critical thinking. So critical thinking they are, sometimes I think that I founded VIRxSYS, I thought I founded the first debating society because we are always discussing things.

I think that makes for good science so I really am honored to have each and every one of you a member of our team.

I would also like to thank Rob MacGregor and Carl June and Bruce Levine and all the team up at the University at Pennsylvania for their collaborative efforts. I would also like to thank Tony Pascorelli, our CEO. He has done a great job in running the company.

That's it. I will leave it now open for discussion. Thank you.

DR. SALOMON: Thank you very much, Dr. Dropulic.

[Applause.]

DR. SALOMON: What I would suggest we do is--I have a couple of announcements. I would like to introduce a new person who joined us on the committee and then take a break, come back and start to discuss this.

So I guess first I would like to note the arrival of Dr. Marvin Reitz. Welcome. Dr. Reitz

is from the Medical Biotechnology Center Institute of Human Virology. Do you want to give us just a quick two cents for what you are doing there?

DR. REITZ: Actually, it is the University of Maryland Biotechnology Institute, Institute of Human Virology. We are an institute that is headed by Bob Gallo and mostly do work related to HIV. I am a molecular virologist. I have been fiddling around with retroviruses for about thirty years or so.

DR. SALOMON: Good. I am glad you are still healthy.

Ten minute break?

[Break.]

DR. SALOMON: I have one additional administrative duty at the beginning of this next session, and that is to introduce Marina O'Reilly from OBA covering for Amy Patterson again this morning. Welcome, Marina.

What I have decided to do here is, basically, go right to the questions, the specific questions from the FDA. I think that what we will do is we will begin a discussion of the questions which were posed by FDA staff specifically about the protocol.

The discussion that will develop is, obviously, specific enough to what you have presented that you will be more than encouraged to make your responses and comments.

I was trying to think of how to do this. You either go through all the different phases of this very complex system that you presented--I am afraid that it would degenerate into sort of an NIH study section which is not what we are here to do.

I think, rather, what I would like to do, in terms of being responsive to the very, very important issues that are on the table here would be to go to the questions and, as the conversation evolves, I think individuals on the committee can go to the details. I think, in this way, we will stay grounded in the specifics of the clinical trial and the major questions that were developed yesterday rather than getting lost as can always happen to a group of academics when they get presented a lot of interesting data.

So that is kind of a chairman's executive decision. If there is anybody who absolutely believes that I have gone inappropriately, I would like to hear it because I don't want to be tyrannical on this.

Is that okay with the FDA staff if I take that strategy. Okay.

**Open Public Hearing**

But, before we begin, this is also a time where we would normally allow public comment. Again, I don't change what we said yesterday, but let me repeat it for the record. Anyone in the audience is welcome to step up to the mike. You were comfortable doing that yesterday. If you were not here yesterday, then please note that you should be encouraged to do so and contribute.

But there is also a time for just a formal public comment before we begin any discussion and it is my understand that Dr. Susan Kingsman would like to start.

DR. KINGSMAN: Thank you very much. I think most of the discussion is going to relate to the very specific questions that have been posed. The only comment I would like to make is whether the safety of the trans gene has been adequately considered because I think there is a lot of focus on the vector system.

I just want to pose the question as to are we delivering, in this therapeutic strategy, a potent and specific mutagen of the HIV envelope

sequence, something that will change its sequence at a higher rate than the normal mutation frequency and that this mutant coding sequence, albeit partial or with low reproductive fitness, in an assay developed in the lab can actually be disseminated in patients with a very high viremia and it is in a region that is functionally important for the envelope.

Even if it doesn't change tropism, it could create new immune determinants. So I am not entirely convinced that there has been consideration of the trans gene, itself.

That is the only comment I would like to make.

DR. SALOMON: Thank you, Dr. Kingsman.

**Committee Discussion Of Questions**

DR. SALOMON: I am certainly not trying to restrict the scope of the discussions that now follow, but I want to try, before lunch--how is that for optimism--to answer the questions and then we will just see where this goes.

The first question is, "Is the VRX496 vector proposed for use in the clinical trial by VIRxSYS designed and manufactured in a manner to sufficiently address safety concerns relevant to

generation of replication-competent lentivirus?" That certainly is something that we discussed at some length yesterday; what is an RCL assay, what are alternative assays, what is the sensitivity?

"Please consider that the vector will be used in HIV-positive subjects. How does this use of a transient transfection system versus a stable packaging line for vector production affect the rate of recombination in a manner that would be sufficiently compensate for the use of one plasmid to encode all helper functions?"

So there are a lot of different pieces we could start off with, but let's start with Question 1 in general. Does anyone want to jump in and I will try and guide it.

DR. EMERMAN: This is Mike Emerman.

DR. SALOMON: Excellent. I didn't know you were on the phone, Dr. Emerman. But you are more than welcome to jump in.

DR. EMERMAN: Some of these questions are related with the RCL. We talked a lot about RCLs which are weird recombinants with VSV-G and things like that. The RCL that they--well, the lentivirus that they don't test for is actually the wild type HIV from their protocol, so they expanding HIV-

infected cells. There will be some HIV that comes out of that.

The previous person who spoke spoke to the real question here. What is coming out at the end of the transduction, at the end of the expansion, that is. So I think there has to be some kind of test for how much HIV is going back into the patient and what is the nature of that HIV. Is it a variant or is it what they started out with?

DR. SALOMON: Thank you. That came across very clear, by the way. I am just reminded, Dr. Cornetta--Ken, are you there as well?

DR. CORNETTA: Yes; I am.

DR. SALOMON: Excellent. You are also coming through very clear. So both of you, please feel free to participate.

Dr. Emerman picked up a theme that Dr. Torbett brought up during the presentation. Dr. Torbett, do you want to just sort of amplify?

DR. TORBETT: I do have a concern with that, given the length of time that the assay goes one. Furthermore, the studies earlier didn't indicate they were looking--in their preliminary work, it didn't look like they were looking for a CCR5 using variant.

I guess I have a little concern. Maybe it is out of scope right now, but it comes back to actually the trans gene, itself, and its ability to derive a different species. So the question I am posing is whether something that would come out, get back into the cells, they would be infused back into the patients, would create additional problems above and beyond what the patient has as his viral load, or her viral load.

DR. MULLIGAN: Back to Sue's point. I think that I would like to see more characterization of what that virus is, as we were discussing during the talk. We really need to look at the entire profile genome, not just the mutations, because it may well be that, as an entire package, the virus has replication competence and may have some unique biological properties.

I think that is very, very key and would help us further assess whether or not more kinds of tests are necessary.

DR. SALOMON: Just for my clarity, Dr. Mulligan, what virus did you mean just now needs to be characterized?

DR. MULLIGAN: The genome that had various

envelope mutations. I think what I heard was that the proviral sequences were never tested in totality. The mutant was taken and moved into an otherwise wild-type background and that mutant seemed to severely retard the characteristics of the virus.

The question is can we go from that data that the background of that particular set of mutant viruses is the same as wild type or have you, in fact, generated something, evolved something, that now has different biological properties.

DR. EMERMAN: Hello. This is Mike Emerman again.

DR. SALOMON: Yes; go ahead, Dr. Emerman.

DR. EMERMAN: I am not so worried about the virus that came out of that Sup-T1 experiment as much as I am concerned about the virus that comes out of the T-cell expansion from the patient. The Sup-T1 is obviously biased because they are challenging with homologous virus to the antisense. So they are going to come up with something there that has to evolve, has to change a lot more.

What comes out of the patient is not going to have to evolve as much because its envelope is

different than the envelope that is used in the antisense which was used from NL4-3. So actually that virus, the break-through virus in that one patient, that is the one I would like to see characterized.

In a sense, they have to know, for each patient, how much is coming out; that is, how much virus are they going to be putting back into that person as part of the protocol.

DR. SALOMON: Yes; I think that is very clear, Dr. Emerman.

DR. ALLAN: This is in the same line which is you have done a lot of work on looking at the escape mutants but only in terms of mutations within the vector. The issue about, well, it is NL4-3 so it shouldn't be any worse than the virus even if you got a recombinant than the wild-type virus. It also goes to what Susan was saying which is I don't think you can predict whether it is going to be better or worse or just as bad or whatever.

The thing is you could get gag/pol recombinants and then the virus that is coming out of those patients' T-cells could actually be replication-competent recombinants because you have

intact LTRs. You could have a gag/pol recombinant.

The issue with that also is, and this was alluded to earlier, too, which is the immune system. CTLs are supposed to very important for limiting viral replication in HIV-infected patients. If you introduce a different gag sequence, a different pol sequence, you may be generating viruses that are no longer recognized by those CTLs and so you may actually get an increase in viral replication in those patients.

So I think that is something else you have to consider, too. So I think, again, which is the same issue which is you really need to characterize what is coming out of those T-cells and those HIV-infected cells.

DR. REITZ: One thing that might be done is to do the Sup-T1 experiment but with cells that would support the replication of an R5-tropic virus like PM1 and then look for conversion, and do the experiment with an R5 input virus and see if you got conversion to any X4 phenotypes because the NL4-3 is a X4-tropic virus.

I think that would give you an indication of whether you could possibly change the envelope tropism of the patients' viruses.

DR. SALOMON: I think the other point I would make directly on that is I am uncomfortable with an experimental result in which there is some evidence for a breakthrough in in vitro that, then, goes and clones 200-and-some clones, which is very appropriate, picks the absolutely most changed clone and shows that it seems to be packaged but it is not very infectious.

So my response is okay. That is one. Now you have got 260 more to go. So not crossing that fine line that I realized of being ridiculous, I just am saying that results on a single clone, what it means to me, thinking about what is going to be going on in this patient, is that there is going to be literally hundreds of opportunities in a relatively short time and then extrapolate that to potentially months and years for all kinds of variants to be developed.

DR. DROPULIC: Most of the variants that we saw were deletions in envelope.

DR. SALOMON: Right. But I think we would all agree that the larger amounts of deletions, you picked the one that had almost 12 percent of the genome replaced, or deleted.

DR. DROPULIC: That wasn't a deletion.

That was a mutation, base substitution. That is the only one I picked because the other ones had deletions in the envelope.

DR. EMERMAN: I really think it is not that virus that really needs to be characterized. It is the one from the other experiment where you actually expanded T-cells from the HIV-infected person.

DR. SALOMON: Yes, Dr. Emerman. I agree. There are kind of two threads going on. We will get back to that one in a second. You are absolutely right. We kind of skirted that for a moment. I agree.

DR. TORBETT: I think there are two parts to this. One is replication fitness and the other is resistance. I think Dr. Mulligan made a very good point that when one inhibits, for example, envelope, they can pick up other mutations which compensate and that is very true in the protease situation. There have been other examples as well, CTLs, whatever.

