November, right, was again the data base is still really incomplete. We get data, we've shown data today from just the NGVL, from Ken Cornetta's group which is great because it gives us data, but I think that Dr. Mulligan's and Dr. Chanock's points are well taken. It's not every case. We don't really see the whole universe and I think in the absence of really getting all the data the way scientists want to see it, it's not reasonable to ask the Committee to kill a vector or to even make those kind of decisions. And I think that you're trying. I mean that was the message in November as frustrating as it was sometimes, that this whole area is suffering in one major way from a data search crisis. We need all the data in one place. And I know that you guys got that message last time.

DR. MULLIGAN: My message was that there is no social redeeming value to that cell line.

(Laughter.)

I don't think -- there's no special properties of that that I can think of that would make me support it the way it appears that I am, but nevertheless, I wouldn't do it right now.

DR. SALOMON: Okay, are we comfortable with that? All right.

The next presentation is from Suzanne Epstein. Dr. Epstein is going to talk about responses to the FDA letter on testing of plasmids. If you haven't already, obviously, looked ahead a little bit here, it's -- we talked about replication competent retrovirus. Now we're going to talk about plasmids. Then we're going to talk about adenoviral vectors and we're also then going to hear some more about adenoviral infection.

Somewhere in the line here I've got to juggle this with lunch and stay reasonably on time so someone doesn't strangle me by late this afternoon. I'll worry about that.

DR. EPSTEIN: Can you hear me? How's that? I'll try and make up some time. I'm going to be talking about testing of plasmid DNA when it is used as an intermediate in manufacturing other gene transfer gene therapy products.

Plasmid DAN is used in a variety of ways in the manufacture of biologicals. In one extreme it is the actual product administered directly to the patient. In the case I'll be discussing today, plasmid is used as an intermediate during the lot-by-lot production process of other products. And then finally at the other extreme, plasmid DNA can be

status is really not very different from any other reagent.

The goals in this area were as follows: testing of plasmids used as intermediates would help achieve consistency of manufacturing of the gene transfer product and would prevent contamination of cell cultures that are used as the product or in making another vector.

What we decided from the March 6th exercise was that we needed to clarify CBER's expectations for testing of plasmids when used as intermediates and also seek advice from the Committee about the reasonableness of our set of recommended tests.

So first to give you some examples of what we mean by this, when plasmids are used as intermediates in production, they're used during production of each lot of a gene transfer product, not just during deriving some kind of a construction of a cell line. Some examples include ex vivo transvected cells, AAV vectors and retroviral vectors in certain cases, certain production schemes by transient

used early in deriving a construct that's used for any of a wide variety of purposes, but then it's a reagent. It's not something used every time and its

transvection.

One thing we noted in the responses to the March 6th letter were that sponsors were confused. There was tremendous in whether or not they reported on plasmids and in some cases they reported only on the final product. In other cases they reported surprisingly only on the intermediate and didn't, for example, provide data in answer to questions 1 and 3 about cellular populations which in those cases may have been the actual product. So anyway, there was confusion as to what our expectations were.

So here's one of the examples. If cells from a patient, this is often a patient-specific population, but could be a cell line, are transvected with a plasmid that contains a transgene, you then end up with a cellular population expressing the transgene product and this is your final material given to the patient, but this vector is quite important and is used every time and will have tremendous impact on the consistency and quality of this production scheme.

Here's another example, production of AAV by a certain method. This is from a paper by Grimm, et al., and in case two plasmids are used, one containing the vector, the intended AAV, and one containing the rep and cap functions and the

adenovirus helper functions. Both are used to transvect a cell line and the cell line then produces the AAV. So again, the intermediate is used every time and its identity and quality will have an impact on the consistency of this manufacturing and whether these cell cultures become contaminated.

There are lots of precedents for regulatory scrutiny of an intermediate. First of all, reagants and intermediates in general, when used to produce biologicals are subject to quality control testing, some of this is in the GMPs in qualifying source materials and so on, and this is quite general. Then specifically, the uses of plasmids I've illustrated for you are analogous to use of retroviral vectors when they are used to transduce cells for ex vivo gene therapy and in that case the transduced cells, not the retroviral vector are administered directly to the patients. There are some other cases where retroviral vectors are given directly to patients, but the analogy here is with the ex vivo case. In those cases, even though the vector is an intermediate, retroviral vector preparations are subject to extensive quality control testing. So what I'll be talking about is nothing new.

What I'll do now is just throw out there

a proposed list of quality control tests, but this is for discussion purposes and is certainly open to change. These would be plasmid intermediates being used, as I've discussed and what I mean here is that each lot of plasmid DNA that was prepared for use would be tested in these ways. So this lot-by-lot testing and we'll come to some one time testing.

Sterility is pretty obvious to avoid contaminating the cell culture. Residual toxic reagents such as organic solvents also could have a negative impact on the cell culture. Endotoxin can interfere with transvection. Then identity is particularly important because of the number of multi-use facilities and we've heard before the discussion of mixups. This could be a variety of types of tests. We're thinking of, for example, restriction mapping, but I'm hoping Dr. Roessler will comment on this because sequencing is a possibility here also. And in a multi-use facility this might include excluding contamination with particular other products. Purity -- these interact -- purity could include ruling out a variety of contaminants and in this case might be an agarose gel electrophoresis. Concentration might be absorbance. We're certainly not specifying particular assays for these things.

And then activity or gene expression can be very important to know that the protein expressed from the transgene is active if you're not doing a full sequence here. So these types of tests are proposed and the sponsor would have to establish acceptance criteria which would depend partly on the amounts being used and so on, what levels of endotoxin might be acceptable, for example.

Note that an activity assay is not necessarily a fully quantitated, validated assay like a potency assay.

Next. We're proposing as a one-time test, full plasmid DNA sequence and homology search for open reading frames. As you probably know from the earlier meeting, this type of analysis has to do with finding extraneous material in the construction that shouldn't be there, looking for rearrangements and so on, fairly gross features.

This would be performed once, not necessarily on every lot although we'll return to that in the discussion because there are different points of view there and it would be performed prior to Phase I because these are small vectors and there should be no problem conducting that analysis, so you should know what your construct is.

Then we'd like to raise the question of whether certain other tests are necessary or not for plasmid intermediates. Residual E. coli DNA, RNA and protein are contaminants that can matter in some situations. They can indicate inconsistent manufacture or a sloppiness, but they may not matter if the product isn't going into patients and it's just being added to a cell culture. Then a potency assay is a more quantitative, formally validated assay of activity and we're proposing instead only an activity or expression assay. These additional tests, these purity tests and potency tests are expected for plasmids that are being given directly to patients, so we're proposing a less stringent standard for intermediates.

And that brings us to the questions for the Committee.

DR. SALOMON: Thank you very much. Can you do one level of clarification while everyone sort of gathers their thoughts and that is you used these words very clearly, potency and activity. Can you maybe just --

DR. EPSTEIN: Give examples?

DR. SALOMON: Yes.

DR. EPSTEIN: A true potency assay would

be a measure of what the product is supposed to do to have its biological effect in the therapeutic setting.

An activity assay can just demonstrate something the product does. To try and give an example, suppose the plasmid is being used to transvect lymphocytes which are then infused in the patient and lymphocytes are supposed to go and kill something. An activity assay might be that the plasmid causes the appearance on the cell surface of that protein. A potency assay might be some correlative killing or an animal model in which the tumor regresses, something like that. So it's much more difficult to provide a potency assay and for a final product for patient use by Phase III you have to at least do your best. For an intermediate, we feel if you are getting, for example, the proper enzymatic activity of insert, that's good enough or whatever.

DR. SALOMON: Good. That's great. Okay, specific questions then?

Are the quality control tests listed appropriate tests to be performed on each plasmid lot? Sterility. Yes? Obviously, right.

Residual toxic reagents, for example, solvents. Now my response there is I'm not quite so clear, so maybe this is where we need some discussion.

Number one, it's not always so easy to assay a preparation for the presence of solvents unless we're talking about gas chromatography or thin layer chromatography.

DR. EPSTEIN: There hasn't been any resistance to that. Organic solvents, phenol or ethanol or whatever that are used in plasmid purification can be detected by means like gas chromatography, very sensitively and it doesn't seem to trouble people to do that.

DR. SALOMON: Well, okay. I'm just bringing it out. Here, again, we make this segue, to have a gas chromatograph I think minimum would be $75,000 to $150,000 investment.

DR. EPSTEIN: They just have a firm do it for you.

DR. SALOMON: Okay, fine. I guess the other question would be if there's phenol or something contaminating it, what's the concern? If I now add it to my T-cells in order to deliver this potency product, I'll affect the T-cells and I won't know it, right?

DR. EPSTEIN: Right. Say there's chloroform in there. You may kill your T-cells or even if you didn't, you don't want to give chloroform

to your patients.

(Laughter.)

DR. SALOMON: I certainly don't want to give chloroform to the patients. I'm just trying to be reasonable here. At some point, you're going to wash the cells I would hope.

DR. EPSTEIN: Yes, but basically, the reason to be concerned in the case of intermediate, would be the health of the culture. It's more of a consistency. In fact, all of this is more of a consistency. In fact, all of this is more of a consistency issue than safety. It's very unlikely you'll put enough of something in to create a safety hazard to the patient. It's more likely that you'll kill the cells and/or mess up, contaminate your production.

DR. SALOMON: Good. I'm fine with that if everyone else is fine with it.

Endotoxin, I think we pretty much all assume that lot testing should include endotoxin testing and that is a danger and I don't want to give my patients endotoxin and it's a real issue in any manufacture. Is there any disagreement there?

Identity. Now that covers a lot of ground. I hope that there's some discussion from the

group here. I'll play the stupid guy for a while here, but in some way identity is important.

(Laughter.)

I'm telling you identity is important and I want discussion on this one.

(Laughter.)

DR. ROESSLER: I'll take the bait there. I think that there probably is a role to consider the value of sequencing of lots and I think that's largely on the basis of a specific transgene in the effect that a specific transgene might have during the production process in terms of an adverse selection pressure it might exert on your E. coli producing strain that would allow for mutations, rearrangements or deletions to occur at a high level that might affect the fidelity of your product and might affect how much of your viral reagent was manufactured post-transvection or how much of your transfusion of interest was produced post-transvection.

DR. SALOMON: So the last time we met, we all agreed that you had to supply sequence identity for plasmids under 40KB, in like 40KB or less. And the interesting thing that came out today, I think partly in Richard's comments was where is that sequencing validation done and how often do you have

to do it in order to stay within the Committee's spirit that you know what's in, right? We all agreed that on the way in you need sequencing because you just don't know what someone helped you with and it's just really hard when people are giving you little vials to know exactly what's in there, right? And your point is well taken that some of it's even selected again by differential growth of the production bacteria.

So we all agree on that, but how about -- how about at the end of production? How often does one have to determine the identity? Is it only on the first day when I give you the vial, you grow it up? So what is an appropriate recommendation from the Committee on tracking identity over time?

