absolute consensus that, prior to initiating a Phase I trial, all of it should be on the table, and I still think there are many aspects of that will be addressed in some of the other questions put forward.

If one says, well, should we not accept sequences that are anomalous or unexpected, one could say that, but of course that discussion will evolve to what level of anomaly or unexpectedness is acceptable. This can go on and on, so that is why I voted nay, to reflect that there was this-- more complexity of opinion expressed.

CHAIRMAN SALOMON: Again, it was gray, I just wanted to make sure everyone understood why you would vote nay. One thing I did not say, and I apologize, is that I do always encourage the audience to participate. This is a public meeting. There is a microphone there, and if you choose to come to the microphone, which I see a gentleman doing, then just identify yourself for the transcription.

I would like to make the same point to our invited speakers, and they should specifically use the microphone there on the left by the podium. Again, I would encourage them to participate, and the only thing is I'm going to try to stay on time.

DR. BYRD: I'm Paris Byrd, from Maxogen in Redwood City, California, and I want to address the issue of sequencing AAV vectors at the production lot level.

To actually do that requires at least one entire production lot. The amount of DNA that is in one of these production lots for AAV would then go for sequencing and you would not have enough DNA to actually do an entire sequence by the standard sequencing methods that are now in use.

The question is would you accept then using it in its plasmid form, which is one step removed from the actual production lot? The size is not really captured for adenovirus, as it would be for AAV, in terms of what is feasible.

CHAIRMAN SALOMON: That is a really good point. I think we should have some discussion of what we are exactly talking about sequencing there, but I think the general intent right now, with this first, question, was just that you had the complete plasmid that you started your experimentation with sequenced, and in a few moments we will get back to talking about should you also have sequenced the master producer line and/or clinical lot. That is a good point. Thank you for bringing that up.

FLOOR QUESTION: In order to answer this question, then, what are we actually sequencing? Will it be the production lot? Will it be intermediates? I'm not quite sure that we have an answer that we would want to live with now, based on this, and one thing that's not captured is are we talking about GLP-validated sequencing or research-grade

sequencing? They are two very different things, and for AAV, we're talking not about $20,000, we're talking in the range of $200,000 to $250,000 to actually dedicate an entire or multiple production lots to actually getting a sequence on the final production lot under GLP.

CHAIRMAN SALOMON: Excellent. Excellent point. Yes?

DR. CHAMBERLAIN: From my own personal point of view, I mean, I think these are good issues and they apply not just to AAV, but to other viruses. What I was voting on, at least in my own opinion, was that the starting material, which in most cases is going to be a plasmid, should be sequenced. I think the question of whether one would also want to sequence a production lot or viral growth, we should come back to on point three.

CHAIRMAN SALOMON: I agree. That is what I assumed we were voting on, and Carolyn, that follows the discussion we had yesterday, that that is what we would be voting on, so this would be the plasmid initially. The idea is that we don't know there is like a gene stuck in the middle that some fellow put in trying to help us along, and got past.

DR. SAUSVILLE: I think that is a key point. I think the spirit of this should be that latitude needs to be potentially available to the FDA, to make the situation

reasonable to each biological situation and to be driven by the science of each vector. The key point is exactly that there is a definable starting point as to where you then evolve the sequence for clinical use.

CHAIRMAN SALOMON: There actually was a question raised there that, I think, might be worth a few minutes of discussion, and that is what are we talking about when we're talking about sequencing. Before we go on to question two, there's a question I had a little bit later in my notes for discussion, but I think it actually is not inappropriate to kick it around for a minute here. I think, as we just heard, there are different kinds of sequencing that we could do, and you could sequence once or sequence 100 clones.

What exactly does the committee think about that? What should we define in general terms to the FDA, sequencing?

DR. MULLIGAN: I would say simply just getting the right sequence, that at this point, as opposed to one of the other questions, it's just that what you bring in to begin whatever process is what you thought it was, and so I think people know how to sequence, and I think the criteria is getting the right sequence, not making any errors.

CHAIRMAN SALOMON: That would be saying that this initial 40 KB sequencing is academic-quality sequencing, where we trust each other. It is reasonable. There isn't a

whole open reading frame that got stuck there and we know if there are some deletions in the starting material.

DR. MULLIGAN: I guess one more point, and it's that I think we keep getting off on what if there are other sequences, and I think we should look at it very philosophically, just knowing what we have in our hands, okay, and we'll address the issues at another point, in terms of if it looks like there are other things we didn't think.

So, it is really having something where you know exactly what it is, and then there is a separate interpretation of whether that is okay.

CHAIRMAN SALOMON: Very good. Agreed. Dr. Anderson?

DR. ANDERSON: Let me just ask our FDA colleagues, is the FDA position that an academic sequencing, assuming that all the data is there and so on, is adequate, or are you going to require GLP-level sequencing?

DR. NOGUCHI: The really correct answer is at all levels, regardless of who does it. We want to make sure the information you supply us is accurate and is controlled and is reproducible. That can be done in academic center or it cannot; it can be done in the pharmaceutical industry or it cannot. It depends on exactly what you submit.

CHAIRMAN SALOMON: I have a series of questions

that follow, sort of what does it practically mean to sequence, I left for later, but I think we will just follow them now.

The second question then is, I think everyone would agree, but if you disagree, that this could be manual or automated sequencing, core lab or contract lab, and if there's any discussion there--it doesn't seem like it. The next question is that you should submit the sequence, and the question now is what does it mean to submit sequence? How should it be submitted?

One of the things that I feel fairly strongly is that sequence should not be submitted as just a run of A's, G's and C's, but rather should be submitted electronically and submitted with comparison to relevant published data banks, and that should be specifically stated. Right now, there is no requirement or even request for electronic submission of sequences.

It would be, I think, valuable if the rest of the committee agrees to make a recommendation to the FDA that at least there be electronic submission of the sequence, not of the IND. It is there any discussion of that? This is just on how to submit sequence to the FDA.

DR. BREAKEFIELD: We were discussing what if you had some contaminated sequence and it was being kind of generated in multiple vectors, kind of the example we saw

with the salmon sperm. It would be nice of these sequences that would then be available to other investigators that are sequencing, you know, that this database would be accessible to some extent.

CHAIRMAN SALOMON: Well, that, I think, we should defer to our FDA colleagues, and a lot of that I don't think could be publicly available, because if a company submits a sequence on their vector, I don't think there is any law that would allow that to be publicly available. Again, I would defer. I don't know if a lot of academic investigators developing new things, intending to sell them to pharmaceutical companies, are going to share their sequences publicly, either. Any comment from the FDA? That is pretty clear, though. Right?

DR. NOGUCHI: That really gets back to the accuracy of the data. We would expect that people know they need to control their own systems internally, and if you have a problem, that you would address that. You can always ask us for help or advice on that, but if you have contamination of your whole system, you need to clean it up. That is independent of FDA evaluation.

CHAIRMAN SALOMON: The next question would be does everyone agree you should sequence both strands of a double-stranded DNA vector or plasmid?

DR. CHAMBERLAIN: I think that gets back a little

bit to what we mean by an accurate sequence. You said what we submit should be accurate and reproducible, but I think a typical academic sequencing lab that is just going to do one-pass, one-strand sequencing is going to have an error rate of about one percent. If you do both strands, you're going to drop that error rate, but even the professional labs doing the genome sequencing that are doing 6X, 10X redundancy are getting error rates of one-in-10,000. I don't know how pedantical we want to get here about error rates, but I think the difference between one strand and two strands is mostly a matter of what your error rate is acceptable at.

CHAIRMAN SALOMON: That's actually what it intended to do, was to reduce your error rate. That was question--that was my last question, is what would be the error rate you would tolerate? As we all know, there's an error rate of about one-in-1,000 bases with automated sequencing.

DR. NOGUCHI: Dan, I think what we're asking here are really advice on the major points. The technical details is one reason why the specific guidance FDA will have will need further discussion and interaction with reality as to what can be done. We really unfortunately need to move and get some very fundamental questions on the table, which we have already alluded to in our previous

discussions.

CHAIRMAN SALOMON: Good enough. If everybody is comfortable, I realize it takes time, but the issue here is we keep talking about sequencing without any discussion yet about what sequencing means. I think I was detecting some people were uncomfortable with it. Let's move on to question two. Vectors greater than 40 KB in size, it's proposed that coding sequence of the gene of interest, transcriptional control regions, and regions including and flanking any sequences that are altered during production of the vector should be determined prior to initiation of a Phase I clinical trial. This is very early.

For this group of vectors, which now are the herpesvirus vectors, the poxvirus, EBV and--should the full sequence be determined prior to initiation of a Phase II clinical trial or later? That is the next thing we need to discuss. I guess the specific proposals are the extent of flanking sequence determination that would be adequate.

DR. MULLIGAN: Just to get the ball rolling, I think how you actually do the sequencing would be a tough issue, but I think I would very strongly push for having a complete sequence of the herpes vector, EBV vector, and I think the issue of do you sequence every new vector is perhaps something I would not have great concerns about.

I think that every vector or the parent vector,

ought to be sequenced, and some are, some are not, and I think this is the genome generation, and I think having information, when you can get that information, is something that is very important. I'm interested to hear what Xandra thinks, but I think with these big DNA viruses, I think there are still questions about whether isolates may have certain sequences.

They could have unusual pathogenic characteristics, and sometimes the biology of the test systems may not identify those characteristics. If we were to see patches of human sequence in a herpes isolate, one particular researcher's herpes isolate, that could be very, very important. It could point us to other pre-clinical tests to see whether there was something important or not important about that sequence.

I just think that this is a generation where, when you can get the information, you must get that information. I think the cost-benefit is a benefit. There is a real benefit to having a sequence, but whether, again--to reiterate, I don't think that you necessarily want to make everyone, every time they put a gene in a herpes vector, do a sequence. I think there ought to be strains that are used by people, and when they use it, if they use to choose to use a big virus, it's not that they're penalized for using a big virus, but if they're using it, it's more complex and

provides more risk.

CHAIRMAN SALOMON: What we have to focus on now is do we accept the basic principle that you need sequence, which means then it is really why do we not hold that principle to a larger vector. That is basically the question.

DR. MULLIGAN: Yes, one more point that might be helpful to have the FDA talk upon, is the proposal here I found is interesting, because it says when you go to a Phase II, then you ought to have it, and I think it would be helpful to have their perspective. My feeling is it is just information. You must have that information before you initiate a Phase I, and I'm curious as to the reasoning about why in the Phase II do you all of a sudden have to have that information.

CHAIRMAN SALOMON: Good point. Okay. Dr. Sausville?

DR. SAUSVILLE: So the question allied to some of those considerations is--one thing about the size cutoff is clearly the really new creations would likely be most frequently, particularly plasmid-derived or things of that nature. I think the question I put out on the table for people who actually work with these viruses, is does the community of herpesvirus workers or poxvirus workers have a sort of repertoire of common strains that could potentially

addressed this, that as long as you start from someplace that you know where everybody came from and there is sequence or information, and then what you are going to be doing or what the individual investigator is going to be doing is going to be editing that in relation to their local and regional context.

That might be, to me, a more defensible compromise that wouldn't call for making this simple distinction on the basis of size.

CHAIRMAN SALOMON: Dr. Whitley has gotten up, because I was going to call on him in a second anyway.

DR. WHITLEY: I will let Dr. Breakefield join this discussion, as well. I think it goes back to what Dr. Anderson said earlier, and that is there are potential risks versus benefits in terms of sequencing the entire genomes of the "herpesvirus family". I don't think it's wise to say herpes simplex is the same as cytomegalovirus or Epstein Barr virus is the same as HHVA. I can well-understand Rich's argument about KHSV and EBV. I think the argument is a little bit less cogent when we talk about herpes simplex and varicella.

Cytomegalovirus is somewhere in between, but it is certainly going to be a risk-benefit analysis in terms of whether you sequence these viruses. It is a tremendous amount of work even in the era in which we live, I wouldn't

deny that, and it will be very costly, more costly than the smaller genomes that we talk about sequencing.

To address the other question that was put on the table, are there prototype herpes simplex virus strains that have been used in the engineering of these viruses? For the two that are currently in clinical trials now, one that Bob Martuz has developed that is known as G207, and the other, which is known as NV1020, which is a variant of a candidate vaccine strain that was studied 10 years ago, they came from different parental lineages.