I think, since this is one of the first demonstrations, it behooves the investigators to go that little extra mile and do full sequencing and find out some of this information. I think that is

fairly critical here.

DR. SAUSVILLE: So, along the lines of what seems to have emerged here is issues ultimately with what we might call characterization of what comes out of the T-cell passage process. Is there any evidence or entertainment of the issue that obviously patients come in very many different flavors. So CD4 cells are going to be quite different from patient to patient ranging on everything from what medications they are on to what coinfections they may have.

So does that enter into some of the variants that might actually come and be generated here and should this discussion also consider that issue. I raise that as a question. It would seem to me it would be relevant in the clinical sense.

DR. SALOMON: Certainly, there is some expertise around the table to address that question. Of the population that is being proposed for this study, it is defined as HAART--I guess the beginnings of a HAART failure. These are not total HAART failures because, at that point, I would think that their CD4 counts would basically plummet. I guess one of the difficulties here is defining exactly what is a HAART failure. We will

get to that maybe later.

So for those of you who are real HIV experts, what will this patient group be like? Are there going to be any commonalities for this particular subset of patients?

DR. ZAIA: If you look at the definition of who is included, it is everyone above 5000. So, assuming that let's say failure is some other level, or any level, while on HAART, you can have anyone from 5000 to 1 million. So the question is is the person at a million going to be different than a person at 5000. I would guess they are different in some ways. Maybe they are different because you will have a harder time isolating their T-cells or expanding them.

But at least it introduces the idea of heterogeneity in the population. So when you are looking at toxicity, you may have a difficult time comparing strata. If, in one stratum, you had enrolled three people who had had a million and, in the other stratum, you enrolled three people who had had 5000--I mean, that is a design problem. But it is still important.

DR. SALOMON: The specific question, not withstanding the quality--I mean, those are

important comments. I didn't mean to trivialize them, but the specific question I was asking was do you think, then, that there would be a similarity? Would you come down at all the kind of viral populations that would be present in these patients because you are defining them as being relative HAART failures?

I guess I could interpret from what you said that the answer is no, that these would really be still very heterogenous groups from the point of the viral species.

DR. EMERMAN: There is one point which is important here which is whether or not they have drug resistance at the time you are staring the therapy. So, if they fail HAART because they have resistance, that is one thing.

DR. SALOMON: Wouldn't that be the reason they would be failing HAART would be drug resistance of some sort?

DR. EMERMAN: They have failed HAART because their virus is resistant to an antiviral. That is one different kind of criteria. I think it was alluded to the fact that one could suppress the HIV activation when you are expanding if you do it in the presence of antiviral.

That is a way, actually, they can get around having new variants come up during the expansion if they include antiviral during the expansion. But that predicates knowing whether or not there are resistant viruses present already.

DR. SALOMON: I thought about that, too, when that came up. Exactly.

DR. EMERMAN: So that is a strategy to get around these concerns we have of generating new viruses is if they can show, out to the expansion, that there are no viruses because they have expanded in the presence of antiviral.

DR. SALOMON: Dr. Sausville and then Dr. Allan.

DR. SAUSVILLE: So then, to expand on that, I don't think that we came to a--at least I didn't think we came to a clear notion of whether or not there would be a relative advantage to include the drugs while the T-cells were being expanded. I think that was alluded to before.

But, as I listen to the discussion, if the goal is to minimize the risk of coming up with yet a worse flavor of HIV during this process, it would seem that the preclinical data is a little thin on considering the merits or demerits of that.

DR. ALLAN: I can see individual variation from patient to patient in terms of when you expand the T-cells, what percentage of the cells are going to be expressing HIV. If you have got somebody who has really failed on HAART and they have a high viral load or a high viral burden, then you have got essentially a high multiplicity of infection circulating into the cells that you are getting the vector in which may increase your chances of recombination.

The other issue that follows with that is I didn't see anything where they filed CXCR4 versus CCR5 expression in those activated T-cells. I think that might be an indication as to how much virus breakthrough you are going get, maybe variability between patients that you have looked at but you are taking out and how many CCR5-positive CD4 cells, versus CXCR4.

DR. SALOMON: Dr. Zaia?

DR. ZAIA: I think one question we have to ask ourselves is why would you analyze the virus coming out of the transduction in these patients. Is it because you want to exclude that lot of cells or do you want to prove the implication that Dr. Kingsman started with, that the design of the study

is flawed; that is, if you found one patient that had that, you would accept that premise that you are selecting for a resistance that may change the phenotype. So it is a slightly different question. I don't think we are asking for release testing. I think we are asking for proof from the sponsor that this is not a poorly designed study from its inception; that is, the target is asking for selection in vivo.

DR. REITZ: In addition to the question of resistance, I think, since this is targeted to antisense envelope, you also have the question of the cell tropism or envelope phenotype of the virus as well because one thing that would be concerning is if you converted a CCR5 to a X4 tropic virus, you would generate something that is at least seemingly more virulent than the R5 populations of virus.

So you might be generating a fitter virus in some sense in that way.

One way I think that you could look at proof of concept is what I had mentioned before, which is to see if you do get phenotypes in a system that allows in vitro testing or growth of both R5 and X4 viruses. I think you could look at

it using that kind of system.

DR. SALOMON: So, trying to capture this. What I hear here is a couple of threads. One thread is that if we assume that you are doing your transductions on patient T-cells that are HIV-infected, which is, of course correct--that is what is going to happen--then I think Dr. Torbett, Dr. Emerman, made a very concrete point that I think we need to start with because that is just the simplest point.

Dr. Zaia makes it more complicated and we need to go there, too. But the first point is I think the committee was saying that you have to look at the amount of--the number of CD4 T-cells that are infected in the input and the virus that those cells carry. And we should go back and say what does characterizing that virus mean, but characterize it in some way and, after eight to ten days of activation with the vector, characterize the number of CD4 T-cells that are infected and characterize that population of virus.

There is a lot more to go there. But, how about starting there? What do you guys think of that statement?

DR. ALLAN: I don't know about days. You

are saying eight to ten days. It may not be enough.

DR. SALOMON: Eight to ten days was the period of time that this procedure will be done after which the cells are taken, washed and frozen and assays done.

DR. MULLIGAN: I think the timing may not be all that important. It is important relative to what the clinical protocol will be. But if you want to see the events that occur, I think what you are saying is you go further and you try to really see what happens when you have these cells infected with vector.

DR. SALOMON: Okay. Now, there what I am doing is I am following what I think Dr. Zaia articulated beautifully. The first point is what is a safety request for the protocol as proposed. The second is getting at the science behind the strategy which I am not saying, in any way, shape or form, isn't equally important, but I am, right now, just trying to start with one point and make a step forward.

So this is just, they do the protocol--at the end of nine days when they freeze these cells down, should we do this? Then we will go exactly

where you are going, and that is longer culture and more characterization. But you wouldn't do that for every single lot; right? If that is what you are suggesting, then--

DR. MULLIGAN: I think we are saying the same thing, that you would, before you ever do this, collect much more information on the kinds of viruses that come. And you would do that for longer periods of time. Whether it would be ever meaningful to test at nine days or ten days, once you decide you are going to do the protocol, I am not sure.

DR. EMERMAN: Can I make a suggestion, perhaps. You would want to know what percentage of cells are infected at the beginning of the expansion and then you want to know what percentage of cells are infected at the end of the expansion and had it increased. Then that would be a criteria for not continuing with that particular lot.

DR. SALOMON: Yes. That is what I wanted to hear someone say. That is what I was suggesting, that that would be the minimum.

Dr. Sausville and Dr. Rao.

DR. SAUSVILLE: At one level, this is

maybe a more exotic form of quality control and release specifications than we are used to considering but is brought by the nature of this in that--I would like to see not only what, in essence, goes in which is easy to define but also what comes out on several levels, but across a reasonable spectrum of T-cell populations that might be expected to emerge from a representative set of patients because, to me, in terms of interpreting the outcome of any clinical trial, I don't have any context in which to judge what comes out of the clinical trial in terms of what the product is going in at this point.

DR. SALOMON: So we can have the sponsor determine the percent of infected CD4 in and the percent CD4-infected out at the end of a nine-day period. Then the question is what would we request in terms of characterization of the virus in and virus out?

DR. ALLAN: You are talking about the virus out--by saying virus out, what you are saying is--

DR. SALOMON: Whatever combination of wild-type HIV and vector comes out.

DR. ALLAN: You are talking about the

breakthrough kind of scenario that was shown with the patient's T-cells.

DR. SALOMON: No, no. I think that what we have heard is that two possible things could happen. Dr. Emerman and Dr. Torbett, correct me if I didn't get you guys right, and also Dr. Reitz. The idea here is that in vitro, during CD3, CD28, activation in high concentrations of IL2, and you can play adjective wars here, but in IL2, there would be a change in the wild-type virus, a selection, perhaps, of an R5 variant or something.

Or there could be recombination with a vector that could create a different species.

DR. REITZ: I think that something like that could happen in vivo, also. But you can probably get some kind of indication on the likelihood of that by looking at it in vitro and then characterizing the virus that comes out in vitro more fully.

DR. SALOMON: So can we be a little more specific just because the word "characterize" does not mean anything to a sponsor or to the FDA.

DR. REITZ: In the case I am thinking of, it would be a relatively simple readout of recombination with the NL3-4 envelope sequences of

just looking to see whether you generate X4 viruses, whether you can show them coming out of the gemish that you have after doing some kind of relatively extensive culturing.

DR. SAUSVILLE: But, isn't that choosing one particular marker situation? I am not a virologist, but when one looks at the efforts to describe populations of viruses and different clades, different epidemiologic scenarios, et cetera, one could potentially imagine a more detailed characterization, at least at this initial get-go.

DR. REITZ: What this would answer, and I think is using a relatively simple readout, is it does this occur.

DR. SAUSVILLE: But would that be sufficient if it were to potentially detect other types of changes and then that gets back to the whole issue of--

DR. EMERMAN: I think there are other techniques that could be used. For example, there is the technique HMA, heteroduplex mobility assay, which can look at the variability within that region of envelope before expansion and after expansion. You can ask, are there different

species there after expansion than before expansion.

DR. MULLIGAN: I think there are two types of things. There are, obviously, these more important biological characterizations but then there is also the vector-specific question which really requires a detailed molecular characterization, pro-viral DNA, PRC sequence, whatever you want to do, but to see what variations you have, what pieces of the vector you pick and incorporate, not that particularly that is going to be worse, in any means, than picking up a new envelope.