DR. ROESSLER: Well, we wouldn't expect that there would be changes in the actual sequence over time and is your question related to product storage? For example, that you're going to make a large batch of plasmids that express rep or cap and that you'll need some stability assay to be performed, but that doesn't necessarily have to involve sequencing.

DR. SALOMON: I agree.

DR. EPSTEIN: I'm not sure you realize

that Dr. Roessler was proposing lot-by-lot full sequence.

DR. ROESSLER: No, not full sequence. I think we just need to consider the role for sequencing of lot-to-lot production material.

DR. SALOMON: That's the question that was asked.

DR. ROESSLER: I don't think that I would -- I would just say that for every gene vector that's being produced that restriction analysis alone is going to be absolutely adequate. I think for the vast majority it probably would be adequate, but I wouldn't discount the added value of doing some limited sequencing, primarily of the transgene insert or flanking regions. I think once again it echoes Dr. Mulligan's point that you have to take advantage of the available technology and changes in technology. Clearly, sequencing is evolved and it's become more rapid and less expensive. And so whenever you have a technology that is moving in that direction, it represents from my perspective added value to the manufacturer and to the sponsor.

I think the one other issue is that in the academic sector, specifically, if you have a sponsor who is going to be responsible for performing the

potency assay, and that assay fails, then they're going to come back to the manufacturer and say the potency assay suggests that there was a problem with the plasmid and as the manufacturer what data or evidence can you provide to me that it wasn't a problem with the plasmid. So I think it's just another series of checks and balances and it may be that my perspective from the academic sector is trying to think through that scenario where we might send a plasmid intermediate to a sponsor for a potency assay or a functional assay and then it may not meet specifications and then we have to problem solve that unexpected result.

DR. SALOMON: Well, at the risk of oversimplifying, right now when we think about what kind of things we'd do with plasmids in gene therapy, one is that we would do ex vivo exposure to effector cells, right? They could be stem cells or it could be T-cells or macrophages, something like that, that would give them a property to target or to kill or to home to some sort of area and maybe produce a growth factor.

The second thing is actual injection of the plasmid directly into a site, in vivo, right, such as the VEGF trials being injected into areas of

ischemia, in the heart or into peripheral, ischemic peripheral vascular tissues. So that would be two major things.

Now injection of the plasmid, I would be comfortable that you had made a large lot of plasmid, sequenced a aliquot of it and so I know what I was injecting. That would be easy. The question I have now is in the experiments where I'm putting plasmid into 10^{10} purified T-cells for a study that I want to do, just for example, do I need to - -and I know that the input plasmid is sequenced because it came from the same lot you would have given me had I wanted to do a direct in vivo injection of plasmid. Do I also need to grab a couple of the T-cells and sequence the plasmid in the T-cells? Is that something that we're suggesting?

DR. ROESSLER: I think that's obviously technically much more difficult and presents much more costly scenario. So I don't think that we have enough information. It's kind of a theoretical anecdote that you raise, but I see where the point is, but I think that from a manufacturing product perspective, once again, it seems reasonable to do the complete sequencing and then restriction analysis and then on a case by case basis to consider added value for

limited sequencing of a specific lot.

DR. MULLIGAN: I think Blake's point about how you can take a perfectly good plasmid and grow it up to a large quantity and have something happen to it is very, very key. So I think the issue we dealt with at the issue meeting was essentially just knowing that you're beginning with the right thing and everyone agrees, I think, at this point we ought to have a sequence. I think I would still go for my interest in seeing lot-to-lot sequencing for the very reasons that I'm not sure you really know that the coding sequence is going to necessarily be the relevant place that would affect gene expression. It could be the upstream sequences or something.

The issue of the post-transvection, I think, is an easy no, except for coming back to that black hole of single genome retrovirus things. There is a context where you have to consider a plasmid intermediate within the cells and that's when you're doing transient transvection that make retrovirus vectors. And one of the things that I think most people don't appreciate is even if you have separate, separated viral functions if you in a transient transvection introduce those separate functions, there's a remarkably high rate of recombination among

the transvected sequences so that I don't know if anyone has really looked at this, I don't know whether you've assayed, want to look at this, but I bet you see there's a high rate of those dangerous single genome packaging sequences.

So you might want to ask people to at least assess whether or not that happens because what you're going to see, I think is that everything is going to link to everything, so you're going to have a little bit of retrovirus vector linked to a little bit of lung packaging sequence. It's not exactly what I would say is the best way to go about making this.

Now AAV is a slightly different case. It's probably less of an issue, but it may nevertheless be worth looking at.

Okay, well, I wanted to say one thing. I certainly don't think it's an anecdote to be doing this. At every transplant meeting you go to now, you have somebody, at least one person, getting up and singing the praises of ex vivo gene transfer to target cells and then infusion back into patients.

DR. ROESSLER: I just meant as an anecdote a single case as opposed to a specific protocol.

DR. SALOMON: So what I'm hearing now is we all have agreed before we even came here today that

we would have to know the complete sequence of the plasmid when it arrived at the facility. Then we talked today about the fact that there should be a lot-to-lot control because in expansion of the plasmid DNA in bacterial systems, there can be changes, right? Although Dr. Mulligan, Dr. Roessler, you guys have given two slightly different statements. You started to sound like you were going to do every sequence should be, every lot rather should be sequenced and then you sort of backed away from it and you were saying every lot should be sequenced.

DR. ROESSLER: I'm trying to make it broader in terms of giving the Committee the perspective that you need to consider that every plasmid is a little bit different and there may be plasmids that are used over and over again that are known to be quite stable in terms of their genetic identity.

So from that perspective, it doesn't seem to make as much sense that you would need to do complete sequencing of that particular component, that particular intermediate, whereas you might have a transgene that has a particularly negative selection pressure on your E. coli strain so that you believe there would be a higher incidence of either

recombinational events, mutations, deletions and in that instance, that particular intermediate component, there may be significant value to doing sequencing. Now whether that's complete sequencing or sequencing only of the promoter transgene or whatever, I think you have to deal with that on a case by case basis and that you can't just assume that complete sequencing is going to be the best way to go at this stage.

DR. CHANOCK: Can I ask a question at this point? Just in terms of the utility of sequencing, I understand that theoretically the question is on a practical level. Is the sequencing tied to the release of any plasmid or any material as it goes forward? In other words, you do the sequencing, but is that tied to when that material is then made available only when someone has actually done the sequencing, looked at it and verified it and moved on or is this more protective in the sense of being able to look back and say all right, we have that information, we now need to go look at that because something has gone awry.

It seems to me those two different tracks have two different implications.

DR. SALOMON: Well, that's important to clarify. Certainly my thinking and the Committee can

modify it, is that we're actually saying that for lot sequencing or pre-manufacturing sequencing that these are events that have to be done, checked off before you go on to go forward. It's not a parallel sort of testing that might occur under other circumstances. So trying to take this and make a practical statement out of it, if you have a plasmid that you're going to make large lots and go forward into trial, then basically the manufacturing facility and the sponsor has to answer the question that you posed, is this a plasmid that maintains its integrity and is not an issue, which means there has to be some data on at least several lots sequenced that would satisfy FDA staff, that this was correct and then if you did, perhaps after that there would be a more limited obligation for quality control of the lot. That might make a lot of sense, vice versa if it turned out that either (a) you didn't have data, but you still wanted to push forward in the trial, you might accept the onus until you do have data to sequence all lots. Or, if you had data that actually showed you had a difficult plasmid that you would have to sequence a portion of lots, if not every lot. Is that -- again, I'm just trying to be practical in terms of a recommendation.

DR. EPSTEIN: I just want to explain why we took a different position. If you have both a good restriction map and an activity assay, so I'm accepting what he's saying and we'll take that back for consideration, but if you have an activity assay that's well controlled and highly specific, then you know the insert both is expressed and has not either mutated or been rearranged to such an extent that it loses its biological function. Minor mutations elsewhere that don't lead to wrecking the promoting and so on, we would simply accept. So if the restriction map shows it's what you think it is, you know the sequence when you went in and now some degree of mutation has occurred, but without losing transgene expression and function, that's where we were starting from.

DR. SALOMON: I would just point out though that there's a problem potentially there because when you infuse it back into the patient or you inject the plasmid into the patient or into a tissue, if a mutation occurs, let's say and most of these are natural biological products. Some of them might generate antigens or other unwanted effectors on other portions of the molecule that wouldn't regularly be anticipated and might really be devastating. So I

think you have to be a little cautious at this early in the field.

Again, I'm not saying that I could take the sequence and go aha, I've got an antigen created here, that's not my point.

DR. CHAMPLIN: Just to clarify, what you had said and I believe our consensus was was that you would want to fully identify the lot, but if that lot is going to be used to treat a series of patients, you wouldn't then need to take the T-cells from each patient and redo the sequence at that point which I believe would be onerous.

DR. SALOMON: Okay, no, I was just trying to be logical and walk it through and I -- we haven't gotten to that one yet.

DR. MULLIGAN: I think the issue with the sequencing is -- my own philosophical view that we're in the age where you can easily do that, like wash your hands after you go in the bathroom or something.

(Laughter.)

So I would still push for the complete sequencing, but on the other hand, it's very obvious that's a very low resolution determination of how pure the thing is because you're only going to get the major sequence. So I mean it's of course -- it's a

dicey type of thing, that is if you had 20 percent of some different thing, when you sequenced it, you might detect and you might not detect it by a good high resolution, a gel assay either. So but I just think it's so simple, if I were a company that was making DNA, I would be shocked if we wouldn't be sequencing a sample from every lot.

DR. CHANOCK: Just on that end, I think the technology is clearly there for -- I mean, I think your point is very well taken. It may be 15 or 20 percent representation of a variant, but the whole SNIP world of SNIP detection has exploded and the technologies are there and the software is there, so I think it may very well be possible if you know, if you do the right aliquotting so to speak, to be able to identify at a certain place that you may have 20 percent sequence that goes off when you look at your standard phred phrap scores off of your ABI sequencer or whatever. So I think that that is pretty much at hand already and it's something that we may want to think about.

DR. NOGUCHI: Just some clarification, Dan, on where you're taking this. It just seems like there's still one part of it is that nobody is routinely, as far as I know, looking for how stable a

plasmid is through the production process by sequencing, so wouldn't you have to say that at this point in time everyone would have to do that first and then we would back off on a case by case basis? Is that what you're kind of suggesting?

DR. SALOMON: That's what I said. I mean in other words, either you have data, you have a stable plasmid which I was picking up on what Dr. Roessler was saying, or you don't. If you don't, then you have to show that it's stable. When you've satisfied that it's stable then you can -- you don't have to maybe do it as often.

I think that the technological issues are well taken. We all have core laboratories now. It really is like washing your hands to send something for sequencing.

I like the idea of incorporating even the newer technologies that more bio-informatics than another technology in the sense of looking for SNIPs and satellites and other groups that might rapidly give you information on subspecies that would address the question Dr. Mulligan came up with. That's a good idea.