Whether other herpes simplex viruses arise from wild-type viruses remains to be seen, but for EBV, the folks who are working with that, there at least four strains of EBV that are being used for vectors. So, it is going to be very complex and has to be very carefully thought out by this committee.

CHAIRMAN SALOMON: Rich, before you sit down, for those of you don't know, Dr. Whitley's doing herpesvirus vector and clinical research with them. First of all, when you say it is difficult to do, how long would it take you to fully sequence a clone? How hard is it to do in real terms?

DR. WHITLEY: I don't know. What would you say, Xandra, from what you have been sequencing at MGH?

DR. BREAKEFIELD: We just sequenced the amphlicons, because we tend to use that system. I have to

agree with you, taking on sequencing of the herpes genome, there are so many repeat elements in it and it's not just the size. I do think that sequencing is getting better and better, but right now, that would definitely be prohibitive to an individual investigator trying to move forward, just because of the difficulty right at this point in time.

If you are going to decide on some strain backgrounds and then sequence what they've manipulated, at least initially maybe, when sequencing methodologies improve, you know, make that a requirement. But, right now, it would definitely slow things down.

CHAIRMAN SALOMON: To both of you, one of the things that I think Dr. Sausville was commenting on would be let's say I want to start--I decided I'm excited. I'm going to do a herpesvirus trial tomorrow. I call one of you up and I get what? Do I get a plasmid that has already been sequenced, even though I know everybody has messed around with it for umpteen weeks or months or years? But are there sort of parental strains? I think that was one of the questions.

DR. WHITLEY: I can tell you what we're doing, and I am going to speak for Bob Martuz, as well, because we sort of have been doing this together, and Xandra can correct me if I'm wrong, but for G207, which is his virus, the inserts into ribonucleotide reductives have been sequenced, the

deletions have been sequenced, and about 1,000 base pairs on either side of the deletion have been sequenced. That is a standard virus that is being given out either by Bob or by the sponsor, who is developing that particular virus for therapeutic indications.

For the viruses that we have made, we have sequenced the deletions. We have sequenced the inserts, so we know basically where we stand with that, and we're giving out the same parental stock. It is one parental stock that has been aliquotted into 1,000 lots, and that's the virus that goes out.

CHAIRMAN SALOMON: The point is that what I would get from you as I started my trial is basically not a fully-sequenced vector.

DR. MULLIGAN: That is correct.

DR. BREAKEFIELD: They kind of come in two forms. They have been cloned into the F-plasmids, also, and typically we digest with like 10 different restriction enzymes, because there is instability and there is a lot of change that goes on in herpes vectors just as their passage. I think if you don't do sequencing, you definitely have to do a very extensive restriction analysis of the genome to make sure you don't have major rearrangements.

DR. GORDON: I just want to say there is a little bit of reverse logic expressed in the question, I believe,

here, too, in the sense of implying that you would want to be more careful with a vector after it cleared its safety trial than before it cleared its safety trial. Here you are saying let's not worry about the full sequence until after we know it is safe in a Phase I trial, and once we know that, we should sequence the whole thing before doing a Phase II trial.

I think there is a reverse logic there, which, if corrected, could allow you to escape some of these problems.

DR. SIEGEL: Let me address that question, not to at all suggest what the right answer to the question on the table should be. The first thing to point out is that virtually all clinical trials are safety trials, certainly Phase II and Phase III trials are products in development, which extend exposure to much broader populations than--you know, if you do a Phase I trial in one dozen people, you could events occurring at a 30 percent or 40 percent frequency that you just don't happen to see.

So, they are all safety trials. Now, the question was raised before about the logic of phasing in a requirement, and I have to say a lot of different types of requirements are phased in during clinical development. The whole notion of good manufacturing practices and of process validation is phased in. There was a question about--Abbey had a question about getting rid of contaminating virus that

was talked about, autoclaves and acid treatment.

If you were making something for production, you would probably have to test every corner of your autoclave to ensure that it did, in fact, function as anticipated to get rid of that contaminating virus, but we don't impose that at any early phase of development for those sort of pragmatic reasons French was mentioning. We might impose some practical standard that you have an autoclave that is known to reach the right temperatures and is appropriately tested, but with a simpler form of validation, and then we phase in requirements with size.

Many, especially in biotechnology, many, many, many products that go into Phase I do not go into Phase II or Phase III. Requirements that come a little bit later, but as you get into Phase II or Phase III development, you both get increased risks because of increased exposure, but decreased costs overall, because you may be screening 10 molecules in a few patients in Phase I for that one you're going to advance, or 10 viruses in this case. The actual screening costs decrease tenfold.

There is a tradition and a logic here, which is that more testing later on can provide a higher increment to level of safety. There is, as presaged by French's earlier comments, plenty of precedent for taking one level of safety concerns in early studies and advancing them as you move on,

one level of controls to address safety concerns early and advancing as you move forward.

CHAIRMAN SALOMON: The answer that Dr. Siegel is giving you is it is not unreasonable to have less of a safety profile from the point of view of not knowing the whole sequence as you go into a Phase I, but as you go to the Phase II or later. One of the issues we ought to deal with in a second is when we think we should have more sequence, and I will get to that in the next point.

DR. SIEGEL: And since you recharacterized what I said, I do want to make clear that I was not making a statement specifically about whether it's reasonable not to have that in Phase I. I was simply making the statement that, in general, it has been seen to be reasonable to increase certain types of controls and requirements through development. I have no expertise on which to base whether or not sequencing of herpesvirus should be done before Phase I and prefer not to comment in that regard.

CHAIRMAN SALOMON: Okay.

DR. MULLIGAN: All that being said, who in the FDA did suggest that in Phase II there should be complete sequencing? I would turn then the question in the opposite direction. Why is there a reason at any point then to know the sequence? I know I have my opinion, but I'm curious, if it's necessary at some point, why does it become necessary

at any point?

DR. NOGUCHI: We are making practical decisions and I think part of the answer is what French said, in an ideal world, all sequence before you do an experiment, but that is not technically feasible in the case of herpes. I think that point is quite cogent that even within a passage, you get multiple rearrangements and we have not even yet gotten to the question of what do we do with sequence that we do have in hand.

Part of this is a balance between what we can, as a group, actually do in a timely enough manner to make product production move forward, as well as the consideration, well, if you took the information being needed absolutely, then herpes and some of the poxviruses wouldn't move until the technology to sequence those accurately come into being. We are going through a series of both risk-benefit evaluations, as well as practicality, trying to move things forward in as safe a manner as possible.

We are transferring some of this discussion to the public domain, so that you can actually understand why we are on some occasions being somewhat arbitrary. Everyone has picked up, in a way, you're sort of reversing your stance on the small versus the large, but the fact of the matter is the large, you cannot get as much accurate

information at this moment in time, and it may take awhile to get that.

The corresponding question is, as a society, which is what this is going toward, should we then prohibit anything moving forward that is larger than 40 KB until we have the methodology to get the basic information, such as sequencing? That does not address it all, but I think that is our basis of thinking.

CHAIRMAN SALOMON: I think that is well-said, Phil.

DR. SAUSVILLE: I just wanted to pick up and potentially ask Dr. Breakefield to amplify on this line of, shall we say herpes biology, because to follow on what Phil was alluding to, one basis for what was called reverse logic is exactly that, the sequence that you get with respect to herpes, with respect to present technology, would not ultimately be as meaningful as the sequence with the smaller.

That might actually be a reason, since I personally don't want to see herpes use prohibited, I think we just need to make this balance, again basing it on the biology of the system.

DR. WHITLEY: I think what Dr. Breakefield said before is obvious, and that is serial passage of these viruses leads to genetic change. We're going to have to be

very careful about that, and certainly the critical trials that we do, that has been built in as one of the standards. The one point I want to make is that I don't think sequence data in and of itself should be taken in the absence of relevant biologic models, and we're going to talk about that this afternoon.

It is one thing to talk about sequence, but do not isolate that discussion from the relevant biology of this virus. We know how it behaves in certain systems and hopefully we can spend some time with those issues later today.

DR. BREAKEFIELD: Really, in my heart of hearts, I think we should sequence everything that goes even into Phase I before it goes into patients. I think the government should provide us the means to do that. This is an important thing to do, and it is just the logistics of it right now that if you have people kind of developing these vectors in academic institutions, they don't really have the resources to carry that out.

I find the situation of arguing for something just based on the practicality of it--that I think is actually a good idea, and I feel uncomfortable about it, and I certainly would abstain from this vote just because of that, but I think it is a very hard call.

CHAIRMAN SALOMON: I think that is really well-

put, and that, I think, is the whole reason we're here this morning, and this has got to be one of the major questions I think we will deal with in this morning's session. What are we really telling the FDA? I think we're really going to have to face this as a hard thing. At one point, we accept the overall concept that we do not want to stop the development of new programs and new technologies.

At the same time, we have a responsibility to the public to do it safely. It is okay if the message to the FDA is that you feel as experts that it should be sequenced, and that then the recommendation to the NIH and the FDA would be to provide alternative ways to do it. I think we have to be ready to make that sort of recommendation.

MS. MEYERS: A few years ago, in the early development of gene therapy, we quickly saw that it was going too far into the commercial sector. By that, I mean the science was so basic at that point, it was much too early to go into to the commercial sector, but it was going there anyway, a lot of it because of the cost, and then the government set up some vector manufacturing facilities for academic scientists. I think there are three of them. Do they still exist? Okay. Which was not enough, but it was something, at least.

The government really should do something to set up a facility that could do this type of sequencing and

cater to the academic scientist, because, if not, then the field will continuously be in the commercial sector before it is ready.

CHAIRMAN SALOMON: I would just point out for the record that the national gene vector laboratories, which do produce vectors for clinical trials and is, of course, supported by the NIH, does not do sequencing of vectors. That is all done by the sponsors or the investigators.

DR. MULLIGAN: I think one piece of all this we're missing is expertise from the real high-tech sequencing people. I think it would have been helpful to have some of those people, because I think the cost of sending it to a contract laboratory is very different than sending it to the Whitehead Institute or someone who really knows how to do large-scale sequencing. I think this is high-tech gene therapy.

We ought to use the highest tech that we have in the country and I guarantee you we will find people who will be able to do this, and I think we would be shocked by the cost. That is, there are companies, there is a whole range of people who really look at sequencing in a very different fashion than any of us, and I don't think they would look at a herpes sequencing project as anything more than trivial.

MS. MEYERS: I think that the public is generally very uncomfortable with the idea of poxvirus as things being

played with in laboratories. If you do not assure the public that it is being done in the safest manner possible, then you're making a big mistake, because one accident will be just a dramatic effect on this whole field. We have already seen, for example, what one bad example has done to this field.

I cannot vote for that the way that is written, because I feel strongly that the first person who takes the first dose should be assured that it is a safe as possible.

CHAIRMAN SALOMON: We get back again to the idea is the sense of the committee at this point that there should be full sequencing of any vector and that this arbitrator division by vector size is just arbitrary?

DR. SAUSVILLE: I remain troubled by the issue that, with current technology, and maybe again there is better people to do this, the meaning of the sequence that is going to be generated for the larger viruses, because they have to be taken apart in order to get the sequence. There is going to be sort of an uncertainty, sort of, factor always operating, and that is in contrast to the smaller pieces.

Again, I just ask the question, and again following on Dr. Anderson's concern about incremental gain for required activities, by just saying we require a certain level of sequence or a sequence above a certain size, are we

ignoring the practicalities of what that information means at this point?

CHAIRMAN SALOMON: I think, I mean, that is a question open to discussion, but the point is, I think what Abbey is trying to make us cognizant of is what we're saying to the public then is we're going to go forward with a Phase I clinical trial with a vector that has been around for years, been passed through several different laboratories and has never been fully sequenced. I'm not going there.

DR. MILLER: Can you just update me on where we are with these viruses and the INDs that are already filed? Are they ongoing? How much of the water is over the dam already? If we have clinical experience already in the INDs that have been filed and have been started, on the herpesvirus and the poxvirus, it's not like the first patient getting the poxvirus is going to be prevented from getting it. The first patient, I assume, has probably already gotten it, but I may be wrong.