DR. SALOMON: Dr. Torbett?

DR. TORBETT: I guess, for a minimum, I think what I am hearing is that we would like to have the same populations going back into the patient and started and the same number of cells infected so the individual is not worse at the go. Otherwise, it will be very difficult to analyze efficacy of the trial.

So, at the minimum, I would think that the envelope sequences, if they are breakthroughs, and this is very important because we are using cells from patients that are presumably resistant to the

current protease regime. So there will be high--there possibly will be replication.

So I think, at the minimum, the question of what is changed in the envelope region by sequencing and, at the minimum, by heteroduplex analysis, is something that should be considered as well as the number of cells going in and out in terms of bookkeeping infectivity are infected.

DR. DELPH: Would it be important to look as well at viral fitness or replication capacity of what is coming out?

DR. SALOMON: I think that what Dr. Reitz was saying is that. He is talking about biological assays of viral fitness. That was picked up as a theme by others. So, yes.

DR. REITZ: Also using that as an index for the possibility of recombination.

DR. ALLAN: I would go another step, though, because it is not necessary to have a replication-competent virus coming out that is a recombinant. I think that all you need to have is a gag/pol recombinant because, if you are introducing that into the patient, at some point later, you may get the recombination occurring. So it is not even just looking for viruses that have

the ability to replicate but you have to start looking at large sequences. So you have to do sequencing across a genome.

It is not just envelope that you are going to be worried about. I agree; that is a major concern. But, also, LTR gag and pol.

DR. SALOMON: Okay. So, trying to keep this in focus. We definitely hear that we need to know percent CD4 going in, percent CD4 infected going out. We also appear to be in consensus that we need some sense of the biological activity of the virus going in, as a baseline, but more importantly, of course, the virus coming out to make sure that just the procedure of transduction activation and in vitro culture doesn't alter the characteristics of these viral species that get returned.

But then, as we go to where Dr. Allan have taken us, and Dr. Mulligan, we need more background information is what I hear the committee saying, as well. I think that is where Dr. Zaia began.

I think, Dr. Mulligan, why don't you pick up on that. What additional information does the committee want that we wouldn't necessarily want them to do every time they did a patient but that

we need now to even think about it as an issue for the protocol.

DR. MULLIGAN: The simple, I think, would be to, even in vitro, to go through culturing the cells for a longer period of time because I am not sure we would ever be able to assess exactly what the test would be during the actual clinical procedure until we knew what we were looking at.

We didn't really talk about this but my conclusion is that there is really not a very good in vivo mobilization assay, from what we have heard and, therefore, we may be limited to these in vitro assays. So, carrying on the cells in culture and just looking for the kinds of things that occur won't necessarily tell you what is going to occur in the patient.

But things may well go on after the nine or ten days of culture. Different things might go on--and just seeing the kinds of variants that you would get I think would be very important.

DR. SALOMON: I agree with that. I was thinking about the experiment that could be done here. There is always a flaw in thinking of experiments on the fly. For that, I apologize to you because I am sitting here for a day. You guys

have done this for a year, probably.

But the experiment I was thinking about was if you took a group, X number of fresh CD4 cells from an HIV-infected individual, activated them in your protocol, go about nine days, wash them, then add in new fresh CD4 cells from a noninfected individual, and sort of do serial passages each time bringing in some fresh CD4 cells from an individual and begin to look at--sort of use that as a real-life sentinel cell, at the same time doing parallel experiments in a very highly permissive cell line for R5 and X4 variants, just to really push the system through a couple of times to see what kind of viral variants are going to be produced and selected.

I don't think you are going to do those kinds of experiments in a SCID-mouse model. I would like to point out that my lab does NOD/SCID-mouse work. I am not in the anti-SCID-mouse population. I think it is an extremely useful model. But I am saying to you my feeling is I don't think these are experiments that could be done in that system.

DR. ALLAN: The monkey studies. There was a monkey study that was shown yesterday, and it was

nice, a lentiviral vector and it was targeting to the brain. You ramp up the concern when you introduce the wild-type virus into the person who is getting the vector.

So my question is, in this particular protocol, I am not certain that it is--designing a situation in a monkey-model system is strictly a basic academic endeavor because the thing you don't want to do is you don't want to kill an AIDS patient with the protocol.

I am not certain, at this point, whether or not that might happen. From what I have seen, I don't know that you are going to make that patient worse by introducing this vector. So the issue then is how do you define--we don't have a model system. The SCID mice aren't so good, while monkeys you have got to do all this stuff to.

But, yes; you do have to finagle a few things, not huge, but I am just saying if you want a model system, that is what you are going to have to do. I am not saying you have to do it. I am just saying that it is available.

DR. SALOMON: Dr. Torbett.

DR. TORBETT: I think what we are asking, I thought we were asking, is whether we could at

least look for the minimum number of changes seen over a certain period of time in culture without--and I can think of many studies in some of our mouse models and our other models that are probably beyond the scope here.

But I think, at the minimum, we need a little bit more information, at least in classic cultures that people have done in vitro. I think, at least myself, that would give me a little bit more reassurance.

DR. SALOMON: To Dr. Allan, I think the point here, to put this in context, is we are trying to focus on the protocol and we are trying to do it in the context of the principles we articulated yesterday. We talked about the relative value of the monkey model yesterday. I don't think that, unless the committee wants to take me otherwise--I don't think that the message to the FDA or the sponsor now is that these guys should go and generate a SHIV-modified monkey model before we would allow a protocol like this to go forward.

Do we agree on that? I mean, there are problems in the field. We acknowledged those yesterday. I thought your ideas were really

interesting.

DR. ALLAN: This is a Chicken Little thing, obviously. It is not going to create a public-health nightmare. But still the issue is the AIDS patient is at risk. The patient may have 600 CD4 counts, may only have 5000 copies per ml of plasma, so it is not that they are in bad shape at that point.

So they are actually, I would think, relatively healthy. And you are going to treat them with this and you could kill them depending on what happens. I don't know what the risk is. But it is the same sort of situation when you say, well, gee; anthrax, we are not going to test postal workers or make them do this stuff because we don't believe that it is--and then you go back--you just don't want to be on a committee where you say, well, gee, a year ago, we didn't think that was going to happen and it happened.

DR. SALOMON: Okay.

DR. ALLAN: I am not saying that you should do a monkey model. I am just saying that there is a level of concern and the question is whether or not you want to take it to the next level.

DR. SALOMON: And I was saying that we have articulated that concern yesterday, and I am testing you now to see whether or not you want to elevate the level of concern higher than we elevated it yesterday to say that you would put that as a barrier, basically, before going forward.

I am saying that I haven't heard anything from the rest of the committee that suggests that this is a requirement to go forward in this area, but I certainly am not arguing with you about what you have articulated as an issue for the field.

DR. ALLAN: I am not saying that you would use a monkey model to test a lentiviral vector that was used for some other reason. But, in this particular case, you are using a lentiviral vector in the context of an AIDS patient who has a wild-type HIV. To me, that is different.

DR. SALOMON: Dr. Zaia?

DR. ZAIA: We are being unfair to the sponsor if we don't set the bar, or at least advise the FDA on how to set the bar. So let's talk about two bars. A high bar may be evidence in the monkey that you cannot change the biotype of the virus in such a way to make the monkey worse.

So let's suppose that were done and you

actually constructed the vector with antisense to SIV envelope. You then went through the experiment and you found that the monkeys did make more virus and they grew faster and they got AIDS quicker. You failed the bar. You did not pass the bar. The bar was set high and now you probably would never bring this to the clinical trial.

That is one bar. That is not an unreasonable bar if you are concerned about what let's call it the anthrax level of concern. But the other bar, then, would be looking at--let's call it the Torbett bar.

DR. TORBETT: It is always a lower bar.

DR. ZAIA: You look at what goes in and ask the question, is there some change in that virus. If we look at, let's say, 200 clones and we characterize the mutations. We look at some fitness and we have this experience. We don't see any more increased fitness from these three experiments we did, let's say. So that is the low bar that you pass.

I guess there is no real way to know what is best. If we are very concerned that the design of the experiment may lead to worse virus, then I think it is reasonable to ask for the higher bar.

It is not an impossible task to accomplish.

Once accomplished, it may give us a lot more--lessen the anxiety, let's say, of the scientific community that is arguing in defense of this study to go forward to advance the field.

DR. EMERMAN: A way to get around all these questions of a worse virus coming out of the transduction is to--I think they proposed was to do an expansion in the presence of antiviral drug, or antiviral drugs, assuming they pretest virus from the patients knowing that--

DR. SALOMON: Dr. Emerman, there is nothing in the protocol at the moment suggesting these expansions will be done in the presence of antiviral drugs, number one. Number two, we have pointed out that if, indeed, these are patients who are, at some level, failing HAART, then the significance of the antiviral drugs is less clear yet.

DR. EMERMAN: But that could be tested because, if, at the end of your expansion, you don't see any virus, then that is fine. That means that they didn't expand a drug-resistant virus. People fail HAART for a lot of reasons only some of which are resistance to the drug.

DR. SALOMON: That's fair.

DR. ALLAN: The thing is, it doesn't fail--it doesn't end when you put the cells into the patient. You put the cells in the patient, you could continue to get recombinants. You could continue to get things that happen later on. So it is not just, this is safe now and we can put it in.

DR. CORNETTA: Dan, this is Ken Cornetta. One of the things that has been disturbing, I think, for me over yesterday and today is not only--I think most of the assays that have been looking for recombinants have been geared for common recombinants. But even looking back at the old MLV data, various cells that can be tested may or may not express various recombinants very well.

It seems most of the people that have presented have looked at one assay using one or two cell lines. I think, in just general, I am not sure folks have spent enough time looking at assays for trying to detect recombinants that really make me feel very comfortable that people have a good handle on being able to analyze both the product and then the transduced cells.

DR. SALOMON: Good.

Dr. Sausville and then Dr. Reitz.

DR. SAUSVILLE: I just wanted to--with all due respect to the monkey models, I would counsel against making that a bar because I think that is investing what is a biologically very informative model with a level of ultimate access in the clinical-trial sense that I just feel very uncomfortable with. I think that I would rather characterize well the product that comes out and address some of these issues of variability in the transduced cell population rather than the monkey situation.

DR. ALLAN: I understand what you are saying, too. But what happens is that you don't know what is going to happen when you put it into a biological system. You can do tissue culture and you can passage it, but that is not what happens in a person that is infected. It is not what happens in a monkey that gets SHIV or whatever. There are studies that are done which, if you take a deletion in nef and you put SIV into a monkey, then you get nef back.

You can put a defective virus in one leg and another defective virus in another leg, and you get replication-competent virus.