All right, again, just kind of plodding forward is the question Dick Champlin had said and the

next step would be do you have, what do you have to do with the -- this will be cells that have been transduced. They put the plasmid in and you get the transduction and you want to go forward into the clinical trial. Do you have to do anything with those? I'm not saying that I think you do, but I just think that's something the Committee ought to make a comment on.

DR. MULLIGAN: I can't understand why you'd want to do that.

DR. SALOMON: Fine. I'm just being the devil's advocate right now. I don't want to do that, no.

DR. SIEGEL: You're specifically speaking of when you say do anything, you mean do sequencing?

DR. SALOMON: Yes. I'm talking about right now, I give you 10^{10} T-cells from a pheresis because I want to put in a granzyme and stimulate it with a dendritic cell antigen for my tumor and then inject it into the patient with melanoma. That's a very scenario. That's the kind of things that people want to do with plasmids right at this second.

DR. SAUSVILLE: Yes, but it seems that that's going to vary in a case by case basis and where you're going to efficiencies of detection, how you

detect it, etcetera, that could be viewed as I think very onerous to actually require that type of thing.

DR. SALOMON: I wasn't, again, I know that that's the way this is going and I think we should comment on it as part of this discussion. I don't think that that's what we should be doing right now. I agree with that.

Okay. Any other comments on identity then?

Purity. So I guess here we're referring particularly to things like E. coli DNA and RNA as well, I guess, you could add that to things like solvents, etcetera, that would go forward?

DR. EPSTEIN: Actually, the second question deals with that. I think it's the second one. We're talking here about general purity. Should you have to do something like an agarose gel to show what you've got, then looking for specific contaminants is where we'd like to distinguish it from plasmid for patient administration.

DR. SALOMON: Okay. Any comments on that?

DR. SAUSVILLE: Agarose is cheap.

DR. SALOMON: Agarose is cheap. Okay. Would that be okay? Is that enough these days? I mean an agarose gel, you sustain with ethidium

bromide, you turn up the integration on most image processing packages. You can always find an extra band here and there. What exactly is it that I'm supposed to show to reassure everyone that I have a pure plasmid?

DR. EPSTEIN: Consistency. You can have contaminants at some level. The cells would be washed and so on, but you're just going to look at the purity of what you're using. And have some reasonable standard.

DR. MULLIGAN: We had a talk, I think, last time or several times ago about someone getting into the details of dimers circles, and that type of thing. What is your opinion on -- I mean that can vary from batch to batch. You have basically the same identical construct, but it may be as a dimer, trimer or some complicated multimer. Have you thought about whether or not you want to have a consistency in that?

DR. EPSTEIN: Well, for plasmids at the later stage, say for patient administration, you certainly want to note the percent that is supercoiled and the percent that's in various forms. I don't know if it's consistent which form is the active one. Do you know whether transvection of cells is always the same species?

DR. MULLIGAN: Well, I think it makes a big difference how big it is and it would make a difference. So if you have different species, it would make some difference. I'm not actually personally all that concerned with it, but I think that it is, it will be different. You'll have a giant piece and that will probably behave differently, depending on how the multimerization occurs. You may have different gene expression potential.

DR. EPSTEIN: So you could simply report all the forms observed and if you are way out of line with your experience of a reference, say you have a reference standard, that would be useful.

DR. MULLIGAN: Again, on these things I would think the investigator and the manufacturer would really want to have a product that's as good as possible, as homogenous as possible.

DR. SALOMON: When we went over these questions with the staff before this, my comment at this part was that we're dealing with things that are production quality issues that I think certainly are beyond my expertise. I mean we agarose gels all the time with plasmid DNA, but I'm just excited when I see a big band around the right molecular weight and then we cut it out and go do our blunt, clone it into

something and go on. And that's not what we're talking about today, so I feel a little bit -- one of the comments that I made back to the staff on that was that did we have enough expertise on the Committee at the production level. That's one of the reasons we asked Dr. Roessler to join us.

Does anyone in the audience want to comment on this? I mean if you see two or three different bands on it, you can't just cut it out and purify it. I mean when you're talking about commercial lots, right? So what do you do when you see these? It's just you mark it down and it's part of the record which is what Dr. Epstein suggests and is that okay with everybody? These are the things that concern me is that the Committee is making some comments on things that I feel, I certainly feel is not in my area of expertise any more.

DR. MULLIGAN: I mean I think a key message that's probably the most important message is that as we march down more biologicals, there's going to be more and more of the issue that these entities are not homogenous like a drug would be and the viruses are clearly the case and it's most important to have the FDA get a sense of how they're going to deal with that philosophical issue and the plasmid DNA

is one of those, but clearly virus preps, when we get into the different ways to purify and the inability to completely characterize the composition of a virus prep and so I think we have to begin to think about the fact that you can't be that specific. And I think plasmid DNA is that guy who talked to us, some expert in making plasmid DNA and I think he had some ideas of how to reduce the amount of the multimer forms and so forth and you want to encourage that sort of thing, but I think the state of the art is that you're going to get this sort of thing and I agree that just at least having a good, accurate description of what you have is probably the best you can hope for.

DR. SIEGEL: This is not an issue for gene therapy. It's present for our protein products, for vaccines and so forth. It's not a -- the question, as worded up there and I'm not sure exactly whether our group wants a different question answered, but the question that's asked up there is what testing should be done, not what specifications should be set for those tests. It's very common for development of complex products that we require a test be done and that the initial specification is that the results are to be reported and reviewed and that over time that tells you, among other things, not just how homogenous

the product is, but how consistent it is and whether your Phase II studies are being done with the same stuff as your Phase I studies and ultimately it collects the data base that as you move into more advanced studies and licensing, you can set specifications based on not only what is considered safe and effective, but also what is considered achievable levels of consistency so that if something unusual happens, you have an indicator and you -- so I'm not sure we really at this point are asking or need to be discussing whether where to set the limits or what to allow or whatever. The question really was I think the one that Dr. Sausville answered pretty succinctly, that agarose is cheap and we should be testing it and accumulating the data, if that's, in fact, the sense of the Committee.

DR. SALOMON: I think that's fine. Again, I was just making sure that we were comfortable. To request tests that make no sense also is onerous and part of my feeling, the job of this Committee is not to support that sort of thing either. So that's kind of why I was questioning is this something you are going to make a decision on or are we just archiving it. I'm okay with that.

Please identify yourself.

MS. CHRISTENSEN. Yes, Janet Rose Christensen with Targeted Genetics Corporation. I think in response to the question that you asked about what are people doing, I think this really dovetails back to what we heard from Mary Malarkey earlier. Specifically, that yeah, you can get a band, you can do the ethidium bromide staining, whatever, but again the controls and the amount of characterization, the understanding of what your assay is telling you and as Dr. Siegel just pointed out about as you move through production and developing those controls I think is a very common type of approach we take in the industry and I think that that's a very reasonable thing as we gain more experience, that I would expect that we should be able to quantify and characterize what we should be seeing and as part of that you're going to have to understand the sensitivities of your assays and it gets back to assay qualification which again, I think, is an important attribute of these types of assays, even very early on. So when we get a question from the Agency specifically well, that's a nice looking band, what is it, and does it have any relevance, we can answer those questions.

I think what you're hearing, certainly from my perspective is taking the approach of Dr.

Siegel and what the FDA has advised us is a very use and I think it really supports the ultimate issues of product safety, product consistency and patient safety.

DR. SALOMON: That's exactly the kind of feedback that I think I'd like to hear more of from the audience as again, as you see appropriate to back up some of the manufacturing experience that we don't have sitting up at the table.

Yes?

MS. SEAVER: Sally Seaver, Seaver Associates and I consult on CMC issues and I would like to back something up and really ask the Panel if they want to do this and that is if you wanted to complete sequencing, is your sequencing -- I know you all do it in academia, but can you validate that method and are you doing it under full GMPs? I think that goes back to Mary Malarkey's talk and I would like to remind you that we do not do full sequencing of the amino assays on every protein lot, even in Phase -- even in clinical trials of our recombinant proteins and clearly a change in amino acid could affect amino genecity.

DR. SALOMON: Thank you. Good comment. Do you want to comment on that, Dr. Roessler?

DR. ROESSLER: Well, once again, a main reason that I said I saw a role for sequencing and I'm not sure that I would advocate complete sequencing along the lines that Dr. Mulligan articulated, was for the quality control aspect that's a necessary part of our role in the NGVL programs, specifically when you sent plasmid material to a sponsor and they do a functional assay and that functional assay fails, then you have to problem solve and having that sequence data allows you the opportunity to get some insights into what the problems may have been. And once again, I think that there's always value in doing whatever you can at some level within the cost-effective constraints to try and verify the identity of the material that you're using to manufacture your final product.

So I think that my perspective may be a little bit different than your perspective.

DR. SALOMON: I think appropriately so, which is good.

The next question would be on concentration. I mean that's kind of a no brainer unless I'm missing something.

Activity in gene expression. And I see this one as sort of now segueing with the next

question and that is should tests be added for potency. Can we have some consideration for a second about activity versus potency assays?

DR. SAUSVILLE: It gets back to this question, do you mean this in the case of ultimate use such as Dr. Champlin brought up, or do you mean in the expected performance in a model system as a description of the product? I clearly would be in favor of the latter. I would not be in favor of the former.

DR. SALOMON: So you're saying that if there was an animal model that was used in the process of your pre-clinical and now you were a -- a year or so later you were doing your clinical study, if you demonstrated potency, it should be demonstrated in the animal model?

DR. SAUSVILLE: Animals, in vitro cells, whatever. I think that would be part of the complete description of the product package and in that sense be in the spirit of potency.

DR. EPSTEIN: There wouldn't necessarily be an animal model if here, the intended function of this plasmid is to transvect 293 cells, for example. So I think we have to back off yet another layer beyond what you're talking about. This is not for

patient use and the intended function is in vitro and is simply for transvection. So I think we should simply the whole thing greatly and look for activity ability to transvect those cells and express what it's supposed to. That can be by flow cytometry or enzymatic activity and it should be a very simple test.

DR. SAUSVILLE: I didn't mean to imply that an animal should be used. I mean one could imagine situations where they might be, but as you say, you want to imagine situations also where that's not necessary.

DR. CHAMPLIN: It seems as if this is a product-specific issue. Certainly, some things where there's a readily detectable functional assay you should probably do it. If you're going to give T-cells that are going to kill a liver tumor that's unique in that patient, there's no readily apparent ex vivo assay that could show that you're going to kill that patient's tumor.

DR. EPSTEIN: That's probably the wrong topic. We're talking about the plasmid intermediate being qualified for use. We're not talking about taking the T-cells -- the potency assay for those T-cells might be an animal tumor model. I want to

back off that we're only talking about showing that this plasmid is good enough to use and --

DR. CHAMPLIN: Let's go back. When I asked you to clarify this, the idea was that activity would be, let's say expression of a gene in the targeted cell and that potency was some sort of cellular assay.

