versus infectious particles, but I diagram here in yellow that actually the majority of particles as we measure them currently, or most people do, are non-infectious after purification.

Then there are infectious units, which will infect permissive cells they are replication defective. These are the ones that would actually deliver and produce the therapeutic trans gene.

Another component, and this is the one that's of concern is the replication competent adenovirus, and with the most commonly used production methodology nowadays, this is an unavoidable consequence of manufacture. And there will be discussions later of how replication competent adenoviruses arise in the current

production methods. But these would be infectious particles and could, potentially, could establish an infection in a recipient.

And then we also measure all these particles together and I'll discuss a little bit more how that's done. But we'll be talking about the technical challenges of these measurements and how we can improve them and the reference material, I think, will help the community at large to better measure these things.

The vector particle measurement, which is looking at all the different particles is a physical/chemical measurement. The most common methodology is to lyse the particles, measure the amount of DNA and correlate that genome number with the particle number. We'll hear a little bit more about that later.

The infectious activity is a biological assay and, therefore, inherently, more variable. There are a variety of ways

to do this, including measuring plaques on a lawn of permissive cells. These are cells that will allow replication of the otherwise replication-defective particle. There are other ways through immunological detection of some of the virus proteins.

Then, finally, the replication competent adenovirus is a biological assay, where you measure the infection on cells that don't complement the vector, that are not permissive for replication of the vector, the intended product, but will reveal the presence of a recombinant replication competent adenovirus.

These are all the measurements that I talked about are very important in terms of safety, efficacy and, also, in terms of an assessment of how well the production process is; how reproducible it is in manufacture of these vector products.

Of course the safety issue is pretty obvious, if you administer a

replication competent virus, what are the consequences you're exposing to that? And we'll have a lot of discussion about that later. The other issue is that exposure to viral proteins can cause some toxicity, itself, so knowing how many viral particles that you're putting into the patient is an important parameter and if there's a sharp elbow in the curve between dose and toxicity, having precise measurements of this is important.

The other thing is inefficacy. Of course, if there are a lot of nontransducing particles in a preparation, you're going to minimize your chance of actually having a benefit of expression of the intended gene. And as I mentioned before, this is a good way to assess a production process.

Well, all of these things, of course, have been recognized very well by the adenovirus gene therapy community for quite sometime. Starting in '93, adenovirus

vectors were first used in cystic fibrosis protocols. And even back then some of the technical challenges of measuring reproducibly, reliably, and across studies were recognized and there were recommendations at that time that are reference standard for vector being produced. There was a long interim during which there was quite significant development in this field, but nobody had really stepped forward to produce this kind of standard.

In 1999, with the unfortunate death of Jesse Gelsinger, there was a RAC safety symposium and there was a renewed call for standards. And there have been other calls for standards along the way, but I'm happy to say that this has gone forward rapidly since that point.

The goals of producing a standard are to have more consistent, safer vectors. As it stands now, particle counts, itself,

is subject to inter- assay variability, probably the best assays are 10 percent. Infectious units assay is even more variable, but there's nothing, really, to hang our hats on, in terms of comparing studies that are done in different preclinical settings, different manufacturers and even different clinical studies. So it's anticipated that our ability to relate measurements much more precisely to one another will really improve the safety profile and comparability of this vector product class.

It also will help us to develop regulatory policy with more solid data, and we can make our recommendations based on measurements that we have more comparability between different clinical trials across those trials.

So there's been formation of a group called the Adenovirus Reference Material Working Group. This is a

partnership between government, industry, academia -- it's going forward under the coordination of the Williamsburg Bioprocessing Foundation. And there's a lot of good information on what the activities of this group are at this Web site. There's also some at the FDA website mentioned here.

But a lot of the activities that were planned are going forward. This has been very gratifying to those of us who've been involved to see the spirit of cooperativity and volunteerism that has gone into this. A master cell bank, has already been donated; adenovirus wild-type virus stock has been donated; master viral seed stock has been made. The participants who will produce and formulate the bulk virus have been identified and the initial characterization and provisional titer of that preparation -- these participants have been identified; vialing, the people who will serve as repository and distribution,

these people have already been identified. And there's an ongoing effort right now to solicit contributions to look at particle infectivity. So, it's very active. I think by the end of this year, we'll actually have a standard to distribute.

And Dr. Beth Hutchins, Estuardo Aguilar, myself are some of the people and Steph Simek, who've been involved in this. And I think it's really a nice contribution to the field. We're very happy and gratified it's going forward. And I think this will have a big impact on our ability to look at these products.

So, now, I'm going to change to a discussion of or to a discussion of our recent changes and recommendations. And this is directly related to the information that we gathered from the March 6 letter that went out to all our sponsors of adenovirus gene therapy, as well as other gene therapies.

I'll discuss our change in recommendation on the particle to infectious unit ratio and the recommendation on RCA limits.

But to put that in perspective, I just wanted to introduce this idea. There's a couple different ways to look at risks associated with different products and I borrowed this term from the people in the field of radiation safety. But I think this is the way we basically looked at exposure to replication competent adenovirus. And that is to say that we want it to be as low as reasonably achievable.

So the recommendations that we've made were based on looking at the information in the March 6 letter responses and looking at the community's production data. We actually solicited data on all production lots, whether they were used or not, why they were not used in the clinic? Why they failed, in other words, a lot

release. And based on that kind of information, we reformulated our recommendations.

And as the field has progressed, as people have gotten better and better at making virus or vector. And as our ability to measure that has gotten better, these recommendations have changed several times over the years.

The other thing to discuss is that, as we currently use these recommendations, we apply them to all product lots, regardless of their intended clinical use. And I wanted to have discussion of the appropriateness of that. That is shifting paradigms to a more risk-based paradigm and the question is what kind of information do we have, what do we need if we want to reconsider this kind of approach, where we just say we want it -- the exposure to be as low as reasonably achievable.

So these recommendations of the particle-to- infectious unit ratio, the reason for this is, as I showed you earlier, that one diagram of what's in a purified adenovirus particle. It's a mixture of noninfectious and infectious particles and the infectious ones can either be of a desired type that will deliver the therapeutic gene or replication competent. But in the past, we've asked that there be per infectious units, less than 100 total viral particles. So this is, basically, you could express this as saying you want at least 1 percent of your product to be able to transduce or deliver the gene.

This previous recommendation, again, had been based on review of production lots in earlier days of gene therapy. The March 6 letter came in, that data was looked at and we decided in the spirit of trying to increase the efficacy or the ability to transduce to change this

ratio. And the recommendation now is that per infectious unit there be less than 30 total viral particles. So another way to look at that is to say that you want more than 3.3 percent of your preparation to be infectious.