DR. WILSON: That was in the graph that was presented this morning, but may have gone too quickly. Herpesvirus, we have two clinical trials ongoing, and poxvirus, we have about 12, although there are, I don't know exactly how many, but poxviruses are also being used in Office of Vaccine as vaccines, as well. This number only reflects those that are being used for the purpose of gene

transfer type of clinical protocol.

DR. MILLER: Not being a gene therapist, there is more heterogeneity in the smaller, in your potential for the smaller, less than 40 KB, than--is that not true?

DR. WILSON: No, I didn't think that it is necessarily true.

DR. ANDERSON: Just out of curiosity, what is the status, what are the requirements in the DNA vaccine field? I mean, there are lots and lots of trials with vaccines, with pox and the rest of it. If those are not being sequenced, then Abbey, what we're talking about is there are already 10,000 patients who have gotten it, and to say, "Oh, we have got to sequence it before our first patient."

DR. WILSON: I don't know exactly what our Office of Vaccine sequencing policy is, but I can be certain that they aren't requesting full sequence analysis of a poxvirus being used for a vaccine.

CHAIRMAN SALOMON: I am a little bit stuck here, because I want to try and give the FDA the answers to the questions. That is about the only thing I'm supposed to get done. At this point, I'm a little stuck because I'm not sure what I should push as the next question. If we're kind of agreeing--Carolyn, if you want to comment, I mean, at this point, I'm getting the sense from the committee, and don't worry, I don't think we're calling this to a vote,

right, we agreed on that. This is just discussion, so everyone should relax. We're not going to try and come to a vote on it, but what I'm hearing from the group is no one really feels comfortable in this idea of a principle applied to smaller vectors versus larger vectors, simply on the size.

Yet, Dr. Sausville several times made the point of sequence is sequence, what is the significance of sequence, and I think that's a fair point that I can't respond, but I think Dr. Mulligan's response to you might be, well, yes, I don't know what every sequence means, but at least it's a damn good starting point for genetic manipulations of materials intended to going into human patients. I mean, if there's a big open reading frame for a transcribed protein, then I think we ought to know about it.

Carolyn, do you have a comment that you want to--

DR. GORDON: I just wanted to comment that I sense, as a quasi-outsider here, that people are looking for information that will mollify them and make them feel more comfortable with using these vectors, and for psychological reasons, perhaps as much as scientific, they have latched upon sequencing as the type of information that they want. I am not all certain that sequence information is, perforce, enlightening, and I just wanted to say that I'm not so sure that it always is enlightening.

CHAIRMAN SALOMON: I think Dr. Whitley has made the point to us, and I totally agree with that, that no one is trying to say that sequencing is the only demonstration of safety or responsibility before going on. He is saying you have to do the animal models and we all agree with that. What we are dealing with, though, is sequence information, a piece of information that should be required, and that is the key.

DR. CHAMPLIN: You know, the issue that Dr. Anderson had raised is what is the relative balance in the small vectors that was a small cost for potentially a large benefit. Here, it is a big cost for potentially a small benefit, in terms of really understanding what is going on. I'm not sure, I think it may be more of an impediment to progress than a facilitator of progress, to make a rigorous sequencing requirement for the larger vectors when it is both impractical and hard to interpret.

CHAIRMAN SALOMON: Well, I guess that is what is sticking me right now. I mean, what is it you guys are saying? I have not got it straight. Is it a large cost for a small benefit or is knowing sequence important?

DR. CHAMBERLAIN: Well, I agree with the previous speaker, that I personally would not be in favor of requiring sequencing determination of an entire large vector, because I think the information you gain is not

worth it. In the case of the smaller vectors--well, let me back up a minute. I think what we really need to get it, as Richard said earlier, is that what you're producing is what you think it is, and the amount of sequencing that you need to do to confirm that with the small vector, it doesn't take that much more to complete the vector and that can be done rather inexpensively.

I don't think that argument really holds up for vectors that are approaching 200 KB, even though our large genome sequencing centers are routinely sequencing tens of hundreds of, you know, bacs (ph.) a day. There are difficulties in giving them new clones. They generally agree to take on larger-scale sequencing projects only as a subcontract, where you pay them, or if they have a collaborative relationship where they are going to get co-authorships out of that.

I don't think they are going to be that receptive to suddenly having these large vectors dumped on them, without them getting anything in return for it, and the important issue, I think, is to make sure that when you have constructed a new herpes vector or whatever, that it is essentially what you think it is, and I believe that can be done by sequencing the relevant regions you have modified and then following that up with extensive restriction mapping.

Until there's some mechanism to make it affordable to do this large-scale sequencing, either through improvements in technology or a huge infusion of new funds from NIH, that we should stop short of making that a requirement.

MS. MEYERS: It seems to me that this whole discussion, the determination of whether it should or should not be sequenced, is based on finance, and that doesn't make any sense. It's just a matter of how much is it going to cost, and I don't know whether I would be willing to risk one of my kids going through a Phase I trial in gene therapy when the investigator has to admit that he does not really know whether the construct of the thing is what he thinks it is, and it should not be a financial cutoff. It should be a scientific cutoff.

DR. SAUSVILLE: I guess I would respectfully disagree with the contention that it's strictly financial, because if it were strictly financial, I would be in your camp. I remained troubled with the biology--not troubled--I am struck by the fact that the biology of these viruses is intrinsically different than the smaller-sized ones, and this gets back to the question of the meaning of the sequence, because if in order to determine the sequence you're breaking apart and there's no certainty that when you put it all back together again, that that is what is going

to go into your theoretical patient, what is the meaning of it?

I think that is where somebody remarked earlier that the behavior of the construct in a relevant biological model and an additional biology needs to be factored into the decision.

CHAIRMAN SALOMON: Just in trying to get just a little bit of agreement on this, would you agree that even though you should not--perhaps it is not reasonable to require sequencing of everybody's vector before the clinical trial.

I know that there's disagreement on that, but is it reasonable that the parent vector has been completely sequenced? I think what I was trying to get at was the parent vectors, in many of these instances, have not been sequenced anytime in the recent past, which means then that you really are not knowing what you're putting in.

DR. SAUSVILLE: But this gets to what I had raised a number of minutes ago, that is there consensus among workers in the field as to what those parent vectors are? I guess we have heard that for herpes, there are two that people use, but for Epstein Barr virus, there are four, and so I think--

CHAIRMAN SALOMON: None of them have been sequenced. That's the problem I'm having. If you would

tell me that they were sequenced, and then it was 1.8 years since they were sequenced and it passed through three different labs, I could deal with that. You're saying, if they're never sequenced, then I really have no idea.

DR. SAUSVILLE: That is a relevant question for our FDA colleagues. I mean, the FDA has a historical, long-standing interest in defining standards for biological reagents. Are we talking about really then, as an outcome of this discussion, asking the FDA to call for, perhaps themselves to establish, these standard strains and then they would be sequenced and that's what everybody would build their biology on?

DR. BREAKEFIELD: I think that sounds like a great idea. I actually think, practically speaking, if you said there are two strains and we want you to get it like from the ATCC and put your thing in this, we would all do it. That would make life very much easier and it would be a very practical solution to it.

DR. NOGUCHI: What I would like to do, Dan, is to try to wrap up this question, and I hope we can get a little discussion on question five. Again, we're running into practicalities. FDA is committed to a very large program for standardization of adenoviral vectors. That is not being done at all simply by FDA. We are in the middle of creating an MOU. We're talking about at least four

reference laboratories who will be doing the characterization, which now will include sequencing.

The production of the vector itself may come in through competitive bidding. We're talking about a multi-component, both academic, private industry and federal government working collaboratively to do this. We cannot put this on any one entity, none of this, because there simply isn't enough wherewithal at any one entity to complete all the tests, all the production, all the quality control that needs to be done.

I think if the outcome is a recommendation that consideration be made for really addressing the issue of these larger vectors, we're comfortable with that, but we are really not comfortable with being given mandates for which we really have no current funding or would ever appreciate getting that. We're committed to many things, but we also need a whole lot of help in that.

DR. GORDON: I just want to say quickly how very good it sounds to try to introduce the principle of sequencing the parent vectors. It has been asserted here that these vectors are biologically distinct from the smaller vectors and therefore it may be more relevant to sequence them that way. That may not always be true for the smaller vectors.

There may be smaller vectors that have different

biology in the future where parent vector sequencing makes more sense or whatever. I think it is sort of a way of jumping that bridge between the below-40 and above-40 KB, and it is a way of introducing some practicability into all of it. I really like that notion.

CHAIRMAN SALOMON: Phil, when we discussed this yesterday, Steve and Carolyn and I looked at each other and said there is no way we are going to get this done before noon, and that doesn't surprise me, but yet I think these are really important questions, and that's why I haven't jumped ahead out of this discussion, because I think this is a really important question.

What I would like to do is take a chairman's prerogative and add another 15 minutes now and about a half-hour after lunch to try and get at some of this, because I think we should go on. We can try going on to question five right now, but I think question three has got some important issues there.

One of the issues, just before we leave this one, what is still bothering me a little bit now is one of the issues was if we accept the fact that you do not sequence the 40 KB, greater than 40 KB vector, and I'm not even going to try and do a consensus this time, because I don't think there is a consensus around the table here on this, but what is begging an issue then is do you ever sequence it? If you

do not sequence it before the Phase I, does the committee have a comment or discussion on do you sequence it before Phase II? Do you sequence it before Phase III? Do you sequence it before licensing?

DR. BREAKEFIELD: I guess I feel that most of the innovative work is kind of done in Phase I, and if it looks promising, hopefully some company or something will move forward with it, and then there would be the funds to sequence it. I think it is more that first step of trying to get into a Phase I that I would certainly like to encourage the academic investigators to be able to do.

I would also like to restate that they should be sequenced even at that level, but right now it is impractical. I agree with Richard that you can sequence big pieces, but these have a lot of repeat elements and you would have to have somebody committed to doing it.

CHAIRMAN SALOMON: But you like the idea that you would have to sequence before going to Phase II.

DR. BREAKEFIELD: I--

CHAIRMAN SALOMON: I mean, I'm not trying to put words in your mouth.

DR. BREAKEFIELD: I--that's what I would--

CHAIRMAN SALOMON: Okay.

DR. SAUSVILLE: I would certainly take the position that before Phase III, which is looking at

marketing that's going to go on for some large number of years, you would want sequence even if there are ambiguities in meaning, because what you're basically defined are patterns of behavior that you're going to expect this construct to exhibit over perhaps decades of use.

The issue of whether to require it before Phase II, I think that's going to depend on the nature of the disease, nature of the indication, the level of--whether a Phase II would be possible if we did require it. I guess what I'm saying is I would still be ambiguous or desire the sequence, but live without it going into Phase II, but would really want it before Phase III.

DR. MULLIGAN: I don't see the consistency of that point. I think you're trying to raise the question that I have been troubled with, that it either is or isn't important to have the sequence, and what is it other than the cash, essentially? We should address it, either it is or it isn't, and if it is at Phase III, I don't understand your reasoning why it is all of a sudden at that point.

I think, as Xandra was saying, it is the cash, that maybe you've got someone to sequence it, but I think that's maybe now the reasonable question on the table, is is it ever important to have that sequenced and maybe divorce it from the cash issue?

CHAIRMAN SALOMON: I think one of the things that

we can maybe just not come to consensus on is I think what Phil is trying to say is if we get too specific here, we're going to also reduce the flexibility the FDA has. Someone will come back and say, well, your advisory committee said you had to sequence it, which might be a dangerous position to take.

Again, I'm making this all public. I may be a little concerned, as chair, to go there, that we'd come out with an official statement here. I think there is enough disagreement and lack of consensus here that I don't think we can give the FDA that sort of recommendation, but I think I've been very straight in the record that I see--I'm a little confused.

The only thing is this whole thing recently, with the gene therapy scandal or whatever, if that happened, if somebody died in one of these trials and, you know, the Washington Post and New York Times published that we had no idea what the sequence was of the vector that went in, I just don't know how that would play the next time Jay and Phil confront Dr. Frist, but I think that's their problem right now--thank God--if the sequence was fully known and something about it had not been noticed, especially if the vector was 200 KB.