DR. EPSTEIN: Oh, I'm very sorry then. I was trying to define potency and get you to understand the concept. For a cellular preparation, potency is the kind of thing I alluded to. But here, we're talking about an intermediate and even the ultimate potency assay, if there were one, would be a very quantitative, very validated version of in vitro transvection because that's all this material is intended to do. I'm sorry, I thought that the definition of potency was unclear. And the easiest cases to give a definition where the product has to serve its biological function for therapy, but here, the real question before us is whether to do an assay with controls showing that this transvection works, or whether to really do a quantitative and validated version of that, a fancy assay requiring showing that the T-cells now have acquired a receptor that mediates lyses in vitro, for examples. Or, can you just show

that the plasmid went in and the T-cells now have a marker on their surface?

DR. CHAMPLIN: Presumably we have shown this for the parent gene and your question is do you have to do this lot-to-lot reassessment, can you kill the tumor in nude mice or what have you and I would think that would not necessarily need to be done if you've shown that you've got expression of the protein of interest and that that -- you had met all of the other criteria that we have discussed.

DR. SALOMON: I guess my concern here, just trying to keep the conversation going to all the different levels it could go, I'm not certain of that, in the sense that we keep repeating the obvious fact that this is a very new field and that there are a lot of rules that begin with manufacture and go all the way to administration that are far from clear. And if that's true, I'm not so certain that I'm comfortable not having early on maybe more than later in terms of potency, so if there are -- if I'm giving it to T-cells and I'm going to take those T-cells and put them into the patient and put the patient through all the different things that I might do including radiation therapy and all that, based on these great plasmid transduced T-cells I'm giving them, then yeah,

dammit, I think they probably ought to have some sort of assay lot-to-lot before I go to the bedside, particularly when everything is so new that we really don't have all the rules in place. So it's -- I actually disagree with you with on that one.

Amy, I know you had a comment and then Richard.

DR. PATTERSON: I think it might be helpful to parse these concepts out because in the dialogue they seem to be intermingled.

Suzanne, you were first asking about or one of the things you were asking about is efficiency of transvection and then someone asked about duration of gene expression, which genes are being expressed and I think those are fundamentally different concepts and they're lumped together here. I think it might be helpful for the Committee to consider them separately. Transvection efficiency, gene expression, is the gene expressed, to what extent and what's the duration?

DR. EPSTEIN: And then the one that people are raising now is you're trying to show that the product of the expressed gene functions as it should and how close does that functional assay have to be to what you're concerned about or can it be a marker assay?

DR. SALOMON: Well, I think -- yes, and I think there that's got -- I don't think we can give you guidelines for that. That, I think is clearly product specific, but if I have a product that well, taking Dick's example, it's going to kill liver tumors, I mean there's got to have been a human model, I mean a mouse model probably for it. I'm hoping, obviously, I think we ought to stop short of insisting on nonhuman primate model for the testing. And so there would be let's say a skid mouse model. I'm just making that up, but there would probably be a model like that and if you did have a model like that, then it probably wouldn't be unreasonable to test that until we become more confident that these sorts of things work.

DR. SAUSVILLE: Yes, but I guess my position would be that although in the development of this product, such a model would have likely been used to gather confidence that things were -- this was a good thing to do. I would be wary about establishing the response of a mouse model as a qualifying issue in the manufacturer's subsequent lots because at least our experience with mouse models is if they range from the health of the mice, hepatitis, I mean, so this gets into very problematic sorts of issues that could

make things very expensive.

Certainly if there was the type of information collected and the expected duration of expression of the gene, the normative properties of the gene product, again, as we emphasized earlier in the morning, much of this preclinical testing and quality sorts of issues are to help explain the behavior ultimately in the clinic and as long as it's available so that there were a problem or lack of expected performance, you could go back and maybe address these issues. It's fine. Bottom line is I don't think requiring animal model behavior is a good thing.

DR. SALOMON: And as I pointed out, what I'm trying to do is push the conversation as far as we can go to try and make sure we get a clear idea of the Committee, of what you want to do. I would also agree that every lot having to be done in an animal model would be onerous and that would be fine. However, I would say though in terms of the pendulum that if it was a simple cellular assay that that would probably be readily doable and so when those kinds of assays were available, a couple day assay of killing or something in a model, then I think it wouldn't be unreasonable to do that at this early point.

DR. SIEGEL: I would note that there's not just an issue of being onerous here, but I think as Dr. Sausville correctly pointed out, potency assays are not dichotomous. They're quantitative assays and a quantitative in vivo assay, you know, you're lucky if you can get within a log or so, base 10 of estimate of accurate estimate. They are just not very useful and I think it is to be urged of all manufacturers and sponsors to be looking for relevant potency assays that can be done in a more reproducible way such as cellular assays.

I'm a bit concerned about time. I wonder if we might want to move off this question.

DR. SALOMON: We're almost done with this one.

Dr. Mulligan, do you have a --

DR. MULLIGAN: I was just going to say I think Amy's point about the transvection piece of this is that any assay of biological potency where you're trying to look at, I thought the DNA's potency is going to be totally variable based on DNA transvection efficiency. It's like an in vivo assay. So I would go very easy on biological assessments. If what we're talking about which is what I think is is the DNA that's been now sequenced a number of times shown to

be the right thing, does it have inherent biological activity it ought to have? I think there's almost nothing you really have to do on that front. Because if you try to transvect it and look for how well it performs, if you did five transvections, you'll get a variation of probably 30 percent and will that convince you that you'll have less potent DNA?

DR. SALOMON: I don't think that would convince me I had less potent DNA, no. But if I was going to do a clinical trial with this reagent in any shape or form, I would like to know if that would be fine to know that there was going to be a 30 or 40 percent difference in the product that I eventually put in the patient.

DR. MULLIGAN: I'm just trying to, maybe in the spirit of moving along, say that's another issue. That's not the DNA issue. That's the transvection issue. You know, you have to show that the procedure that you're going to use with the DNA is reversible, but that's different than the DNA issue.

DR. SALOMON: Yes, I understand that though. I have to say to this particular issue every time we've tried to clarify it, I get less clear where we're stopping it, because we start talking about potency assays and then we are talking about -- yeah,

if you just want to talk about the plasmid DNA, then we're done.

DR. EPSTEIN: We're not talking about the final ex vivo cells or the AAV or retrovirus. They have their own potency assays. They will fail their lot release criteria if this plasmid doesn't do its job. What we're trying to do is avoid tremendous rates of failure.

DR. SALOMON: All I'm saying is in the lot of clinical uses of the plasmid, it's the plasmid into a cell and that's your product.

DR. EPSTEIN: Right, and we're talking about qualifying the plasmid, but the cell is subject to assay before it goes into the patient, including potency assay when there's a correlative of what it does.

DR. SALOMON: Fine. So you got some free discussion on that.

DR. EPSTEIN: Right, that was not the question being raised. But thank you.

DR. SALOMON: Then I think we're done. The last one is full plasmid sequencing and homology analysis appropriate as a one time characterization test and such analysis could use plasmid from the master cell. I think we've covered that adequately.

The other thing we did when we presented this kind of as a discussion before, we all agreed that time would be -- that this would be a real killer to keep all of this on time and we're seeing that this is very difficult to keep this all on time.

So the question here would be that I'd like some feedback right now. Obviously, lunch -- we'd be right on time for lunch.

(Laughter.)

We haven't done the presentations on adenovirus. So Phil, do you want to comment?

DR. NOGUCHI: Yes. What we would like to do is we certainly would like to have Dr. Chanock present his extensive knowledge about the adverse affects of replication competent adenovirus and Dr. Bauer has agreed that I could condense his to a very short, just statement that his talk would lead into it. Dr. Bauer will actually update us at our next Advisory Committee meeting because it will be at that time even more information on this collaborative effort between academia, industry and the government in terms of producing a standard for vectors. But Dr. Chanock's talk is actually could be done in the afternoon as part of the clinical and compliance issues because it's very relevant to the clinical

situation.

DR. SALOMON: So would a good plan be to break for lunch now and begin the afternoon session with Dr. Chanock's talk?

DR. NOGUCHI: Yes.

DR. SALOMON: Okay. Then I want to see us all back at 1:15. Thank you.

(Whereupon, at 12:39 p.m., the meeting was recessed, to reconvene at 1:15 p.m., Thursday, April 5, 2001.)

AFTERNOON SESSION

1:35 P.M.

DR. SALOMON: Sit down and we can get start with the afternoon session.

(Pause.)

Can we have everybody sit down, please, so we can get started? We've got a pretty busy afternoon here and I appreciate the Panel already sitting down and all that. That's great.

Okay, so this afternoon we're going to pick up what we interrupted for lunch with some slight modifications that I will explain in a second, but essentially going on to some, the clinical issues in adenoviral infection and to start that I'd like to introduce Steve Bauer who is going to make some comments to put this into context.

DR. BAUER: I just had a few short quick comments about adenovirus and what we learned from the March 6th letter response and some of our changes and the first one is we are going to recommend from this point forward that the ratio of virus particles to infectious unit be less than 30 virus particles per infectious unit for adenovirus lots and I know there's been a lot of discussion of this at various forums in recent times so I wanted to make that announcement.

The other is that our recommendation for RCA which had been based on a radio with infectious units, we're now going to change to a specification of less than one RCA in 3 times 10^{10} viral particles and that's in response to a lot of discussions about assays and their precision and accuracy.

And then finally, I wanted to just set the stage for our next speaker, Dr. Stephen Chanock who has agreed to come and talk to us. The specification that I just mentioned, less than 1 RCA and 3 times 10^{10} virus particles is going to be used for clinical lots, at least currently, regardless of the clinical indication. And the appropriateness of that stance is what, I think, the next speaker will address.

The background issue is are there clinical indications for which this recommendation might be too stringent and/or are there clinical indications for which that might not be stringent enough. So without further ado, I'll turn the floor over.

DR. CHANOCK: Thank you. I thank Stephen and Phil for inviting me to speak this afternoon. When I was first invited I wasn't quite sure what I was going to do and coming this morning I wondered whether I was supposed to be the comic relief before lunch, but then they moved to after lunch, so now

hopefully while everyone is digesting I'll be able to provide a little bit of comic relief, but more importantly, the clinical questions and particularly the clinical issues that come up with an issue that we were discussing earlier about lots, what is the consequence about what we would consider in a very practical terms of lot failure, in other words, too much of an infectious load being challenged or being infused into an individual who is clearly at risk.

So in order to do that, what I'd like to do is talk about several issues. I'll take the first slide, please. And we'll start with this slide. And there are a couple of very broad, important points that I think are very important to the comments that Steve just made in terms of really trying to set a bar, per se, and having it be more specifically addressing the actual host who is going to be receiving the gene therapy product and that really comes to the critical point is the host immune function is really what's crucial about adenoviral infection and I'll take us through adenoviral infections in normal individuals as well as individuals with immuno-compromised systems such as bone marrow transplant recipients, patients with HIV infection and then primary immunodeficiencies. So

there's a very important issue that I'd want to jump right into as just a primary point and that relates to primary infection and we know that there are many different serotypes of adenovirus and immunity appears to be specific to the different serotypes of which they're well into the 40s now. And we know this is very important because this really provides for recurrent risk for exposure to different adenoviral infections. Now the question is what specific serotypes are being used as the backbone for the generation of the specific gene therapy vehicles. I think that's a very important question and I know that there's been a big debate in the literature about going beyond using serotype 2 and 5 for reasons related to immunogenicity.