Now, recognize that infectivity assays are problematic, and we'll have more discussion of that later. Keep that in mind, but that's -- that was the rationale for this change.

The other change was in the limit of how much RCA should be present in clinical lots. And the previous recommendation was that there be less than RCA and 10 to the 9th infectious units. And, as I've said several times, with recognizing that this is a problematic assay compared to the assay for vector particle, we've changed that. And the new recommendation is based on particle number, and we also dose on particle number, so I

think this is also in harmony with that. I think there's better precision in this measurement and this way of expressing it.

So the current recommendation is now there be less than 1 RCA and 3 times 10 to the 10th vector particles. And this was derived -- multiplying this number by that factor of 30, so that's how we came about getting that number.

Finally, I'd like to discuss application of this recommendation. As I said, this is currently recommended for all adenovirus vector lots regardless of the clinical use. And in a trial where there doses of 3 times 10 to the 13th vector particles, this recommendation would say that the potential exposure was up to 1,000 RCA. Now, you'll hear data later that there have been substantially higher potential exposures to RCA based on our previous recommendations and, as I said, this field has been going forward, our recommendations

have been changing as the technology's changed and improved, but I think that it's important to realize that this would be the current level and in the past, the levels have been higher.

So, if we wanted to think about changing from this overall type of risk minimization paradigm of as low as reasonably achievable to a risk-based recommendation, we need to consider what kind of information do we have and there's some literature on this. There's clinical experience with wild-type ad infection and Dr. Phyllis Flomenberg will give us a talk later about that.

At the last BRMAC, Dr. Steven Channick gave us a nice talk about that. And I provided for the committee members the transcripts and slides from his presentation last time, as well.

There's clinical experience with gene transfer studies and I've asked Beth

Hutchins and Dick Sublett to come and tell us about that and to relate to us what the status of the patients was in terms of their immunity and their potential exposure to replication competent adenovirus.

There's also some notable adverse events. I already mentioned the one, you know, the death, the unfortunate death of Jesse Gelsinger. And also an early notable adverse event in Ron Crystal's cystic fibrosis trial. And these still remain somewhat mysterious, but there have been questions whether or not they could related to RCA, but I think retrospective analysis of some of the lots using a standard will help us understand that sort of thing.

But the other thing is, what information would we like to see. If we would like to say, for instance, that for certain routes of administration, certain patient populations and so on, we can try to make more a risk-based recommendation than

this universal application of our current recommendations to all clinical lots.

And some, if you look at the data that came in with the March 6 letter, there are, actually, a wide variety of experience of how many clinical lots were made and how many had to be discarded because RCA. Some manufacturers had as high as 40 percent lot rejection, some had zero. And there's a lot of adenovirus vector biology that is involved in that. But if we have an ability, a better ability to quantify RCA and have some confidence that different clinical applications can have different standards, we could, perhaps, not be so wasteful in our adenovirus lot utilization.

So, I wanted to just spend a few minutes talking about what Dr. Steve Channick's talk -- well, since he's not here, found his talk very informative, last time. But I mentioned that some of the things that we can use to look at risks of

RCA exposure are what happens with the wild-type adenovirus infection and in different patient populations. And the previous information is focused -- the best studies, I think, are in the arena of bone-marrow transplantation. And in that setting, adenovirus, including types 2 and 5, which are used for gene therapy vectors can be a significant cause of morbidity and mortality in bone-marrow transplant.

And I think one of the take-home lessons is it's important to consider immune status in terms of infection or reactivation of a previous infection, and latency of viruses is an important issue.

Some of the lessons that have been learned by the field and that were communicated to us by Dr. Channick include, these: The neonatal adenovirus pneumonia is significant. There are sporadic, severe, localized outbreaks. If you look, these aren't really immunodeficient people, but

they're immunonative. If you look at SCID population where there's a severe immunodeficiency, they're at high risk for adenovirus. If you look at a syndrome like DiGeorge, where there's immune defect, there have been case reports of fatal hepatic necrosis.

In the realm of solid organ transplant, adenovirus infection of the transplanted organ has been recognized as a problem and perhaps been tied, recently, to rejection of cardiac transplants. The source of that could be reactivation in the recipient or the donor.

Then in AIDS patients, there's been some recognition that adenovirus can cause some problems.

So in contrast to that, there has been a lot of clinical experience with adenovirus and we'll hear about that, as well, or with adenovirus gene therapy products with relatively good safety

profile.

So, with those kinds of considerations in mind, we're going to later discuss whether or not recommendations regarding acceptable levels of RCA should be the same for all different clinical uses. I explained these two different ways of looking at risk, either minimizing it to the greatest extent possibility or look at risk-based. And then, I think, I pointed out that the status of the patient in terms of their immunity is an important consideration.

The other thing that's not on here, but I think is important, will be discussed later is route of administration. With the exception that we will discuss ex vivo transduce cells and whether or not RCA measurements should be done on those as well.

And then if we would like to move towards a different recommendation for RCA

exposure, what kind of information should be gathering. And I think having a standard will help us look retrospectively at what's been given and correlate clinical outcomes there and we'll also be able to increase out precision and accuracy of future lots. So I don't think we necessarily have all the data that we want to do this now. That's a point of discussion, but I think we could think in terms of, you know, gathering it and what we need to do that.

An with that, I'll conclude, thank you for your attention and I'll take any questions.

DR. SALOMON: Thank you, Steve. I had a couple questions and there may be some questions from the rest of the group. The first one is, are we going to come -- are we going to get some more information a little later in the day about going back to the standard reference set and exactly how that's going to be used? Because, if not, I

think that would be something important to just to give a little more detail on.

DR. BAUER: Well, Dr. Aguilar will talk a little bit more about that in his discussion, so, if you haven't gotten the answer to whatever questions you have at that point, you know, bring it back up.

DR. SALOMON: A second thing is, are our speakers today going to also talk about -- there are some developments in the vectorology of the adeno, as well as some new strategies for producer cell lines, and other production techniques that could significantly alter, for example, the ration of replication-competent virus and also, hopefully, increase the efficiency of production of infectious viral units. And that also could have, I think, significant bearing on our --

DR. BAUER: Absolutely --

DR. SALOMON: Discussions.

DR. BAUER: Absolutely, there are

developed and developing alternative ways to produce the vector that are much less prone to this recombination event, that ends up in RCA. And you'll hear from our speakers what that kind of recombination event is and how to avoid it. So, we'll get some information on that later.