So I don't know that an in vitro tissue

culture system is going to be able to tell you that. I am still wondering whether--because the monkey-model system is not that difficult to engineer, really, because you can still use the env, because if you use the SHIV system, you don't have to redesign the antisense env. You can use that.

The only thing you have to redesign is really the gag/pol. So it is not that big a deal. I am not doing it so I can say that.

DR. MULLIGAN: I was just going to say that we may not want to equate the level of concern with whether we do the monkey--I think that is maybe what Ed is saying. I would look at it that we have the level of concern, I think. the issue is if we were really to analyze the data that we would get from the monkey, would it really satisfy, for sure, our concern one way or the other; that is, this data could be very, very helpful.

I am sure everyone would love someone to do this and test it. But if you really ask this question which we always ask is how relevant is the model system, how meaningful is it, I am not sure, in this particular case, we would get any clear consensus, even though we might get a clear

consensus that the level of concern we have is very high.

DR. ALLAN: It wouldn't tell you yes or no, it is safe or it is not safe. What it does do is it give you a lot more information in terms of safety in a patient. It gives you a lot more information.

DR. SAUSVILLE: But, on the other hand, if you are going to pursue the point, if the animal model were to faithfully replicate the clinical scenario, you would have to have some sort of control where the animal is observed for the same period but with a non-changed virus.

You are talking about a scope of an experiment that we just usually don't require, I don't think, in any therapeutic area. I am concerned that that could ultimately block progress if that were set as a bar for every type of therapy of this sort.

DR. ALLAN: I'm only suggesting it in the sense of treating an AIDS patient with an HIV vector. I am not suggesting that in other types of therapeutic modalities but only in this particular case.

DR. DELPH: I am obviously not competent

to make any determination as to whether in vitro testing is going to give you better information than monkey models, how much better the information from monkey models is going to be. But I do think that there is a level of concern there, which I share. And I also think that this is a new area. This is completely new territory as far as we are concerned in terms of putting lentiviruses into HIV-positive patients, an HIV-positive patient.

I think, for that reason, we need to err on the side of caution. So I would urge that the bar be set higher rather than lower. I don't know what the tests are that you need to get to that bar and whether they are worth doing. But I would err on the side of caution.

DR. SALOMON: As someone who is representing the AIDS community for us, my interactions with the AIDS community in the past wouldn't have prepared me for that statement. I just wanted--if you could kind of deal with that. Do you think that what you have just said would represent, obviously not every person in the AIDS community. No one is trying to be that absurd, but it seems to me there are groups in the AIDS community that feel that a patient failing HAART is

a young person, innocent person, dying.

The relative risk here of generating a new virus and spreading it into the community, which is what you should be concerned about, would be very low providing you had patients who adhered to appropriate behavior during the trial not to bring this whatever was happening inside them, if anything bad was happening, to another patient.

So I guess I am a little uncomfortable. I just hope that we think about that for a minute. The best way to kill an idea is a committee. At some point here--I don't want to go too--I just want to make sure that I raise the issue for discussion here. I think I am being clear.

DR. DELPH: Obviously, I cannot speak for the HIV community and, obviously, there are going to be very different opinions from mine. But I do think that if you look at--there are people who really have virtually no options at the moment and who are in dire straights.

But I have no idea what the relative risk that you are talking about, how quantifiable that is. So it is very difficult for me to say at this point is the relative risk worth it for those people or for the rest of the HIV community. I

guess what I am saying is that, yes, we need to balance the risks and benefits but this is completely new territory and whereas, I think, we have familiarity with antiviral drugs, it is a lot easier to say what you need to do and to assess those relative risks.

Right now, I have no way of assessing those risks. And, from what I have heard, I don't know that many people here can quantify that risk for me.

DR. SALOMON: Just to share with you kind of where I was from, just to finish the thought. I went through this over the last five, seven, years with xenotransplantation. Today, we still don't have a very specific measure of relative risk and so this equation of risk and benefit has been as much a part of those debates as we have now been having here.

I think that one has to think about--one of the things that came out in our xenotransplantation considerations was the idea of individual risk and public risk. Individual risk can be handled very well by a consent form. The risk that a more rapidly, more fit, HIV species could come out in an individual and lead to a more

accelerated death can be explained in an informed consent and put in the context of, we have done our best to select a patient population with HIV that really has no option.

If I wasn't convinced of that, if we were talking about relatively healthy people being pulled off the street, that would be a different issue. But providing that we would assure ourselves later that the clinical-trial design chose an appropriate population, that is individual risk.

So, the only thing that goes to public risk is that then there would be transmission from that patient in the trial to the public, anyone that they would be in contact with in any context. That is manageable and small if the patient population is mature and compliant. That is where things get gray.

DR. ALLAN: I am concerned about the patient. I guess, in contrast to xenotransplantation, I am little bit more concerned about the patient here. With xenotransplantation, the patient is going to die within a week, month, or whatever. An HIV-infected patient who has got a 600 cell count and 5000 viruses, I don't know how

long they are going to be able to live for. Do you know? A couple of years?

DR. DELPH: Longer than that.

DR. ALLAN: Maybe longer than that.

DR. DELPH: Somebody with a viral load of 5000 and CD4 cells of 600 is really not in dire straights.

DR. ALLAN: No. So you are jeopardizing the individual health. The other point about xenotransplantation, just to take the other aspect, is we do require, in xenotransplantation, that they do an animal-model study, that they put pig hearts into baboons. We even set a bar in terms of primates in xenotransplantation.

DR. SALOMON: Okay; but just remember that was a benefit bar, not a risk bar, in the primate.

DR. ALLAN: We are doing the same thing if you require a macaque model. You would be looking at efficacy as well.

DR. SALOMON: Okay; just the discussion was on risk.

Dr. Zaia and then Dr. Reitz.

DR. ZAIA: I think that I am persuaded by Dr. Sausville's point that a monkey model as the bar may not be adequate for two reasons; one, it

may not answer the question and it may be too impractical for other studies as they come along in the future.

But I think that there still is an intermediary situation, that the sponsor carries a burden to show, and that is the fitness of the virus. So if there were, say, 100 isolates analyzed and 100 of them were unfit, or whatever the word is for that, and there were no better-fit viruses in that, at least you have assured the patient who is undergoing the consenting procedure as well as the FDA that it is a highly unlikely event to occur.

I think maybe that is all we can say.

DR. SALOMON: And that was the consensus earlier

DR. SAUSVILLE: I further make the point that, even if you had a very successful monkey experiment, you would still have to do that for the product that you are going to put into the person. So that is why I see this is a product-characterization issue at one level.

DR. SALOMON: Dr. Reitz?

DR. REITZ: I would just like to agree for a slightly different reason with Dr. Allan about

there being--I think you have got to think about risk to the patient more than the population because it seems to me the likeliest phenotypic conversion in this particular situation would be the an X4-tropic virus which could be fitter within the patient but it is also a virus that is less easily transmitted, almost never transmitted from one person to another. So I would be more concerned about the patient than the population.

DR. DROPULIC: Could I just answer that?

DR. SALOMON: Okay; she has been standing there, too. Go ahead.

DR. DROPULIC: I just want to answer that comment by Dr. Reitz. We will restrict the patient study population to X4 strains. So we will demonstrate that the patient has X4.

DR. SALZMAN: This is Rachel Salzman, STOP ALD Foundation. I want to comment about the bar setting. We talked about the high bar, the low bar, the in-between bar. When you start talking about a high bar for just the HIV subject patient population, that causes a little bit of problem because there has been so much discussion here that the non-HIV population may become and HIV-positive population.

So when you start saying, well, the bar for HIV studies is this and the bar for non-HIV studies is that, then there is kind of a logic flaw. So, of course, I am kind of coming more from the perspective of you invest all this time and effort into accomplishing and meeting the standards of the high bar and what you have learned, ultimately, just doesn't seem like it is of the value for what you have invested into it.

So just remember that the non-HIV population, technically speaking, could become potentially HIV and we wouldn't want to exclude them and make them have to meet these higher bar standards.

DR. SALOMON: I don't necessarily see the logical flaw, to be honest.

DR. SALZMAN: The logical flow is every time that it has come up saying, well, let's put the HIV patients in this category, and then the HIV specialists say, well, just remember that the non-HIV people can become HIV-positive. So suddenly you have done--

DR. SALOMON: There is nothing illogical about that. That is real.

DR. SALZMAN: Right. Exactly. So my

point is that if you are going to say, okay, the high bar is only for studies that you are going to treat HIV patients, well, then, by definition, the high bar has to be for non-HIV patients because they could become HIV patients.

DR. SALOMON: Exactly. I don't think anyone suggested an illogic on that. That's all.

DR. ALLAN: But what I think she is saying is that she is afraid that what we are going to do is--

DR. EMERMAN: --we are not doing that in the non-HIV people. If they get infected later, that is a totally different story. We are talking about what goes into those people.

DR. MULLIGAN: Another point that may make you feel a little more comfortable is that the mobilization sorts of things we are talking about are dependent upon what HIV infects, too. So, in many of the cases, you may have a particular interest in. Although it is true that you could be infected by HIV, you may be doing some cells, trying to transduce cells, that are not susceptible to HIV.

So it wouldn't necessarily mean that it would completely cover all kinds of activities. So

if you were doing muscle gene transfer, for instance, even if you were infected by HIV, in principle, you wouldn't be infecting muscle cells.

DR. SALZMAN: Right. I am coming at it from the other end, just saying--I mean, I think you see my point. I understand what you are saying, also. I am just saying I have concern when you are saying, well, we have these standards for the HIV population that are strict. What I am saying I like having the more middle bar and that then you are saying everyone could potentially be HIV positive, so potentially all patients should have to meet those higher standards. That is all. It just seems to me the value is not necessarily weighing what you are investing into it.

DR. SALOMON: Dr. Wilson?

DR. WILSON: I wanted to make one point to clarify regarding the last comment which is that if I had been following the train of discussion, I think Dr. Allan's proposal to raise the bar is specific to this particular protocol because of the use of the antisense envelope where there is data suggesting that that would drive changes in the HIV envelope.

As Dr. Mulligan was also saying, not only

is it an issue of different target cells, but presumably that issue wouldn't arise if you are not treating HIV with that type of--you wouldn't be putting into a non-HIV-positive patient population that type of a vector.

MS. KNOWLES: I am hearing the scientific concerns from others on the committee and I agree with those concerns. But I have some other safety concerns in terms of the proposed trial. I think that the proposed tests in terms of in-process and QC during manufacturing are vague except for the RCL assays and that those other tests ought to be identified and detailed.