The clinical side, particularly, the immuno compromised hosts really in adults it's much more important to think about reactivation because in adults it's much rarer that a primary infection is taking place, whereas in a child, particularly a younger child, primary infection is taking place, so I think there's a pediatric/adult dichotomy that we need to factor into this as well in thinking about who are the hosts and who is potentially at risk per se.

And then that reactivation is really

determined by the underlying events, in other words, alterations are changes in immune function, not only from the point at which you start, but then what happens during the course of that such as in a bone marrow transplantation, particularly an Allogen A transplantation with HLA mismatch, T-cell depleted cells. That's a very high risk for having reactivation of adenoviral infection as opposed to someone who may have an autologous transplant with no manipulation whatsoever of their marrow. Those risks have very significant implications with respect to the likelihood of developing infection, plus the question of co-infection and the ever present of which I think there is very strong data to really argue against, but I at least want to bring it up, the question of the oncogenic potential based upon the animal models and the information that's seen in other systems, but not in humans, per se, for oncogenic adenoviral infection.

I might just add at this point that I really have not been convinced or seen anything in the literature to suggest that chronic or persistent adenoviral infection has clearly been linked to any known human cancers at this time and if anyone can come forward in making that point I would very much like to see that data. I think this question has been

studied for an extended period of time.

Next slide, please. So really the adenoviral serology is really based on a number of standard references of sera that have been generated with the primary epitope being the capsid protein, one that's not necessarily going to be eliminated in the standard approaches right now towards generating adenoviral vectors and particularly it's a different question when we talk about adeno associated virus, reflects the heterogeneity of the adenovirus genome which we know has the ability to evolve and when we look at the sequencing now that a number of the different serotypes have been sequenced, have been classified into these so-called DNA homology groups. And this is very important when you think about the question of recombination and there are these anecdotal cases of recombination between serotypes that are of the same VNA homology group. For instance, there's a well known reported case in an HIV individual that had serotype 7 in 37, apparently have a recombination in vivo and I think that's a very important question to consider.

And then also, we know that the serotypes are associated with pretty specific clinical manifestations and we'll review some of those.

Next slide, please. So just taking the large DNA groupings, we think of the A subgroup as really being associated with common upper respiratory infections that affect us all, that we certainly see in military recruits or in prisons or adult populations that are in very close proximity as well as in the pediatric setting. We know that a large percentage of children who come in with apparent strep throat turn out not to have strep throat and adenovirus certainly is an important contributor and plays and important part in that.

Now the B subgroup is very important, particularly in the immunocompromised population. We know that in, for instance, particularly the allogen A transplant recipients, hemorrhagic cystitis can be an extremely disabling and actually a very dangerous long-term complication because of the propensity to have continual bleeding and hemorrhaging and an inability to really stop that.

Similarly, the respiratory tract with pneumonia. We know that there are endemic URIs in the tonsillopharyngitis, certainly with the subgroup C and then the very epidemic keratoconjunctivitis and I want to just pause there for a second and talk about what we know in terms of the transmission of adenovirus.

We usually think of as a relatively stable virus that can be transmitted from individual to individual by droplets, by cough or by touch and certainly we know with the D group that we certainly see the keratoconjunctivitis or so called, associated with swimming pool outbreaks where one person can go in a pool and everyone else who gets in that pool for the next or two is certainly at risk for developing that infection and certainly those in the audience who have children and have gone to a pool or you've had that and then three or four days later are beset with that, certainly understand that.

The conjunctivitis and the pharyngoconjuctival fevers are also very important, particularly the pediatric setting. And the gastroenteritis is really a much more complicated story. When the adenovirus was first described people thought that it was an important cause of diarrheal events, particularly in young children and I think that that's waned as we've gotten better at identifying other pathogens. It appears to be sort of dropping on the list of pathogens associated with clinically significant diarrhea, particularly in younger children. But when you look at the immunocompromised hosts, i.e., the transplant

recipients, or HIV population, that's where the gastroenteritis, the F pops back up and we certainly know that in HIV there are a number of studies of individuals with HIV infection who have chronic gastroenteritis in which the Fs are isolated and are presumed to be responsible, or at least partly responsible.

Next slide, please. So how do we detect that in a virus clinically? We take material and we inoculate into cell lines and we look by a number of different effects and what many laboratories use now are fluorescent antibody staining per se, but we also use direct tissue detection and this is particularly important in the immunocompromised host where you're addressing questions of either pneumonia of hepatitis in an individual who is getting sick very quickly with what we would describe as disseminated disease. And we either do an in situ hybridization, Southern blot analysis or PCR. It's not that easy, necessarily, to make the diagnosis of an adenoviral infection per se. You have to look for it and you have to have a diagnostic virology laboratory that's thinking about it and not every laboratory clearly is and I would say that that's a point we may want to come back to in terms of linking where and who is going to be

following up and looking specifically at specimens and individuals who may be receiving these base products.

Next slide, please. We know in the healthy child, roughly 80 to 90 percent of children have antibodies to 1 or more serotypes between ages 1 and 5. Serotypes 2 and 5 which are the backbones that have been used for a number of the vectors that have been commercially and/or academically advanced at this point, 2 and 5 fall into that 80 to 85 percent, so we know it's a very common adenovirus out in the general community, causing up respiratory infections and many, many children develop antibodies to it.

We know that mild illnesses generally last less than 10 days, usually on the order of 3 or 4 days. There's a latency in lymphoid tissue as well as adrenal tissue and an interesting thing that's been published, sort of buried in the review about two years ago was when they looked at a number of individuals and saw this serologic profile and then went back and looked at the actual lymphocytes isolated from a small subset of individuals. About 75 percent of individuals who were serologically positive were positive by PCR and their lymphocytes, but not symptomatic at the time. So in other words, there's this idea that adenovirus once infected can certainly

be maintained in the lymphocyte population as well as we know that important targets are respiratory epithelial, particularly for regeneration, for making new viruses as well as for infection. And then certainly, the renal and particularly the bladder epithelial are other very interesting targets.

Next slide, please. So we know that adenoviral infection, particularly in the normal host, particularly in children, sort of here we look at the decreasing frequency of pharyngitis, a very, very common cause of pharyngitis and similarly conjunctivitis. Gastroenteritis should probably be down about the equivalent of pneumonia and from some of the early 1960s and 1970s studies of these large sort of sweeping prevalence studies of children with pneumonia through the United States, pneumonia represented between 5 and 10 percent causes associated with adenoviral infection.

Next slide, please. So really, we know that there are sort of calculable attack rates in the general population and we now have these numbers that have been published and have been verified and confirmed by other groups, not only in the United States, but certainly in Western Europe this has been looked at and it's roughly about 40 per 100 person

years below the year age 1 where you know that somebody is infected and that there is an ascribable clinical manifestation. Now whether there's actually a culture that links that is a whole other question and as individuals get older you see a decrease in the infection rate, but we still see that it's substantial, roughly 14 in 100 per years for people above age 10. And we know that acute diseases are the most important thing, particularly in the upper respiratory tract. Roughly 5 percent of URIs across the population, 8 percent of childhood pneumonias and adult pneumonias probably a little bit less than that.

Why don't we go on to the next slide? So now I want to, having taken that sort of background, I wanted to take a step back and really think about the immunocompromise population where we know that many, many people have been exposed to adenovirus, the question is is there a difference between adenovirus infection and adenovirus disease when we now launch into looking at, for instance, the reported experience in bone marrow transplant, in HIV-infected individuals and primary immunodeficiencies. And indeed, there is. I think it's important to just pause and use this definition as we look at the literature so that we understand two questions. One is who actually has

reactivation or at least the ability to be able to see and isolate that they have adenovirus from a sterile site which is a palpable risk in a palpable number that can be generated in any number of studies and then out of that a subset, generally on the order of anywhere from 20 to 40 percent of individuals who will actually what we consider to be clinically significant disease, in other words, there are clinical symptoms that are linked to the isolation of the particular pathogen at that time. So if you want to turn this around, in other words, there's a good percentage of circumstances where we may find adenovirus is isolated from urine or from sputum or from the gastrointestinal tract, but yet it doesn't link very closely with a clinical event that's taking place. So there is this disjointed nature that I think we have to take into account when we look at these numbers and are trying to calculate what would be estimable risk that we would use or apply in trying to come up with particular guidelines at this time.

Next slide, please. So what are the clinical syndromes that are associated with adenoviral infection, I just want to give them sort of generically and then we'll start to look at what the literature really tells us at this time. Well, the

disseminating disease is really defined as having two or more of the following and this is one that we worry about. This has mortality rates of anywhere from 30 to 80 percent, depending upon the host. In other words, what's going on in the patient, what's the status of their immune system, are they in that terrible ablated stage, 15, 20 days post-allogenic transplant? Do they have a primary immunodeficiency? That's very different from an individual who may be just minimally immuno suppressed. In other words, they may have just a perturbation of one part of their immune system, but not a complete loss. Pneumonia certainly is a clinical syndrome that we worry a lot about as is the fulminant hepatitis and pancreatitis. Colitis and gastroenteritis certainly in the transplant population. As I mentioned before, the hemorrhagic cystitis and I just put up here for the sake of completeness, the encephalitis, but this is exceedingly rare in this population and I don't think this is something we should really concentrate on. These are rare case reports and I think that there are some issues about whether that should really be applied in any given model.

Next slide, please. We know that the distinct serotypes have been associated or presumably

cause disease in the immunocompromised hosts. Serotypes 5, 11, 34 and 35 and I just underscore the importance of 5 showing up there have clearly been associated with infection and immunocompromised adults and if we just look at, for instance, a series of 46 patients with Adeno 35, I mean this is sort of looking the other direction. Clearly, a number of HIV infected individuals have problems with 35 and 35 is strongly linked to the hemorrhagic cystitis problem. Bone marrow transplant and renal transplant as well as severe combined immuno deficiency and then a few individuals who are otherwise healthy, although that's always a very difficult question of what's going to happen, what diseases are they evolving and at that time we have not characterized per se.

Next slide, please. What lessons have we learned from the patients with primary or secondary immunodeficiencies? We know that there are sporadic neonatal adenoviral pneumonia which can be very severe and they are very localized outbreaks with a fairly high case fatality rate in newborn nurseries. We see less and less of that now that we're better and better at being able to recognize and cohort neonates in the NQ. SCID population, in other words, patients with severe combined immunodeficiencies and the absence of

B and T cells, the patients who are at significant risk in that circumstance. There's very high morbidity, mortality. Small sets of case reports of either hepatitis and pneumonia with extremely high fatality rates, 80 percent or greater. The DiGeorge syndrome-case reports of fatal hepatic necrosis and then certainly now we move into the solid organ transplant where we know both the infection of the transplanted organ as well as reactivation in the donor and these kinds of cases and reports have clearly increased over the last 5 to 6 years in the literature and if you just try and look at those very carefully, part of it, I think is the reporting bias of people beginning to catch on and look for this, but there's no question that there is a clear morbidity and some mortality associated with particularly in solid organ transplant, individuals who are receiving particularly severe immuno suppression.

