measure -- how to use the ruler basically. And it goes back to what Estuardo was saying in terms of, we're creating, in essence, an arbitrary unit. The method is not so critical right now. In terms of the reference material, we are, in fact, including the diffusion calculation correction. That's being incorporated into getting that into the initial number for the reference material.

And down the line, then we can look at the issue of methodology. But we're creating a unit is what we're really doing. We're defining that unit right now, and that's why, in the end, laboratories, different companies, whatever, can use this reference material to validate their method. Their method may be different, but the unit will be defined and as long as they can report back units that mean the same thing, then we'll all be able to reference relative to each other regardless of the methodology

that they're using. In the end, it may mean that people move towards very similar methods, but it doesn't force people to do that automatically.

That's also true for not just the infectious unit, which varies a lot but, also, for the particle, the physical particle number itself. Because even though people do OD260 SDS methods, those methods, depending upon exactly how you do that, how you lyse the material and make the measurement vary also and I think the FDA has commented that they see variability even in that approach.

So, even though you would think the physical measurement should be the same for everybody, in point of fact, a lot of us recognize that there are definite differences there, and so the reference material will also define the physical unit, which I think will also be very useful to the field. So I just want to point out

those two issues that we're defining units, not so much how you measure the unit.

DR. SALOMON: Yes, good points -- still, however, you have to admit that the definition of a unit, then, would allow us if we set, then, standards based on units, that would be very useful. Yet, how one handles the reference material when it gets to the different labs in the field will also have a lot to do with how accurate the unit determinations are.

So there's still going to have to be a significant amount of specification on exactly how the assay's done and that gets back to Dr. Sausville's point.

Is there another comment, and then we'll go on to the next talk?

MR. MURPHY: Chris Murphy with Genzyme. I just wanted to kind of clarify: The change in the particle to iu ratio that's being proposed -- is that going to fall in line with the addition and, I guess,

correction with, you know, the calculation with Fick's Law? And the reason that I ask is, you know, currently it's not uncommon to get a particle-to-iu ratio using a Spearman-Carber calculation without correcting for the diffusion of virus up around 30 or 40. If I correct for diffusion of virus, I can bring my particle-to-iu down to 1-to-5, that sort of thing. I guess what I want to kind of verify is that is this change already going to be implemented now regardless of coming to a consensus on the titer assay?

DR. BAUER: I can respond to that. I think the methodology -- we have not, in the past told our sponsors the way that they need to do these measurements, but, of course we look at how they do the measurements. So, if you have a measurement and a system for calculating the infectious particles that, you know, we have looked at and accept, so I think, in effect, we will

be applying those correction factors. I think that was it.

DR. SALOMON: Okay, then the next speaker I'd like to introduce is Dr. Beth Hutchins, Director of Process Sciences for Canji. The title of her talk is Replication Competent Adenovirus Assays and Clinical Data for rAd-p53 in Cancer Patients.

DR. HUTCHINS: Can everybody hear me? All right. I'm going to give a little bit of an overview of how RCA assays are typically done to just give you some better feel for, actually, the variation in that area and then talk, specifically, about adenovirus p53 vector and our methodology and then data from patients relating to RCA shedding and et cetera.

There really are two sources for RCA: One is that it can be created during the actual construction of the vector and this can happen with the most common methodology and really it was the

traditional methodology until more recently. And that is the large fragment method of recombinant adenovirus construction. And that's where the recombination takes place in the production cell line. And it does not matter what type of production cell line you're using. It doesn't matter if it's a 293 cell oops -- did something just cut out or, okay, or PER.C6 or any of the more truncated E1A complementing cell lines that are now becoming available -- you want to adjust something? In any case, if you allow the recombination event to occur in the production side and then try to select out viral plaques from that, what you'll find is whether you do serial plaquing or not, you don't eliminate multiple things in the construct.

The newer E. coli recombination methods, where you do all your plasmid (?) recombination and then you select a then you select a single plasmid from the bacteria

and use only that single plasmid to do your transduction into the production cell line can eliminate this as a source of RCA. Most of the constructs, though, that are in the clinic these days were still made by the old large fragment method of production or construction.

The other source of RCA is -- during production that is recombination frequency between an overlap in the E1 region of the cell line versus the vector backbone itself. And the thing is, the frequency of that recombination isn't really known and it, actually, has to be estimated for every combination; that is for the overlaps that exist between your specific vector backbone and your specific production cell line. Now the newer cell lines try to address that by specifically eliminating this -- these types of overlaps.

In the field today, RCA testing is a bioassay involving either one or two cell

lines. The indicator cell line is most commonly the A549 cell line or a lung carcinoma. And detection can be by a variety of methods: Either cytopathic effect or immunostaining or PCR, as the end thing that you're measuring. When you rely on cytopathec effect, generally, the assays are set up with a confirmatory step to show that the specificity is not something that -- some infection event of some other virus that just happened to occur during the assay that is, in fact, an RCA.

There's no guidance on, you know, which to do this and you'll find that there's a variety of methods out there. I think FDA can comment on that for the committee.

Nonetheless, all of these assays are set up and qualified to be sensitive to 1 pfu or IU or infectious unit of RCA at some confidence level, hopefully, at the 95 percent confidence level. And, in essence,

what you're doing is a plus/minus or quantal assay. And how you get quantitation then is based on the sample size that you test. So, you don't get out from the way these assays are typically run out is I have X-number of RCA in my material, what you get out is I have one or I have less than one in whatever amount I test.

And this goes through an example of this type of method of quantification. So, you test at different amounts of sample. 5 times 10 to the 8th; 1 times 10 to the 9th; 5 times 10 to the 9th, 1 times 10 to the 10th vector particles. And you get negative results, no positives detected at the two lower levels, but you get positive results at the two higher levels of particles assayed. And what you then estimate is that the amount of RCA is less than 1 in 10 to the 9th or greater than 1 in 5 times 10 to the 9th vector particles, which, if the clinical dose is 10 to the

12th vector particles, then it does contain somewhere between 200 and 1,000 pfu of RCA is what we estimate. There's no exact number that's coming out of this, it's a range.

Part of that is the 1 pfu detection is also, of course, limited by the distribution of virus in a sample. This is where the amounts of replicates of the RCA bioassay can become quite critical. This last calculation is corrected based on your handout, somehow my math was not that good that day.

Now, the construct and the data that I'm going to talk about more specifically relates to recombinant adenovirus that expresses the wild-type human p53 gene. The backbone of this vector is an E1a, E1b, protein IX deleted cassette with also a partial E3 deletion that completely inactivates the E3 expression.

These deletions were created to

allow the insertion of the p53 expression cassette in the E1 region and we purposely put the protein IX deletion in to decrease the overlap between the vector backbone and the 293 production cell line, which is the cell line that we use for production of this vector. That decreases it, actually, from about 1,000 base pairs down to 200 base pairs, just for background information.

We had -- we've done pretty much of the routes of administration that Dr. Bauer showed earlier. The data that I'm going to talk about relates to these trials and these patients. We've done intratumoral injection, with 72 different subjects; intrahepatic artery infusion, with 50 different subjects, with doses up to 7.5 times 10 to the 13th particles, though most of -- a good majority of those patients got 2.5 times 10 to the 13th particles; and also by the intraperitoneal route, with 54 patients and the dose ranged in that case up

to 7.5 times 5 to the 13th particles; and the majority of those patients got 7.5 times 10 to the 13th particles in a dose.

So, the bioassay for RCA that Schering Plough (?) uses to test our production lots of the p53 adenovirus is, again, one of these quantal bioassays, and this one has a CPE readout. We use a two cell line method because the p53 gene has a particular effect on the A549 cells that we wanted to avoid and so the indicator cell line, though, is still the A549 cell line. It's a four-week -- typical four- week assay. We use PCR to confirm any positives so that we know that we are detecting RCA, if we do detect it. And it is sensitive to one pfu of RCA.

Now, it's sensitive to one pfu RCA, but because of plasone (?) distribution, when you want to validate the assay and show that you have 95 percent confidence level for detection of something,

when you do triplicate tests of -- or you do an N of 3 on testing your material, we can, with 95 percent confidence, detect 4 pfu of the control spike. If we wanted to have 95 percent confidence in one pfu detection, the end jumps up quite dramatically and it's not practical to do that. And the only RCAs we've ever been able to detect, either through a process -- a validation study or that come up in actually assaying production lots are, really, the dl327 backbone, that is the p53 expression cassette's been kicked out, the E1 gene looks normal, the E1 region looks normal again, but it still has the E3 deletion, that is part of the parental backbone from which the vector was originally derived.