DR. MILLER: I think that's the best point, I mean, if you believe doing the sequencing would prevent the

deaths or severe untoward outcomes in these studies, such that you would not want to expose patients, then yes, you should sequence. If we're saying that we don't think, with these large viruses, that there's going to be enough benefit gained to potentially make both Phase I and Phase II trials at least able to be done in a reasonably or safe and sort of broad-reaching--not broad--innovative patterns, I mean, Phase I and Phase II are still looking for evidence, you know, safety plus some preliminary evidence of efficacy before you take it to a large Phase III trial.

They're more likely to be investigator-sponsored or small-group-sponsored, as compared to a Phase III trial, where you really need a big support. So, I mean, I'm saying that if you feel that it is truly that sequencing is going to save it, then I think we would all say vote, but I don't think the feeling of the people--that that is going to be the major vector on whether this is a safe or not safe thing to do.

DR. SAUSVILLE: I would agree, and that is the basis for the potential perceived lack of consistency that you pointed out, in that when you get to the level of Phase III and what is entailed by Phase III, by definition implying marketing, then at that point I think it is reasonable to demand the sequence, but to not stymie investigation and to allow this broad reach is where I, at

least, was trying to make the balance.

DR. MULLIGAN: I think there's just a lot of opinions upon the usefulness of sequencing information. I think, if you talk to the genome people, there's one point of view. There are certainly people like John that do not think the same way. But I think this is exactly the issue, what is the value of having sequence information? And I think there are just two camps. There are some people, and I am in that camp, that thinking that having that information is better than not having it, and I think there's a good chance that it can be helpful in the future.

Other people will say, though, it is just too complicated. What if you miss the thing? But you'll never catch the thing if you do not have the sequence. I'm going to stop talking, but I think the reason I'm so really positive about pushing this is that I think this does have a very important implication for the evolution of gene therapy in the future.

I think the whole discipline ought to think about having some perceived rigor that it may not have had. I think that although you could even say this is a symbolic type of thing, it is just having information. I think Abbey appreciates that, that having the field say, look, we may not be able to understand information, which is always said about the genome project, but we have the information.

I think your point is very well-taken. If there's a death due to a herpesvirus vector and no one can figure out what happened, and, in fact, people said this never occurred before in the preps that we had before, you might be concerned that there was some variant herpes strain. What would you do? I would almost guarantee you that someone would ask the people to sequence that herpesvirus vector.

DR. TORBETT: I have somewhat of a philosophical question. It would be really useful to have this information, but if there is no way to access that data and compare it to your own parental strain, then the data remains meaningless. There has to be a way of taking that information, comparing it to what you have, and know that your particular strain is within a certain realm or not.

I think that is something that has conflicted me during this whole discussion, not so much with the smaller viruses, but with the larger viruses, and perhaps the committee would like to comment on that point.

DR. MULLIGAN: I think that we've been drifting from a compromise. You know, the first thing was sequence your herpes vector. The second thing was that is too tough, let's use something that has been sequenced. I think you're getting to the point of maybe that, in practical terms, does not make much sense, you know, once you're slightly off the

mark, you're completely off the mark, and I think that is a good point.

DR. TORBETT: I am in favor of sequencing, but what are you going to do with the information once you have it, and I think that's what I think some of the committee, at least I feel, is somewhat conflicted about, what can you do with that information if there is no way of figuring out what it means or even having access to other pieces of information other investigators have?

I know it's not the FDA's point to comment on these, because these are proprietary pieces of information, but that somewhat conflicts me here.

CHAIRMAN SALOMON: I think that one thing that you could say is if you find sequence information that is, let's say, base pair changes that are conservative or not, change an amino acid here or there, at least you can now begin to see whether or not, for example, there is immune response in your animal models reflecting these changes. You could look to see whether or not you might be--I think more critical is whether there are whole sequences that have been adopted.

This viruses, as you well know, have survived in evolution by grabbing host cell genes and incorporating them and then using them functionally. The idea there could be a chemokine that was in there or a fake Class I molecule, all of which are examples in CMV and EB virus and HHVA, and I

think those are the things that Dr. Mulligan and I are concerned about.

I have to say I continue to be more on the bullish side of sequencing, but--

DR. SIEGEL: As a nonexpert, let me ask, though, particularly about that last risk. Those larger changes, such as insertion of a major open reading frame that might be a Class I or a chemokine, to what extent would restriction mapping or other techniques less fine than sequencing likely detect those or exclude them?

CHAIRMAN SALOMON: That is question three. I thought we would get at that at lunch--after lunch--not at lunch. Excuse me.

DR. SAUSVILLE: Right. It bears, though, on how compelling it is to do sequencing.

CHAIRMAN SALOMON: I agree, Jay. That is really a good point. I'd like to kind of wrap up this morning with the idea that we will spend a half-hour after lunch trying to get at question five and then three. Is that okay, Carolyn, as an order? We sort of did four, but the question here would be if you are in the camp that was advising the FDA not to do the sequence, and there is considerable sympathy for that as a recommendation, are we then suggesting there is no sequence, or are we going to go back to that we should do 40 KB, just not--

DR. SAUSVILLE: No, I would say--I mean, the question is written here, you know, the parts that are relevant, I would categorically agree we should have that sequencing.

CHAIRMAN SALOMON: I just wanted to get this part on the record.

DR. SAUSVILLE: We're talking about what is not in those parts.

CHAIRMAN SALOMON: Obviously, I'm just trying to end here by making sure that we do not miss getting on the record a critical point here, and that is does everyone agree, regardless of whether you want to sequence the whole vector or not, does everyone agree those areas of the vector that have been specifically modified by the investigator, particularly the insert and/or the promoter regions and the linker sequences, they should be--are we at least agreed on that?

Do we agree that it should be 40 KB, that you take the middle and go 20 KB in either direction? That was my suggestion, just that I didn't like the idea that someone working on adenovirus got punished by having to do 40 KB sequencing, but someone working on herpes did not. I am being partially facetious, but at some point here you should decide how much flanking region you're going to include in this, and I suppose we ought to leave it at that.

Well, again, I think we've pretty much summarized this morning's discussion. Do you want any more summary, Steve, Carolyn? Are you okay with that, and are we okay with the idea of a half-hour after lunch to deal with question five and three? I have been told no more, though. Please. Okay.

It's 12:30. I would like to start again at 1:15. Thank you all very much.

[Whereupon, the committee recessed for lunch at 12:32 p.m., to reconvene at 1:15 p.m.]

[1:29 p.m.]

AFTERNOON SESSION

CHAIRMAN SALOMON: I would like to begin now with the afternoon session. However, as I said before the break, I am going to put in 30 minutes of discussion on topic one now, before, and then everything will just be pushed back a little bit, and I apologize to the speakers and to the audience if they had timed their afternoon and I'm messing it up. I think that I feel comfortable, after having a chance to just think about what we did this morning, that no more needs to be said about this morning's discussion.

I, in discussing it with each of the FDA people--people got the sense that they heard what we were saying and I think we've done a responsible job representing the diversity in the community and there are no complete answers, and I think I am content with that. So, let's go on and deal with question five, and I am going to try and, so it is 1:30 right now, just see how we go on five, and if we're not getting there, I'd like to sort of skip on just so we can briefly discussion question three, so we will see how we do here.

So, question five is--I have to go back and forth, because I have my notes on one sheet, but I have Jay's version on another. Hold on. Okay. Should unexpected sequence and/or open reading frames be identified during

analysis of the vector sequence? What additional steps should be recommended?

This is now picking up, I think, where we were at, at the end of the morning session, specifically what do you do with sequence? I think this was asked by several members, Dr. Sausville, Dr. Torbett, so what do you do with the sequence if you have it?

If unexpected sequence is identified during analysis of a vector sequence at any point now. Let's not get hung up whether it's before Phase I, Phase II or Phase III. What should you do? For example, A, expression analysis of the reading frame for RNA and protein; B, if sequences identify open reading frames, analysis should include retrospective studies of the subjects, for example, an antibody response or, I would imagine, a T-cell helper response or a cytotoxic T-cell response to the potential protein.

If sequences identified include transcriptional control elements, should we look at expression pattern of the vector sequences? Is there any discussion of that? What should we do if unexpected vector sequences are found? Again, a resounding silence here. That is fine, then we go on to the afternoon session. They'll be happy with me.

The point here is that I'm not going to let you out of it this easy, though. We all agree that there are

going to be sequence differences, because even if there's nothing else going on, most of these vectors have spontaneous mutations. There are errors in the sequencing procedure. There are homologous and non-homologous recombination. So, I think we have to face the facts that whenever the sequencing is done prior to licensure, there is going to be sequence differences, deletions or insertions, and we have to say something about this. We can't just be silent.

DR. SAUSVILLE: I think the answer to the question is it is a resounding "it depends" really. I mean, you know, clearly, it also depends when in the sequence it is detected and also the nature of the event, because we all would agree that if it were in a production lot for something that was going to go into clinical trial and the change affected the actual gene of interest, that clearly would be a basis for rejecting the lot or trying to fix it or something along those lines.

If, on the other hand, it is in a portion, particularly for those under-40 KB entities that we likely will have lots of information, if it is in a somewhat less well-defined place where it is not obvious that it is going to affect anything, I don't see anything needs to be done about it. I think the point about open reading frames is germane, and that even if it is a vector-related open

reading frame, one could imagine some type of immune response, et cetera. I would not necessarily even say that that should be changed.

I guess the more detailed answer is that then the sponsor should seriously consider whether the change detected would impact the nature of the clinical question being asked or considered.

CHAIRMAN SALOMON: Okay. That is one way. French?

DR. ANDERSON: First off, the reason I came in late, as you know, is the big issue that we were discussing earlier was sequencing, and the point was made, Rich made, that there is all kinds of expertise in the genomics program, and 200 KB, come on. That is sort of an afternoon's work, but it is a big deal for us. So, Phil Noguchi and I, sitting together at lunch, and Phil and I, as we often do, say, well, how can we get around this problem, and one issue would be perhaps if the Human Genome Project could pick up these big viruses, both in terms of gene therapy, as well as vaccine development and so on.

So, I called Francis Collins, and that is who I was on the phone to when you got started. Thank you for delaying, by the way. I saw you talking up here, but it took a little while to get through. There is, of course, a procedure to go through, so we will get together and go

through the procedure. There is a reasonable possibility that this would go onto their program, and therefore there would be a sequencing of the significant vaccine gene therapy vectors, viruses.

Okay. Now, in terms of answering this issue, we are all going to agree on this if it is important, you have got to do something. If it is not important, you don't do anything. I will now argue against what I initially said. When I first was talking, it was as a gadfly, to try to open up thinking. I will now respond in this context to what rather horrified you in saying, well, salmon sperm DNA is curing cystic fibrosis.

The way that could take place, in fact, is that that piece of salmon sperm DNA happens to be homologous to an enhancer region or locus control region or MAR, and SANR, an insulator or something else that then allowed the cystic fibrosis gene to be able to function better in that particular context. If you do not have the sequence, you will never know that, and then when somebody else makes exactly the same vector, they think, it will not work.

Having the sequence really is vital, but then the issue comes down to when do you do it? Your starting material very well might be exactly what you think, but it changes as you go through all the production and all the rest of it. The fact is, one needs to have as much sequence

information as possible, and then what you do with it is just what Dr. Gordon said. You have got to figure out what you're going to do with it, but if you don't have it, you can't do anything.

It is a balance, but the general principle, the FDA has to be left flexible to be able to use its own decision on a case-by-case basis of what policies they are going to set forth in terms of what is going to be required and not required, but balancing between cost and benefit as much sequence information as possible, as late in the production process as possible, is a good thing.

DR. GORDON: I think sequence discrepancies fall into two categories, and the resolution at which you find these discrepancies would be irrelevant if they were categorized in this way. One is in which you expect them to have some functional significance, and the other is when they don't. When they don't, I think it would be a mistake to advise that anything be done because I think we could end up chasing our tails with errors and other minor anomalies.

When things are functional or potentially functional, I think the appropriate action to take is to determine if they are, in fact, functional, and use the appropriate test mechanism, depending on the situation, to determine if they are actually functional. Whether or not you should insist that they then be removed if their

function is apparently extraneous to the purpose of the vector, is a far more complicated issue.

I would say that dividing these sequences up into ones that examine--when examined, determined to be potentially functional and non-functional, are in two categories, and I think we can deal with the potentially non-functional ones by saying we are going to log the information for the moment, but not take action.