DR. SALOMON: And the last question, I'm sort of playing a role now, as chair, but, you know, clearly the field has been under the shadow of the Gelsinger case, publicly, if not also within the experts involved. The obvious question here, of course, is when that's driven so much of the concern over the safety of the adenovirus in the last two years, until recently, I understand, that for legal reasons there were -- there's been not a lot of information that's been able to have been shared. At this point, are we going to have any information on the replication competent retroviral titer and particle titer, et

cetera, of those preparations that were used in that case?

DR. BAUER: To my knowledge, the status hasn't changed and we're not able to disclose everything -- I don't know they'll -- any of my FDA colleagues have a different answer for that.

DR. SALOMON: Well, that may be something that other members on the committee may want to comment later but, again, sort of just playing a role his as chair, I would say that the discussions and the recommendations of the committee will all have to officially be taken in the context of if we don't know what exactly happened in that case, and I'm not, you know, saying that right now there aren't good reasons why we're not getting full disclosure on it -- I was just saying, without knowing that, at the same time all of us around the table knowing that, you know, there was a patient death involved, I

think we have to make sure that officially, at least my view of this is, that that has to modify anything that we come to conclusion today. Any questions from anyone else, Mahendra?

DR. RAO: I had a quick question about the monitoring on the patient side. How do you distinguish between the activated virus versus donor virus that you've put in to the patient if the seratype is the same, I mean, from wild-type?

DR. BAUER: Yes, that's a good question. There are hallmarks, molecular hallmarks of recombinant virus that you can look at. And we have asked people if they have indication of a replication competent adenovirus arising in a patient to look at the molecular structure of that. So you could distinguish that between a reactivation of a latent virus that had already been there and a replication competent adenovirus.

DR. SAUSVILLE: So, there are two potential, as I see it, components to the issues that we will be discussing. One is a set of problems, such as the generation of replication competent that may occur at a given frequency, but affect a very special patient subset. And then there's the more general types of toxicity that may reflect the range of normal, quote/unquote "host responses" to adenovirus co- proteins, et cetera.

Does the agency have a perspective as to which of these components is most important or should dominant the discussion, as it were, because I think they lead us in somewhat different directions.

DR. BAUER: I think they're hard to separate those out, and I think that both are important, but I hadn't really thought this through before, but thinking about the replication competent adenovirus as an established infection is probably the area

that I would like most feedback on at this point. I think it is unclear and there, you know, there is some information on adverse events with -- in preclinical models saying that innate immunity and an immediate or very quick response to the adenovirus co-proteins is an important toxicity. But the relationship between that and the adverse events in the Gelsinger case are probably -- that -- since that was a very quick relatively fast event, that probably reflects the kind of response that would be innate immunity and not so much an infectious event, but I think we do want to discuss infectivity of adenovirus products. Does that help.

DR. SALOMON: Abbey? I'm just going to let Dr. Mulligan respond, because I think he's going to respond specifically on this, and then go to you.

DR. MULLIGAN: I would echo the importance of Ed's point. If you look at

the Gelsinger case, I would think that it's not going to be because there is replication competent adenovirus that there is difficulties. But it may be there is just more adenovirus than a human has ever seen. And so, I'm struck by the issue on the focus on the replication competents as opposed to other things that track with real recombinant adenovirus particle. So, for instance, if there was proteins that are associated with the virus particle because of the cell type in which you propagated the vector, could that account for differences between the toxicities that you might see. This is exactly the kind of thing I would think in the Gelsinger case could be an issue. That is, you're not only putting in many, many particles but, you know, could it be that there's something in those particles, some contaminating host protein?

Certainly, I know about retroviruses and in the case of

retroviruses, it's very, very clearcut depending on how you grow the retroviruses, you get different host proteins that incorporate in the code. Now it's a very different system than adeno, but I think that's an issue, perhaps, not for, you know, this conversation, but I think it's key.

While we're on the replication competent question, I'm curious whether you went into the very old literature that looks at the helper, the e1-like capacities of different cells, too? One of the questions I was thinking, as you were talking about how you calculate the amount of RCA is, you know, is there a possibility that there are human tissues uniquely, a pocket of pituitary cells or something, that actually have e1-like function and, therefore, you would have replication competent adenovirus vector in vivo, uniquely in those cells? And Marshall, you know, might have something to say about this. But that would be

another way to look at this where all of a sudden the issue's not an issue of having a hundred or a thousand replication competent adenos, but 10 to the 6th or 10 to the 7th depending on what tissue you hit. So, I'd like to hear if there is something, you know, do we really know that that can't happen is there any precedent for it?

DR. KETNER: Yes, there is data. In the teratoma cell line, there are complementing e1a functions that allow the virus to grow. That's old data from -- it's quite old data -- state of the cells and I don't remember exactly which way it went, but one of the two states of differentiation of teratoma, human teratoma, did complement the e1a functions.

DR. MULLIGAN: That's interesting, from the tumor application, of course, you know, where, you know, if you had some undifferentiated strange tumor type and you were trying to direct infection, would it be

possible that you would have replication, so it just calls into question, you know, how you'll actually measure and whether it is relevant to try to see whether there's pockets of tissues where you'll get replication competents.

DR. SALOMON: Well, I think that's very interesting. Part of what you're saying, though is not that replication competent adenovirus wouldn't be an important thing to measure, but you're actually suggesting it ought to be measured also after infusion of the viral vector, perhaps at several different times. And that's not something that was mentioned.

DR. MULLIGAN: More just the appreciation that their might be an interest in trying to push towards looking at this question of compensation. And the other issue is, whether or not, unfortunately that complicates potential regulatory guidelines where, maybe the status of the vector

becomes much more important. Is it doubly, you know, how many different complementing events in the cell would you have to have to actually give something that would look like replication compromise.

DR. BAUER: Well, one thing, I can say is that you'll hear some data later about monitoring for virus shedding. Or in the peripheral blood and looking at what is there and how long it persists. So, the model that you're putting forth that you have complementation by e1-like activity in the cells, I think we would probably see that by that kind of monitoring. If you look at a virus that is increasing in titer or that there's a lot of shedding or unusual persistence, and you say that, then you could look at what is the structure of that virus. Is it what you put in that replicating for some reason? To my knowledge, there isn't any evidence that that sort of thing's going on, but I think

that is something to keep in mind, and it might be affected by, as Dr. Horowitz said, different kinds of cancer.

DR. MULLIGAN: The other thing is that in the simplest case, the virus that is essentially replicating in particular tissue, when assayed in culture, would not be replication competent, so --

DR. BAUER: Right.

DR. MULLIGAN: You might have a local effect of having replication competents, but you'd never really see it as such.

DR. BAUER: Right.