The specification for the 58500 p53 adenovirus clinical product is less than 40 pfu of RCA in 7.5 times 10 to the 10th viral particles. Each batch is tested either in triplicate at 2 times 10 to the

9th particles, which is, actually, the way it's been done for the -- about the last 2 years. Or it's tested earlier the first years that we were doing this, it was tested and not at one but at three different levels.

Based on the assay confident levels for the triplicate test at just 2 times 10 to the 9th particle, if we find -- if there are no positives in -- of the three tests, or there's 1 of the 3 is positive, then the batch meets the specification. If two or three of the tests come up positive then the batch fails the specification because there would be greater than or equal to 40 pfu in that 7.5 times 10 to the 10th particle amount.

And this data just represents the summary of the validation data, looking at the confidence levels relating to this triplicate testing. And so, you can see that we have 95 or better than 95 percent

confidence in testing -- we can detect the 40 pfu in 7.5 times 10 to the 10th or, because we're testing at 10 to the 9th for pfu detection limit. And that this confidence declines dramatically, which is why zero or 1 can meet the specification but more than that, obviously fails the specification.

Now, if you look at or as we look at our protection lots over a period of a number of year, what we find is that a certain percentage, roughly 10 percent consistently fail the specification. And -- which means that they would have greater than the amount of 40 pfu in the 7.5 times 10 to the 10th vector particles; and about half, based on testing at the three different levels come in right on this -- right where you end up in -- if you look at the 7.5 times 10 to the 13th vector particle dose, you would have about 4,000 RCA pfu maximally. And about, the other half would

have ten-fold more than that. So at our highest dose levels, we could have put -- we could have dosed a patient in a single dose with as much as 40,000 pfu of RCA. Now, we can't say exactly how much we dosed with except that it's in a particular range, just because of the way the assays are set up, so you need to keep that in line. But it is, obviously, more than the numbers that Steve was talking about earlier.

Now, I'm also going to just briefly the type of methodologies that we were using to monitor the patients clinically and to look at various biological samples from them and these methods included both specific and nonspecific methods. So, one nonspecific method is an ELISA that detects the hexon antigen. This does not distinguish intact virus from just parts of the virus, and it also does not distinguish product from RCA or wild-type.

Another type is an infectious

assay on 293 cells. In this case, we're using fluorescence -- a flurocytometer and fluorescence to detect infectious adenovirus. Again, this does not distinguish the type of adenovirus that is an RCA a product or a wild-type infection, but it does say that it is an intact infectious virus and not just pieces of virus that are in the biological sample.

We also had a variation of this assay where the sample was placed onto A549 cells. And, again, this detects infectious virus, but it only detects either RCA or wild-type and does not detect product because the product cannot replicate on an A549 cell.

And, finally, we had PCR assays that you could use those to specify what you were detecting, that is, you could specify that it was RCA or wild-type adenovirus or the product, but one caveat of that is that you're detecting DNA and it does not tell

you that that DNA represents an in tact virian. So, these methods detect different things, but they do allow you to get certain valuable pieces of information, depending on the method you're using.

The types of biological samples I'm talking about are either urine, feces, sputum, saliva, any variety of things and, also, several of the methods have been used to monitor serum samples, blood samples.

Of the studies that I mentioned earlier and the patient numbers, here listed by the various routes of administration and then the four different methods. These are the -- just looking at a variety of biological samples but just now looking at the issue of shedding. You'll see the only method that specifically you can look at RCA, these 2 methods, actually, but in this assay, we found, in 63 different samples we -- or from 63 different patients, we did not detect any shed RCA. This one positive here

is product, not RCA. And these -- these were all product-related positives here. Now, you can see, also, it's a very low rate of shedding, but no RCA's been detected in any of the samples we've analyzed.

So, what are the types of populations we're studying? This vector has actually been developed for -- was being developed for specific cancers. And so all the patients were cancer patients. All these subjects were initially selected to be antiadenovirus positive prior to entry onto the studies and, of course, not to have any evidence of an active adenovirus infection and what we did know that the neutralizing capacity of these antiadenovirus titers before they received any of the adenoviral p53 vector varied considerably in the population. But always, consistently, increased and increased dramatically with dosing with -- after administration of the vector.

And for the subjects were -- the very few subjects we had were adenoviral shedding was detected, we were not able to identify any adverse clinical sequeli.

This is just to give you an idea of the type of information you can get from monitoring patients looking at their ---- antibody response, this is now for patients enrolled in the ovarian interperitoneal administration study and this is a multiple dosing phase of -- portion of that phase 1 study, and in this case the patients we all receiving 2.5 times 10 to the 13th particles or 7.5 times 10 to the 13th particles, I think about half and half in this case. They also did, at the same time receive chemotherapy.

So -- and the way the drug was dosed is there are five days in a row where the drug is administered each day at that does 7.5 times 10 to the 13th particles, and then 4 weeks later another cycle of 5 days

of dosing occurs. So, this is referring to one cycle of dosing, another cycle of dosing and another cycle of dosing. And you can see that this is now average data, and I have to say that the arrow bars are not -- are fairly wide, but it gives you a sense that you do see some drop in the hemoral response but, of course, memory comes back and you see that rise again and it continues to go up and up and by the time you're out past cycle three in later studies we did up to five cycles, of course the neutralizing capacity is quite dramatic.

Now we do also have evidence, though, that we're still getting transgene delivery and expression at cycle three and we had very limited samples that told us the same thing for out to cycle five. So, even though there's this very large neutralizing hemoral capacity in the body, we were still getting delivery of our vector.

Just to get to the summary points,

the bioassays that are used are quantal, but they are sensitive and they can reliably detect RCA, at least in replication deficient vector products.

The more precise quantitation than this quantal method where you get, sort of, this range of RCA that you can estimate in your material is impractical in this bioassay mode, because of the amount of testing that was required. The amount of cell culture that would be required is what becomes impractical.

If the desire is to have a specific quantitative number come out of a particular method, you would have to consider real time PCR as an option, looking for quantitating more specifically. No one's doing that right now, routinely, but that would be another way to look at the RCA issue.

We were not able to detect any shedding of replication competent adenovirus

in our clinical subjects. There was not PCR positive for RCA and no infectious assay-detected RCA. And where we did find shedding, there were no adverse clinical sequeli identified of the shedding's really very low amount and less than, you know, about maybe 1 percent by the infectious titer assay that was used to look at that. And I think that's basically it. So, I'd be happy to entertain questions from the committee.

DR. SALOMON: Thank you, Beth. I'm remiss in not having allowed the panel a break, so -- one option here, would be to do some questions, I think, while it's fresh in our mind and then have a short 15- minute break if that's okay with everybody.

I had a couple questions. Some of them may come up with others, so let me just ask one of them and then we'll see how things come along. One of the things I find very interesting is this whole idea of what

are the molecular mechanisms that generate the recombinant -- the replication competent adenovirus through recombination in these models. So you have some evidence here that you gave that the RCAs were rising by replacing the p53 transgene cassette with E1, did I get that correct?

DR. HUTCHINS: Well, the RCAs that we've been able to detect their structure is all that the E1 - the p53 expression cassettes not there and the normal E1 region is there, but the rest of the viral backbone which has this E3 deletion, that's backbone on which the vector is based is the same. So, you don't have a substitution just at the place where, in fact, the large fragment recombination was supposed to be taking place.

DR. SALOMON: Right, so, again, I'm not an adenovirus -- so this could really be stupid, but what -- so what I'm trying to understand is when you constructed

the vector, you did a number of different things; you deleted E1a, you deleted E1b, you deleted E3, and you made changes in protein IX to reduce the homology (?) right?

DR. HUTCHINS: Right, from a practical point of view, what's done is or what was done in this case is the dl327 adenovirus, which has the E3 deletion in it already was the starting point. And then the expression cassette was constructed through bacterial plasma technology. And so, you have sort of the front-end of the virus constructed in bacteria and you have what really is going to become the back-end of the virus be from a virus from the adenovirus dl327. That virus is digested using specific restriction enzymes, claw-1 (?) is most frequently used to get thee large fragment. And, basically, is what it does is you end up with the adenovirus but from the end of E1 on, and what you're doing is asking these things to recombine, now

you're really, just the part of the virus that has the expression cassette and the front-end with the ITR to recombine in the producer cell line.

This is the most traditional way that this is done, regardless of whether you have an Ad2 backbone and Ad5 backbone and what other elements you're putting together, that's what's traditionally done. It gets more complicated when people have additional deletions, like in the E4 region, so they change what the back-end is.