CHAIRMAN SALOMON: I think that could be a beginning of trying to come up with some kind of consensus. You have to the sequence at some point, remember we are not getting into exactly when this is, and if you've got that sequence now, you analyze it, and you analyze it for mutations that have no clear biological or other functional significance. Those are catalogued. Then there could be two other kinds.

There could be insertions that create new open reading frames or there could be new sequences that might be promoter-enhanced or other transcriptional elements. Is that fair?

The question I have for the experts is how good is genetic analysis of sequence for the identification of those kinds of differences? I mean, how good are we at guaranteeing that we catch all the open reading frames, and particularly I am interested in how good are we at

identifying transcriptional enhancer and other promoter elements from sequence.

DR. BREAKEFIELD: I would say that it seems like when people do sequence analysis for transcriptional control elements, they always find them. I think it's hard to know when they're active or not, and that's a more difficult issue. On the other hand, if you come across an open reading frame of any substantial size, I think even the people who find them, they're going to see if they make RNA just like--since we can't refer back, I mean, I think they would naturally want to make--whether it was expressing a protein and whether the protein was antigenic. It is hard to believe somebody would not want to do that.

CHAIRMAN SALOMON: I think if you find an open reading frame and it that is clearly identified, I don't think we need knew to waste any time discussing what to do there. I don't think there's anyone around this table who can't figure that out. The FDA can, unless you guys disagree.

I'm more concerned with, number one, how good are we at identifying these sequences that would be for promoter enhancer and other transcriptional elements, and if so, then what do we do, because your point is very well-taken, you know, there are all these different short sequences that could be potential interaction sites. What do you do? Do

you have to make reporter gene constructs of each one of these now and look to see whether or not they're active in transduced cells or not?

DR. TORBETT: Given that, as Dr. Breakefield noted, promoters and enhancers seem to be everywhere and most of them are context-dependent. That means they are defined as what cell they're in and if they function, and I think that kind of opens up a large Pandora's box, but I think noting that they are present and have a potential function is worthwhile.

The next point of getting at the function could very well be somewhat problematic, I think. Again, not all promoters, not all enhancers, function equally well, and again it is many times cell-type specific. I would like to have the comments from the rest of the committee on that.

DR. BREAKEFIELD: I would say that, just like Richard Whitley talked about with the herpesvirus, there are certain things you expect of certain viral vectors in terms of what cells they are going to, in fact, replicate in, and presumably those types of biologic assays would be done and might be looked at a little more carefully if there were some potential sequences in there.

You might say, well, gee, does it really show the same spectrum of infectivity that we saw before and I don't think that's that complicated to do and, again, I think

should be probably done.

CHAIRMAN SALOMON: One of the ways we could say is that if you find sequences that may be representing these sort of control elements, that some work be done in the model systems for each of the vectors appropriate to each of the studies to look at genomic expression, right, transcript numbers, et cetera, to see whether or not they are significantly different than what might have been predicted from native or wild-type species.

DR. BREAKEFIELD: I think, since we are really thinking about issues of toxicity, that could be done, too, but I think you need to see that the infectivity spectrum of this virus, the types of cells that it infects and if it is replication-competent, what it replicates in, is like what all your toxicity studies before showed you for that virus completely--have the same ballpark.

DR. O'FALLON: From the simplistic standpoint of a statistician, if we were talking about Phase III studies where we have randomized these subjects into different therapeutic arms, what we are assuming we have done is give the same therapy to everybody that is in the same arm. You're describing a situation where apparently we will discover that that is not the case later on, and you are saying, well, it doesn't make any difference when this is discovered. Of course it makes a difference when this is

discovered.

I would assume if it was discovered before treatment was administered, it would not be administered, that something was not what we thought it was. We're faced with some extraordinarily complicated analysis problems, I would gather, after the fact. If we discover in a randomized clinical trial, that what we thought we had administered is not what we administered, how on Earth are we going to make sense out of that?

We're certainly going to have to study as much of it as we possibly can in order to make sense out of it. So, to not follow up on every lead that we discover, I would think, would make us in an intolerable situation, from the analytic standpoint. We would not see them being able to bring to us anything that they could explain if they did not try to follow up on that.

CHAIRMAN SALOMON: What we're going to going into in the afternoon session, of course, is preclinical models, which is very important to keep that in context, so if there were some tumor-inducing promoter region that got stuck into this, one would hope we might find it, but that is a discussion for later.

DR. MILLER: The more basic question from the regulatory standpoint is not so much what are we going to do with the information, but have you already or is there

already in place the responsibilities of how you report this and when you report it, because I think that people who are looking at this scientifically, when they find something, an abnormality, what I'm sort of wanting to know as a non-gene therapy person, is at what point are they going to report it and how much--how soon into the studies, as soon as they find it out, do they report it first and then do all the analysis, something like an adverse drug reaction, something you have 24 hours to report if it's life-threatening, something not like that, but is that the idea from this or is this something that you're going to say to the sponsor of the IND that they figure it all out and then they include it in their annual report, or is this something you feel should be put in more--earlier on--because I think that helps us shape how much you require, because if after they show that there is a difference and if there is an abnormality and then it is reported and then an analysis plan is then proactively worked on, that gives more comfort than if you say, okay, you can, you know, to the sponsors, figure it out and then we will hear about it down the road. So, I wanted to get that clarified, how that works.

DR. NOGUCHI: In fact, the reason we're bringing these discussions out here is we're literately learning all about these things in almost real-time here and bringing them out for discussion. Our basic concern is exactly what

Dr. O'Fallon referred to, is if there something there you didn't know, then that is not the product that you thought it was, and that should be reported as soon as possible.

Typically, what we do is when we get the information, we work directly with the sponsor to create a plan to either study it further or to change the protocol, any of a number of things. What we're looking for here is to try to get some more generalizable principles so that we can, in fact, just as has been stated, if it's not an open reading frame, our concern and our need to have a plan in place might be less or it might not, depending on how the discussion goes, but if it's an open reading frame, I think what we're hearing is that there is an expectation that you have the open reading frame, what protein is it, and do some more analyses.

That should be done, I would think, as soon as possible. It's not something that you leave for an annual report. Gene therapy is a very embryonic field, despite having been around for over 10 years. Every new piece of information literately can help shape how future studies are done or even how the current study is being done. All the questions you're asking are kind of we are trying to help frame our own staff for how we should proceed and we're asking advice on that.

DR. MILLER: I think that is more specific to each

different vector in product, just like as we saw this morning, where we saw that road map of how--one of the presentations this morning, they actually did this and they showed what they found as they went through their analysis, which seemed like a very reasonable approach to the detection.

I assume that was done based on some discussion with the agency back and forth. I think it's more important to sort of set a threshold for when you report and how quickly, so it is very clearly stated.

DR. SIEGEL: We do have a regulation, it's at 312.32 in the Code of Federal Regulations, that does require 15-day reporting of information which extends beyond adverse events if, in the judgment of the sponsor, it can be animal data and it can be product data if, in the judgment of the sponsor, it has significant potential implications for safety in humans.

Beyond that, there's much less specificity because the type of information is so much broader. We're much more specific in our regulations about reporting adverse events than reporting each new piece of information that develops regarding a product as it develops. However, we work closely with sponsors, and as Phil indicated, there is a fairly high level of expectation that important information is reported, reported promptly.

That doesn't mean, though, that if it is not, it is a violation. I do know that we know that we hear everything that happens when it happens.

CHAIRMAN SALOMON: I think that my comment is on regulatory sequences that might be identified. Today, I think we have some really very good technology in DNA arrays to look at transcriptional regulation of genes, and I think that is one of the things that would be relatively straightforward, I think, for a company or even for an individual investigator to do, to do some simple DNA arrays right now, most of which are commercial.

Yes, they can get expensive. No one needs to tell me that, but the technology is there and I don't want to get hung up on the expense issue, but I think that if transcriptional elements were found that were not expected in the original sequence, I think it would be a minimal thing to ask for some sort of experimental data looking at transcript regulation in those transduced cells or tissues.

DR. CHAMBERLAIN: I think it is still a real tough issue because it depends on what you mean by a transcriptional element. If you're characterizing a vector and you find that the CMV enhancer has somehow integrated into it, then that is going to be pretty obvious, but as Xandra was saying earlier, I think with current DNA sequence technology, for most insertions, you're really not going to

know whether you have a regulatory sequence or not and it really gets to be a very tricky issue.

The other problem is again, as we heard, the context. If you're designing a vector for muscle gene therapy, let's say, you're never really going to know if some of that vector leaks into the spleen, that you may have an enhancer that only works in spleen and not in muscle and things like that. I don't know what the test system would be and how you would go about doing that.

What I might suggest is that a lot of this depends on when you find these sequence alterations. Obviously, if it is early on, before you have started your clinical trials and the simple matter is simply to go back and remake the vector and make sure it's correct, if for some reason you find an alteration and it's very difficult to remake the vector, then I would think one would be expected to go through the typical pharm-tox preclinical studies that are required of any vector where you have made a corrective manipulation and proved that this alteration is not causing problems.

Maybe where the critical issue comes in is if you've already been doing a lot of clinical trials and something arises in your stocks that wasn't there originally, what do you do then? I think at that point then the responsible thing to do would be again make this

decision, can you go back and clean up your stocks and go back to using the correct thing or, if you feel you do not want to do that, then you should be expected to go back to the animal studies and repeat your pharm tox studies the same way that any vector would be expected to go through the approval process and show that this new change is not having adverse consequences.

CHAIRMAN SALOMON: We essentially agree. I was dealing with when you find a transcriptional element that you recognize, then you should be forced to show some genetic transcriptional regulatory data. I realize that it can be complicated because it could be cell-type specific in a tissue and then do you go in with a laser dissection microscope and all that or did it leak to the spleen. It can't be perfect, but at least what you're saying I agree with, that you should go back to whatever you established as the models that were approved on the entry direction for the initiation of your trial and go back and show that what you expect is what you expect and not that there is now suddenly a tenfold difference in expression of a gene of interest.

DR. NOGUCHI: Dan, I think we're getting the feedback that we need on that and while I would personally like to continue, we could probably spend a day here, but we do have another whole session. If we could move to three, we would appreciate it.

CHAIRMAN SALOMON: Let's spend the last 10 minutes here and go to question three. This is an issue of changes in vectors during the manufacture process leading up to the trial. You have sort of gotten in through your original trial. You've done your proof of concept. You've demonstrated a potential clinical utility. You're gearing up to do your Phase I or Phase II. Again, we promised not to get hung up on that, and as you roll into it, you now make a master production cell bank, and the questions in three now are meant to briefly suggest what should be done at that level.

And this now gets to the issue that this gentleman raised in the morning, of, well, wait a minute now, it is one thing for me to sequence my plasmids, but for an AAV vector, for example, if I now have to do a whole production run and sequence it, that is a serious issue. We don't have a whole lot of time to discuss this, but is there like any key points that the group wants to make on this issue in a few minutes?

DR. NOGUCHI: Dan, just a clarification, I think we have to some extent beaten the sequence and--

CHAIRMAN SALOMON: I'm not talking about sequence now.

DR. NOGUCHI: Okay. I mean, here we're talking about as you're producing lot after lot, some of the lots

may not be exactly the same. What are the most sensitive ways that we can detect that?

CHAIRMAN SALOMON: Phil, given that our time is short, what would be the one question you want us to comment on then in this time, you or Jay?

DR. SIEGEL: Just to frame the broader question, then I'll let the others frame it more specifically, but the issue we're talking about here, I believe, is not so much a change in the masters cell bank, which hopefully you establish later on, but from production run to production run, each time you expand a virus many logs to produce a lot of clinical material, you run a risk that, at some early stage in that production run, there could be a mutation, and so that lot of clinical materials in substantial portion are different from other lots, and then the gist of these questions is about how best to assess that sort of genetic stability. Is that not right? How can we focus best in a five-or-10-minute discussion in terms of what we could use?

DR. BAUER: I think the best focus would be if we could have a discussion of what kind of techniques could be applied. I think that Jeff Chamberlain talked about some of those issues earlier in his talk, but to expand on that would probably be the best.

CHAIRMAN SALOMON: Let's pick that up. If you have to do an analysis of a vector lot during production,

can we do restriction mapping and, if so, how should that be done? Is there what we're talking about? I don't want to use the "S" word, the sequencing work.