Also, I am concerned, and this is a little further down the line, but there is very little information regarding the dosage schedule after the initial dose and what parameters are in place for continued treatment, et cetera.

DR. DROPULIC: Could I just answer that?

DR. SALOMON: No. Don't answer that because we will get to that in a second. Those are very important points so don't let us forget that. We are getting close to what I think could be a consensus of this, and this is so important to the whole field, not just to your protocol.

So, trying to capture this here, I think we have all agreed that we need to know the percent of CD4 cells going in that are infected, the percent that are coming out and some characterization of the virus before and after to look at what is happening during activation and in vitro expansion.

I think that we also have all agreed--stop me if I am wrong, here--but we have all agreed that before you really even go forward in new clinical protocol, we want to see more data for longer in vitro culture, naive T-cells, T-cell lines selected to reflect different sorts of biological tropism, X4, R5.

I think the point came that we should be more careful about different kinds of cell lines, not just one kind of cell line. So I think we all sort of follow that. As for the monkey studies and where we set the bar, I think that in the final discussion here, and again I would encourage a vigorously defended and articulated minority opinion, Dr. Allan, but I think that the final sense here is that a monkey study, even for this protocol, is too high a bar, particularly based on the fact that I, too, am not convinced, as Dr.

Sausville said, that it is clear that the monkey study will answer the question.

I, at the same time, as a scientist, would look very favorably on such a project had it come across to me in a different role as a study section. But I would have to see that one and really be sure that it would work before I could come back on a regulatory advisory committee and suggest that that is where the bar should be set.

I think that that was the sense of the majority of the committee. Did I capture it right?

DR. CHAMPLIN: I believe so. I was just going to make one little point. We talked about longer-term culture with toxicities in mind, but efficacy is also dependent upon the lack of emergence of recombinants that would not be affected by the antisense. So, if this proved to something that readily occurred in a high frequency of samples, that that would suggest that the strategy was unlikely to be successful in vivo.

I am not sure, as a nonvirologist, how predictive those types of assays could be, but one would like to see some assessment of long-term sensitivity to the antisense approach and the lack of emergency of emergence of resistant virus.

DR. SAUSVILLE: I would heartily endorse that position because I think it gets to the essence of knowing more about what you have got coming after the T-cell incubation procedure before going on.

DR. ALLAN: The only thing I am going to say about the monkey model at this point is that it is not an academic exercise. It actually goes right to the heart of efficacy. It goes right to the heart of safety. It is a straightforward experiment and, without that, I think you really have to--and this is something we will probably get to later which is patient selection.

So if you are not going to do the efficacy or the safety issue in the monkeys, then do we want to only select patients that we know have a short life span. In other words, maybe their CD4 counts are 50 or 100 or something that--or their viral loads are a million, something that would give you some sense that they may be in crisis, because you are actually treating someone is relatively healthy.

So that is my concern here.

DR. SALOMON: I think that that was corroborated by everybody, that patient-selection

issues are relevant here.

DR. SAUSVILLE: I just had a question for Dr. Noguchi and the FDA staff in relation to this issue of efficacy because, again, while efficacy is certainly a criterion for success--i.e., marketing--my impression was that efficacy, as any type of bar to enter a phase I, is not defined as a regulatory issue. Would you care to address that?

DR. NOGUCHI: We would put it slightly differently, that in many cases the question of efficacy is certainly not the primary consideration that we have when evaluating first time in man. There unusual circumstances such as this one and is the reason for the extended discussion where the question of safety in this particular case and the question of efficacy does become one of those real, almost beyond FDA balance, because part of the issue that has been discussed here is is this experiment just wrong at this time or is it right at this time, or are there other things that can be done.

Part of that must be in the context of potential benefit. If there is no chance for benefit, because of the nature of the vector coming from a disease-causing agent, one might really want

to pose the question, maybe this should not be done at this time because there is no chance of efficacy.

In the absence of being able to say that, then I think we need to consider to do these very careful discussions of the nuances of risks, possibly future benefits, potentially no benefits at this time.

So the long, roundabout way, is it is not the primary determinant of whether to go forward at this time by FDA in terms of that and, for these early trials, first time in man, it is always primarily based upon safety. But the questions of efficacy do enter in the discussion. They may or may not influence the final decision, but they do play a part.

DR. SALOMON: There are some details in Question 1 that I would like to go to try and organize it but not, of course, restrict anyone from bringing up anything else. I think one issue we need to deal with is the vector. Implicit here, Dr. Rao reminded me a moment ago, is we have got to go back now and say how about this vector.

It is a very interesting strategy that has been proposed and described, I think, very nicely

by Dr. Dropulic this morning, to use a single helper plasmid, obviously, a very distinct strategy from that employed and described in detail yesterday by Dr. Verma and Didier Trono and Luigi Naldini, et cetera, where each generation actually broke it into more and more so now we are into four-plasmid systems.

That's fine. It is two different strategies. So one of the things we have got to talk about is the vector and what safety issues are there.

A second of three things that I want to talk about is to use a transient-transfection system versus a stable packaging cell line. We have heard discussions of that yesterday and we should talk about whether or not that has major safety implications and put that in the context of where the field is today.

The third thing we need to talk about, I think, is we need to come back, at the end here, to deal with Dr. Cornetta's comments which I take very seriously, and that is concerns with the RCL assays because that is something we grappled with yesterday and I think we all admit that that is far from perfect.

I think Dr. Cornetta could maybe start us with that in a minute or two. So can we start with vector, transient versus stable, and then RCL assays. I think that will kind of get us through Question 1.

Vector; one plasmid helper versus four-plasmid or three-plasmid systems, transient.

DR. MULLIGAN: After the heavy conversation we just had, I am not sure that this is all that big an issue since we talked about it yesterday in great detail. I would weigh in that this certainly, I would consider, the safest vector for this clinical trial, as we have mentioned several times. I think that could be possibly the case.

I think, on the other hand, it is not very different than the transient transfection approach that others talked about. So, whether you use three separated pieces that all go back together after you transfect them or use one that is already together I don't think makes a big difference.

I guess I would say that looking at the packaging construct in some detail, it has these fancy bells and whistles but I didn't see a characterization that the features that it has

actually do what they are intended to do. I am not sure, in this group, we really want to harp on this but psychologically the fact that investigators put three or four poly-A sites and pauses between the gag/pol and the VSV-G makes me think that they think that it is important that there isn't transcriptional read-through.

They may want to comment on that, but--

DR. DROPULIC: Could I comment on that?

DR. MULLIGAN: I am not finished. Let me just finish. I saw no evidence from any of the information that anyone ever looked, for instance, at the RNA species after transient transfection being a dinosaur in the vector field, and I have seen everything that can go wrong, not according to theoretical principle.

Certainly, in this case, when you have all those bells and whistles, all those sequences, you can have cryptic donors and acceptors, you can cross poly-A sites so they are not functional. Transcriptional pauses don't always work when you put them where they ought to be put.

So many things can happen. I just think it would be nice to actually, for the FDA at some time--maybe we don't need to discuss it now--to see

the information that validates the fanciness of the vector system. All that being put, it is probably comparable to the other transient-transfection systems.

Just on the vector side, other than this issue of whether you want a mobilizable vector or not, it is a garden-grade variety vector like the other vectors people have. So I don't think there is a big issue there.

DR. SALOMON: Dr. Dropulic.

DR. DROPULIC: I think we could those studies if you wanted us to do them to validate the bells and whistles, if you like. But we didn't have any concern--I mean, actually we got the vector from a commercial supplier. It came with those bells and whistles already there.

DR. SALOMON: I guess, from my point of view, I agree with Dr. Mulligan's concerns. I had the same concerns. I like the idea of this high-level molecular discussion and we put a pause site here, we put a poly-A site here, we put an ATG stop code on.

Okay, but, I guess I grew up in the Midwest. It is sort of like, show me and I will believe you. I think that the problem is that--I

am not quite a dinosaur in that I have come to it more recently, and what bothers me continually is that there are all these sort of glib statements of, this will work, or, this will work.

I am not accusing you of being any more guilty of it than all of us. It is all fine as long as we are doing stuff in vitro and we are arguing with each other about whether you like my work and will publish it. But it is a real issue when you start talking about doing a human clinical trial.

I just think, as a principle, what I am defending as a chair, here, is yeah; I think if you are going to tell us you have got all these bells and whistles in the vector, then all I want is some reasonable evidence that those bells and whistles do what they say. Otherwise, they are irrelevant to me and I don't need to know about them.

DR. MULLIGAN: The other thing is just on the fancy models and pictures of all the recombinations that have to occur which, again, I have lived a life of this, that is all well and good as long as the original materials are as advertised.

DR. DROPULIC: It is validated, the

plasmids.

DR. MULLIGAN: I don't exactly mean that. I mean after you do a transfection and you have these two constructs, those two constructs will become one construct. Therefore, your DNA substrates for the events you are talking about are very different.

So, again, I think I am just echoing your point that, at the end of the day, it probably doesn't make much difference and it is probably just as complicated with three- or four-part things. But it doesn't give you confidence, basically, when you don't see the backup for the features of the vectors and it a difficulty in the vector field over the years.

We probably started this off the worst by making very fancy vectors and then realizing it is far better to get rid of things than to add things, and it is far better to have simplicity. But it is important that we get a sense of confidence that people are looking at these issues in a general way in a critical way.

DR. DROPULIC: We have always wanted to do the right thing. So, if that is what the committee requests of it, we will be happy to do to satisfy

the committee.

DR. SALOMON: I think one of the things, again, that maybe we could note here is that when the RAC reviewed it, one of their statements was, wow, this is just a first-generation vector and then you come back and say, well this is--that is where I was getting with Dr. Verma yesterday in trying to articulate the fact that this use of the word generation--again, it is word problems.

So what I am saying is that I agree with Dr. Mulligan, also. I don't think there is anything inherently wrong with your strategy. You guys have got to deal with this. I don't think that a four-plasmid vector system and a transient system is necessarily safer than the strategy you took, necessarily.

You got up and said, well, we did this and we did this because--and that is your argument to me that it is as safe, or safer, maybe. Dr. Verma and Didier Trono, these guys did their four-plasmid system. They did one. And then they did two, and they did three and four, all based on the premise that that would be safer.

All we are asking is that that all be backed up scientifically. It is not a

responsibility of your company more than anyone else, is all I am saying..

DR. ALLAN: Can I address the other issue about the vector is that you have an intact LTR. I know the reasons why you want to use an intact LTR so that you can get differential expression, so that you don't get the expression of antisense unless the cell gets infected with wild type. I like that. That is pretty cool. I like that.