DR. SALOMON: So, I guess the question I'm asking is, in this process of engineering the vector -- I mean, part of my thinking here is, if we could figure out the nature of creation of recombinant adenovirus, I mean, replication competent adenovirus through these events, we could suggest that that be part of the criteria upon which one would accept a clinical vector in a trial.

So, you made all these changes, you reduced it to 200 base pairs instead of 1,000, yet, you still got recombination with E1, so is that telling us that these are not --

DR. HUTCHINS: We think it was there right in the beginning --

DR. SALOMON: ----

DR. HUTCHINS: The virus material because of the way we did -- we created it, that it was always there and no matter how hard we tried to, basically, subclone it out by serial plaquing, we didn't -- we don't achieve that, at least not in ten rounds of serial plaquing.

DR. SALOMON: Okay. So there were -- there was already the backbones to create the replication competent adenovirus in the process of engineering the original transgene?

DR. HUTCHINS: That's our hypothesis for this particular vector, and

we think it's a pretty common experience. Now, at the time that we were using that technology in the earlier nineties, we didn't really recognize that that could be a consequence of what we were doing. As we have more data and really begun to understand better what -- the consequences of what we were doing, we now moved to a method where we do everything in E. coli, select the specific thing that's the intact vector and that's what goes to create the intact viral -- the viriant, so -- and now that source, would be limited, and now the only way you could get RCA is through recombination of events that occurred during production.

DR. SALOMON: Okay, well, that would explain something Estuardo said, then, too. Good. Ed.

DR. SAUSVILLE: But to pursue that direction of thinking, we, in past meetings, have set out the general idea that vectors

of certain sizes should be sequenced and there should be precise definition of what goes into a product. And we stated that for higher molecular weight, or higher sized genomes, larger genomes, there can be a point of ambiguity in relation to the gene of interest as opposed to the background.

So, what this leads to is that, if recombination frequency is going to be the major determinant of this in the next generation, do we need to firm up the sequence definition, flanking the proposed target gene and try and use that for a basis of hedging our bets as to what the recombination frequency would be? I place that on the table. I mean, I'm not an adenovirus expert, either, but it seemed that might go part of the way to dealing with this.

DR. SALOMON: And that may be something that we want to take up right after lunch when we start going into the

questions. But I certainly would welcome any comments.

DR. HUTCHINS: Well I have one, and we're one of the groups that fully sequenced our virus early on and submitted that information to the FDA and the -- there's limits of detection to what you're going to be able to see in there if you have a low-level variant when you're doing this sequencing, both strands, full length. To detect very small quantities of some other molecular variant, you would have to do a different type of analysis and study than you do when you're just trying to sequence the material and just say this is the sequence of my product.

So, sequencing of your vector, the requirement that I think Dr. Sausville's referring to in terms of your earlier discussions and recommendations, is not, by itself, the answer to address this issue.

You're talking about some other

type of molecular analyses where you have to really look at and I hope the committee does not recommend this today, but you would have to look at, you know, a large number of clonal isolates of your vector from some kind of production lot or a number of production lots and then try to analyze those and that would be, you know -- one of the issues, I think, that's being raised today is, well, okay, we could maybe collect that type of data, but if the risk of that material in your product isn't very high to begin with, what is the necessity to do that. You know, what's the benefit that's gained from that.

DR. SALOMON: I would say that's exactly what you need to be telling us today. That's exactly the kind of reality check from people doing this that I want to hear and that the committee wants to hear and the FDA wants to hear. Dr. Ketner and then Dr. Siegel.

DR. KETNER: You mentioned that you constructed your E1 replacement to minimize the overlap between 293 cells and the recombinant. So, there are cell lines which are engineered to have no overlap, I mean, PER.C6, I think, is intended to be that way. I wonder whether you've had experience growing your viruses on PER.C6 and whether, in fact, the RCA level drops to zero, as in principle, perhaps it ought?

DR. HUTCHINS: Your vector backbone still has to match the PER.C6 cell line construction. So if I took my current p53 vector and grew that in PER.C6, assuming I even had -- okay, the way -- I could still, potentially, get recombination because my vector backbone does not, at least the way it's currently set up, does not currently match the way the PER.C6 construct is. So, what Crew Cell (?) will tell you is, if you want to use their PER.C6 system, your vector backbone -- they'll give

you the -- or they'll offer to build it for you -- the specifics that you need to know so that you don't have that overlap. You actually need to know what the E1 region is in your producer cell line, so then you can make that match- up.

But then, again, if you still do large- fragment recombination as the way to create that vector and you make it in a PER.C6 cell line, you're still going to end up with variant molecular variance in your material. You would still have to create that change, that matched backbone, outside of the producer cell system, such as through an E. coli bacterial system, and then put the selected thing in to not have RCA. And there are other cell lines that are designed the same way.

DR. KETNER: Given that, I mean, given that this is in principle a possibility, maybe we ought to at least discuss the notion that the RCA levels be

reduced to, I mean, to zero by choice of appropriate sub strains.

DR. HUTCHINS: I would argue that that's the point of your discussion today, what the risk is and what is the necessity.

DR. KETNER: Yes.

DR. HUTCHINS: In countries where that's the requirement, they don't have clinical trials going on.

DR. SALOMON: Well, then that's -- that's good. I mean, we -- that's what we need to discuss, I agree. Dr. Siegel.

MR. SIEGEL: In discussing the sensitivity of your assay and the ability to use the virologic the viral culture for precise quantitation, you referred to issues of practicality and number of replicants. With the quantal assay, if you have a single hit, kinetics then -- with enough replicates if you, and you have a plasone distribution, you an get a rather precise quantitative in most systems using, you know, the, say, 96

cell plates or whatever there's not that much practical limitation to getting reasonably precise -- I wonder if you would speak, since I also don't work with adenoviruses, what are the limits? How many cultures can be done with how much effort? Is there a problem doing larger numbers? You said you did three replicates --

DR. HUTCHINS: Yes.

MR. SIEGEL: Because more would be hard?

DR. HUTCHINS: Yes, typically, when people are testing near the level of the FDA guidance in that 10 to the 9th, 10 to the 10th particle range, because there are -- as Estuardo mentioned earlier, the more -- or the higher the concentrational particles that you're putting onto your culture, the more likely you'll see toxicities that will actually affect the assay. This is, you know, this is just a byproduct of using cell lines and high

concentrations of the material.

So, this means that in order to test, say I wanted -- decided that I wanted to test 10 to the 11th particles each time I did my assay. In order to -- and also still kept my pfu sensitivity to 1 -- and I would have to use a larger number of cells or more wells or roller bottles or flasks in order just to look at that 10 to the 11th particles, and now, if I want to do that with a certain percentage confidence level in my ability to detect that 1 pfu, I've got to do that same number of flasks x-times each time for each lot.

And, you know, I have to say that I'm very happy to be affiliated with a corporation where our lot sizes were quite large, but I know that many institutions, the lot sizes aren't that big, and so that -- that can be a real burden.

The other thing is that because it's a quantal assay, the quantitation comes

out of the amount you test, not -- because you're only checking one or none, basically, right? So, it's yes or no. And so the amount you test drives the quantitation in this bioassay method. So, that's the other reason why most people only test to about 10 to the 10th, because the amount of cell cultures that's involved.

And the other point that I should point out is that when you do this test, you don't just do the material by itself. You do the material by itself, the material with a spike, so, I mean, you already have these other controls built in that just in a single assay, you have other cell culture, not just the culture involved with the actual replicate numbers of the unspiked product that you're testing.

DR. RAO: We talked about a reference standard earlier. So if you were to use the reference standard here would you just use it as a spike of the wild-type

instead of what you used, or would you have some other method in your system? Or would you use the reference standard?

DR. HUTCHINS: Well, I would not be using the reference material directly, because I don't want you to use up all the stuff we produce. What I would like people to do, and what we're going to be recommending is that people create an internal reference material that they tie to this material that they'll be able to use. But what the reference material will do is define the unit, the infectious unit, so now when I say I'm spiking or I'm making sure my RCA assay can detect one infectious unit of RCA, it'll be the unit's that's defined by this adenoreference material. And I'll make sure that my RCA assay is validated based on that -- the ability to detect that unit. So, does that clarify that point for you?

DR. SALOMON: Dr. High.