DR. SIEGEL: You've sequenced and done whatever else you want to on your starting cells. Now you've produced something to give to humans. What do you need to do in process and at the end?

CHAIRMAN SALOMON: Southern blotting, PCR, quantitative.

DR. BAUER: The bottom line is with the techniques that are available, such as restriction mapping, Southern blotting, PCR-sequencing one lot, whatever it is, are we confident that those catch the events we want to see and how concerned are we with that, and what would be the techniques, first of all, to apply to that?

CHAIRMAN SALOMON: We have a lot of virus at ten-to-the-ninth viral particles, but what do you guys want to know about that?

DR. ANDERSON: I think what is clear is we don't know any more than you know and that is what you wanted to find out. That is the reason for having an advisory committee. If the advisory committee can't advise you, then you know as much as there is to know. Certainly as much information as you can get--and you already know if you do a restriction map you will find big things, and as you be more

refined, you will find smaller things.

I think what I am comfortable with is that the FDA recognizes that there is a balance here, that you can't just say you have got to tell us everything, and I am comfortable that you are as aware of the issues as we are.

CHAIRMAN SALOMON: French, I think the issue that they want us to address is also is there a limit--I mean, we have to recognize the fact that any of these viral products used in a clinical trial, in vivo or ex vivo, is going to have a percentage of recombinant or deletional mutations or whatever in the mix in these ten-to-the-ninth particles. Is there some sort of a limit to that that we want to advise them for allowing--we're talking now production runs?

DR. ANDERSON: I am very aware of what the issue is. There is no question that that happens. Those of us who have been, in part, responsible from looking at data from production runs are very aware of it, and my feeling is the FDA knows as much as we know. In fact, you know more than we know, when you get right down to it.

If we had lots of time, we could talk about it all day, but I don't think we have anything useful to tell you that you don't already know.

CHAIRMAN SALOMON: That's going to make for a short day.

DR. SAUSVILLE: I would just want to know if one

is comparing, for example, a master cell bank with what is produced ultimately to be used in a clinical trial, why would we want to have any different criteria than what went into setting up the master cell bank? If sequencing is as easy as it is thought to be in certain size ranges, why not sequence the product lot, as well, at some frequency?

DR. NOGUCHI: Not to prolong that, but I think that was one of the things that Dr. Bauer was bringing up. If we really wanted to detect a low level of genetic variation, sequencing 100 clones would probably not even begin to be adequate, and so from a practical point of view, even if you could sequence 100 clones, could you do it in a several-week period and then analyze it throughout all the areas, do the back-crossing? While it is an ideal situation, what we're really asking here is other than sequencing, what are the genetic tests that can give us an indication of how good that lot is or how reproducible that a lot is?

DR. GORDON: I think certainly sequencing 100 clones would not be very helpful. That would only give you a one-percent resolution. I think the test applied would have to be appropriate to the situation. If you are looking for infectious particles, for example, that can be an extremely sensitive test. Where that is not relevant, there are PCR, quantitative PCR methodologies--there a lot of

methodologies that would be more than sequencing.

I think one of the other issues, though, is what level of detection would you find to be unacceptable? If you found some mutants, at what level does that mean you have to do something or not? Every lot is going to have something in it, so I think the more relevant question is how to intervene in the context of getting that information or if to intervene.

DR. BREAKEFIELD: Well, I was just trying to look through this list and see what I thought would be reasonable. I think certainly a restriction digest with a lot of enzymes, you want to make sure there's no big change, like French said. With these vectors, you can't really PCR across all of them, but PCR is very sensitive and it could pick up if there were some changes, some more subtle rearrangements, maybe one in 1,000, like a restriction digest might be one-in-100, if you were really lucky--this might get you down to another level.

It wouldn't be comprehensive, but it would just give you some idea if there had been some major rearrangements. You would have to do like PCR sets across the region, and also I was struck by what Jeff said, that in some of these like gutless antibodies, you might get rearrangements and if you actually kind of did quantitative PCR and compared the relative ratios across the genome, you

might get an idea whether there had been a loss of something in a major fraction, you know, and could be fairly sensitive, and then some kind of relatively straightforward gene expression profile, like you were talking about, seems like something that could be done fairly straightforward, and at least we'd be covering a little bit of the gross changes, you know, some minor changes and then some gene expression changes which we certainly want to know about.

You have to make them doable, you know, and sequencing isn't going to help you in this case.

DR. CHAMBERLAIN: Yes, I agree with those points. I would just follow up a little bit to keep in mind that when we're talking about production lots, you're going to be somewhat limited with the material that you can deal with, and so there are so many assays that you can do, and the restriction mapping, southern analysis, PCR methods, are ways, are probably the most sensitive methods that we have that will go in and pick up major abnormalities.

I don't think any of these methods are going to pick up more than about a one-percent variation in your sample. I don't really see any way around that. The ultrasensitive assays that we have referred to a little bit, for example, with adenovirus, there are replication-competent adenovirus assays that will pick up one-in-ten-to-the-ninth and all that, but those are extremely unusual

assays that require a specific and selectable biological event, and there's not going to be any assay comparable to that for random genetic variation, and I think we're just going to have to live with the idea of testing your vector as extensively as you can before you get into the clinic, and then just live with the limitations of the assays, that as long as it is 99 percent the way it is supposed to be, that you will have to live with that.

CHAIRMAN SALOMON: I think, for interest of the time, that is as well-put as I can do it. I think we have to realize the fact that these are biologics and I think that is the spirit of the committee, if no one disagrees, that if you look at an erythropoietin production run or recombinant human growth hormone or any of these production runs, you know, then you would say, well, yeah, we need then sequencing of ten-to-the-ninth molecules of EPO before we will accept it, and that is kind of what we're doing here.

I think we have to accept the fact as physicians and the public needs to realize that these are biologics and there's going to be variations there and it is going to have to be part of the consent process, I would suggest.

DR. MULLIGAN: I think, though, even out of one percent, there are certain contexts where one percent would not be detectible, say placqued assays or PCR, subtle differences, point mutations that make mutant proteins. I

think those are contexts where you would want to perhaps, in preclinical work, at least, say what are the chances that, at about one percent, I will get a mutant hemophilia sequence, so I think these are all context-dependent, but those sort of issues are very key.

Also, I think that for the question of how heterogeneous is your virus prep, those things, I think, should be under the preclinical stage. So, if you want to look at adenovirus vectors, say gutless vectors, I do not think it is unreasonable to sequence 100 or 200, do some sort of find restriction or restriction mapping with four cutters or something just so you can present, you know, we don't think this is a likely thing that is going to happen because we looked at 100 of these, so we know it's not going to happen with frequency.

I don't think you would actually do that from lot-to-lot, you wouldn't test it that way, but I think we should, as Xandra said, make sure that the test we do, however gross the tests are, would pick up real rearrangements. I know in the Wilson trial, there are gross rearrangements that were trackable back to an earlier point, in some of the virus seeds, and those things we have easily the methods to make sure that does not happen, and so those tests should definitely be on the final product that goes into the patients.

The other thing I would mention is like with retrovirus vectors, is that some of these vector systems have a literal inherent variability, and that will never change. If you look at your retroviral particles, you will, if you sequence enough things, see that there are differences. I think the FDA and everyone has to be comfortable when you're using these complicated things, there are just biological processes that are not perfect and we may see more and more, with other vector systems, that the same thing does happen.

It is possible we may have to cope with accepting a certain level of natural biological difference that we have.

CHAIRMAN SALOMON: Obviously, I agree with that. I think we have come to some consensus, in that for each of the vector systems that you approve for clinical trials, we're going to have to look initially at--in the preclinical phase, to get some sort of indication from the investigator or sponsor whether or not, if you have 16 passages of a producer cell line, that suddenly 20 percent of the viral particles are recombined and mutated, and if there is that much drift, then it is probably a very reasonable question for the FDA to either say go back to the drawing board and come back with a more stable system, or impose a more strict post-manufacture monitoring, as opposed to let's say a

system that came along around the same time that had a much higher degree of fidelity over the long-term, but in the end, it is a biological product.

Okay. Now we get to go on to what we're supposed to be doing this afternoon, and I again am grateful for the patience of the speakers who are to start the open public hearing. Without any ado, I believe I'm okay in introducing Dr. Janet Rose-Christianson, of Targeted Genetics, who has been invited to speak. Please.

DR. ROSE-CHRISTIANSON: I believe all of you should have a copy of what I'm going to be reading, but I would like to just go over some comments. Targeted Genetics Corporation is a gene transfer product manufacturer, as well as a clinical trial sponsor. Our products encompass both synthetic and viral vectors, delivering a variety of genes for therapeutic and prophylactic use.

Therefore, we have a very keen interest in the topics being discussed at this advisory committee, product characterization, preclinical animal models and long-term patient follow-up. I would like to address these topics individually.

Regarding product characterization, from that perspective, we believe that the currently available CBER documents provide sufficient guidance at this point and in this area. We believe that gene transfer products can be

handled in a manner analogous to the approach previously used for other formerly-novel biologics, such as recombinant DNA and monoclonal antibody products.

We also believe that a gene-transfer product has the potential for meeting the definitions of a well-specified or characterized biologic. Regarding preclinical animal models, we do support the judicious, but not gratuitous, use of animals. Toward that end, we support the use of appropriate animal models, using rodents whenever possible.

The need to use non-human primates should be carefully evaluated before any study is initiated. Each animal must be necessary to achieve the study objectives. As more information continues to be garnered pertaining to specific vectors, we would propose that a consideration be given towards platform preclinical studies. For example, adeno-associated virus, or AAV, is the vector that we're using to deliver genes targeted to treat several different diseases, such as cystic fibrosis and hemophilia.

Clearly, preclinical studies must mimic the anticipated route of delivery in the clinical trials. However, we believe that there should be some consideration given to the biologic behavior of the vector class itself. This would reduce the need for costly and redundant studies, yet provide sufficient information to initiate clinical

trials.

We would ask the committee to consider the use of smaller confirmatory studies to evaluate biodistrubtion, for example, of the same class of vectors produced by similar methods and delivered by the same administration route, despite a different transgene.

Long-term patient follow-up--we have amassed significant experience with long-term patient follow-up and really thank you for the opportunity to share these experiences. This includes the lifetime follow-up for retrovirus vector trials performed in the early 1990s, as well as adeno-associated virus vector and non-viral trials, which is required by the RAC.

To summarize, long-term patient follow-up is challenging at best and impossible at worst. Although our experiences have been shared with the FDA, I would like to tell you about some of the problems we have incurred in performing lifetime follow-up. Number one, once a patient has completed the study, there is often little motivation for the investigator to keep track of each subject. The value of performing this follow-up is difficult to ascertain, because to the best of our knowledge there has yet to be a report issued from the data collected by the FDA to date.

Number two, autopsy specimens, in general, have

not been available because the request for an autopsy was denied or the investigator was not promptly advised of the death, therefore the patients had been interned, cremated, et cetera.

Number three, we have encountered several barriers to patient follow-up, the most problematic include, A, a lack of investigator motivation to perform follow-up once the site is closed to active follow-up; B, from a scientific perspective, many of the gene transfer systems do not result in the long-term persistence of the transgene and transduced cells. It is, therefore, difficult to justify performing expensive and complex testing on autopsy samples in situations were there is little, if any, likelihood of finding vector sequences. Further, the false positive rate of these type of PCR assays at high sensitivity levels makes it probable that positive signals will be seen at some frequency, further confounding the issue of true long-term persistence.

C, patients are often lost to follow-up; D, the costs associated with long-term follow-up, especially when it is a lifetime requirement, these costs are attributable to sponsor and investigator time, the need for continuing regulatory filings and FDA interactions, and long-term database management; E, to date, we're not aware that there is a compelling scientific data indicating that lifetime

follow-up is really useful.

Although the data are presumably submitted by other sponsors and investigators, there is yet to be any data or analysis released to those supplying that data. Going forward, we offer the following suggestions for consideration in addressing long-term patient follow-up. Number one, define patient follow-up requirements considering the following: the vector class, the level of the effective dose; the disease being treated; the maximum number of patients needed to be follow-up, not just everyone; route of administration and the treatment being investigated.