DR. SALOMON: Abbey, you've been put off a little bit, I'm sorry.

MS. MEYERS: Patient. If there were something that happened in the Gelsinger case that is important for the purpose of discussion today, and especially would help us in the public health mission that we're supposed to have, I don't

understand why we can't talk about it. Now, if there's proprietary information, can we just meet in private without the public to discuss what we know? And if it's not proprietary and there's some other reason, what is the reason?

DR. NOGUCHI: Well, it is a good question, I think on a practical basis, Dr. Wilson has actually recently published or is in press a couple of more- detailed analyses of the events surrounding Mr. Gelsinger's death. In fairness, none of that really seems to shed any great light or insight as to what to really look for in terms of questions of whether it's RCA, whether it's an immune function, whether any of those are absolutely critical. I think the available data would suggest it's really a set of immune, cascading immune responses to the massive viral load that really is most closely associated with his death and the spiraling events that we saw. But

that's really not anything that I think is any different than what was discussed almost a year and a- half ago.

So I, your point is well taken, but I don't think we have anything that we feel would add substantially to our discussion here on that. That's part of the issue. The numbers of adverse events that are critical in terms of being much more than expected are relatively rare, and they're in a few different cases. And that's part of the difficulty that we're facing here in that we don't have enough information, even on those cases, in order to be able to compare.

I think that's one of the reasons that we have been focusing on creating a standard, which should have been available, probably, earlier, but now, by focusing on the standards, by focusing on all the different events that can occur and all the considerations, such as packaging of novel

proteins, I think we can do a better job in the future. But right now, we just, unfortunately, don't have a lot more that either privately or publicly we can add on the Jesse Gelsinger case, vis-a-vis leading us to a new approach or a better approach.

MS. MEYERS: So, for the purpose of today's discussion, then, the Gelsinger case, there's nothing being held back that we don't know about?

DR. NOGUCHI: That's correct.

DR. SALOMON: Phil, one of the concerns I have is, if we go forward, let's say, and at the end and get into discussions of sort of a maximal viral particle load that could be administered in a clinical trial -- which is one of the questions, indirectly here -- do we know what Jesse Gelsinger got, in terms of number of particles? I know -- I've -- do we know what -- did we really know what he got? In other words, was there an error? Did he get

more than we think? I mean, that's the kind of thing, I guess, that my initial comments were aimed at, that I just see that as coloring our discussions somewhat. Not necessarily implying that anyone's holding anything back, but just not know exactly what happened there is an issue for me.

DR. NOGUCHI: Well, that point is well taken and I don't have those figures right on hand, but it's clearly of the nature of close to 10 to the 13th particles, that's total particles and the transducing units was -- Steve, do you remember exactly what that way? It's an -- it's an excellent question, we just didn't prepare for the --

DR. SALOMON: Right.

DR. NOGUCHI: I would like to suggest that a lot of these questions are of a more general nature and we'd like to make sure that we get all the prepared talks in here, some of the questions will be answered, many will not be, but that's part

of what the more general discussion for the afternoon is about.

DR. BAUER: I just had one more comment in response to Dr. Sausville's earlier question and thinking about -- seeing the discussion that's going on now and where do we want to go? I would like to focus more on the relationship between, you know, the risk for RCA infectivity and exposure in patients, rather than toxicity.

I think the story that the viral proteins have toxicity, you know, is pretty well acknowledged and that the acute -- very acute toxicity is related to that dose. There might be a range of responses that we don't appreciate how to, you know, predict that when we first, you know, administer the product. But I think if we could focus -- it's -- it's hard to say these are totally separate, of course, but we can focus more on the infectivity risks.

DR. SALOMON: Last question,

Dr. Kurtzberg.

DR. KURTZBERG: Is there any data regarding the state of the patient whose receiving the adenoviral vector, in terms of their innate immunity or previous exposure to wild-type adenovirus or whether or not they're shedding adenovirus at the time of the gene therapy?

DR. BAUER: When patients are enrolled, they're -- most of them, in the past, have been assessed for their immune status with regard to antibodies and to adenovirus and they're also looked at in terms of their -- whether or not they have a current infection, by adenovirus or other viruses, so those patients aren't -- these products aren't administered to those patients. So you don't want to give something to a patient where there's a helper virus already in the patient, for instance, so.

And you'll hear some data later

about what was immune status and what was the outcome of the immune system response in certain patient populations, to the vectors.

DR. SALOMON: Thank you very much, Steve.

DR. BAUER: Mm-hmm.

DR. SALOMON: So just to put this into context, then, our first obligation as a committee by the end of this afternoon is going to be to respond to the FDA's staff's specific questions. And those are outlined in your book -- your panel. And that's -- that, at some time we're going to have to just focus on answering those questions because that's what the job of this committee is to do.

However, as is evident from the discussions that we just had, there are other issues and it's not necessarily meant to imply that those issues aren't as important in thinking about adenovirus. So, to the extent that we can separate questions

that we specifically have to answer from more general discussions of topics that are not on the docket today, then that would be extra value for the -- for the FDA staff.

So, at some point here, I don't find any particular problem with the idea that there may be alternative things to talk about. If you'll forgive me, ever-so-often focusing us down on that. I think we'll have a fairly reasonable day, in terms of time, but that's maybe famous last words, so --

MR. SIEGEL: I just want to clarify on that, actually, we would much appreciate, and will benefit, time permitting from any discussions on any topics related to adenoviral safety. And I think what Phil was talking about in terms of general questions was, simply, that we think it might be useful to have questions for clarifications to the speaker-specific points after each speaker. But in terms of

some of the general discussions we're getting into, it might be best to have all the speakers first, because I think they'll inform of those discussions.

DR. SALOMON: Yes, absolutely. Okay. Well, then, it's my pleasure to introduce a new friend. Dr. Estuardo Aguilar-Cordova from the Harvard Gene Therapy Initiative, whose going to talk to us about history and overview of adenoviral vectors.

DR. AGUILAR-CORDOVA: So, I was asked to just give a general overview of some of the adenoviral characteristics, which I think will be pertinent to the discussions and understanding. I know it will be very repetitious for some of you.

Also, then, to give a little bit of the nuts and bolts of what it means when people talk about titers for replication competent detection. And, finally, to give you a little bit of background on the

standard that is being developed by a working group with great participation by Beth Hutchins and Steve and Stephanie and others.

So, historically, the adenoviruses were identified in the early 50s from an adenoid and they're an etiological agent for some of common cold- like symptoms and other inflammatory responses in various tissues, depending on the seratype, but they have not been identified as an agent of any tumerogenic potential in human tissues.

