recombinant assay might have certain merits.

The advantages of an RCL assay is, of course, it is going to detect--again, this is in vitro, and I really want to stress that-- most of the time when I think about concerns for RCL, it is the ultimate RCL, it is the RCL in vivo that is going to potentially cause disease, but the advantages for the RCL assay in vitro is to guard against RCL.

I am not saying we shouldn't do that, we should, but I do think, under the quality controlled environments, it is ever going to be detected.

What are the disadvantages? As I have said, it is not predictive against the emergence of RCL in vivo, and it is not informative. It is not informative in many important respects in my opinion, as it relates to recombinants that will likely be formed.

It doesn't give you any information about the composition of recombinants, if they are formed. It doesn't tell you anything about the functionality or the replication potential, and I think that is a key word which I will come back to, potential of the recombinants.

It doesn't give you information about how the host will interact with the recombinants, nor how the recombinant will interact with the host.

Finally, I guess bottom line, it doesn't, in my view, tell you much about the risk that the vector will pose to the treated person.

[Slide.]

PCR assays. The advantage is they can detect vector and/or packaging-specific DNA, as Dr. Kingsman just pointed out. The disadvantages relate to specificity and the similar points I made about biological specificity for RCL assays.

[Slide.]

The advantages for a gag-pol recombination assay would include the enablement of the monitoring of vector stocks for what I called pre-RCR. Specifically, these recombinants represent a coding region with a functional gag-pol, and would likely contain or be flanked by LTRs.

So, what is the significance of this pre-RCR?

[Slide.]

The significance is several fold. It shows gag-pol-vector recombinants are produced, so

it tells you that you are generating a recombinant with functional gag-pol.

Without functional gag-pol, and I depict that as this structure, RCL cannot be generated in primary transduced cells, and importantly, nor can that recombinant, if it lacking this structure, or the vector if it is lacking a recombinant which contains this structure, lead in vivo over the course of time to the emergence of RCL.

I think this is a different wording of what I have just said. Functional gag-pol is required for the recombinant to generate RCL in vivo.

Thus, my point, in vitro monitoring for functional gag-pol-containing recombinants provides a tangible way to analyze lentiviral stocks in vitro for their potential to generate RCL in vivo.

[Slide.]

I have summarized or I have attempted to summarize all of what I have just said here. My hypothesis is that recombination will be formed and that instead of going this pathway, the blue box is supposed to represent the in vivo situation, where you take this vector which contained recombinants that would not be RCL, you place it in the

individual. You don't know what is going to happen with respect to recombination in vivo and you don't know whether it is going to lead to RCL.

Alternatively, if you use the gag-pol recombination assay, my hypothesis is that it could serve as a surrogate to predict the risk of this vector stock for generating RCL in vivo. Thus, you avoid the unknown pathway.

[Slide.]

So, this is where I was going to start. Believing that genetic recombination would occur, we wanted to understand how recombination could compromise or could generate forms that would pose a risk in vivo.

At this time, tat transfer assays, gag transfer assays, RCR assays were negative, so we devised an approach to detect, but more importantly, to enrich for the presence of the recombinants, and our idea was that if we could detect recombinants and enrich them, then, they could be characterized both genetically and biologically.

[Slide.]

Here is the approach. This represents a HeLa cell into which is integrated this genetic

structure. It contains an HIV LTR and importantly, puromycin. The point is that we transduce using a lentivector, HeLa cells to introduce this structure and screen these HeLa cells for sensitive induction of puromycin resistance by tat expression, so that with a single copy of virus in this cell, sufficient puro could be expressed, that the cells would grow and confer resistance in the presence of 5 micrograms or more per mL of puromycin.

[Slide.]

The notion was that if we could use this cell line where LTR was turned by tat, it might provide a more sensitive means to both detect and then select and enrich for recombinants. So, we generated these vector stocks, and the hypothesis was that if the vector, shown on the ends, and the packaging constructs, shown in the middle, in this case tat is highlighted because the key element that would need to be present in any recombinant is tat.