Additionally, we would like to suggest that we need to ensure there is some sort of sunset and/or review provision to reassess the need to continue long-term patient follow-up. Would a maximum of five years be appropriate? For example, we performed trials involving the delivery of the aerosolized AAV vector for treating cystic fibrosis.

As our patients have mild-to-moderate disease, it is likely that many will survive for 20 more years. How long should we follow these patients, especially in Phase I studies were a no-effect dose was administered? Is this really cost effective?

Number two, delineate the relationship of long-term patient follow-up and product registration. Will trial

completion and closure be a requirement before a product can be registered? If so, this could be a very huge issue. Three, issue an epidemiological report of the long-term patient follow-up data each year. To date, we're not aware of this information being made available. It would truly be useful to all those involved in this research to have access to this data.

Four, assess the cost-effectiveness of this reporting with respect to patient safety. What has been learned so far?

Five, specifically define the expectation of sponsors pertaining to long-term follow-up and what is considered acceptable.

Six, harmonize any long-term follow-up with the RAC. Otherwise, there will be additional costs associated with providing different reports with differing monitoring strategies to two HHS agencies.

Seven, use vector system and route of administrations as two major criteria for follow-up. How many patients must we treat before we can stop performing long-term follow-up? Can given classes of vectors be excluded from long-term follow-up at some point? It would be also useful for the committee to provide the rationale for long-term patient follow-up.

As you may know, long-term follow-up is not

required for patients who participate in clinical trials of other non-gene-transfer drugs that are known to modify DNA or vaccines that are remarkably similar to current gene transfer vectors, yet have escaped similar regulatory scrutiny. Is there a scientific basis for this type of follow-up being performed exclusively on gene transfer patients?

To conclude, we believe the length of time for following a patient who has received gene transfer agents in a clinical trial should vary and be based on defined parameters. We believe that these parameters should take into account the phase of the clinical trial and the disease being treated. As an increasing number patients are not imminently facing death due to their disease are enrolled in these trials, the length of follow-up should be modified or appropriate to the disease.

I would like to thank you for your time and your attention.

CHAIRMAN SALOMON: Thank you. I should point out for, unless just so there's no confusion, the first part of your comments will be this afternoon. The long-term follow-up issues will be dealt with tomorrow morning.

I would like to invite the second speaker now, Dr. Russ Lyons from Genetic Therapies, Inc., Novartis.

DR. LYONS: I want to thank the advisory committee

for giving me a few minutes to talk about some of our preclinical data. My comments will be limited to comparison work that we have done in mice and monkeys with adenoviral vectors, specifically an AB3 vector that is deleted in E1, E2A and E3. We do have plans to extend these studies to gutless vectors based on the findings that we have achieved with an AB3 vector.

To give you background on what the study designs look like, the route of administration is IV. The does used in both species was three-times-ten-to-the-twelfth particles per kilo, and we included four monkeys in the study and 30 mice. The time line is shown here. The important time points that I want to point out are the one-week time point where we took a liver biopsy on all four monkeys in necropsied 10 mice.

We did the same type of analysis at one month, again liver biopsies on all monkeys, necropsy on 10 mice, and then all animals were terminated at the two-month time point. The dots along the time line indicate other observations that are made. We do every-other-day observations during the first week, including hematology and serum chemistry, and then weekly observations throughout the study.

To show you what the clinical pathology looks like in both species, one representative enzyme, AST, both

species peak at about two days after administration and resolve within one or two weeks and continue to be normal throughout the study. The platelet drop that has been seen by other investigators, we see as well, with nadir at four days after administration and resolution by one-to-two weeks, and they remain normal throughout the study.

What I do want to focus on are the microscopic changes that were observed in the liver, and I have summarized these here at each of the biopsy or necropsy time points. At the one-week time point, the most prominent feature is extramedullary hematopoiesis in both mice and monkeys, although we do see a few changes with increased mitotic figures and increased actin immunoreactivity in inappropriate cells, and the mouse and the monkey look very similar in terms of the number of animals presenting these findings.

By the one-month biopsy and necropsy, the more prominent features are prominent sinusoidal lining cells, which we believe, based on immunostaining to be stellate cells or EPO cells. We also see now apoptosis in about half of the animals, a cellular infiltrate, more animals presenting with increased mitotic figures, and now many of the animals with actin immunoreactivity and the beginning of a laminin immunoreactivity. We also begin to see the onset of hepatic fibrosis.

By two months, the majority of animals show hepatocellular apoptosis, as well as these indications of an ongoing process of hepatic fibrosis. What I would like to summarize in terms of our comparison of mouse and monkeys is that we do see very significant species similarities, both in the acute effects, the time and durations of either an elevation of liver enzymes or the transient thrombocytopenia mimic each other quite well in both species.

The sub-chronic liver changes that were seen in both species are extramedullary hematopoiesis, activation of the stellate cells, and this is the inappropriate smooth muscle actin standing, activation of the stellate cells progresses to a morphological transformation to myofibroblasts, and we believe this is the mechanism that is initiated, leading to hepatic fibrosis.

What I didn't mention where nuclear inclusion bodies. By the two-month time point, we see nuclear inclusion bodies that are fibrillar in nature, that have not been described in monkeys or mice by anyone else with any other compounds. So, these are still under investigation. The differences that we see in mice all pertain to the sub-chronic liver changes. The hepatocellular damage appears to be more severe in mice than in monkeys.

There is a more prominent cellular infiltrate. The distribution of the findings and effects in liver seem

to be more patchy. We have also detected nuclear inclusions in non-parenchymal cells. The nuclear inclusions that I referred to up here all occur in hepatocytes and in mice we also detect nuclear inclusions in stellate cells.

Another finding in mice that we have not at this point tried to duplicate in monkeys is the activation of oval cells. Oval cells are liver stem cells that are activated in response to severe hepatocellular injury in instances where the hepatocytes can no longer or no longer have a regenerative capacity.

I would like to conclude with this summary of findings. We believe the acute and sub-chronic changes are similar in mouse and monkey. The acute changes in clinical pathology are dose-dependent in both species. The sub-chronic changes are dose and time-dependent. The effects progress over time and the microscopic changes cannot be identified with standard H&E.

Many of the changes we found required immunostaining and analysis at the ultrastructural level with TEM. The liver fibrosis does not correlate with any clinical pathology parameter. All the clinical pathology parameters are normal by the one-and two-month time points, yet there is a chronic process of active hepatic fibrosis.

So, our conclusions with regard to AB3 vectors for systemic use are that mouse is an appropriate species for

assessment of adenoviral vectors. We did not detect anything in the monkey that was not present in the mouse. The long-term microscopic evaluations are needed, regardless of vector persistence. In the monkey, we have not been able to detect vector in the one-month and two-month biopsy, yet the process of hepatic fibrosis is continuing.

The surrogate markers of liver fibrosis will be needed to monitor patients treated with adenoviral vectors, since we do not have any clinical pathology correlate at this point.

Thank you.

CHAIRMAN SALOMON: Thank you, Dr. Lyons. That was extremely on point to this afternoon's discussions about models. I think these two speakers have made us a nice transition into the afternoon session, which is entitled Preclinical Safety and Efficacy Testing of Gene Transfer Vectors. I think that really the big challenge here is to-- I think of us accept, particularly as we have evolved the discussion in the morning, that safety issues are critical for allowing regulatory decisions on gene therapy trials.

We also were reminded that these kind of preclinical models are critical measures of safety. In other words, just doing sequence analysis, which I think was clear to all of us, is not going to guarantee safety. It doesn't mean it's not important, but is not going to

guarantee safety, and so these have become very important. At the same time, insisting that every gene therapy trial have a primate model study is something this committee would have to consider very carefully, as the effect on trying to develop trials in academia would be devastating, if that was required.

I think it is really important now to consider these sort of issues, is when is the mouse model equivalent or even better than a monkey model? When is a monkey model required, and if a monkey model is equivalent, what kind of additional information we can get from it? With that, I would like to introduce Dr. Anne Pilaro, who is going to do the FDA introduction on preclinical models.

Dr. Pilaro?

DR. PILARO: Thank you, Dr. Salomon. I'm going to speak you today about some of the uses of animal models in development of drugs or biologics or gene transfer vectors and focusing today on vectors for gene therapy. The initial steps in the development of a new gene transfer vector can really be broken down into three areas, and that is the characterization of the product, looking at the manufacturing and the quality control issues, some of which we touched on this morning, the demonstration of biologic activity is proof of concept that the vector is doing what you think it does, and safety, and that includes toxicology

testing in animals, as well as biodistribution.

The purpose of doing the bioactivity studies in preclinical models, especially models of the disease, are really to establish the rationale for conducting the clinical trial in humans. What we really hope to get out of these models is an understanding of the feasibility of the gene transfer, the duration and levels of gene expression and the degree of functional correction that is needed to give an important clinical benefit to the patients.

We also hope from the animal studies to be able to optimize the dosing and the regiment for the clinic. What is the optimal schedule for administration of these vectors? What is the optimal route of administration?

We also, from doing these preclinical activity studies, frequently get additional information about what species is more appropriate for conducting further testing, including toxicity and biodistribution studies. The goals of the preclinical toxicology testing are really no different from the goals of testing for other biological therapies or drugs.

What we want to be able to get out of these studies is to be able to recommend the initial safe starting dose for the clinical trial and a safe dose escalation scheme for humans. We want to be able to identify potential target organs for toxicity, which leads us to be able to

identify appropriate parameters to monitor for the clinical trial. For example, if you see a liver toxicity in the animals, you would want to be monitoring patients for serum levels of transaminases. You also want to be able to identify any patient populations that may be at risk from the intervention.

Using the hepatic example again, you would want to exclude patients with any previous evidence of hepatic damage. The biodistribution studies, we will hear a little bit more about tomorrow morning, but they're really designed to address two issues, and the first is the dissemination of the vector to the germ line and the potential for any transfer to future progeny.

To date, total gonadal tissue has been identified and assayed. It is not been germ cells themselves. The other purpose of doing biodistribution studies is to look at non-target tissues. Does the vector stay where you put it or does it go to a non-target organ and what are the consequences if the gene actually gets there? This gives you information that lets you plan a better toxicology or preclinical safety trial.

Both of these issues may be addressed in the same preclinical study. What you want to get when all your preclinical work is done is an idea of what is the relationship of the dose of the vector to the biological

activity and what is the relationship of the does to the toxicity and how far apart are those two, what do the curves look like, do they overlap or is there a good separation that gives you a good margin of safety?

You want to be able to understand whether the dose or the route of administration or the schedule of administration can affect the toxicity or the bioactivity, and you want to be able to identify the risks for the clinical trial. The bottom line is that animal data are important because prior to availability of human data, the toxicology and the bioactivity or pharmacology data provide the sole source of data on which safety assessments can be made.

I want to touch for a minute on some of the models of animal diseases that model human disease that have been very useful and been evaluated in some of the gene transfer studies. These include the Wobbler mice for ALS or Lou Gehrig's disease, the W/WV mice in Fanconi's anemia, the sparse-fur mouse model for the ornithine transcarbamalase (ph.) deficiency, the MDX mice in muscular dystrophy, the cystic fibrosis knockout mouse for CF and several non-gene-modified or non-knockout models that are actually spontaneously occurring, including the Watanabe rabbit for hypercholesterolemia and hemophiliac dogs and various mucopolysaccharide defects in dogs, cats and rats.

These models have all been utilized for preclinical efficacy studies and, in some cases, safety studies and have really proved very useful in gene transfer research and forwarding the field. I want to present a couple of case studies now on how preclinical data have allowed clinical trials to go forward and what the different areas have been.

The first case study will focus on using an animal efficacy model to support the study of a gene therapy for cancer. The disease is metastatic breast cancer. The gene defect is not really known. It may include several. It may include alterations in BRCA-1 or in c-fos expression or P53 down-modulation or others. The clinical outcome is usually very aggressive disease. It is spread through the lymphatics. It mets to the lung and to the chest wall, peritoneal cavity.

There is poor response to chemotherapeutics and the medium survival is less than 40 percent over a five-year period. The gene therapy approach taken by this particular group is they used a retroviral vector, expressing the multi-drug-resistance-1 or MDR-1 gene. They took CD-34 stem cells, transduced them ex vivo with the retroviral vector, and planned to reinfuse them into patients after serial high-dose chemotherapy.