They are a linear double-stranded DNA encapsulated in a protein shell. There's over 100 in the adenoviral group, two different characterized adenoviruses. And the wild-type has been used as a vaccine in military recruits, actually, of seratype 4N7 (?), and I think we'll hear a little bit more of that indicating some of the safety profile that is known in this type of viruses, at least when interaclly (?)

delivered. It's important to understand a little bit about the characterization -- physical characterization of the adenoviruses and it's an icoshedral. And this is really quite critical: 13 percent DAN and 87 percent protein and that's how, back in the early sixties it was determined that 1-OD (?) of adenovirus is roughly equivalent to 1.1 times 10 to the 12th viral particles, by comparing the protein characterization and then the OD readings and so, and it has been borne out by other types of analysis in the future that in fact that does hold true and that's a fairly accurate quantitative measurement. The gene structure and organization of adenoviruses are -- has two ITRs and two origins of replication. The transcriptional units are 5 "early" genes; 2 "delayed early" genes and one major late transcript. What's important is that these are what has been primarily lated in the vector constructions and I'll

go through that a little bit in a minute.

They were able to be manipulated with vectors with two critical characteristics of the virus: That it can package up to 105 percent of it's genome size, so if one can take a little bit off, one can also put a little bit back in. And, also very important, it can be manipulated as a circular form, put back into the cell and it'll go into the linear transcriptional unit.

So, the early antiviral vectors were considered replication deficient. One could package up to about 8 kilobases (?) of foreign DNA by doing E3 deletions and E1 deletions. It's relatively easy to produce it in high titer, it can infect a wide variety of tissues and it has high expression in non-replicating tissues.

So there's been an evolution of adenoviral vectors and, again, this will be, perhaps, important in our discussion of what

the importance may be of RCAs and such. Original vectors, one would stick a gene of interest in the E1 region and that would be replaced, and it could have various deletions in the E3 region. The E1 region, as I mentioned earlier, is a transcriptional activator that would then lead to expression of E3 for the other early transcripts and then, ultimately, to a cascade that would start the transcription of the late transcripts, most of which are structural proteins that would serve towards creating new viriants (?).

However, as mentioned by Dr. Mulligan a little bit ago, there are multiple cellular proteins that have been described that are able to basically pinch hit for the E1 functions, including IL6 is one of the critical ones. And that can, in fact, transactivate some of these early genes, which could then be toxic to the cell. We know that the E2 product and the

E4 products, some of the E2 and some of the E4 products, are toxic to the cells and, in fact, there's an E3 product, which would be transactivatable because there are enough papa B and AP1 sites in that promoter, which could be upregulated and in different cell types. And there's a protein that are called the adenovirus death protein, which serves towards lysis.

So there are multiple ways in which a vector not being replication competent can still cause significant tissue damage and it can be observed in almost all cell types in vitro, where, if we put sufficient concentrations of replication deficient vectors, we will observe cytopathic effect.

Now, the first-generation vectors that I've been mentioning here are E1 deleted and they can be either E3 deleted, partially deleted or not deleted at all. So-called second-generation vectors, went

farther and deleted either the E2 or the E4 regions and transcomplimented in the production cell. I will show you some data that may or may not have any effect on their toxicity.

And more interesting, we get in the gray area which, what I'm calling here Generation X, I just -- because it doesn't really matter, but these are prototyped by the Onyx virus, which is, in fact an E1A positive cell, E1B minus, and that supposedly is effective only in P53 negative cells because the E1B would, then, bind the P53 under normal circumstances and absorb it out, so letting the cell go through the replication cycle. So, putatively, when there's a P53 minus cell, the E1B would not be necessary in that case and this virus would then replicate more efficiently in those cells and it can, again, be E3 plus or minus.

This is very close to a wild-type

virus and this is what's been used in all the Onyx trials. There are Generation X.1, which are where they put either the E1A or the E1B with tissue-specific promoters and then, theoretically, this would only replicate in the tissue where the promoter is active. But as we all know, many of these promoters can be leaky, especially when they are outside of the genome context. So, again, very gray zone as to whether these products are, in fact, replication competent adenoviruses and their measurement of what is an RCA in these would be a complicated issue.

And this, perhaps, should have been called Generation Y, because it's completely different. And these are the helper dependent vectors and the helper dependent vectors are those in which, basically all of the genome of the adenovirus has been taken out and only the ITRs and the packaging sequence remain.

So, as I was mentioning in the sort of Second-generation vectors, some data that was by O'Neal, *et al.*, in human gene therapy back in '98, and what we can see here, this is platelet count and it's been a fairly repetitive observation in vivo, in humans that there is at least a transient thrombocytopenia that's observed and in some cases a consumptive thrombocytopenia after adenoviral vector delivery. And we can see here that the deletion of an E4 or an E2 doesn't seem to generate much of an advantage with regard to the causation of thrombocytopenias.

Also, when we see here the elevation of ALT, what we can see is this is 1 times 10 to the 11th, 1 times 10 to the 12th, all three of these experiments were done at 3 times 10 to the 12th and this is 1 times 10 to the 13th. Down at the bottom are doses, the effects were not noticeable, either with a first- generation or

second-generation, only in the 3 times 10 to the 12th window was there a differential of any statistical significance, but once one gets to the 1 times 10 to the 13th, again, that significance is gone.

And what's also of interest to note is that the difference between almost no effect to a complete, very significant rise is only a ten-fold difference in as far as the quantity that was delivered to the -- to the animals.

That's not to say that all of these things make absolutely no difference. Here we have what I call the Generation X.2, or the helper dependent vectors and what we see in the liver enzymes in this case. This is the first-generation vector, one can see as the dose increase, there's an increase in the liver enzymes that then drops down, so it's self- limiting. And that was not seen with this helper dependence, even at the same number of particles.

So these are all equivalent particles, you can see the level of -- the duration of expression from this first-generation vectors was very low, whereas the duration of expression from this later helper dependent vectors was much longer lasting, this is 8 weeks in this case. And -- but, also, that's not to say that this is always the case. We have seen, in some of our monkey studies, where occasionally a monkey would develop immunity against the transgene and limit the effect at all. But this does start to address a little bit on the -- at least at these low doses of incoming viral proteins that maybe it's not the low-dose of incoming viral proteins that's causing that acute increase in liver enzymes, as here we have the same number of viral particles and we can see with the first-generation there is some more acute toxicity. It may have to do with the early gene expression that we see from these

vectors.