And then the HIV population has been a very interesting population for a number of reasons. We've been able to identify new serotypes from the HIV population and then the ever-present co-infection with other pathogens which is a very important question in adenoviral, particularly with pneumonia that there may be other pathogens that may have kicked off a

pneumonitis or a type of infection in the lung that then has the adenovirus reactivation.

Next slide, please. So let's just concentrate on bone marrow transplant. If you look at the different published studies, the mortality is anywhere from roughly 20 to 60 percent with risk factors being the very young and the older patients who are at greater risk for poorer outcome. Graft versus host disease is a very important risk factor for reactivation of adenoviral infection, particularly the acute GVH, but it certainly can be seen in the chronic and then the conditioning with T-cell depletion and in a particular, HLA mismatching. I think they're very important things.

Now the risk for adverse outcomes, we know that individuals who have multiple sites, those patients who have disseminated infection, as I put that list 3 or 4 slides ago of two or more sites. Those individuals are the highest risk for a poor outcome. We know that serotypes, for instance, 2, 5, 7 and 9 are particularly important for pulmonary disease in the younger patients and 11, 34 and 35 in the hemorrhagic cystitis. And then this ever-recurring question of co-infection with opportunistic infections.

Next slide, please. Now when we actually look at some of the studies, for instance, we go to the Flomenberg study, adenoviral infection occurred in 21 percent of 200 patients who were undergoing bone marrow transplant. And of that, 6.5 percent overall, or in other words, one third developed clinically significant disease, so just taking that paradigm that I was trying to convey in that slide a little bit earlier, we know that from that study, particularly, that the isolation of the virus for multiple sites and the presence of GVH were very important risk factors, as well as infection appeared more common in children and this comes back to a point that I made with the very first slide and that is with children who may be immunologically naive, this very important question of primary infection I think is all the more pressing because of the clinical implications in an immunocompromised child are quite literal, quite significant. The time of onset in children, we knew that these things come on much sooner, whereas in adults you see them over a period of time. Again, this may have something to do with reactivation versus primary infection.

Next slide, please. From the big Mirza study of 1300 adults, in that situation they looked at

specifically adenoviral disease; 6 percent versus 1 percent in the autologous setting. They did not find that GVH was a risk factor. They had a lower case fatality rate than in some of the other studies, but again, I think it was very important. If you look at the Shields study going back even further, about 5 percent and what I think the message here is, even though it may have been lower numbers earlier on, we're getting more aggressive as a community with respect to bone marrow transplant, longer, more intensive therapies that put patients at greater risk and probably more commonly used in the last 10 years than they were the 10 years before or the 10 years before that. So I would use that as one way of understanding that and not to say that we've gotten worse, per se in treating, just that we are better at creating the circumstances where somebody is at greater risk for developing that infection as we've pushed the envelope of immunosuppression per se.

And then certainly the Blanke study of 13.5 percent among T-cell-depleted allogenic bone marrow transplants. There again, a mortality of roughly 50 percent GVH and co-infection were not contributory in that study. So there are certain current, such as GVH, which show up in some studies

and don't show up in other studies and I think that we have to sort of factors those into what specifically is being done in those particular transplant populations.

Next slide. So when we look specifically at children, I think this is very important that the adenoviral disease is about 18 percent in children which is a higher number than we've really seen in adults, particularly high in individuals who have significant GVH, but also individuals without GVH so I think in the pediatric setting it's still more of an open question of the importance of GVH. We saw a lot of adeno-12 in this particular study which is uncommon in the normal host and the most important thing that the authors of this study really suggested was preconditioning, but I again point to the fact that this was done 12 or 13 years ago.

Next slide. And then now we look at a retrospective study looking in the last decade and we see that -- I'm sorry, that's a mistake. That should be 6 percent adenoviral disease, not infection, in the pediatric population. I just noticed that. In that setting, it was really restricted to mainly patients with hematologic malignancies which raises this other question, what's the underlying condition that the

child or the adult is receiving the transplant for? Is that a contributing factor. And certainly having an underlying suppression or loss of a immune function is very, very important. And as we looked here, down at the bottom, the type of graphs seen at the mismatched or matched unrelated donors, appeared to have a higher likelihood than the HLA-match and the Autograft. Fits with the model that we'd seen before. But as you can see, the numbers are floating about 3, 5, 10 percent at most of individuals in a cohort of transplant recipients which develop significant adenoviral disease and I think that's a number that continues to be relatively consistent.

Next slide, please. And then the question is where is this disease? Well, when we look at this Hale study, the hemorrhagic cystitis was really a very significant problem. We know that 7 of the 13 individuals died, but only one of them clearly died as a result of the adenoviral infection that was associated with significant hemorrhage and other complications.

Can we really implicate other risk factors such as total body irradiation or type of graft? Certainly by different kinds of statistical manipulations these things are brought up, but again,

I think that's not the purpose of the discussion today, but clearly these are underscoring the importance of other events that are taking place in these populations.

Next slide, please. So the hemorrhagic cystitis is certainly something, I think, that we all have to pause and think about because we know that there a small cadre of otherwise healthy children who develop hemorrhagic cystitis with adenoviral infection and it can be a chronic debilitating problem. But when you then put in the circumstance of a bone marrow transplant and an underlying disease, it is a particularly difficult disease and entity to treat and this is one that I think we really have to watch very closely. At the same time, we're able to actually monitor by looking at urine samples and specifically culturing urine samples for adenovirus.

Next slide, please. So really what do we take from this transplant literature? Well, for the older individuals we really look at this question of reactivation and I think most people would agree that it's reactivation in those populations who have a defect or a set of defects that have been introduced in the adult population.

In children, there clearly is this risk

for primary infection and you still have the possibility of reactivation in children and I think that that's something that still has to be underscored, but the younger the child, the greater the risk for a primary infection per se. We know that case reports of primary infection can be devastating in particularly young infants and we may really, as we begin to bring bone marrow transplant into the infant population, children under two years of age expect to see increases and talking with some of the pediatric transplant centers and being at some of their meetings in the last year, there clearly are sort of anecdotal references to this, but no one has put this together in a large enough series, anyone who would have the gumption to get up and show in front of an advisory panel per se, but that's something that we need to be concerned about and over the next year the Panel should particularly have a close eye on that.

Next slide, please. So in the adenoviral setting and particularly in the bone narrow transplant population, we know that the primary infections are things that we worry about, particularly in the younger children. Reinfection is clearly another issue that I've just barely touched upon, but nosocomial transmission is clearly a very important

question and the question is who is susceptible to that reinfection or exposure to a serotype that they may have had, immunologic response to and have lost it due to their underlying either disease or therapy and then certainly reactivation which we know is very important.

Next slide. Treatment, unfortunately, at this time is still relatively limited. We don't have good antivirals that are clinically in hand that have progressed at least to a Phase III or to licensure in the United States at this time. Ribaviris and Ganciclovir have each been used. They are rare anecdotal cases of successes, but I think the overwhelming experience is that these are not primarily successful therapies for adenoviral infection and in particular adenoviral disease. Intravirus IGG has certainly been used and there are again anecdotal cases, but I would -- it's safe to say that our treatment options at this time are extremely limited, so an immunocompromised host who develops this significant infection with adenovirus is really in a very perilous state and much of their, I think the reason for survival or success of getting someone through really has to do with supportive care and really most importantly the reactivation and the --

and really, the reimplementation of their immunologic system being reconstituted.

Next slide, please. Now in HIV, we see enough people in the 1980s predicted that adenovirus would be a very significant pathogen in the HIV population and it really has not been. Other than the chronic diarrhea, it really has not been a significant problem in the HIV population which is something that I think most of us would not necessarily have predicted 15 years ago per se, in just thinking about the transplant and/or cancer paradigms being applicable to HIV. We know that there are a number of individuals who can excrete adenovirus, particularly in their urine and there's this famous case of the question of recombination between 7 and 34 which I think is a very important point that we have to at least be aware that this has been shown in vivo or at least suggested in vivo.

Next slide, please. So really the issues again come back to this same slide for the sake of time. I think I've emphasized them very strongly. The state of the host immune function of the individual and I think when we're thinking about gene therapy protocols, this is very important. It's probably a very different risk in an individual who is

undergoing cancer therapy or in conjunction with chemotherapy as opposed to an individual who may have an underlying congenital disease or primary monogenic disorder in which there is no known defect in the immune system per se, but this is a vehicle to be able to approach neurodegenerative disorders. I think those are two very different poles. The exposure to primary infection, certainly as we get younger becomes more of an issue as well as the reactivation in terms of what other concomitant therapies or changes are potentially taking place as a result of the natural history of the disease or therapies or supportive therapies that are being offered to the individual at that time.

Steroid, corticosteroids is always an issue that's brought up and there's very, very little data to really link corticosteroid usage and the development of adenoviral disease and the reactivation. That's a topic that has not really been addressed and I have never really found anything satisfactory in the literature to be able to address that. But that's certainly something that I think is important for this population.

Next slide. So really the future issues we want to look towards the development of new

antiviral therapies and I know of several different approaches that are going forward, as well as the use of cytotoxic lymphocytes is certainly being addressed in several academic centers right now.

Early detection is very important and then personally, my own laboratory is very interested in this host susceptibility factors, looking at things at the genomic level and asking the question are there certain SNIPs that are going to predispose or protect an individual who is at high risk for developing adenoviral infection, but I think those are still some way away. So I don't think that these, the host susceptibility factors are really available in any meaningful way to apply to any of the things that need to be addressed by this Committee at this time.

Next slide, please. So really, in my mind, there are some very important things I'd like to end on, sort of as points for discussion and thinking about and that has to do with the use of adenoviral vectors and the question of adenoviral infection, whether it's Iatrogenic or whether it's a natural co-infection per se in the gene transfer protocols, really has to do with the response and site of the inoculation because there are certain places we know that the adenovirus replicates particularly in the

respiratory epithelium and we know that pulmonary disease can be a significant problem. And similarly in hepatic cells so the inoculation into each of those places, I think, raise very important questions. The state of the host immune function is very important, both with respect to the changes that are being undergone at that time that are either iatragencic or disease related, but then also this very, very rare case, I think, at least gives us pause to at least consider this question of recombination events and thinking about really what kinds of things could potentially go wrong and there's no real data in the animal literature to really validate this per se with respect to gene therapy reported studies per se, but again, it's a theoretical question that I feel morally obligated to at least toss that out for discussion.

Next slide. So why don't I stop there and see if there are any specific questions and I'm sure we'll have discussion.

DR. NOGUCHI: Dr. Chanock, what is the experience in terms of adeno infection in patients with chronic hepatitis? Does it add to any risk? Is there any literature on that?