The hypothesis behind this approach is that MDR-1

expression by the stem cells will confer resistance to taxol and other marrow-toxic agents, allowing the doctors to give higher doses of these agents and potentially increasing the anti-tumor affect. The preclinical studies in support of this came both from in vitro and in vivo data.

The in vitro data were done as proof of concept studies showing that they could get effective gene transfer and resistance to taxol in both murine and human stem cells.

What they found in these studies is that there was a higher level of gene transfer observed in the murine than the human stem cells by about 30-to-50-percent higher. They did see no inhibition of cell proliferation or altered cell phenotypes after flow cytometric analysis. They also saw that the gene was functionally active. They used rotamine-123 eflux and flowcytometry as a measure of MDR-1 function and they actually saw that following transduction, they had an increase. Although it is somewhat modest in the human cells, it did support their hypothesis.

In vivo studies were then planned, serial bone marrow transplants in lethally irradiated mice with MDR-1 gene transduced stem cells and escalating taxol doses were done to select for the MDR-1-positive cells. The safety issue behind this trial was would transfer of the gene or expression of the MDR-1 gene actually affect engraftment or reconstitution or the function of these marrow cells.

For this particular study, there was no formal toxicity testing conducted. However, what this group did was they built the toxicology endpoints into the efficacy study and followed it out over the duration of their study. What they saw was there was no adverse effect of the gene transfer on engraftment or on reconstitution of marrow populations.

They had a beneficial effect on survival in these mice. They had resistance to escalating doses of taxol. They were actually able to treat these animals at LD-100 values for taxol and all the animals survived. There was also support from the literature that this group used that showed that transgenic MDR-1 mice had no adverse effect on engraftment.

In this first case study, what we have seen is an animal model of efficacy was also used at the same time to gather safety information, and this gave us a better understanding of what was actually occurring in something close to the clinical setting.

In the second case study, I'm going to compare toxicity of vector in monkeys and mice, such as we just heard from Dr. Lyons. The diseases is hemophilia-A. The gene defect is a mutation in the Factor VIII gene. This results in either defective or absent Factor VIII production.

Clinical outcome, this is an X-linked disease that affects males only. There about 15,000-to-20,000 males in the United States affected. The severe form of the deficiency results in factor levels of less than one percent of normal, and this is associated with increased morbidity and mortality in these patients.

Uncontrolled bleeding events can require exogenous Factor VIII to stop them. Patients can be treated prophylactically with IV Factor VIII, but it is very expensive and very difficult to do. They have to be given frequent injections. The repeated bleeds into joints can lead to significant joint disease? Bleeds into the-- intracranial bleeding, rather, is fatal, and so there was a need to develop a therapy to treat these patients.

The gene therapy approach was to take a mini-adenovirus or gutless adenovirus that we heard from Dr. Chamberlain this morning carrying the Factor VIII CDNA. It is given by IV infusion to target the liver. Preclinical studies showed that about 90 percent of the vector uptake was in the liver after IV dosing. The vector itself contains a liver-specific promoter region, so it is only expressed in hepatocyte tissue.

Patients can be monitored for gene presence and for expression by measuring the levels of Factor VIII in the plasma, and this is done by an ELISA assay. The goal of

this study was really to see if correction of Factor VIII levels could be achieved in a range that might be therapeutically beneficial, and the hypothesis is that correction to as low as one-to-five-percent of normal could actually lead to an improvement in phenotype.

The efficacy data were obtained in hemophiliac mice, the Factor VIII knockout mouse models on a black six background. Doses from 2.4-times-ten-to-the-eleventh to eight-times-ten-to-the-twelfth vector particles per kilo were given IV. Detectable Factor VIII plasma levels were present in the plasma out over six months and the longest mouse actually went out to 247 days. Peak Factor VIII levels were actually present in levels that are therapeutic. They were 164-to-892 nanograms per ml, while the normal level in humans is approximately 200 nanograms per ml.

In the hemophilic mice, they had restoration of clotting time to within normal limits. Similar results in terms of the factor levels of production and the duration of expression were also seen in normal C-57 mice or normal bulb-C (ph.) mouse, so on a different background, you got the same effect. The toxicity studies for this particular product were conducted in normal mice. Animals were dosed with four-by-ten-to-the-tenth to four-by-ten-to-the-twelfth vector particles per mouse by IV injection.

This is a single injection on day one and they

were followed out in this study for 30 days. Toxicities included a transient dose-related decrease in platelets at day four and a dose-related increase in liver enzymes, ALT, in particular, also on day four. This is again similar to what you just saw from Dr. Lyons.

On histologic evaluation of the different sacrifice time points, there was minimal microscopic pathology in the liver. We were able to determine that the no-effect level dose for toxicity in this group of mice was four-times-ten-to-the-eleventh vector particles per mouse. This group also did preclinical toxicology studies in cinemologous (ph.) monkeys using two monkeys per group, three dose levels, separated by half-log. They also, I should point out, used the dose of 4.3-times-ten-to-the-eleventh, which is the maximal dose plan for their clinical trial.

Again, they saw transient dose-related increase in platelets and an increase in ALT on study day three. Histology in these animals was not done until study termination at day 30. At that point in time, there was no distinctive toxicity observed in the liver. These livers were no different from the control animals. The no-effect level dose in this group of monkeys was 1.4-times-ten-to-the-twelfth vector particles per kilo.

What we actually did was go back and compare the

data from the different studies in mouse and in monkey and look across them to see what are the similarities or the differences. This is looking at data from individual animals in terms of monkeys and mean values for different groups across a couple of different studies for the mice. Factor VIII levels were pretty comparable in the mouse studies between the balb-C(ph.), the C-57 and the Factor VIII knockout mouse.

The range of Factor VIII levels seen in the GLP tox study was 202-to-490 nanogram per ml. In the two monkeys that actually were at the highest dose group, peak Factor VIII levels were seen of 88 and 24 nanograms per ml, so they did not quite reach the same levels as the mice did. ALT was also elevated in both groups, but this is still within normal limits.

Pointing out to the monkeys, the elevation was barely one-and-one-half times over background. The elevation of the mice was a little bit different. There was one outlier that actually had to be discarded because the sample was hemolyzed. The other two animals in that group had elevations that were just outside normal limits or within normal limits.

The platelet value is a mean value for three animals in the group, for the mouse, and it was 44,000 at day four and that was the nadir that was reached. At day

three in the monkey, the nadir that was reached was 15,000 or 34,000, so similar to what was seen in the mouse. When we actually went back and recalculated the no-effect level dose for the mouse, it was previously presented as vector particles per animal. When it was gone back and calculated on vector particles per kilo, it was determined that it was virtually identical to that which was observed in the monkey, 1.6 versus 1.4-times-ten-to-the-twelfth vector particles per kilo.

I do want to point out that these data have been publicly presented before at the RAC meeting and we did request permission from Genstar Therapeutics, their representative is in the audience today, and they were gracious enough to allow us to present these data today. The last case study that I want to present to you is how safety data in animal models compare to actual safety data in humans.

This is a somewhat old set of information here, but it is still one of the best representative sets of information we've seen with different species, compared to the human. The disease is cystic fibrosis. The gene defect is the mutation in the CFTR gene resulting in defective chloride secretion and defective mucus and bacterial clearance in the lung.

The clinical outcome is chronic respiratory

infections and digestive disorders, sterility in males and death by the age of 35-to-40, although this is beginning to creep up a little bit. The gene therapy approach for these initial trials in CF was to use an adenoviral vector carrying the CFTR CDNA. The plan was to instill this through a bronchoscope into one lobe of the lung so that if any toxicity was seen, other lobes would be spared.

You could also monitor for the presence of the gene in airway epithelial cells by doing brush biopsies and analyzing them by PCR and RT-PCR. In looking at the safety profile for this, animal models of the disease were not appropriate for obtaining information about the pharmacology or the efficacy. The CFTR knockout mouse does not have the same lung pathology as the human does.

It has a secondary backup chloride channel in the lung that functions in place of the missing CFTR. As a result, it has the digestive disease, but no long pathology, so it is not a really useful model to get activity information. Basically, what was done here was in vitro studies were used for efficacy. Safety studies were done in different animal species.

The preclinical safety or toxicology studies showed a dose-limiting inflammation in the lungs. This was observed in all species tested, be they rodent or non-human primate. It was dose-related and there was a very sharp

threshold. There was only a two-to-five-fold difference between the no-effect level dose and the toxic dose. The original clinical trial was conducted starting off at a dose of two-times-ten-to-the-seventh platforming units, or PFU, of the virus instilled into the lobe of the lung.

A dose-limiting inflammatory reaction similar to what was seen in the animals was observed at the next-highest dose level, which was two-times-ten-to-the-ninth PFU. This trial was stopped because of the toxicity and re-evaluation of the data was done at this point in time by both us and by the groups involved.

What we basically did was went back and conducted an analysis of all the different species that were used, and what the no-effect level doses were. When we actually looked across the species, the no-effect level dose was between 1.7 and 2.4-times-ten-to-the-ninth platforming units per meter squared. The doses here are normalized to total body surface area, since lung surface area, which would be what we would expect the toxicity to be based on, scales to body surface area, and this is a more appropriate way of comparing the data across the different species.

What is important to note is that at this point in time when these data were obtained, the no-effect level doses in the animals were approximately equivalent to the toxic dose that was observed in the human. The toxic dose

in the human was 1.2-times-ten-to-the-ninth infectious units, in this case, per meter squared, as compared to the PFUs per meter squared up here.

We're right about in the same range and, as I mentioned, there was a very sharp threshold here. What this wound up doing was the clinical trial was reevaluated and then redesigned. A smaller volume was used to treat patients, since that was one of the contributing factors. A second generation E1E3-deleted vector with some small changes in the gene sequence to make it safer was also used.

A spray-gun approach, by which the director rather than being instilled into the lung, was actually applied locally in a tiny droplet formation. Single and repeat administration dose cohorts were included in this trial design. The clinical data from this trial showed that a single administration was well-tolerated up to 3 million PFU. It was a dose-related, positive gene transfer.

Actually, that should be three-times-ten-to-the-eighth PFU. Repeat administrations were tolerated up to two-times-ten-to-the-ninth PFU per dose. They actually did more higher dose levels in this trial since they had shown in the single-dose levels that they were safe.

Gene transfer was positive after the second administration, but not after the third repeat dose in the highest dose group, and this was due to development of

antibodies. These data have been published and have been used by permission, as well.

In summary, what we can say from the studies is that safety data can be obtained in a well-designed efficacy model where you can answer two questions at once. Mouse studies can provide similar information as studies conducted in monkeys. We have seen that now in two separate instances, and no one species may be predictive of toxicities in humans. Not all toxicities may be seen in all species and even humans may not be predictive of toxicity seen in other humans.

Our next three speakers today are going to give us a little more information about preclinical models for gene transfer. Our first speaker, Dr. Estella Jones from the Division of Veterinary Services at CBER, will give us an education on the use of nonhuman primates in biomedical research. Our next speaker will be Dr. Katherine High from the Children's Hospital, Philadelphia, who will talk about answering questions in an animal model of the disease that could not be answered in a normal animal model. Our last speaker will be Dr. Richard Whitley, from the University of Alabama at Birmingham, who will speak to us about the use of aotus monkeys to address the latency issues involved with herpesvirus.

I would like to thank you and introduce Dr.

Estella Jones.

CHAIRMAN SALOMON: Dr. Pilaro, before you sit down, just to make sure that, if we're going to use a jargon, that we are all familiar with it. Can you just be a little more specific as to this no-effect level dose? There were lot of numbers thrown around and you were using it to compare the different models, so please explain that a little better.

DR. PILARO: In pharmacology and toxicology jargon, there are actually two no-effect level doses. The NOAEL is no observable effect level dose. This is the dose at which you have no pharmacologic or biologic activity. What I was discussing today, calling the NOAEL, is the NOAEL, or no observable adverse effect level dose. This is the highest dose a vector or drug or biologic, whatever it is that you're studying, that can be given with no discernible toxicity or no difference from control animals.

CHAIRMAN SALOMON: You compared them, then, in trying to come up with a way of using this to objectively compare the value of different animal models, you compared the NOAEL--

DR. PILARO: We compared the NOAEL, the no observable adverse effect level dose.

CHAIRMAN SALOMON: To another parameter that you didn't show on the slides, which was the therapeutic dose.