DR. HIGH: With respect to some of

the questions that we are asked to answer this afternoon, I wonder if you could tell us something about this group of cancer patients that you presented data on? You have about 200 people with non ---- and head, neck and colon cancer and ovarian cancer and so forth. Are there requirements that these are all people who are relatively early-stage? Do you know anything about their --

DR. HUTCHINS: They were all --

DR. HIGH: Immunologic status --

DR. HUTCHINS: They were all relatively late stage, heavily pretreated, I mean, these were phase 1 studies, so this was definitely -- this was not a neoadjutant type of situation, this was, you know, they've already exhausted a lot of their other options. Nonetheless, they had to have relatively good performance status, so that we could distinguish disease-related effects from, presumably, product- related

adverse effects.

So, but they are, you know, treated -- on the other hand, we have evidence that at least many aspects of their immune capacity were quite well in tact. Hemural immunity as well as cellular immunity from cytokine profiles and some other data that I'm not presenting today.

DR. SALOMON: Marshall.

DR. HOROWITZ: Is it appropriate to ask about the 50 patients that had intrahepatic artery infusion with an average of 2.5 times 10 to the 13th --

DR. HUTCHINS: Thirteenth.

DR. HOROWITZ: Times the 13th, yes. Would it be appropriate to ask about toxicity and chemokine and other measurements and expression in liver, is that expanding too much?

DR. HUTCHINS: It is appropriate to ask that -- we have reported, for instance in the December '99, I guess it was

RAC Safety Symposium, we reported in some detail our safety data -- clinical data, and also preclinincal studies related to that and that route of administration and dosing. We, in fact, did see dose limiting toxicity. You notice that in our ovarian trials we heavily focused -- ended up with the 7.5 times 10 to the 13th particles.

Now that's by a different route of administration, that's tolerated quite well in patients. But at 7.5 times 10 to the 13th particles, by the intrahepatic arterial route, that is not tolerated in patients. But the effect was on -- was a cardiac toxicity, in fact, not a hepatic toxicity. And we went down then to being able to establish that 2.5 times 10 to the 13th viral particles was a safe dose.

One of the things that -- we did not reinitiate our IHA protocols after the Gelsinger death, although we could have. We had permission to do so. But for a variety

of reasons, including our own concern about how close were we to, you know -- the difference between 2.5 and 7.5 isn't that big. And while we felt very confident that we were defining our particle dosing consistently and, you know, accurately, we just felt that it wasn't worth the risk at the moment until we had more information. So we started to focus more on the ovarian IP route of administration after that.

DR. HOROWITZ: Did you measure chemokines circulating at that time?

DR. HUTCHINS: Actually, in the IHA studies we did and I'm hoping, very soon, that manuscript will actually be published, but I think Dr. Bob Warren has actually presented that type of data at several forums, including at the Recombinant DNA Advisory Committee. We do see cytokines IL6 (?) and such in both in serum as well as in tissue, we've looked at that quite extensively. I mean, there's definitely a

local immune reaction in liver and there's a systemic immune reaction, but there's no evidence to us that RCA is related to that. I think that gets back to general --

DR. HOROWITZ: Right --

DR. HUTCHINS: Viral toxicity.

DR. HOROWITZ: These questions are not so much for RCA, but for the total dose --

DR. HUTCHINS: Right.

DR. HOROWITZ: And if I may ask, were any liver biopsies done and evidence of expression of the transgene in the liver?

DR. HUTCHINS: Yes, yes. And there was, consistent -- once we got above a certain minimum dose, we had consistent transgene expression in both tumor and non-tumor samples from the liver.

DR. HOROWITZ: Thank you.

DR. SALOMON: One question that I had, let me see, is just a simple one, you know, from my calculations and these, again,

are flawed when I'm up here, I'm not usually thinking as straight as I should be, but I think it means that about 50 percent of your production runs would not be acceptable if we adopt the new guidelines that FDA staff has suggested?

DR. HUTCHINS: Well, if you read the guidance, there's always the -- there's always the ability to collect data to support a different specification and then discuss that with the agency and determine if they agree that your data supports that different specification. That is, in fact, the type of thing that we did -- to have a different specification than the less-than 1 in 10 to the 9th that was in the original guidance. So, we have preclinical studies that support --

DR. SALOMON: Well, my question though are you okay that this is going cut -- is this going to cut out 50 percent of your production runs, and is that okay?

I mean, I have no idea what that means.

DR. HUTCHINS: If it becomes an absolute rule, yes, it would, but I -- I think the FDA needs to comment here on how they would apply that.

DR. SALOMON: I'm sorry, Alison, did I get that wrong?

DR. LAWTON: Can you just clarify that, Beth? If it becomes an absolute rule, my understanding is, yes, it would cut out 50 percent of the lots --

DR. HUTCHINS: That's correct.

DR. LAWTON: And the question is, is that acceptable?

DR. HUTCHINS: No.

DR. LAWTON: Right, thank you.

DR. HUTCHINS: You need to understand we're pro -- we produced the vector --

DR. SIEGEL: But --

DR. HUTCHINS: At a fairly large scale. You're talking about vector runs

where we had 10 to the 16th particles produced at a time, not that we would make purification batches on that same scale, but, I mean, and the RCA level would be consistent in that entire, you know, viral culture batch, so that would not be acceptable.

MR. SIEGEL: I think, though --

DR. SALOMON: And that's what I was trying to get you to say.

MR. SIEGEL: There needs to be clarification. We've not proposed a rule, we're proposing a guidance.

DR. SALOMON: Right, exactly. And, I mean, I just wanted some feedback on, you know, what the field thought of that, you know, guidance before we get into it this afternoon. Dr. Flomenberg and then Dr. Sausville.

DR. FLOMENBERG: Phyllis Flomenberg, Thomas Jefferson University. You mentioned that you prescreened all of

your patients for antibody to adenovirus, was that sera-type-specific antibody?

DR. HUTCHINS: The test was -- the assay was designed, you know, the adenovirus type 5, but how specific are -- we did not then go back and look at whether those antiadenovirus titers that we measured were only --

DR. FLOMENBERG: So, it wasn't a neutralized assay?

DR. HUTCHINS: To ad-5 versus to any other adenovirus isotype.

DR. FLOMENBERG: So --

DR. HUTCHINS: We wouldn't care, actually. That wasn't what we -- the question we were asking -- we were only looking at the ad-5, but to say that it was only -- those responses were specific to ad-5, I can't say that.

DR. FLOMENBERG: Was it a neutralizing assay?

DR. HUTCHINS: We did both kinds

of assays, the requirement for entry did not discuss neutralizing capacity, just that they had to be seropositive.

DR. SALOMON: Dr. Sausville.

DR. SAUSVILLE: So a question that the introduction of the clinical trials data that you alluded to in the cancer patients and also in your response to the question about viral replication in both liver and tumor, do you routinely quantitate the expression of coxsackie adenovirus receptor in tumors and does that influence or could that influence the potential perception of infectability of a given lot -- given -- depending on the use of the material?

DR. HUTCHINS: We did look at that in a number of our Phase I studies. Actually, the IHA study and the ovarian IP study. In the IHA study, there was a relationship between CAR level and expression. In the IP study, there was not in tumor tissue. Why that is I can't say,

but besides the fact that there's CAR and, of course, the secondary integren receptors are required for internalization, there are still undefined receptors that most of us believe exist and we don't know what they are, and so there can be other mechanisms for entry of adenovirus into tissues.

And just to correct a point: When I was talking about detecting expression of the p-53 gene, we don't believe that the vector was replicating in those tissues. You were saying, just to clarify --

DR. SAUSVILLE: But, I guess it ultimately raises the question, when one uses a standardization procedure that's based, presumably, on a given report or cell type it has a certain receptor and entry mechanism. Is it clear that that's the same receptor and entry mechanism that might either mediate a toxic or therapeutic outcome in the various contexts that the virus would be used.

DR. HUTCHINS: I'm sorry, you're asking in terms of an infectious titer assay?

DR. SAUSVILLE: Right.

DR. HUTCHINS: You -- it would not really be feasible to set up an infectious titer assay to represent the human condition in terms of the variety of tissues and, you know, depending on the indication you were interested in, the route of administration, whether this was a direct in vivo administration or an *ex vivo* situation, I don't think the intention is to say that infectious titer assays should reflect all aspects of what you're going to be running into, that's not going to be possible.

I think it comes back to what is the -- what is the practical outcome of a reference material? If you -- if we define infectious unit and it's going to be based now on the wild-type virus that we have the most clinical information about from a

safety perspective in terms of what's known in terms of the natural infections. That -- it goes back to the point Estuardo made that arbitrary unit will help us determine the relative activity of everything else. I don't expect my p-53 adenovirus to be as infective from that unit perspective, regardless of how I assay it, as ad-5 wild-type. It -- I don't expect that.