So, from this first part of it, we can see that adenoviruses can be converted into efficient transfer vehicles. They're not inherently dangerous as a class of vectors or vehicles. Not all adenoviral vectors have equivalent toxicity profiles, but some of the safety mechanisms that we might have heard or read about may not be that significant. The dose of vector is related to the toxicity of serve, that is -- that's very clear and reproducible, the more we give, the more toxicity we observe.

And I will now show you that standardization of dose specification is necessary and that it hasn't been there to date.

So, characterization of viral vectors are two things, generally speaking: Purity and strength. And lack of contamination by advantageous agents, including replication competent virus as

part of the purity issue. And the strength is the active concentration for toxicity and efficacy, just very loosely defined. And I'll go through some of the things that we do here.

Physical determination, as I've mentioned is the most common method utilized at this point, is absorption measurements and, as I said, from the early 60s it's been known that 1OD 260 is about equivalent to 1.1 times 10 to the 12th viral particles. This has been confirmed by other methodologies, as well. So, that's a very quantitative measurement.

The next is the biological determination, that is the infectious units. And we've heard a little bit from Dr. Bauer here about how, right now, we're content to get 3.3 percent activity, that's -- the other way of looking at that is that there's 96.7 percent nonactive in each one of these preps.

And how we determined this -- it has some physical characteristics and I'll go through some of these. But the key issue here is the likelihood of the vector and cell ever meeting in your detection system, so that you could ever see it. And there's some functional characteristics of the system whether the cell that you're testing it on has good receptors for the adenovirus and it's detected. Most people are using either HeLa A549s or 293 cells for detection systems, so I won't go through these. All of these are highly susceptible to adenoviral transduction. But I will go through some of the physical characteristics.

Typical titer set up has a culture dish, some cells at the bottom, adherent cells, and then one puts a mixture of virus on top of those cells. The collision between the virus and the detectors cells here is mandated by Brownian motion of the

virus, a concentration gradient towards the cells or external forces, and I'll go through each of these.

But Brownian motion is random and for small molecules within a liquid at this temperature, it's basically negligible. What that means is that, by Brownian motion, those viruses will never move from where they are, and these cells will mostly just see the viruses that are right here next to them, but they'll never see anything that's up here.

And with the -- four or five years ago some experiments to show this and that is by putting different volumes of the same virus dilution onto some plates, we could observe the number of positive -- this is betagal, so we could see little blue cells, and these are averages over many, many wells. And what you can see is that it doesn't matter how much volume we put on top of it, the number of positives of serve was

the same, which is what we predicted by just physical characteristics of the system, which, if we translate that to titers, we can see there's a significant difference in calculated titer, so how one sets up the assay will have a significant impact of what titer is calculated.

We then did some centrifugation experiments calculating the displacement of the virus, if we were to centrifuge the plate, and that was just calculated as a distance between the pressures, RCFs put in, the time and the sedimentation coefficient. And by doing that, what we can see is that now we've brought down by spinning at 90 minutes for 1,000 RCF, we calculated that the distance traveled by any viriant would be .4 centimeters and we knew the depth of each one of these wells.

So, at 50 microliters per well, all of the vectors should have come down and the same is true at 100 microliters per

well. But at 200 microliters per well, the depth was greater than the distance traveled. So we didn't expect all of the vectors to come down. And, in fact, that's what we saw. These are the static conditions and the vector particle-to-infectious unit ratio went from 22 to 82 under static conditions.

When we centrifuged the vectors, we observed that there was exactly a two-fold difference between 50 and 100 microliters, as expected and not a two-fold between 100 and 200, again, as predicted, the titers are much higher, as you can see, we're now at 1.5 times 10 to the 12th versus a low of 9.8 times 10 to the 10th, and if you remember where the toxicity thresholds, a ten-fold difference is very significant in whether there will be toxicity or not. And the viral particle-to-infectious unit now are between 5 and 9.

And I won't go through what all

these calculations mean, but this is using the fixed law of diffusion and Stokes' equations and by that we can then calculate what is the maximum expected observed hits and what would be the true value behind that. And by doing that we come up with what we call the normalized and standard titer.

And if we calculate it whether using the static conditions or using the centrifuge conditions, we come to very much the same number of titers and we see that the true value of the viral particle-to-infectious unit ratio in that preparation was really closer to 1.3 to 2.4 and 1.3 to 1.9, very, very tight numbers.

And based on that, we conclude that the majority of the particles that are being produced in these high-quality productions are, in fact, infectious. And it is only a consequence of the detection system that we come up with the empty viral

particles or non-infectious viral particles that are often talked about.

There's one other little bit of information that's critical within this and that is that there's an original titer and there's a clinical titer. And it's important for people to keep track of the titer, not only at the point of production but, also, at the point of the clinical distribution. This was a little experiment, it was published back in "Nature Medicine," back in 1999. And what it showed was that when these vectors were being transported to the clinic in dry ice, even during a short period of time, the PH of the buffer that they were being transported in would drop, the virus would precipitate and one could lose seven logs of titer in a -- in a very short period of time, within hours.

So that shows some of the problems that may be attached to quantifying these viruses, and also some -- why it might be

very important and now then, why do we need to standardize and what is being done for the standardization?

We need to standardize so that we have something to measure it against and we can all be talking about the same things. People talk about platforming units, CPE units, all kinds of different units and we in our laboratory, when I was back at Baylor, we had made a production and sent it to six antiviral laboratories to be titered, and there was a two-log differential between the lowest and the highest titer that we received back from the people that had experience in titering adenoviruses.

It's very important to have something that really -- is meaningful. And when there's a threshold of toxicity between patients, and one group may base their starting dose or their continuing dose, based on another group's toxicity observed, if that has no correlation to each other's

titer, then it really, it's very meaningless. So, it's crucial for managing the manufacturing process but, also crucial for maintaining consistent, quality controls and dose escalation studies. And, ultimately, of course, from the producer's standpoint, it will be critical for ever having a true product.

And, lastly, I'd like to say that the standard doesn't need to be perfect at this point. Since I've already pointed out to you how there is a lot of difficulties in where and how to measure these things. And that, hopefully, will continue to evolve, but if we have a fixed point, then everything else that's around it can be related to that fixed point. And if that fixed point is there as we talk about this square, we know that it's one down and one to the left, when we talk about this square, it's two right and two to the -- two down and two right. That, also let's us measure

what's the difference between this point and this point. So that will be an equivalence so that we can all be talking about at least comparable units.