But, on the other hand, if you have an intact LTR, it gives you a great chance for recombination because you have got LTRs there. So it is like you are between a rock and a hard place because you have increased your chance that you are going to get recombination, copackaging recombination, all these things with your vector.

So you are trying to balance these two things and I am a little uncomfortable with that.

DR. MULLIGAN: The recombination issue, I thought this would be dealt with in the mobilization; that is, if you drew the conclusion, as we kind of did yesterday, that we didn't want mobilization, then you are, obviously, in a quandary relative to the whole concept here.

Tracing the logic, it appears that some of

this may depend upon how much of a safety feature it is to have inducible antisense. I know that was emphasized in the talk. One of the conclusions that that is important is that you have the LTR present.

There are some other ways you could do it, but you certainly have to, otherwise, make a brand-new vector system. I didn't even want to get into that but I think that is an issue--I don't see particularly making antisense in an uninfected cell. I haven't seen, again, any data of how that would be dangerous. I may be interested to hear that. But I agree with you, that is the feature that is totally related to the mobilization question.

DR. DROPULIC: The efficacy of our vector is not only relative to the antisense payload. As I mentioned, it is a combination effect. Having the UTRs helps the tracking, helps competition for packaging. These are all attributes that help inhibit HIV.

DR. MULLIGAN: As Ronald Reagan would say, "There you go again." This is the same issue of there is--and I read the protocol very carefully--the issue of whether or not antisense worked better

when the RNA is localized. The data was not there for that.

Again, if that is the case, we need to see data that is rigorous and supports that.

DR. DROPULIC: I can tell you one example. We have a collaborator in the Boston area who has done some challenge experiments with a SHIV. It is the D12. Now, the SHIV D12 is very interesting because it contains both the tat and the rev and the envelope from pNL4-3, and yet it is in an SIV-type backbone.

So what our collaborator had done is transduced cells with our vector and then challenged both pNL4-3 and this SHIV onto the cells. He found three logs inhibition of NL4-3 while two-fold or so inhibition of the SHIV.

Now, the SHIV is very interesting because it has got tat and rev that would also stimulate the vector LTR; right? There is not much difference there. When you think about it though, the level of expression is similar. It should affect and express from the vector.

The only thing that you can think about that is different between the two is colocalization. I suppose I am not articulating

that very well, but the differences in inhibition between SHIV and NL4-3 in transduced cells with a vector is indicative of some sort of differential effect.

DR. SALOMON: What I don't want to do now is--this is the path into a study section discussion. I would love to have it, to be honest with you, but that is not the job today. That doesn't trivialize the value of the science you just described or the issues that Dr. Mulligan has brought up.

Transient transfection versus a stable line; I have not heard anything in the last two days that makes me more or less comfortable with one or the other, provided the same sort of safety characterizations are done with both. In fact, my sense from everything I have heard from everyone is that we are probably not quite--the field is probably not quite ready for stable lines yet, that everybody sounds like they are working on them but that there are issues of titer and truncated LTRs rather than self-inactivating LTRs and different sorts of strategies.

Is there anyone who disagrees, basically, with the statement that--I don't think that is a

go/no-go criteria to use a transient. I think that the field could be moved forward now relatively safely with a transient expression system.

DR. MULLIGAN: I think so but, in fact, I think there was a consensus by everyone that the stable packaging cells did offer theoretical--

DR. TORBETT: I tend to agree with that, but in the absence of data, I think it is an even call all the way around. I see no data either way, again, with the one-plasmid versus four. Again, without the data, it is a personal judgment.

DR. SALOMON: All I was trying to get at was I think the advice that we can give the FDA on this point is that we wouldn't tell you to refuse a transient at this point. I just don't think there is anything here that suggests that is less safe than a stable.

So that brings us to Dr. Cornetta's comments and our discussion yesterday on RCL. Ken, do you want to just start us off, sort of restate your issues and add whatever you want to the RCL issue so we can get through that?

DR. CORNETTA: I just was looking at what has been presented and what is out there. I am just not very confident that we have something that

has been anywhere close to being validated for detection. Some of the things that are presented may not address two issues; one, when you are talking about amplification, you need to consider the effects and efficiency of the amplification cells.

Since we are not sure exactly what these recombinants might be in regard to both what their LTRs may be and also what the envelope may be, we need to be thinking about how efficiently might this recombinant infect the amplification cell and then, also, if it does infect the amplification cell, will it really be amplified.

That came to some issues, for example, yesterday talking about the PERT assay and using MLV as the amplification control. MLV probably would be amplified very efficiently in 293 cells and so you may well be setting the bar too high in trying to figure out what is your positive control.

I am not sure I have an answer for these, but I just don't have a lot of confidence right now that we can feel that the material that is being tested has been in a very rigorous way to date.

DR. SALOMON: Thank you. I think that that nicely states, also, the kinds of discussions

that we ended up with yesterday where I felt that the consensus we had reached at the end of yesterday was that, right now, still the RCL assays, and I am not contradicting what Dr. Cornetta just said, still the RCL assays are what we have.

So we have to think about maybe ways to enhance the quality of the RCL assays, and I think Dr. Cornetta gives us a couple of suggestions along that line. The fact that there are alternative assays, the PERT assay, looking for the gag/pol recombinants, I think we all agreed that that ought to be pursued and, yet, the fact that neither had really been that well validated made us all a little uncomfortable advising the FDA that that should be a requirement.

Are we sort of all agreeing on that sort of thing? Ken, are you comfortable with how I said that?

DR. CORNETTA: I think so. Just talking about your prior point about whether we use packaging cell lines or using transient transfection, the way we produce virus in the vector system is going to change very rapidly. But one thing that may well be the determinant of where

to go with these new systems is what are we really going to say qualifies that end product for our clinical use.

That, I think, needs to be a major focus and I am not sure we are there yet.

DR. SALOMON: One issue, just to be specific, is choice of cell lines. Just given that we are dealing with the VIRxSYS protocol, what process did you do to decide that the H9 cell line was your cell line of choice for RCL assays? That was the only one you tested and you liked it, or did you test ten others? You didn't show us any data for validating the use of the H9.

DR. LI: NL4-3 is a very classic T-cell-topic virus. You can infect H9, Sup-T1, whatever you can choose with all similar sensitivity. However, the reason we chose H9, FDA requires that all the reagents that we are using has a CFA. So we can't just make a virus stock.

So we bought a commercially available pNL4-3 virus stock with identified PCR50. It was provided by the manufacturer and where the titer was sensing the H9 cells. So we want to verify this one virion particle and go back to the parental cell line where the virus was produced.

DR. SALOMON: So that would tell you that the wild-type virus from which you made the antisense envelope sequence would be caught if it flipped, if it reinserted in the right direction, recombined. Then the H9 would be the ideal. But that, to me, isn't a very good rationale for its use for detecting replication-competent lentivirus emerging from this very complex recombinant-prone transduction system.

So I might argue that you should probably test a number of cell lines and, in addition, consider the use of target cell lines that, let's say, express tat. What was the one, CC--it was one of the cell lines there. Someone help with me. Is it rev that is expressed at low levels? Carolyn?

DR. WILSON: C8166 that has HTLV1 tax.

DR. LI: Can they have a clarification here? We are talking about two places where we do the RCL test. One is for viral production. In there, there is no HIV. There is nothing, except the--

DR. SALOMON: No; I am talking about the RCL testing of the T-cell-transduced product at the end of the nine- to ten-day period before infusing it into a patient.

DR. LI: I mean, there we do not use H9 because you can't the H9. The cell already has HIV. The cell is from an HIV-positive patient.

DR. SALOMON: Right; so what do you use to detect--

DR. LI: We use 293-T because--

DR. SALOMON: Because that won't be infected. Okay. I admit to a confusion on my part on that one.

DR. LI: Okay. So we know VSV-G can, if any recombination happens between the gag and pol and VSV-G, it can be very productive, in fact, for the 293T-cells.

DR. SALOMON: Any comments from the committee? Is that going to be sensitive enough, the way they are going at this? I am still not totally clear here, because I kind of blew it, didn't I?

DR. KAPPES: I see what that approach does is it takes us back to the question of you may have generated a recombinant. We don't know what it is. We haven't characterized it, but we know it likely does not contain VSV-G environment. My point is there still is a void of information as to what recombination may have generated.

DR. ALLAN: Are you on Question 2 and, if you are on Question 2, where are you on Question 2?

DR. SALOMON: You don't have to make it worse.

DR. ALLAN: You are talking about assays for RCL, and there is the virus production and then there is the stuff that goes into the patient; right? If you are talking about, like this one says LTR gag/pol recombination of media--

DR. SALOMON: No; let me clarify. That is where I blew it. I should be talking about, right now, virus production in which case my earlier comments were okay. When I blew it was when I went and talked about what was coming out of the viral product which we need to get back to. But you are right. That is Question 2. I apologize to everybody. I am just human.

DR. BORELLINI: I have another question on the assay. What is the effect of the vector that is in large excess and carries the antisense for HIV on the amplification of the RCL?

DR. LI: We did a spiking experiment, used the bulk harvest which has lots of virus, our viral production. And the spike positive HIV. Actually, that is required by FDA. You have to prove your

viral production does not inhibit your positive control. And that was characterized. There is no difference with the viral production bulk harvest liquid, solution, whatever you want to call it, or just simply the H9 cells media. There is no difference, no comparable difference.

DR. SALOMON: So I think we are at the end of Question 1. I still believe--again before I made the misstep--I still believe that you should probably go back and test several cell lines, not just the H9.

DR. MULLIGAN: I think the question was if you are using a therapeutic vector that is supposed to suppress the emergence, how does that affect the detection of HIV. So I am not sure how to interpret the results you just presented. That is it doesn't work in this case, or what? Do you get my point?

What HIV you are putting in; could you maybe go back?

DR. LI: For the spike?

DR. MULLIGAN: Yes.

DR. LI: It is the bulk harvest. The vector we use for the cell transduction is processed and concentrated. For the bulk harvest,

we just use that as a culture--it is very diluted. We are not using that for transduction purposes. It is the very raw first harvest from the producer cells after DNA transfection. We just want to demonstrate, because that is the step. FDA requires you to do the RCL test.

DR. SLEPUSHKIN: I am Vladimir Slepushkin. I am responsible for production so maybe I can explain better. In the first step of the production, one of the first steps of the vector production is collection of the supernatant that contains vector. That is what is tested in this QC test for RCL.

In this supernatant, vector is in a very diluted concentration compared to the final product. Therefore, I think it doesn't impede sufficiently wild-type replication.

DR. SALOMON: Then I believe we are through Question 1. Do we agree with that?