And there are a couple or reasons for that: One, the gene of interest can have an impact on infectivity and cytopathic effect or expression of a gene measured by immunostain, however you decided to do your end-point measurement for infectivity. Many of us are well aware of the affects of different genes that we put in the expression cassettes on infectivity measurements, which can impact or is not accounted for in the diffusion corrected calculation.

The other thing is that it's

somewhat crippled in terms of its ability to replicate even in the context of a complintation cell line like the 293 cell because we cut out some other things that an enhance replication of the virus. So, the relative IU unit, however that ends up being defined by the reference material will assist us from looking at these things across the field, but one, I don't think you should expect that my recombinant vector A and my recombinant vector B actually all have the same infectivities. And, in fact, the issue, then, of the vector particle-to-infectivity ration will probably have to be revisited after this, if you start looking at the complications of that issue. I mean, that's not a subject for today, but it's -- potential outcome of having this reference material defining infectious unit for us.

DR. SALOMON: I think that's a good place to be, I mean, you're defining

that the task today is to look at sort of advice on standards for the preparation. And what Dr. Sausville's pointing out is another issue that's going to have to be dealt with is what happens after you give it to the patient. We've kind of touched on that, already, before. Last Comment?

DR. MULLIGAN: Let me go back to the method you used to make the original vector. I'm not sure I see the reasoning -- is there any data that suggests your contention that that's what accounts for the increased helper?

DR. HUTCHINS: I'm sorry, you're talking about the large-fragment methodology?

DR. MULLIGAN: Yes --

DR. HUTCHINS: Of -- I would contend that a viral plaque does not -- I know this is really against traditional virology -- but I would contend that a viral plaque does not necessarily contain just one

viral entity and that's what we're relying on to pull out of what you've produced during construction, the thing that you then want to ultimately amplify.

DR. MULLIGAN: Well, I mean, I assume most people would do a multiple plaque purifications.

DR. HUTCHINS: And they have and --

DR. MULLIGAN: Yes, I mean, I would just make the comment, I doubt that's the case and the alternative is that, in fact, the vector you have for whatever reason has a different recombigenic potential than other things. And I think that it's very hard, I mean, I don't think there's any good -- that very directly attributes the recombination potential to an adenovector's -- in a linear fashion to the amount of overlap, it's more complicated. But there's a sense that that has to be the case that there's going to be trend towards

more recombination. But I think it's equally likely that different precise, unusual contexts of foreign sequences juxtaposed to vector sequences will lead to different amounts of recombination, that's why ---- question --

DR. HUTCHINS: I didn't say that that wasn't necessarily true and I didn't present data today supporting why we think there are two possible sources of RCA. The fact remains that you could have recombination during production. And you would have to examine that very specifically for the vector you're looking at and the production cell line you're looking, okay. So, in that sense, you are correct. But there is data to support the other -- the other way of creating a source of RCA and I'm not presenting that today. That's all I can say. And, obviously, no, I have not made that data available to you and we hope to do that soon, but not today.

DR. MULLIGAN: But this is key because Ed was asking questions if we every want to think about giving input on how one goes about making original adenovirus construct, which I probably don't think we'll do, this is important to have a sense of how likely is it that that really is an issue. And it's also important if there will be constructs that, in fact, tend to have a greater propensity for recombination, even when they're put into PER.C6 or other kinds of fancy patching cells.

DR. HUTCHINS: I guess, in respect to your --

DR. SALOMON: Let me interject --

DR. HUTCHINS: Mission today, though --

DR. SALOMON: Yeah.

DR. HUTCHINS: I don't think the mission today is to discuss how to eliminate RCA or sources of RCA. I think the mission today is to discuss what is the risk when

you have RCA, because the reality is, regardless of what means we might have to not have it in the future, it -- we do have it in today's clinical lots, every single one of those 58 active INDs, you could be sure there's RCA there.

DR. SALOMON: What I was -- let me interject -- I think that it's important to also protect the speaker in the sense that, you know, the deal here is that Beth and Canji agreed to present a certain set of data and you've done that. And I'm not saying that the line you're taking now isn't relevant to the overall field, but, you know, she's not going to be able to show us that data and she never agreed to show it to us.

DR. MULLIGAN: Yes, the issue, I think, is that in the briefing document, the FDA indicated that when they looked at the answers to the March 6 letter, they had a sense that, I think the term was,

"routinely" people obtained less than such and such and, therefore, the FDA made, I think, a probably reasonable argument that that's a reasonable limit ---- and so, I mean, maybe there should be some discussion on the reasoning behind that argument. I mean, it seems straight forward that if most people can attain that, maybe that is reasonable.

Is that, indeed, the argument, that you are making? Because it will be very relevant in how you set that and we need to know precisely how you came to the recommendation, and if it is precisely because there was, in your look-see, there was a routine capacity to get that level -- that low level of RCA, then I assume that means that you think that that's a reasonable basis for setting that as the limit. And that's relevant, then, to people happen to be not routine.

DR. BAUER: No, that's precisely

the way this recommendation came about was looking at the data that we had and saying, I'll use your terminology, routinely people could achieve that level, but I did mention in my talk there was a range where some manufacturers had zero, some have as many as 40 percent of their lots rejected.

And the other point that's relevant is this is a recommendation that can have, you know, qualifying considerations that are applied to it. So --

DR. MULLIGAN: That means that, I hate to harp on this, but I think it's very key -- that means that the feeling of the FDA is it's not unreasonable that if the state-of-the-art -- if the common state-of-the-art can achieve something, that that should be the standard.

DR. SIEGEL: Absolutely, we -- you know, for any of a number of types of contaminants, you know, there's a long

history in biotech products of setting different standards, say, like, for e-coli dna present or for LPS present, you know, there are two approaches to setting a standard and I think Steve outlined both and both have good reasonable logic. One is to say it's reasonable and feasible to achieve a certain level and you should achieve that level. Another is to associate a certain level with a risk and ensure that the level is set below the risk.

The reason one often goes to a -- there are two reasons one might choose the first to set a standard based on -- one might be one simply does not know what level there's risk at, where the other might be the assumption, as with radiation, that there is a -- there is no cut point that risk exists at any level, it's just proportional to level and it should be kept lower.

And, I guess, one of the things we

hope to get out in discussion here is whether we should be moving more toward risk-based levels, as opposed to achievability-based levels. A risk-bask level, one might imagine, would be different in different populations, one might imagine would be more based not on the per--total-virus particle, but per-dose given, for example. And there might be times where we want to combine the two and allow -- we're working with guidance -- and allow, in certain populations or in certain dosing regimens, a different limit might be appropriate and that's -- I think that's the gist of what we hope the discussion will guide us toward.

DR. SALOMON: Okay, I think that this is a good time to break, and this is -- what I know that some you don't know is that there's no public comment that's been requested. Now, we will call for public comment anyway, as part of procedure, but we

have an hour that, you know, we're taking up with some of this discussion, which I think's been good.

So, what I'd like to do is take a ten-minute break now, with the idea that we all know 10 minutes will turn into 15, no matter what I say. And be back here and then Dr. Flomenberg will give us her talk.

(Recess)

DR. SALOMON: If we can kind of bring everybody in. Cognizant of the fact that oftentimes some of the most interesting things get done during these breaks. And I certainly learned a couple things that will come up later. So, I don't see anything bad about a little longer break, but I'm going to get in trouble with time soon, so --

Couple of just real quick things, and I mean really quick: One, I'd like to welcome Dr. High to the table. She, unfortunately, had some transit problems, again, we've all been there and don that,

but anyway, welcome to the Committee as a new member. Also, Dr. Simek?

DR. SIMMICK: Simmick.

DR. SALOMON: Simmick, has joined us. Dr. Noguchi had something that he had to do and Dr. Simmick has been involved actively in the adenoviral working group within the FDA, so now we have three of the FDA staffers directly involved in and we'll take advantage of them later.

Okay. And I made one egregious -- yeah, well that's your job, you know, you get paid the big bucks for this. I made one egregious mistake and I apologize to Dr. Sublett. I just missed it, there was Ad-5CMV-p53 in the title of two things and my brain skipped over them. So, anyway, the next speaker, before Dr. Flomenberg is Dr. Richard Sublett, Director of Quality Systems for Introgen. And the topic of his talk is -- where is Dr. Sublett? Oh, sorry. Absence of RCA Isolated from Patients

Treated with INGN 201 and Ad5CMV-p53 Construct, Dr. Sublett.