This is the last slide and, unfortunately, one can't tell very much. But there's a moral in here. And this is just a quick note about talking of multiplicity of infection. It's a fairly meaningless term and we're all very used to it. It's a classic virology term, but as you can see here in these two diagrammatic wells, they're both a multiplicity of infection of one, we have the viruses here. I guess it would be .3, but this is much more likely to reach the cells than this one is. And, yet, they're the same MOI. And when one goes fishing, one numbers the number of Marlins probably a multiplicity of infection of 10 to the 16th and 1 and there's 10 to the 16th of them, it's very hard for me to ever find one of those so, I

would say that using the terminology of multiplicity of infection is not very useful in characterizing these viruses. Thank you.

(Applause)

DR. SALOMON: That was excellent, Estuardo. So, one of the things that just occurs to me is that I found it very striking that there was such a difference in the titer when one did something as simple as move the virus from a production facility to the bedside. And that's a concern, actually, we've had in our own laboratory with retroviral vectors, that many of them fall apart at rather small PH changes, and it sounds like the adenovirus is prone to the same thing.

So how do we -- number one, have they figured out a way to address that issue, since that would be dramatic? And, secondly, related to that, how does that fit into how one would manage the standard, because it could be quite a problem, if at

different times, you standard was changing because of the conditions.

DR. AGUILAR-CORDOVA: Yes, and so, the first answer is, yes, that the way to manage that has been figured out. And it's fairly simple, you know, one just doesn't -- one protects it from dropping the PH in this case, either by a physical barrier or different packaging, formulation makes a difference and such. And, you know, our industrial colleagues, in industry they have a lot of experience in this. And what they do is, they usually validate shipping conditions and they validate that the product at the end has the same characteristics as the product in the beginning. A lot of us in academia had that kind of experience in the past, so we weren't quite as aware of these things.

And for the standard, it has been done vialing and it was an issue that's come up in our many discussions -- the vialing

system, the formulation of it and the shipping -- storage and shipping, to validate that it has the same characteristics.

DR. SALOMON: Dr. Mulligan.

DR. MULLIGAN: Could you walk us through the exact context where the standardization would be helpful? I can definitely see for detection of RCA how helpful having this would be, because you're looking at truly presumably the same thing, wild-type versus a wild-type. In the case where you have -- people have vectors that are different -- that are not growable on, you know, 293 cells, so doubly deleted or more sophisticated things.

I'm not sure I see how this would help guide standardizing the dose given to patients because, you know, the characteristics of the growth of these things in different cells is different. How would you see that this would be helpful for

that application, which is a very important application to make sure that some people aren't giving, you know, 1,000 times more virus than we know to be safe?

DR. AGUILAR-CORDOVA: So, from the standard of particles, if one is working with an antiviral particle, a particle is a particle, so it doesn't matter how it grows within a cell. So it still will standardize the measurement of viral particles. In addition, to that, if one is working with what the majority of vectors are today, which are simple, that will grow in 293 cells and such, or whatever production cell line one is using, they will usually call a CPEF, a cytopathic effect on those cells. And so one does not need to be measuring, necessarily, whatever the transgene is but, rather, the physical effect on those cells.

And at least one has -- and the replication competent will cause the same issue. So, at least one has the ability to

quantitate it with regards to something else. It's unlikely that one would have a vector that would be that much more efficient than a wild-type in causing the cytopathic effect.

Now, if you get farther towards, say, a helper dependent vector, in that case, the standard would probably only be useful in as far as the particle number.

DR. SALOMON: So, getting back -- the standard is going to be wild-type adeno?

DR. AGUILAR-CORDOVA: This first standard is going to be wild-type adeno.

DR. SALOMON: And it's going to be adeno-5?

DR. AGUILAR-CORDOVA: It is an adeno-5, yes.

DR. SALOMON: Right. So, how big a problem will that be for vectors based on adeno-2 or 4?

DR. AGUILAR-CORDOVA: For vectors based on adeno-2, it'll be almost no problem

at all because they're very similar viruses.

DR. SALOMON: Right now, are the criteria for the clinical trials that are ongoing as specific as would be suggested by some of your discussions? Such as, when you do the titer counts, you have to centrifuge at so many RCF for so long in such a volume to do the sort of particle counts? I mean, how much standardization do we have today on that? And that may not be -- let me direct it first to you Estuardo, but then, perhaps, to the FDA staff.

DR. AGUILAR-CORDOVA: To my knowledge, there is no standard protocol and, in fact, there is this system, including the physical calculation which we term NAS titer, or normalized adjusted standardized titer, that was proposed a few years ago, but, at this point, I think that there is no standardized protocol, and that's why a lot of the numbers, even that were received in the March 6 letter, which

is now, one has to do the best that one can to interpret those, but a lot of those numbers are just not comparable.

DR. SALOMON: Steve, Joyce, do you have comments?

DR. BAUER: Yes, I have a comment about that. It might be true that if you looked at different vectors from different people and, you know, gave each other your vectors, you'd get different titers, but we're also looking at the fact that it's one manufacturer whose done one set of preclinical studies and they're dosing -- in their preclinical and clinical studies based on their own measurements. So, at least within their study, they are, you know, achieving a fair degree of reproducibility.

The question is, how would that compare across studies and that's an important thing for us to make some of these recommendations and considerations about toxicity but also, I think that the dose

that you start with in the clinic is always based on a preclinical study with your particular vector preparation, so that the comment that Estuardo made about basing doses, I think that's true to the extent that you might look at other studies to base your starting dose in the preclinical model, but then you would use that dose and your own experience in a preclininical with your own product to determine what your dose range in the patients will be.

DR. SALOMON: And we won't get into it today but, of course, preclinical models are either small rodents or large animal models. And I don't want to say the obvious, but for the record, you know, going from a dose in a nonhuman model to a human model has got it's difficulties. And most clinical trial designs are done as dose-escalation studies, particularly at this stage in gene delivery, so, you know, at some point here this dose issue is

really -- is going to be critical.

DR. BAUER: I think, also, one of the benefits of having the standard is it will, in a way, bring people to use the same methodology, you were asking about that, but I think that's going to be an important outcome.

DR. SALOMON: I think that's great, yeah. That's why I wanted to make sure that we got enough discussion of that. Dr. Lawton and then Dr. Mulligan.

DR. LAWTON: I was actually following up on the comment that you just made and one of my questions was, from a practical perspective in the future, once the standard has been set and the methods have been set, are you assuming that everybody will, indeed, be working to that so that you have that measure, and that that will be a requirement that those methods are used so that you understand it across all of the different companies that are doing the

work?

DR. BAUER: I think we haven't really seen what the outcome will be once we have a standard or -- let me just say one thing, we refer to it as a reference material, because the word standard in regulatory parlance has a specific meaning, so -- when the reference material is available, we haven't said that we're going to require everybody to do the assay the same way, but my belief is that, in effect, that's what will happen because there will be a titer assigned by a consortium of laboratories an agreed-upon standard and I think, given the kinds of consideration that have just been pointed out. I think people are going to have to use the same methodology, but if it -- if that doesn't work out, you know, we might go to saying, well, you need to do it this way so we have confidence that we can compare titers.