DR. CHANOCK: There are a couple of very small studies that suggest that reactivation of

adenoviral infection in the course of chronic hepatitis, whether it's with a known B or C or whether it's chronic active hepatitis without a known pathogen, it may contribute to that, but I would say that that literature is extremely rudimentary and I'm not sure there's a lot we can do to generalize on that, simply because what we're looking at are case reports, basically, where someone sees something and says yes, this may mechanistically make sense, but I don't -- I'm not aware of an extensive literature on that.

DR. SALOMON: One of the things that I'm trying to now put this back into the context of the way it was, we were going to try and present this in the -- so we're not going to talk about the replication competent adenovirus issue because that's going to come up later, not during this session but in a subsequent session, but I think everybody should see first just so that the record is clean for later that this thinking about what the implications of adenoviral infection in different patient groups that you so expertly presented today is very relevant to our thinking about the quantity of replication competent adenovirus that might be contained in a gene therapy trial. But I'd like to take a moment, you do

bring up a couple other issues about adenoviral therapy that might be worth mentioning and the question I had with regards to this reactivation. So I'm used to it as a transplanter with CMV infection and CMV disease and reactivation, so this is just all -- I'm used to this one. This is easy.

But what we're saying here is there are two things that we're interested in in this session. Tomorrow, we're going to talk about long-term follow-up. One of the ways that we tried to think about long-term follow-up an dhow that impacted on regulation was the idea if you had a non-integrating virus, that long-term follow-up was maybe less of an issue than with an integrating virus. However, if this non-integrating DNA, double strength DNA virus, the adenovirus is actually capable of reactivation later, then I have two questions. One is maybe that's not true. Clearly, just integration is not the measure of long-term follow-up and then the question is is the production of adenoviral vectors being done in such a way that they are not taking on latency, that this is unique to the wild type virus. That's one question I had for you. And then the second is closely related and that is, if that's not true and if you have long-term persistence of adeno-DNA and

adenoviral vector exposure, how much possibility is it that every year you get an adenoviral infection and you get recombination finally?

DR. CHANOCK: Let me start with the second one. I think the second is -- they're both excellent questions. The second one I don't think anyone has data at this point to really answer that sufficiently and that's the kind of thing that I would think that as we go forward with these trials that we would need to continue to monitor because I think that is a real concern and a real question.

The issue of using a very immunogenic adenoviral serotype such as 2 or 5 is the backbone which we know generates a very good immune response that presumably is for life in most in vitros unless there's some kind of insult or diminution of their immune function, but that doesn't mean that closely associated by DNA homology groups, adenoviruses may not undergo a recombination event and that HIV case is one that just points that out in my mind as something to think about. Again, I want to emphasize that's occurring in a very particular individual who's at very high risk, who the presumption is at least in our current understanding of adenoviruses is that that person probably has very high titers and was

continually infected per se with adenovirus. And that's a very important question because I'm going to slide into your first question because then it comes back this issue of how do we make sense of this paradigm that's been put forth of adenovirus not being an integrating double stranded DNA, but yet that Columbia data suggesting that IPCR in the lymphocytes of individuals who have been infected, that they may be asymptomatic, but a good percentage of them have adenoviral sequences. That kind of study is, I think, provocative. It hasn't been carried to the point that you would say that all the controls are done to be sure that some portion of the adenoviral genome hasn't been necessarily integrated or hasn't been picked up by some other pathogen, whether it's EBV, any number of other things that we know can go underground, so to speak, in lymphocytes. But I think that's the kind of question that we need to go back to the community and look at much more closely and that people who are interested in this have to at least address that and think about that because the answer really is not available at this time. I think that's the kind of thing that although I recognize that this body is trying to help guide and set guidelines, but at the same time we can also identify questions that we hope

experts who are looking at this in the community would say yes, we can apply our system and analyze the appropriate data or animal system, whatever to be able to address these kinds of questions.

DR. SALOMON: I think my comment to that and we can get some more comment is just exactly that. I think one of the responsibilities, at least that I take on as chair is that I don't want us to be making advice when there isn't the information and the community to make it, so it's exactly what you said.

DR. MULLIGAN: I'm interested in the question of how many virus particles would it take to initiate an infection and I love the swimming pool --

DR. CHANOCK: I thought I was going to get away for voting before that.

DR. MULLIGAN: I love the swimming pool issue and that is what I'd really like to know is every time someone jumps in the swimming pool when you have these outbreaks, what's the probability that they will get a disease from this because the amount of virus particles, I was trying to calculate how many liters a swimming pool was to what the concentration of virus would be to give you a small number.

The suggestion is it's a very small number that's necessary, right?

DR. CHANOCK: Correct. And you know, in the swimming pool the question is how much of it is the water and how much is it the lack of hygiene in the dressing rooms and the close proximity of people who are barely clothed, touching and bumping into each other. I mean you can imagine the hospital epidemiologists are very interested in this kind of thing and it continues, it's a classic board question and things put before people in training. But what I think is also very important is not all adenoviruses are the same in terms of their infectivity, as well as their tropism for both tissue and subsequent development of disease. Two and 5, we know, certainly have been certainly seen in sort of small, endemic respiratory outbreaks in military recruits, prisons, centers where a number of children are kept, for instance. And so there is some information on that. The question is how many actual particles, I don't think anyone really knows that answer. The animal models and particularly the cottontail rabbits that are used for infection, those kinds of systems are helpful, but in terms of being able to actually calculate what's the viral titer of actual replication competent adenoviruses that it takes to engender an infection, that's still very much of an open question.

DR. MULLIGAN: What about a single cough? Is there any experimental work that says how many virus particles are put out from a single cough of an infected person?

DR. CHANOCK: Actually, going back a number of years there were studies of that nature and that's the infectivity of a cough? I would have to go back and look. I know there's very strong information for respiratory, stentitial virus and parainfluenza 1 and 3 which are big problems in the pediatric setting. I'm not sure that those studies have been carried on in adenovirus per se, but I think that information may be available. I just don't have it in my fingertips.

DR. MULLIGAN: I think that the question on this is just if you want to set a certain limit, does the limit always have to be zero, that is, how many -- if you thought that one or a couple of virus particles had a certain measurable frequency of giving -- you should be very, very serious about setting those limits far off.

DR. SALOMON: I was actually laughing when you said that because I like the swimming pool concept myself. I think that the agreement that we sort of had going into this is that there's no question that this is really an important thing to talk about, but

that we didn't think that we had, we really were setting it up for this discussion yet.

So I think I'm going -- unless my -- the FDA tells me no, go for this -- no.

DR. MULLIGAN: I'd just like to ask them what is the topic here?

DR. SALOMON: What we were -- there's two things. That's what I was trying to explain before. I didn't do a good job apparently. Initially, what we wanted to do was get into this sort of new setting of how many replication combinant adeno could contaminate a clinical lot. And it was decided just because of the interest of time and the important of that discussion that we wouldn't get into that right now, that we would make it a separate committee discussion later and I said that, but I guess I didn't make it clear enough.

Nonetheless, I think that we felt very strongly to have -- to go on with the presentation, changing its focus a little bit, in that it's really a beautiful introduction into the afternoon's topic about clinical issues in gene therapy, because it's the way of saying that really, our understanding of the behavior of the wild type pathogens that we've made into vectors and their behavior in different

situations and their biology, like this issue of are there episomal, double-stranded DNA or is it integrated in some. I don't think any of those -- is really the kinds of directions this committee and the whole field need to go into. So I think we'll take it as an introduction and realize we won't get to discuss all of the -- because I think there's a lot of interesting things to talk about here.

DR. CHAMPLIN: Quick comment. I'm impressed that this is really a safe virus. You really have to have a pretty profound immune deficiency to get sick here and even more than half of the bone marrow transplant patients do just fine with this virus and in the absence of profound (The document referred to was marked for immune deficiency, it doesn't cause serious disease. So not to say we shouldn't be concerned about it, but of the spectrum of viruses that one can think about this would seem to be on the safer end of the spectrum because the immune system seems very effective to deal with this particular virus.

DR. SALOMON: Well, remember though again, this is kind of segueing into the discussion, we have the sense that it's a safe virus because we're not seeing a whole lot of sick patients say in our bone

marrow transplant patients, but there's a whole lot of protection between them and people with adenovirus. So we don't really know how many particles are floating by. So if 6.7 percent is of the patients are getting -- is the incidence of getting infected and having disease is actually because one viral particle got sneezed out on the parking lot, floated through the ventilation system and into your transplant patient's bedroom, then it's a damn serious virus in an adenoviral gene therapy trial. I don't think we can answer that question.

DR. CHANOCK: I think there is some information that, in fact, this is not as infectious as for instance measles or chickenpox which, in fact, can have that where someone can be on a ward one floor away and be highly at risk and develop it because somebody coughed and it went through the ventilation system. I think the point is very well take that it's remarkable that this is such a ubiquitous virus and so many people see it and in many ways we're lucky and it's important to recognize that that many immunoviral compromised patients are not coming down with it, the majority are not. But again, what I would want to leave as a very important point is really age, I think is an important thing that we really have to think

about and I realize that's always a difficult issue when you're trying to set up programs, but for the clinical implications, you know, of adenovirus in a very young child, they're probably very different than an adult because someone at age 20 who may be undergoing a gene therapy protocol has seen most or all of the adenoviruses that are going to be used as the background multiple times, over and over and they're probably going to be able to handle those whereas the very young child is a very different question.

DR. SALOMON: I'm ready to go on, except Abbey, I didn't mean to cut you off. Is it okay?

MS. MEYERS: Maybe somebody can answer this later on this afternoon, but they've been using the adenovirus all these years in gene therapy. I remember there was one experiment with cystic fibrosis where there was a very severe reaction. I'm wondering if somebody can tell us what the results are, what were the adverse events in adenovirus experiments and was there any pattern?

DR. SALOMON: Again, I don't think that's exactly where we want to go this afternoon, but I think Dr. Chanock is the world's expert on this, but he can certainly your question briefly, I think.

DR. CHANOCK: I know there have been a couple of instances where individuals have received an adenovector and had an acute pulmonary type of infection where it looked like they had pneumonia or indeed actually had pneumonia and of course that's a risk that you have to face, for instance, in a patient with cystic fibrosis, but I would take the more philosophical step back that I think that for many of the reasons we talked about before, this is a remarkably hardy and very useful vector and I know, you know, we have to be very valued in pushing the envelope and if we don't we're not going to make the next steps because as you know, gene therapy still has a ways to go before it really is a defined and truly successful therapy and I think that those kinds of risk benefit analyses, again, I would fall more on the side of using an adenovirus knowing that we have that risk in certain patients, but as long as they understand and everyone else understands, those are questions we can talk about alter.

DR. SALOMON: Yes, I think we'll have to stop there and realize there's a lot to talk about with adenovirus. That was a good introduction.

I'd like to introduce -- no. I've been reminded by my better two-thirds that I now have by my

obligation to open this up to anyone in the public who would request to speak. This is the open public portion and no one had asked to speak, but I'm still requesting if anyone would like to.