DR. SUBLETT: I'd like to thank the Advisory Committee for inviting me to speak today and talk about Introgen's experience with RCA and our Ad5CMV construct.

Very briefly, we're going to discuss replication competent adenovirus levels in INGN 201. We have data from a semi-quantitative assay and also from a qualitative assay and we're going to compare those and establish the levels of RCA that we believe we have in our product. We're going to talk about patients who have treated with INGN 201, the numbers of patients, doses, and essentially give an estimation of the RCA exposure to these patients.

Then I'll talk about our attempts to isolate RCA from patient samples, and I'll very briefly discuss the immune status

of these patients.

INGN 201 is another ad5-p53 construct. It is an E1 substituted adenovirus also, and E3 partially deleted construct. And within the E1 region, there is a p53 expression cassette. It is produced in the 293 cells that we've been talking about quite a bit today. And you can get double homologous recombination events between the flanking regions of the 293 host cell and the expression cassette, which result in low levels of RCA in the product.

We've already talked about this in great detail, but this is just a cartoon showing how you can get the double homologous recombination. We've used two assays over the past several years. Most of the clinical lots that we have used were released using a qualitative roller-bottle CPE assay, or cytopathic effect assay. Lately, we have started working with a

semi-quantitative plaque-like assay to get a better handle on the actual levels. Both of these assays were looking at 3e10 viral particles and, in general, we believe we have approximately one RCA and three E10s, we're testing right at the limit of detection.

Back to the cartoon -- this is a kind of a silly cartoon but, essentially, this box represents a vial of our product. There are a few RCA in our product. And our ability to detect those, largely depends on how big our net is. For logistic reasons, our net is about 3e10 viral particles. So, if we take one swipe through this box, we may or may not pick up one of these red balls. And that's a very simple- minded way of looking at it. But to address Dr. Siegel's question of, well, can we just -- we're testing 3e10 viral particles, what about 3e11? Already the assay we're working with is 40 tissue culture plates,

and to increase that to several hundred tissue culture plates is logistically a nightmare. And, in fact, most of your QC assays would become invalid because of one reason or another with that size of an assay.

The consequence of testing at the limit of detection, now, is lots with the identical RCA level may test as positive or negative. So, you're not going to get good consistency. Also, if you do multiple tests of the same lot, you may get inconsistent results.

This is a comparison of the two assays. In the first column, well that's just the lots that we've prepared. The second column is the number of RAC plaques that we identified in assays on these lots. And we have compared that with the CPE data that we obtained on those same lots. The level of sensitivity for both these assays is quite similar, with the semi-quantitative

assay we had 8 out of the 14 were positive with either 1 or more RCA plaques. With the qualitative assay, 6 of the 14 were positive. What you see highlighted in yellow is where you have disagreement. We had, in both columns, positives that were negative with the alternative assay.

Also, please note this is a little bit of our problem lot B2029701. This lot was released using a CPE assay where it tested as negative and, in fact, this is the highest level of RCA plaques that we've ever seen. So, these results really just are not consistent. And, again, I want to emphasize this is a consequence of us testing at the limit of detection. I would like to say we've used this lot extensively in the clinic. It was one of the lots we used a great deal. That's because this assay was not available at the time the material was produced and released for clinical use.

We have tested a total of 35 lots

of INGN 201 at the 3e10 viral particle level. These are the results of the plaque titer assay. And about half our lots test with zero plaques. And then we've also seen 1, 2, 3, and 4 and then we've had that single lot where we saw the 7 RCA plaques. Again, what I want to emphasize is, I don't think these data argue strongly that these lots really have fewer RCA than these lots. The assay's not that sensitive. And, again, that's why we're calling this a semi-quantitative assay. We just aren't testing enough viral particles to make this a quantitative assay.

Our conclusion is that all of our lots contain some RCA and the level is consistently low, approximately 1-to-2 RCA and 3e10 viral particles. One think I should emphasize here, when I -- we're talking about viral particles tested, but this is an infectious titer assay, so these are, essentially, pfus or infectious units.

So the number of viral particles is greater than that and that goes back to talks we've heard earlier today. This is just to emphasize, this is a very small number of RCA we're talking about.

Using these data, we can come up with an estimate of the number of RCA that we have exposed patients to. Through June of last year, we had treated 406 patients. We've treated about 100 patients since that time, so these numbers are out of date. About -- over 2,800 doses, and we have given a little over 3 times 10 to the 15th total viral particles to these patients. The RCA just says vp, but that really should be pfu administered is probably on the order of 2 times 10 to the 5th. So, the total RCA exposure in these patients is low, but it is definitely there and we don't want to pretend that some patients were not exposed to RCA and others were.

This is more of the same type of

information. The maximum dose that we have given in our studies was 3e12 viral particles. This would be an RCA exposure of about 150, again this say pfus and not vps. One patient had received -- had gone through multiple cycles, he had received a total of 4.6 e13 viral particles, so over 2,000 RCAs have been administered to that patient.

So now that we've discussed that, I want to move on to our clinical experience. In Phase I clinical trials, we've done -- I don't want to talk in great detail, because there's been a variety of Phase I clinical trials. Most of these were cancer of the head and neck. As a rule, most patients were treated by intratumoral injection. The dosages ranged wildly or not wildly, but, widely, since this is a Phase I clinical trial -- we were in control every time we dosed a patient, it just varied.

The doses were, essentially, 2.5 e12, up to 1e12 viral particles and in these

patients, essentially, we looked at urine and plasma and, also, some oral rinse samples. And these were tested for the presence of RCA by replication on the A549 cells. These cells are not permissive for the -- our construct, which is E1 deleted. So, this requires -- this is specifically looking for the RCA. We looked at acute samples and chronic. The chronic would actually go out to close to 12 months after treatment was concluded. And the assay sensitivity in these studies was less than 10 pfu in a 0.5 mL sample. And in 3,200 patient samples, we no evidence for RCA in any of these samples.

We also have data from three Phase II clinical trials, looking at plasma, urine, feces, and oral rinses from these patients. These patients were all cancer of the head and neck. To address an earlier question, these were all late-stage patients, so they were all quite ill.

We did PCR for Hexon and p53 sequences and we also looked for CPE. It gets a little complex: The plasma samples were all prescreened by PCR before the CPE testing was performed. All the other samples were tested by both PCR and CPE. And I'm focusing on the CPE results, again, going back to Beth Hutchin's talk, the PCR, certainly, can detect the presence of DNA, but we do not know if that's from intact virus or just a DNA fragment, so I'm focusing on the CPE data here.

The CP test was performed, initially, on the 293 cells, which would be permissive for the E1 deleted adenovirus, our product. If it was positive on the 293 cells, we then passaged that material onto the A549 cells, which are not permissive for the E1 deleted adenovirus.

Okay the three studies -- the first one was T201, again, had neck cancer. The dosing was 4e11 to 2e12 viral particles.

There were two treatment cycles; one, was treatment on days 1, 2, and 3, the alternate cycle was 1, 3, 5, 8, 10 and 12. We collected the samples for biodistribution studies pretreatment and on day 28. So, in this case, if it was this treatment cycle, it would be 25 days post- treatment; on this one, it would be 16 days post- treatment. This trial treated 107 patients.

202, similar subject profile. The dosing was lower in this study it was 1e10 to 4e10. Treatment was on days 1, 2, and 3. Again, the samples -- the biodistribution samples were collected either pretreatment or on day 28, 47 patients were treated.

And then, finally, we had study T207, this one, daily dosing was 1ea2 viral particles, they all received the same treatment. They were dosed either on day only or days 1, 3, 5, 8, and 12. The biodistribution samples were more extensive in this study. They were collected every

other day following the last treatment of the cycle, through 15 days post- treatment. I also want to say that this is a treatment cycle, but the patients did go through multiple treatment cycles, or they could go through multiple treatment cycles. And most of them did go through multiple treatment cycles. And that's true for all the studies. Would you go back, I'm not quite through with that slide.

Thirty-six patients were treated. Also, in this study, we looked at the immune response, adenobody response to the virus. We also evaluated at least one household member from each patient's family that was treated in the study.