DR. SALOMON: Dr. Mulligan, do you

have a comment?

DR. MULLIGAN: Yes, you raised this interesting question of the E1A plus E1B deficient vectors. How has the FDA looked at those in terms of RCA? I would have thought from what you see in papers that, you know, you can get virus titer, you know, in normal 293 cells in some of these. There's certainly some controversy about this but there's certainly a number of conditional adenovirus vectors where you will see, although it's reduced -- that is, things that have a preference for tumor cells or whatever, you will see some growth. How would you possibly get virus that passes even the old tests?

DR. BAUER: I think that's a good question. The approach has been that we realize these replication selective viruses, in fact, it's a selective, it's not an absolute barrier, as you pointed out, but the approach is to say we asked to develop

better assays to measure the degree of selectivity. And the other thing is from the preclinincal and clinical studies, we do keep in mind that these vectors are a different class, in effect, and, therefore, ask for increased clinical monitoring and that sort of thing. So --

DR. MULLIGAN: That's interesting because depending on how much you believe in how well they work, you might have a lot of --

You know, they're shooting in a lot of these virus particles. So, I mean, basically, they're as exempt from the normal standards for traditional vectors, is that right, in terms of helper.

DR. BAUER: We don't have a good handle on ways to measure it, that's true, but we respond to that by different clinical monitorings and different considerations for the preclinical studies.

DR. SALOMON: Jay, did you have a

comment?

MR. SIEGEL: I have a question for Dr. Aguilar-Cordova. So, you show data suggesting that the sensitivity of an assay to infectious particles can vary vastly, depending on how the assay is done. As you know, we've set a limit at that 3 percent -- 3.3 percent of -- at least, of particles should be infectious, I guess that was tightened up from 1 percent. And that was based on what seems achievable in current experience.

Is it your conception that, actually, a much higher level is typically being achieved and that those numbers reflect insensitive assays for infectious particles?

DR. AGUILAR-CORDOVA: I can only speak to some of the data from our own productions and, in those cases, the majority of them are achieving that and better. Now, having said that --

DR. SALOMON: The majority are achieving 3.3 percent or are you saying you're achieving -- because in one of your numbers, it looked like you were getting over 50 percent in one case?

DR. AGUILAR-CORDOVA: Well, not, let's see, I think I showed data that showed that the Vp-to-iu ratio was in the neighborhood of 1.9, 1.3 so up close to that, I think. And, again, I believe that some of that is just due to detection ability. But having said that, there are some constructs in which -- even with our own system and, as Steve mentioned, very reproducible system within house. Some constructs have a little higher Vp-to-iu ratio than others and we haven't quite figured out why yet?

DR. SALOMON: But, Estuardo, I also was confused by that, only in the context of what Steve had presented and what you presented. It's a ratio of 1.3 -- I

---- mess up the math, particularly, when I'm up here not thinking straight, but 1.3 would be almost 75 percent, wouldn't it?

DR. AGUILAR-CORDOVA: It would be about 50 percent -- close to 50 percent.

DR. SALOMON: Yes, so --

DR. AGUILAR-CORDOVA: 1-to-1 would be 50.

DR. SALOMON: So, that's a whole lot more than 3 percent.

DR. AGUILAR-CORDOVA: Right.

DR. SALOMON: So, I'm having trouble with --

DR. AGUILAR-CORDOVA: Remember, though, the data that I showed you -- what it also described is that from the very same virus vector preparation, we were able to have titers, I believe it was from 9 times 10 to the 12th to -- I mean, 9 times 10 to the 10th, to 6 times 10 to the 12th, depending on how we set up the titer assay. And so, in one instance we're almost

100-to-1 and in the other we're at 1.3-to-1. It's the same vector preparation, it's just detected in a different way. And so, that just emphasizes the actual point, that it may not be an intrinsic characteristic of the vector preparation, but rather a consequence of your detection system.

DR. SALOMON: I can't think of any better way of articulating why were here today. Marshall.

DR. HOROWITZ: Do you take off the input virus after a period of absorption?

DR. AGUILAR-CORDOVA: We have tried that, yes. And under static conditions, we basically see no difference in titer, if the virus is taken out of the well and placed on a different set of wells 12 hours later and, I think it was 24 hours later, as well, and the titer did not change. Indicating that the majority of the virus was still in the supernatant and also indicating something about the stability of

that virus.

DR. HOROWITZ: Yes, so what you did is the supernatant, replated, has the same titer the second time around as the first time around?

DR. AGUILAR-CORDOVA: Correct.

DR. HOROWITZ: Saying that the extraction is relatively low, because that's what I was going to suggest. And that's another way, although more tedious to really see what's there, until titer and exhaust, I should say, the supernatant. By repeating titers, you obviously realize there's more virus there than you're scoring the first time?

DR. AGUILAR-CORDOVA: Right.

DR. SALOMON: Dr. Sausville?

DR. SAUSVILLE: So, I think this discussion, though, illustrates a point that Alison Lawton was leading us to in the sense that, it's one thing to describe a reference standard, but it's -- but I think we're

going to have to go a lot farther than that. There's going to have to be a, I think, a consensus, potentially, at some point as to how the assay's done, what the cell types, et cetera, so it's more than just a standard, because what this discussion is showing is that the concept of titer is really very context and assay dependent, and so it seems that without defining those things, you know that a standard is going to be relatively meaningless, actually.

MR. SIEGEL: On the other hand, it's clear that a standard is the first step toward evaluating what's the best assay. And, often, standards are sent to multiple labs for evaluation is where you determine what assays are sensitive and what are not.

DR. SAUSVILLE: Right, so I certainly take your point. And the standard is the first step. I just would certainly hope that we don't regard it as the last step.

DR. SALOMON: Yes, that's a key point, I think a couple of us have made it in different ways, so, that's great. I mean, we owe that to Estuardo for focusing us on that. Any other comments or questions at this point? I always, and I apologize to the audience, not having earlier said that we -- you're part of this discussion, as well, that's the purpose of these public meetings.

So there is a microphone there. All I ask is that you identify yourself for the purposes of the record, but I would welcome any of you at anytime during this place to get up and make a comment.

DR. HUTCHINS: Hi, Beth Hutchins of Canji. As far as the reference material goes, the real purpose of this reference material is actually to define a unit. We're actually going to put the markings on the ruler and then later we'll figure out what's the best way to measure those --