DR. TORBETT: I guess I am a little confused because if it was expressing, wouldn't you knock down the number of possible recombinants in your readout assay? I think that is what Dr. Mulligan was getting at. The answer probably is yes? I think I just heard that is an MOI-dependent

phenomenon. I think that is what he was saying. Is that correct?

DR. HIGH: But the answer we got is that it is okay because it is done on the bulk harvest and the concentration is dilute. But it must be done at the end, also; right? It is not just done on the bulk harvest, is it?

DR. LI: That is not a requirement.

DR. SAUSVILLE: That is the issue, the characterization of the final product is, as we started all this out, problematic.

DR. SALOMON: Right. So our job isn't, however, to express our angst. Our job is to give them some sort of specific guidance on what we should do. I am being a little quiet because this is getting close to the edges of my experience and expertise. So I am looking to my colleagues here. You have got to know when you know and when you don't.

DR. SAUSVILLE: Correct me if I am wrong, but the point that--the specific issue in the question was for the manufacture of virus. There, they are doing what the FDA requires. What was just articulated though was that the concentrated material at the end of the day, after it has been

through T-cells, et cetera, what I, and I think a number of us had in different ways, have issue with is that that entity remains somewhat undefined.

There, I think your point is quite well taken, that one would like to know, and one could imagine infecting different cell types as part of that. Again, whether this fits into the requirement guidance, et cetera, I think it would convey confidence that the product is going to perform in the way we think it is.

There, it would be a dilution issue among others to ferret out the different variants that might be present.

DR. CONDE: Could I just make a comment? I am Betty Conde, also with VIRxSYS. I work on QC. The reason why we test the end-of-production cells and the bulk harvest for the RCL assay is because that is what the FDA requires. So we are trying to meet the requirements as stated by the FDA.

The reason why, as Xuexia and Vladimir mentioned before, it looked like the bulk harvest wasn't affected is because it is about 100-fold less concentrated than our final product.

DR. SALOMON: I suppose if I understand it, then you know that you have come to the lowest

rung in this particular area. What I get is you have a viral containing supernatant that you want to test if you have replication-competent lentivirus. So you put it on H9 and then you say the supernatant; right? Very low MOI at this point which is a big problem because we have already seen, with your vector, at least, and I am sure it is really true with everyone's, that the lower the MOI, you begin to get grayer and grayer.

So you do it at a very low MOI and you do six passages now. So if replication-competent lentivirus is happening, then, theoretically, it should be coming out passage to passage and anything left over from the early inoculum is long gone by the sixth passage. I am okay with that. And six passages to amplify it--well, I would again defer to my colleagues, but some of the stuff we do with endogenous retrovirus, six passages is pretty reasonable.

So you get amplification. And then you go back in and you do PCR for VSV-G and for--that is end. And also gag.

DR. CONDE: And also gag; right.

DR. SALOMON: You don't detect anything; right? You get zero signal.

DR. CONDE: Right.

DR. SALOMON: That is replication-competent lentivirus-negative.

DR. CONDE: Right.

DR. SALOMON: So now these guys are going--well, wait a minute. Later you are going to take 200 MOI and go to T-cell transduction, do the same assay at 100 MOI. I think that is what Dr. High was suggesting.

DR. CONDE: Yes. But we also test the transduced cells at the end, the same way, using an RCL assay.

DR. SALOMON: That's okay. The question here is knowing that you don't have any detectable RCL at an MOI that really doesn't represent the MOI that you are going to go on to your T-cells with an issue to anybody. It is a test. It is a crude test. It doesn't convince any of us absolutely that there is no RCL in that prep, is all I am saying.

Then the question is, okay, but leave us alone because we do it again after the transduction. Is everybody okay with that? Because, after the transduction, of course, life is different. You are not going on to H9. You are

going on to 293. You have got a background of wild-type HIV. There could have been recommendation. So a premise you are trying to say here is that your assays are relatively equivalent so it is okay because we were going to reassure you after the transduction.

I guess my concern here is that this 293 assay after the transduction is more problematic and less sensitive, but maybe that is wrong. That is what we need to discuss.

DR. ALLAN: The 293 assay is just to detect whether you have got VSV-G; right? It is not to detect whether you have got any HIV-related sequences. So, again, why are they testing bulk rather than the purified virus prep?

DR. WILSON: Perhaps I could clarify that because I am not exactly sure where that impression that we only require testing of the bulk harvest came from. So, just to clarify, that is not something that we require, recommend. I think that we would determine that on a case-by-case basis, what the appropriate stage for testing would be, whether it be a bulk harvest or a final product.

DR. LI: That was the document based on the murine leukemia virus.

DR. BO: Mark Bo from GTI. I may have missed this. What is the positive control, then, in the 293 test of the cell?

DR. LI: That question has been answered many, many times because we do not want to create an HIV VSV-G virus. I don't think anybody wants that around.

DR. BO: So there is no positive control?

DR. LI: We do not have RCR-positive--you can't create a positive control to predict an unpredictable event.

DR. EMERMAN: Hi. This is Mike Emerman.

DR. SALOMON: Go ahead, Mike.

DR. EMERMAN: One suggestion is, instead of H9 to use primary CD4 cells which would also amplify the HIV but then you come back with your PCR assay for VSV-G. So what you are really worried about is recombination between I guess anything that is VSV-G and HIV. Even though you would be getting replication of HIV in that culture, your test would be is there any VSV-G still around.

By using 293 cells, you are getting rid of all of the HIV. That is certainly a fair test, I think.

DR. SALOMON: Dr. Emerman, you are saying as long as they do the 293 test and maybe do a CD4 test at the end of the transduction that you are okay with the current strategy?

DR. EMERMAN: Yes; I am not sure what the 293 test is actually going to--yes; I am okay with that.

DR. ALLAN: I think the reason for the 293 is because, if you have got $10^6$ HIV particles and you have got one particle of potentially recombinant VSV-G, and you just threw it on CD4 primary cells, you are just going to get--you may not see VSV.

DR. EMERMAN: I think if you had $10^6$ HIV, you wouldn't want to be putting that into a person, anyway.

DR. ALLAN: Well, no; this is what is coming off the transduced cells, so you are going to get some--you may not get a lot of HIV, actually, but you would get some probably because of the breakthrough studies we saw earlier. So I would expect you would see some HIV.

DR. EMERMAN: Right. But, again, that is what you are worried about, is there something really, really bad in that stuff. One thing that

would be really bad in that stuff is something that had VSV-G in it.

So you are amplifying something that is actually more realistic. And you are also amplifying in the same cells which it is going into in the body, which is primary CD4 cells. Whether or not something grows in 293 cells is somewhat irrelevant. Those are not the relevant cell types.

DR. MULLIGAN: He is getting at the issue of how you preamplify before you test for the VSV-G.

DR. EMERMAN: Yes.

DR. SALOMON: Right. I heard that. I guess, trying to synthesize it here, I am still uncomfortable with the idea of testing the low-MOI bulk supernatant because I am not convinced by anything that the 293 VSV-G assay that you are employing to test the final T-cell-transduced product and, even accepting Dr. Emerman's excellent suggestion that you do it with fresh human CD4--I like to do that and that is something you should be doing.

But I still am not convinced, because of the sensitivity of the VSV-G assay and some of the unknowns like the absence of a good positive

control, which is now anticipating a little bit more the discussion in this second question--so I would still like to know, coming into the high MOI, 200 MOI, bulk, ready-to-go into the T-cell mixture, that there is no RCL.

I would like to know that before it goes into the human T-cells.

DR. SALOMON: Dr. Torbett?

DR. TORBETT: I guess it is a question of the percent they are testing of the sample, whether it is a low or high MOI. I kind of thought they were testing 1 percent of the sample of 3 percent, it shouldn't make a difference. But maybe I am wrong here.

DR. LI: We do test the 5 percent of the total harvest when the total harvest is 6 liters. But, above 6 liters, we test 300 ml.

DR. WILSON: I need to explain. That 300 ml is really specific for MLV vectors using an RCR standard that was developed for MLV vectors with an amphitropic envelope and qualifying your RCR assay for the sensitivity of detection of that particular standard.

We have not addressed that issue for lentivirus vectors.

DR. LI: So what should we do? That is the only thing out there we can follow.

DR. WILSON: For now, I would go with the 5 percent rule because we really don't have the tools and we haven't had extensive discussion to go with a model like we have for the MLV-based vectors with the 300 ml rule.

DR. SALOMON: That 5 percent, I believe, again looking for confirmation or discussion, that 5 percent should be the concentrated vector to be a model for what you are going to put on the T-cells a few minutes later.

DR. LI: Let's clarify one more point. I think that the rationale we do on the bulk harvest, because it is a transient system, you have a chance of DNA contamination. Most likely, RCR will happen in that step than later. You go through purification. You get rid of all your DNA--most of your DNA by benzonase treatment. So the cleaner product.

So if RCR will happen, RCL, if you like, it will happen in the more dirty, like you say, step. That will be the first step. That is the rationale behind it.

DR. SALOMON: That is an interesting

point. Again, I think we have to be very cognizant of production issues in the kind of advice we give. So I think that is very good. What I am sitting here thinking is, okay, fine. Then take the 5 percent, benzonase treat it, purify it and test it.

DR. LI: You mean after it goes through all the purification and test it again, 5 percent.

DR. SALOMON: I say that. But everybody here who knows me knows I have never made a 40-liter batch of anything and benzonse treated it and purified it, so it is perfectly okay for someone to say, easy for you to say but it is hard for me to do.

But I need to hear you tell me that.

DR. LI: The other thing is I think we have to really remember the vector is not our product. Our product is vector-transduced cells. That; we are vigorously testing that with DNA PCR, RT PCR, and biological assay.

DR. SALOMON: Dr. Cornetta, you are someone here that does this all the time. I know Dr. Mulligan, also, and maybe others. I don't mean to exclude anyone. Ken, do you have a comment? How do you come down on this?

DR. CORNETTA: Again, I would like to see

more data. You are talking about two potentially different products if you are talking about the undiluted or unconcentrated material versus the concentrated material. I would suspect--I guess I would tend to like to look downstream. It may be that the initial product, which is not concentrated, may have inhibitors to transduction or something else in there, too, that you may also have some more interfering particles, or something else, that might change how your readout is for detecting of RCL.

So, again, that can be done fairly quickly once you are confident of what your RCL assay is. But, again, those are the kinds of thing that you would like to look at to see what kind of level of detection you are getting.

It is a very difficult issue since we don't really have a good positive control to be able to follow through there. Again, there seems to be a concentration or a tendency to try to look at one cell line for detection. I am not sure that is good. I think, from what Mike Emerman was saying, looking at CD4-positive cells are probably good at looking for HIV recombinants that may have the envelope for CD4.