Just looking at the CPE data for these three trials: T201, we had 459 samples were tested for CPE, 8 of these were positive on 293 cells, this is -- would be our construct or permissive for our construct; none of them were positive on the

A549; 201 a similar results, 175 samples were tested; 3 positives on the permissive cell line; none on the nonpermissive cell line. And then, on T207, we had 880 patient samples, not -- this does not include the household member samples, 18 positive samples. We did isolate two CPE positives on the A549s, but both of these were sera type 11 adenovirus, it was not related to our construct. Before we move in, all of these samples were tested by southern blot analysis to look for genetic rearrangements of the virus and there was no evidence of any genetic rearrangement in any of these. They all appeared to be identical to the original construct.

So the results is, we could isolate virus or its DNA in body fluids up to four weeks post- treatment. This includes the CPE treatment. We, I think day 25 or so, we actually could get a CPE positive on the 293 cells in at least one or

two cases. The only CP positives on the A549s were not related to our construct at all. And while I did not show the data in the household members, there was no evidence of horizontal transmission of the virus.

The immune response -- this slide might be a little complicated to follow. This was only on Study T207. We had data from 29 patients pretreatment and then 22 after 1 cycle and 9 patients after cycle 2; there are also some data after cycle 3, but this shows for the point I'm trying to make.

The ELISA is an antihexon. ELISA, the neutralizing is actually just the ability to inhibit a plaque formation or I should say CPE in an assay. We did have a number of patients had low titers at the beginning of the study, about half of them had no titers at all. Somewhat fewer had neutralizing titers. Two patients actually had very high titers at the beginning of the study, this was not an enrollment criteria.

And one of these patients here, is in fact this patient right here, so not only did he have very high -- I say, he, I don't know if it's a he or a she -- had very high antihexon titers but, also, moderately high neutralizing titers as well.

After the first cycle of treatment, which could be either one or multiple doses, most of these patients were starting to mount an immune response and this was both the ELISA and the neutralizing titer. And then, after two cycles, only one of nine didn't respond. This patient is the same patient, so one out of the nine patients did not respond with either an antibody titer against hexon or a neutralizing antibody titer.

So, our conclusions is INGN 201 does contain RCA; in our assay that is 1-2 RCA in 3e10 viral particles. This is very near the limit of detection so this number is fairly soft, but based on looking at a

number of lots, we're fairly comfortable at this level. We think the levels are fairly consistent from lot to lot, generally only a single lot tested with seen, they've always been less than 10 RCA.

As a consequence, patients treated INGN 201 have been exposed to low levels of RCA. We have not been able to isolate RCA that had been derived from INGN 201 in patients or household members and we've looked fairly extensively. We've see no adverse events that appear to be related to RCA in these patients. Most patients treated with INGN 201 developed antiadenovirus antibody and this is both antihexon and neutralizing titers.

And, finally, we are not comfortable with the current guideline of 1 RCA in 3e10 viral particles, we would like to see that reconsidered based on topics that have been discussed earlier, and these are largely risk factors. So, we think the

patient population, the indication of the product, and the available safety data should be taken into account. Thank you very much. (applause)

DR. SALOMON: That was excellent. I had one quick question. And that is, if we know molecularly that replication competent adenovirus would require reinserting the E1A --

DR. SUBLETT: Mm-hmm.

DR. SALOMON: -- or the E1 segment, rather, couldn't we -- wouldn't it be simple to design an assay using quantitative real-time PCR for the E1 segment and so, obviously, there's something stupid about this question because you guys would have done this, so, you need to educate the chair.

DR. SUBLETT: Well, I'm not sure I can answer it well. One concern that's been raised, and this may be discussed in the next talk, although I'm not certain, is many

of these patients, there are latent adenovirus that, as the patients proceed are started to be shed and looking for the E1 region and we have a concern that looking for the E1 region may not be due to our construct at all, but may be due to these latent adenoviruses that have become activated.

DR. SALOMON: I'm assuming you know the E1 sequence in your producer cell line, or you can go back to the lot and do this?

DR. SUBLETT: There's some overlap, although I'm not sure that I'm qualified to address how much it is. We have discussed this assay, but I think there is merit in that argument.

DR. SAUSVILLE: The other issue is that PCR wouldn't tell you anything about the function of the product that that came from so --

DR. SALOMON: I guess what I'm

saying, Ed, is that if -- if the molecular mechanism of creating a replication adeno is now recombination allowing insertion of this, then, if you could quantify the number of particles that have this inserted element, that would actually relate directly to the number of RCA in the prep.

DR. SAUSVILLE: But it would not only be the number of nucleic acid events that you could detect by PCR but the functionality of them, because, supposing, you could detect that, but they were nonfunctional viruses, it wouldn't be -- it wouldn't be an effective virus.

DR. AGUILAR-CORDOVA: That is part of it but in addition, during the process of production, you have the -- that sequence coming from the cellular DNA and it can be stuck or carried with the virus. So, at the early stages, because there is small pieces of DNA that are on the outside of the virus or maybe they're on the inside, as well.

So, at the early stages of -- or with the very actual product, that's a difficult one to decide where the cut-off is. However, through a cycle of amplification and most of these tests do go through a biological amplification, as well as the -- or can go through the biological amplification as well as the molecular amplification -- and they can become a little bit more feasible. And such assays do exist and people do run them.

DR. MULLIGAN: I don't think that was a dumb question. If you had PCR primers across the junction, you know, obviously, you could generate something that would show that, and I think it would be useful, even though, of course, you're not showing it as functional, but you're directly -- as directly as possible measuring the frequency of the event and you might be interested.

DR. SALOMON: That's what I was thinking, where you'd have primers that would be above the junction in the 293

indigrant (?), which would tell you that it was DNA that came from the producer cell line and others that were crossing the junction in, you know, so you'd have -- you're have your viral construct and you'd see where it got reinserted, but --

DR. AGUILAR-CORDOVA: In the producer cell line, you would have exactly the same sequence that you would have in the recombinant because of the crossover, but once it goes through one noncontaining cell lot, then it's --

DR. SALOMON: Dr. Rao.

DR. RAO: I just had the same question that we had for the earlier speaker. If you adopted the new standards of the recommendations, am I right that all the lots that you have would be then --

DR. SUBLETT: Approximately half the lots that we've tested would have been negative at that level, but first of all, we find rejecting half the lots commercially

unacceptable and this is a commercial enterprise, although we certainly -- many of us are motivated from trying to do a little bit of good here. But, also, and this is one thing that testing at the limit of detection means is the lots that we rejected, in our view, have the same RCA level as the lots that are acceptable and in some ways, it's become very artificial.

DR. RAO: Can I extend that question? So, taking, presumably, what are higher levels of RCA than what are current proposed guidelines -- in any of your immune-compromised patients that have been used in this trial, have you seen anything -- I mean, you presented three studies with patients, which suggested that there was no evidence of any effect of RCA?

DR. SUBLETT: That is one of the things, I was trying to point out in the last slide, and maybe I didn't do so well. I think you do need to take the patient

population into account. There have been reports on the severely immunocompromised patients and, perhaps, those should be excluded from using adenovirus constructs with these types of RCA issues. But the patients that we're looking at do not appear to be that severely immunocompromised that we have any evidence problems to date.

DR. SALOMON: One of the things that came up in the break was a question that, have there been any documented cases of replication competent adenovirus in any patient group causing a disaster? I mean in the sense that a lot of us in the retroviral field have been sensitized by work in non human primates and a couple patients where we had RCR?

DR. SUBLETT: Not to my knowledge, that hasn't happened. It does get muddy, in all honesty, because both Beth Hutchins and I are really looking at similar patient populations, they're similar constructions,

they are late-stage cancer patients and, in fact, the death rate is fairly high in these patients. But it has been associated or believed to be associated with the underlying disease that may make seeing some of these things hard to see, but I have not heard of any case where anyone really felt that RCA was a contributing factor in any of the adverse events.

DR. SALOMON: I think that's an important thing for the Committee to keep in mind. Are there any comments from anyone else on the Committee on that, in terms of the literature? Yes.

MS. MEYERS: Do you know how many of these patients are still alive?

DR. SUBLETT: I do not know the number, but I do know it's fairly few from the -- certainly, the Phase I trials, it's very few. There are a few from the Phase II trials, but these patients, typically, were having life expectancies of 3 to 6 months

when they entered the trials. And, in fact, most of them have passed on.

MS. MEYERS: So, it's clear, then, that these patients really don't live long enough for you to see what the long-term effects would be?

DR. SUBLETT: Although in one patient, we followed him for -- or more than one patient, we followed them for a year and we haven't see evidence of RCA. But the number of patients that we've followed for that long is fairly small.

MS. MEYERS: Mm-hmm, thank you.