Yes? Just identify yourself.

MS. CHRISTENSEN: I'm Janet Christensen with Targeted Genetics, please excuse my voice. I'm not trying to imitate Lauren Bacall. It's just coming out that way.

I wanted to take a couple of minutes just to address some of the issues that were raised this morning about quality assurance and quality control. I realize it's kind of wedged in here at kind of an awkward time. But I think there were some good questions raised by the committee about complexities of quality control and quality assurance and the issues on investigators and sponsors as they're trying to develop these new technologies.

I've had the pleasure and sometimes I reflect on that, yes, it's been a pleasure, in the last 22 years of being direct, very involved in quality assurance and quality control and I got involved in the entire recombinant DNA process back in the early to mid-1980s. At that time, I would say that the recombinant DNA issues and activities back

then were probably pretty analogous to where a lot of the gene therapy and gene transfer issues are today and even though there's a lot of different types of concerns about documentation and what appear to be very, very onerous issues, it's not reinventing the wheel here.

Back in the 1980s it was well, gee, biotech is different, we don't need to follow GMPs because we're different. Well, at the end of the day the answer is guess what, it's not different. The issues are the same. The documentation systems, the way that companies and investigators can structure their quality program can be an added value to not only the study, but the patient and the product as well. They don't have to be highly complex. My view on this sliding scale for GMPs is that you have GMPs in Phase 1, but they may not be as complex. They may not be as detailed. The compliance issues for quality control and quality assurance in my view help to validate the clinical trial. They help to ensure that the product and the result that you're seeing from the patient, albeit safety or efficacy or whatever, are really founded in science by reducing variables. So I think in viewing, excuse me, in viewing the whole issues about quality control and quality assurance, I

thought Mary did a great job in kind of giving a good framework for that today. But I'd like to assure the committee that there are a tremendous amount of resources available for the industry. I think it's been raised and I think it's a very good point that we somehow need to bring those two together, either through ASGT, meetings like this, the outreach program the FDA is doing to ensure that we can get information to these groups earlier and investigators earlier rather than later, so we can keep maintaining some momentum with the industry.

Thank you.

DR. SALOMON: Well said. Okay, anyone else?

All right, then the two photographers jumped up. I thought my God, they're going to address the audience on --

(Laughter.)

You're more than welcome, too.

(Laughter.)

I'd like to introduce two more people to the table, old friends, Karen and Weiss and Patricia Keegan for the afternoon, and Dr. Salewski, I'm sorry.

DR. WEISS: I was just going to say as Dr. Keegan is walking up to the podium that as you know

we're shifting gears a little bit this afternoon to talk specifically on clinical issues and more specifically to issues on clinical trial conduct, issues that deal with monitoring of the clinical trial, oversight functions of the sponsor of the whole clinical program. The presentations this afternoon will be two. First, Dr. Keegan will continue on with the responses to the March 6, 2000 letter that specifically asked our sponsors to address their monitoring and oversight functions and some specific issues related to the pre-clinical program. Then Mr. Salewski will follow to talk about inspections that were done at various clinical trial sites and after that we can open it up then for some discussion and we have some focus questions for the committee regarding trial conduct. So with that let me introduce Dr. Patricia Keegan who is the Deputy Director of the Clinical Trial Division to discuss the additional responses to the March 6th letter.

DR. KEEGAN: Okay, thank you, Karen. What I'll do is review a little bit of the background and the process and then our review of the responses to the letter and the process that has continued beyond the initial set of responses.

Go to the next slide. In way of

background which is obviously redundant to this committee, but part of the issue with regard to the March 6th letter was the death of the patient participating in a gene therapy protocol which was a highly unexpected event and in reviewing the circumstances surrounding that adverse event there was an inspection conducted of the clinical study site which revealed deficiencies in the conduct of the clinical trial, including failure to adhere to the clinical protocol, failure to report on modifications to that protocol to the appropriate bodies and failure to provide all relevant animal safety data.

Next slide. Based upon the concerns raised by that inspection and the events surrounding that event, FDA determined that there were certain actions which should be undertaken to further assess the scope of this problem and those actions were really two fold with regards to clinical protocols and clinical trial conduct. The first was a series of unannounced inspections of a limited number of randomly selected sites participating in gene therapy studies and Dr. Salewski will review that process.

I will discuss the March 6th letter one of the aspects of which requested information on the clinical trial monitoring program from all IND

sponsors as well as requested confirmation of adherence to reporting of all relevant animal safety data.

Next slide. I'm going to review what was in that letter, in part, because I actually needed to review it several times in looking through the responses and in talking to my reviewers because it's clear that very few people carefully read the contents of the letter and availed themselves of the references cited in the text and therefore the responses really didn't address the question, but the question as originally asked was as follows: that the sponsors provide a two to three page summary of their procedures in place that ensured that the clinical trial conduct was appropriate. In particular, it asks that the summary of procedures that were in place to ensure that there was adequate monitoring of the clinical investigations and to demonstrate that the trials were being conducted in accordance with both the regulatory requirements for the IND regulations, good clinical practices and the written protocol.

Next slide. It further stated that these procedures would be those that would ensure that the monitoring was adequate to demonstrate that the rights and well-being of the human subjects were protected

and that data reporting, including safety data reporting was being made in accordance with those regulations to the IND sponsor to the Investigational Review Board and to the NIH Office of Biotechnology Assessment and that it was complete and accurate.

Further, that the procedures demonstrate that the IND sponsor had adequate oversight over the clinical investigation and in order to address that question we specifically asked for an organization chart which identified the individuals responsible for the oversight of the clinical study and a summary of his or her duties.

And in those instances, where the IND sponsor had transferred some or all of his regulations to another organization, we asked that -- we have a summary of the procedures that demonstrated that there was adequate oversight and for the CRO that there be verification or for the monitoring body that had overtaken or some of these obligations from the IND sponsor, we asked the sponsor themselves to verify that they were aware that the obligations for oversight were being appropriately met and that they were to provide a summary of the CRO's oversight procedures.

A separate item in that letter requested

confirmation that all required animal safety data have been submitted to the IND or if there was some areas of clinical studies which had been of -- of the animal safety studies which had not been appropriately submitted, that they be submitted at this time in response to the letter.

That the animal studies in regard were those which suggested the clinical -- significant clinical -- I'm sorry, that the results from the animal studies that we were requesting confirmation had been submitted were those animal studies that suggested that significant clinical risk might exist and that those studies were required to be reported in writing to the FDA and that all investigators should be aware of their obligations to report such studies within 15 calendar days after initial receipt of such animal studies and that IND annual reports are intended to include a summary of major preclinical findings.

The March 6th letter was sent to 156 individuals who were holders of 276 total IND or master files. The number of letters were less than the number of files because certain individuals held more than one file. The responses to date as of March 8th of 2001, we have had, we have received responses

to 200 INDs. The total number of INDs are smaller than the total number of sponsors and master file holders for certain reasons. In those instances, as I've explained in the briefing document where active studies were on-going and it was no response to receive those INDs have been placed on clinical hold. In other instances, INDs had been previously withdrawn or there had been an error in terms of the relevance of a certain master file to the March 6th letter and so there are some differences in terms of the number of responses.

The response to the letter has been reviewed and comments communicated to 165 IND holders regarding the adequacy of the clinical monitoring program.

Next. For those 165 INDs, we noted that there were really sort of two categories of initial response. There were a number of studies, approximately 30 or 15 percent of the total active INDs at the time that the March 6th letter was sent out where the sponsors replied that they had completed all studies, no further development was planned, no further studies were planned, no patients were in active follow-up and those sponsors chose as their response to the March 6th letter to indicate that they

would either inactivate or withdraw their IND. And generally, I think there are only two exceptions, didn't provide further information on the clinical monitoring program.

I'm sorry, go back one. In the remainder of the INDs where the studies were active and we have reviewed the initial responses, it's clear to us that there was some confusion about what we were asking for because most of the responses really did not contain adequate descriptions. They were deficient in their description of the program. I'm not saying that the programs themselves were deficient, just that they didn't contain enough information to describe the programs.

With regards to those 165 INDs where we've completed all review and made communications, there are 26 INDs where the description of the program has been reviewed and an in some cases has involved review of multiple submissions and resubmissions to the IND and we've determined that the program as described is adequate to fulfill good clinical practices.

There are 139 INDs under which there are 212 protocols which have or are being conducted where the description of the clinical monitoring program is not full or complete. For six of those, the INDs were

actually withdrawn prior to or inactivated prior to that March 6th letter and we don't anticipate receiving additional information on those.

There are 27 INDs where the sponsors asserted that all the clinical studies have been completed, no additional patients continued under follow-up and they have chosen to withdraw or inactivate their INDs and again, we don't really have any information on their programs at this time, but should they choose to reactive the INDs that will be a condition of their reactivation, that they provide complete and full detail on their monitoring programs at that time.

There are 106 INDs which remain active, where there is not complete information, sufficient information to assess the adequacy of the monitoring program and for those 106 INDs all the sponsors have been contacted and provided with a description of the deficiencies. And that gave an example letter in the background materials as to the kinds of information and the level of detail so that it would alleviate the confusion of the initial, more summarized letter.

There were, as of March 1st, 35 INDs that remained under review. Those INDs have been cursorily analyzed, but either the information has not yet been

collated and entered into our database and/or we are in the process of requesting that the sponsor send additional information. So we haven't completely closed them out in terms of the first cycle.

I would also summarize the experience since the March 6th letter in terms of new gene therapy INDs and we have held that the INDs, all gene therapy INDs should contain this information, including those submitted since March 6th. There have been a total of 32 new INDs submitted since March 6th through March 8th of this year. Five were withdrawn prior to initiation, there were 16 active INDs which provided at least some of the information regarding the clinical monitoring program and a few of those we've requested additional information to tie up some areas which need further detail. There were 11 INDs which were placed on clinical hold for failure to -- generally, for multiple reasons, among them failure to provide information about their clinical monitoring program as described in the March 6, 2000 letter and again, those sponsors have received a more detailed letter regarding exactly the type of information we would like to see.

Next. In terms of the initial response to the March 6th letter, the major issue really seems to

be that we -- although we thought we were being clear, we obviously weren't and most people essentially missed the boat on what we were asking for and simply failed to provide an answer to the question being asked. In particular, most individuals failed to describe the procedure for monitoring adherence to the protocol and to GCPs. Most of them failed to describe their auditing procedures, for auditing the primary study information and verifying the accuracy. And interestingly, many -- virtually all of the sponsors, with the exception of some of the industry sponsors, really failed to understand that we did indeed mean that we wanted to see an organizational chart of the individuals who were responsible for this program.

Next slide. What did we get? We did generally get a description of the procedures that investigators use at the time of implementation of the protocol that they hoped would ensure that the protocol ran smoothly. For example, the type of things that we would receive would be the investigator would generate an eligibility checklist and would agree to fill out the checklist prior to entering or registering a patient on to study. That is different from the type of information we expected to see with regards to monitoring which documented that, in fact,