But then again, if we are looking for VSV-G, it may be that other cell lines are better. So I think people need to be expanding what kinds of cells they are eventually testing but, again, looking through the process of where you are testing, you may get different results depending on where you test that and at least some initial work should be to justify where your testing is going to be.

DR. SALOMON: Dr. Mulligan, Dr. Zaia. I know you guys also have experience.

DR. MULLIGAN: I would just say the concentrated product, with all the other caveats of how to do the amplification, but the concentrated product. You should copurify--whatever you had in the unconcentrated should coconcentrate, I would think. That is the source of the virus you are using for the infection.

DR. SALOMON: Dr. Zaia, do you have any comments on that?

DR. ZAIA: No comments.

DR. LI: Excuse me, because after you guys say it, I have to go home and do it. So I would ask you a question. For the concentrate, remember our product is an anti-HIV product, whatever the

positive control will be. After they concentrate it, if we do on the HIV-permissive cell line, the positive control won't grow because it will have anti-HIV activity. What am I going to use? How will I even validate my assay without creating an RCR, which nobody wants to see HIV VSV-G RCR.

DR. MULLIGAN: I don't know. I think this is probably being nonproductive to go through this in any detail, but it may depend upon the eventual multiplicity of infection at which you test the product. That could be prohibitive if you have to test a lot of product and you can't use a high multiplicity infection.

But that would alter, I assume, the efficacy of the effect of your construct. So, depending on how you did the infection, you might have to use more and more cells to do the infection. But that might be some way to go.

DR. LI: You are saying to do very low MOI transduction to--

DR. NOGUCHI: Yes, but these are the kinds of discussions--

DR. LI: So then we go back to the bulk harvest diluted

DR. NOGUCHI: Rather than get into details

here, these are the precise kinds of interactions that we invite you to share with our regulatory staff who will, in fact, work with you to determine the proper conditions.

I will just say that, even if your anticipated product is going to be the transduced cells, we require quality control of all components of that which includes the vectors and characterization and so forth. But we really would welcome further interaction directly with our staff.

I think it is unfair and counterproductive to try to work out the details here.

DR. SALOMON: I agree and I think Dr. Mulligan was starting to get that, too. I guess the only part that I don't totally understand yet is at that point all I want to know is if there is replication-competent lentivirus. I don't care whether wild-type HIV grows in these or not. There hasn't been any wild-type HIV yet. This is the concentrated supernatant. So tested on a number of different cells would work for me.

Let's talk about the VSV-G assay since that is part of this RCL assay. So now we are switching--oh; I'm sorry. Please.

DR. KHAN: Arifa Khan, FDA. I just wanted to mention something about the considerations that were taken when coming up with the 1 percent and the 5 percent. That really was specific to the therapeutic vectors that were generated using the MULV-based vectors.

A lot of things were taken into consideration. Basically, the bottom line was that the 1 percent really must at least reflect the volume of the vector virus that is being used in your transduction. Everything does have to correlate eventually with the actual dose, with the volume that is really going to be affecting the transduced cells going back into the patient.

The 5 percent also takes into consideration the number of cells used to generate the volume that is going to be used, at least in one human dose equivalent, used in the transduction.

So the numbers just didn't come out of thin air. For the MULV-based system, all those factors were taken into consideration including the total volume of the lots and the volume used in the transduction. So I think we will have to take those things into consideration for these

particular situations.

DR. SALOMON: Thank you. I think we will take Dr. Noguchi's and Dr. Wilson's guide here very literally and that is these are details. This is not what the committee is really supposed to be doing. I think we stay on a higher ground. I appreciate that.

So I am going through Question 2 here, whether we like it or not. By the way, I skipped a) because I think we answered that yesterday, and we have answered it earlier today, "Should an in vitro assay for detection of functional LTR-gag/pol-LTR recombinant be used as a lot-release assay?" I think we all agreed that that has not been a validated assay and shouldn't be a lot release, unless someone wants to disagree strongly with that.

Nonetheless, I think that we all recognize the scientific value and encourage that it be done.

DR. LAWTON: The only comment I would have on that if, in this particular case, it is a sensible assay to develop and validate, then it may be appropriate.

DR. SALOMON: So it brings me to b) which is the RCL infectivity assay of sufficient

sensitivity and is the positive control for the assay adequate for determining the sensitivity which then gets back to the comment we got also from the audience and sort of where I was going a minute ago.

So let me go back there; ten copies in 10,000 cells, by TaqMan PCR--you will have to correct me here. We do TaqMan PCR all the time in our lab and we can detect ten copies in a million cells. So I don't understand an assay--there has got to be something wrong here. Either I am being dumb, which is very likely, or you guys need to go back and work on this assay.

DR. LI: The TaqMan assay, each well you can put 500 nanograms of DNA. More than that, and the sensitivity gets affected. This is when they sell the instrument, what it can do, unless you can do it differently or you have a way to control your background signal. For ten copies, actually we can claim one copy. But anybody knows, one copy, you run into the statistical problem because you have to run nine, ten, twelve replicates in order to catch that one, and also you do have a false-positive possibility.

If we use that as a lot release, that will

run into a lot of problems. In terms of cell number, I would like to know how you do the million cells for one copy in the TaqMan in that one well. How do you run that? May you have a custom-made plate or something.

DR. SALOMON: No. We use the TaqMan technology in a somewhat different cycler where we can go do multiple dilutions going down from 1 microgram of DNA as cells. But I will have to--for this point, I will just say, fine. If you don't think you can do it on technical grounds, I am not a TaqMan, such an expert that I am going to argue it in this. I will do some of my own homework on that one as well.

DR. WILSON: Perhaps a different way of framing the question is if the qualification assay is done on 10,000 cells and the total dose is $10^9$ cells, is that of adequate sensitivity.

DR. SALOMON: No. I am not comfortable with that at all. So I think that, unless someone disagrees, you either have to have a more sensitive TaqMan assay, so I need to go back and figure out why there is such a disparity between your sensitivity and the one I am used to, in a different system, though.

Or you need to have a different assay because--see, that is kind of what scares me because if it is an insensitive assay, then the risk of moving RCL into the patient is all that more great.

DR. LI: No. The thing is we also go through six passages, remember, for the culture. So, after that, we do TaqMan again. So six passages of amplification.

DR. SALOMON: In this system, now, you are amplifying HIV, wild-type HIV, very likely, in addition to your RCL. Therefore, I am concerned now that there may be issues of competition, for example.

DR. LI: No; 293T is nonpermissive for HIV replication. We specifically try to catch VSV-G pseudotype.

DR. SALOMON: That's right. You are using 293 in one assay and 293 in another. Our collaborator and guru on TaqMan just stepped up.

DR. LONG: Zhifeng Long from GTI. I just wanted to clarify one thing. I mean, in TaqMan PCR, you can easily do 3.2 micrograms DNA per 96 well reaction. We do that all the time. We actually can do up to 6.6 micrograms. So nanograms

is not a limit that you can put into a 96-well PCR.

But that is not the point. I think I want to be fair is that, while in the assay system, they do a six-passage amplification, so they are not required to test $10^6$ cells because you know, if they can verify or qualify their system in a way that they can show the original input one virus somehow a positive control in $10^9$, they will six passage it. At the end, even using 10,000 cells, they can detect that positive control virus, that would be an elegant way of showing that the assay is sensitive enough

I don't really see that 1 or 10 copies in 10,000 cells is the limit because they amplify six passages. What I see is whether your PCR assay is an adequate assay. Actually, the PERT assay is more sensitive because it detects all spectrum of retrovirus. Or you may want to think of other assays like P24. But, in your case, if you mix with the HIV virus, you probably don't want to use it. So I think you want to think of some other way to verify your system separately with a positive control virus that is distinguished from your wild type.

DR. SALOMON: Okay. There are a bunch of

different things there. Thank you, Dr. Long. One of the issues is to use a control virus. I think that all the sponsors, not just VIRxSYS, have stated that they are not comfortable making a VSV-G control virus. I am okay with that. I don't think we need to go there unless someone on the committee feels really strongly about it.

DR. ZAIA: I don't feel strongly, but we saw an example yesterday that it can be done with the VSV, with the mutated accessory protein virus. If it is good enough for a cell genesis, why isn't it good enough--

DR. VERES: I have to qualify that that was pseudotyped. That was not HIV with a VSV envelope cloned into the virus. That was a chimeric attenuated HIV which was pseudotyped. I had both the HIV envelope and the VSV envelope on it. So it is only for a first round of infection.

DR. ZAIA: But it is still a virus that has VSV-G envelope. It may not be exactly what the virus would be like--

DR. SALOMON: But it doesn't have VSV-G DNA or RNA.

DR. ZAIA: No, but--

DR. SALOMON: That is what they would need

as the positive control for this assay.

DR. ZAIA: To further spreading. If you don't have an amplification step, you don't need to have it spread.

DR. SALOMON: I guess I would be okay with, at this point, using VSV-G RNA and VSV-G DNA. I guess the idea in the amplification is they can do it all with DNA. You don't need RNA. That probably is reasonable. So the question, then, would be Dr. Long's comments--I didn't totally follow this part, Dr. Long, is that you felt, because of the amplification, the sensitivity of 10 copies in 10,000 was okay because, after six rounds of amplification, you really have dramatically gone--you could have detected 1 in a million, is what you were trying to say; right?

DR. LONG: Yes; that's right. I think we have experienced six amplifications is ample to amplify a single virus from the beginning. The fact is that here it is a different system, that you need to validate whether the cell line, like Dr. Khan, I think, mentioned about whether you use appropriate cell lines to show, demonstrate, your assay system is sensitive enough to detect RCL. I think that is the key question.

The PCR part, you are right. You can put more than 10,000 cells in a well. But no limit here. You can put 1 million there. But I think the assay is okay in terms of using the final endpoint detection after six passages. More key is whether the assay is capable of detecting RCL without a positive control.

DR. SALOMON: Okay. I think that is definitely some progress on this.

Dr. Rao?

DR RAO: I just had an additional question here. All of this is only detecting RCL which has VSV-G in it; right? None of the other possible recombinants which might be because of the wild-type HIV where there is stress, where you are selecting the envelope, none of those will be deleted at all so you have no detection for what else might be happening other than the VSV-G.

DR. SALOMON: I think that is the point that Dr. Long made, Dr. Kingsman, Dr. Kappes and Dr. Cornetta. So the question to the committee is that is a general area of discomfort. I think we all share that. VIRxSYS shares it. They have come up with their strategy. CellGenesis, Oxford Biomedica, GTI, for that matter.