DR. SALOMON: Would the sensitivity of your assay be significantly improved if you went to 10 to the 11th or 10 to the 12th viral particles, instead of 10 to the 10th? I think this is a question that came up to Dr. Hutchins, as well, and so I just want a reality check, also, on whether that would be, yes, but it would be difficult to do commercially?

DR. SUBLETT: With our assay, I mean, the answer's clearly, yes. The standard deviation on ours, essentially, we averaged about 1.3 RCA per assay, and if you look at the standard deviation, it's 1, so we have, essentially, 100 percent error in this assay. That would -- if we increased the assay size ten-fold, which would be difficult, that standard deviation would probably drop but, in fact, if we average ten RCA in the assay or if we tested 3e11, I guess it would be 13, you would still have a sizeable standard deviation, because that's still a small number of plaques to get a good statistical number. But it would, clearly, be better.

DR. SALOMON: So, just so, again, just so we have a clear reality fix here, the problem with these assays is that you're tending to go to the borderline of their sensitivity, even really beyond them, frankly, because it's just a ridiculous

amount of supernatant to be able to do the assay right?

DR. SUBLETT: Well, more -- no, it's actually more complex, even than that. Putting on more virus on the same number of cells is not so much a volume issue -- is that you run into toxicity from the virus that is not related to replication. And so, if you go under -- in the presence of Estuardo, I had to use the word MOI, but at the concentrations -- at the concentrations we use, and the exposure times that we use, and the cell number, and the virus number, if you're over 200 MOI, you start seeing toxic effects that interfere with the detection of the RCAs, so the, you know, the really easy solution is just to throw more material on and it doesn't work. You have to scale it up. And that's when it gets to be hard to handle.

DR. SALOMON: Point made. Any other comments? Estuardo?

DR. AGUILAR-CORDOVA: I think another thing to keep in mind besides you could just add more volume, I mean, get rid of your toxicity with that MOI. But the other point to make is that all of these are RCA assays that we're hearing about are based on the wild-type that's been titered in the laboratory that's reporting them. So if they're based only on the sensitivity of whatever their assay to detect RCA, so it's sort of a circular argument and we really don't know, and that's where the standard comes in. We really don't know how that relates to anything else from anybody else.

DR. SALOMON: Thank you very much. Then we go to the last, but not least talk of the session, which is from Dr. Flomenberg on discussion epidemiology of adenovirus and its impact in certain patient populations.

So, by the way, just to give sort of a plan, I thought we would finish with Dr. Flomenberg, have a brief discussion,

break for lunch, and then come back and tackle the questions and end the session.

DR. FLOMENBERG: Good morning -- good afternoon, by now. It's an honor to be here today. Can you hear me?

I was asked to talk about adenovirus infections in immunocompromised hosts and also talk about the issue of persistence and reactivation of infection. I think the main points that need to be understood about adenoviruses is that they are truly ubiquitous. Nearly everyone's exposed to these viruses in early childhood and nearly all adults have evidence of prior infection with the endemic seratypes and I would say that just about everyone in this room has antibodies to adenoviruses truly ubiquitous infection.

As was implied, the clinical manifestations do vary according to the age of a patient and the immune status of the host and, as we know, certain seratypes are

associated with distinct syndromes.

As we can imply from the fact that adenoviruses are associated with epidemics, they are -- they do seem to be easily transmissible to susceptible individuals, which would be individuals without neutralizing antibodies to that seratype, which should be protective. The seratypes that are associated with the epidemics are seratypes that are the nonendemic types: Ad3, Ad7, Ad8. And these can be spread relatively easily amongst susceptible individuals. We really don't have any information, however, what the minimum infectious dose is.

In healthy individuals, I'd like to just point out, adenovirus pneumonia may occur, primarily in children. It causes about 10 percent of the pneumonias. The disease is more severe in younger children and infants, and it can on occasion cause fatal infections in healthy children, rarely

healthy adults. It also is associated with extrapulmonary symptoms that usually do not correlate with any viral- specific histopathology. And things that have been seen in these patients include meningoencephalitis, hepatitis, myocarditis, nephritis, neutropenia and DIC. And the mechanism for some of these extrapulmonary symptoms is not entirely clear.

People have questioned whether it was toxin mediated. From what we now know about some of the acute reactions to the gene therapy vectors, maybe some of this is an immune-mediated phenomenon.

Let's move to adenovirus infections in immunocompromised hosts. These can, clearly, range widely from asymptomatic shedding, as has been mentioned, to fatal invasive or disseminated disease. Disease may result from several different mechanisms. These patients may acquire a primary infection, maybe the

result of reactivation of endogenous infection in the patient, as well as transmission of infection from the donor organ in the case of solid organ transplants.

One of the groups that's been the best studied is the bone marrow transplant patients. These are, certainly, the most immunocompromised patients that we deal with. And there, again, is a wide range of clinical syndromes ranging from pneumonia to gastroenteritis, hepatitis, hemorrhagic cystitis, nephritis, encephalitis, and myocarditis.

It is clear from a number of studies that the incidence of infection is significantly higher in the pediatric population compared to the adult population.

The mortality of invasive disease varies, but is in the range of 50 to 60 percent in the bone marrow transplants patients. The risk factors for invasive

disease include, clearly, the allogeneic transplants are at much higher than the autologous and, in particular, as the field has evolved, patients who have T-cell depleted transplants seem to be at even higher risk, as you'll see, when we talk about some of these studies. Essentially, these patients have a -- they're naive to adenovirus.

Other risk factors include the presence of graft versus host disease and two or more culture- positive sites.

This was one of the earlier studies, done in 1985 from Seattle. They reviewed over 1,000 patients who were bone marrow transplants recipients. At this pint of time most of these patients had unmodified grafts from related match donors. It's not clear what proportion of children were in this study. The overall incidence of adenovirus infection was 5 percent and the incidence of disease was 1 percent. And

in the individuals that developed disease, the mortality, again, was rather high, 50 percent.

So, this was about the status of adenovirus infections back in the mid-eighties. As transplanters became more aggressive and we -- they started to use transplant more high-risk patients and used T-cell depletion, what we have seen is an increase incidence of adenovirus infections.

This is the study that I was involved with out of Milwaukee, published in 1994. This was a smaller group of patients, but look at the difference in the patient characteristics. Virtually all of these patients had T-cell depleted grafts. These were naive patients, essentially. Half of the patients had these high-risk, unrelated or partially-matched grafts and the other thing that is unique is 40 percent of these patients were pediatric patients. We reported a much higher incidence, both of

adenovirus infection, 21 percent and disease, 6.5 percent and the mortality was about comparable.

So, in this very different patient population, the risk was higher. But, again, the converse is two-thirds of these patients developed shedding or infection without evidence of disease and did well.

There was a much higher incidence of adenovirus infections in children compared to adults, 31 percent versus 14 percent. There was a much earlier time of onset in the children, as well, in both the endemic types as well as the AD11, 34, 35 were the most common seratypes isolated and I think that's been a general trend and it's not entirely clear why we're seeing a lot of these groupie seratypes in these patients.

Similarly, a 1999 study out of Kentucky reviewed a similar patient population where they had 40 percent of their transplants were T-cell depleted, not

as high an incidence of children and their incidence of infection was 12 percent, again, very consistent in all these studies, the incidence of infection's higher in children. Their incidence of disease was relatively high, 7 percent with an overall mortality of 73 percent, it was hard to sort out in this study, what was attributable to adenovirus. So, again, in a higher-risk patient population, we are seeing more adenovirus infections, as well as disease.

This was the other study in your handouts from the U.K. It's a little harder to evaluate. They evaluated over 500 bone marrow transplant recipients, again, about half had T-cell depleted grafts and unrelated donors, but there was a much higher proportion of children in this group. They documented a much higher incidence of adenovirus infection, 17 percent, but I think I would have some differences of opinion in terms of their definition of

disease versus shedding and the mortality was lower. I think, again, that -- I don't think they distinguished very clearly the difference between shedding versus disease. So, I would say in most of this, the more recent studies about a third of the patients go on to develop disease.

Moving on to solid organ transplantation, adenovirus infections, typically involve the donor organ. They have been the cause of hepatitis in approximately 3 percent of the pediatric liver transplants. That incidence is a lot lower in the adults. They cause pneumonia in about 1 percent of the lung transplants and hemorrhagic cystitis in about 1 percent of the renal transplants, and the mortality is a lot lower in that patient population.

Congenital immunodeficiency syndromes, as was mentioned -- patients with severe combined immunideficiency disease can develop very severe infections,