Then, tat would be expressed if this recombinant was formed and integrated, up-regulate the LTR and puro, conferring resistance, allowing us to grow this to large numbers and analyze the outcome.

[Slide.]

Before showing the data, I would first like to point out that I realize that there are what I am calling state-of-the-art vector components, third generation packaging construct, SIN vectors, and what I am calling the trans-lenti vector, which I will talk more about later, but as I just described, the system we first started using requires tat.

It requires tat to up-regulate puromycin selection. That was the design of our approach. So, those initial experiments were done using a packaging construct which contained tat and rev. It is a second generation-like packaging construct.

The vector contained GFP as a reporter, and three components were transfected into 293 T cells, vector stocks were produced and titered, and 10^7 infectious units of these virions were used to infect this LTR puro cell line to screen for resistant colonies.

What we found is in the absence of nevirapine, which is an HIV-1 specific RT inhibitor, approximately 1,000 colony-forming units were formed, and what does that suggest? It suggests that indeed recombination occurred between

the vector, again flanking the ends, and the packaging construct. It doesn't necessarily indicate that a recombinant, as I have depicted, was formed with a gag-pol reading frame, only that tat was present.

Importantly, with nevirapine, we find no resistant colonies, indicating that this is specific or dependent upon HIV-1 reverse transcription.

[Slide.]

To try to address more specifically the question as to whether recombinants are formed that contain functional gag-pol, we used the approach depicted here. This is the first step I showed on the last slide. If this recombinant is present, and if the gag-pol open reading frame is functional, what we would expect is for that infected cell--now, remember what we have done is grown this out, there could have been very few of these originally, but through selection we have grown them out, selected them, and we have a purified culture of recombinant-containing cells.

If this recombinant-containing cell contains a recombinant, it is functional in gag-pol, it should produce virions, that if

pseudotyped as I show here, we transfected VSV-G into this cell culture, could be mobilized and mobilize its own RNA.

So, what we did in following through on that experiment was transfer the supernatants produced from this cell line, and again transfected not just with VSV-G, but as I was alluding to when I made a comment to Dr. Kingsman earlier, tat and rev, to maximally stimulate and thereby detect the presence of these recombinants.

We took the supernatant, applied them to naive LTR puro cells, and detected 540 colony-forming units, indicating that indeed in the original transduced cells there were recombinants produced, suggested through the process of reverse transcription by this control, that contained not just LTRs, but open reading frames for gag and pol and tat.

[Slide.]

So, from the expanded culture--and this isn't the expanded culture, of course, but it is what I have used for illustration--we extracted the high molecular weight DNA and analyzed the five prime end by PCR amplifying this fragment, and that is shown here. This is a proviral control.

[Slide.]

So, we have a fragment that looks like the appropriate molecular weight, and by sequence analysis, indeed, what we found is that the vector sequence was joined to the packaging construct sequence, and of colonies we picked and analyzed, all 10 contained this sequence as you might surmise based on the requirement for an open gag-pol reading frame.

[Slide.]

If we use the same approach, that is, PCR amplification of a DNA fragment to amplify recombination on the 3-prime end of the vector, we also found joined in between the vector and plasmid, and I depict that differently because I think it is not just an interesting, but an important point with respect to DNA mobilization and recombination.

This is the 3-prime end of the vector, so up here somewhere, reverse transcription initiates and generate the strong stuff DNA, the RU5, which jumps or translocates to the 3-prime end of the vector through the sequence homology with R.

So, now you have a stranded DNA and the vector RNA. The polymerase continues reverse

transcription and what we have found by our sequence analysis actually switches templates into the packaging construct RNA, and we found of 10 analyzed, four different forms. They are depicted as a recombinant that crossed over into the 63rd A of the poly(A) tract, the 53rd and 47th, and this position I believe was at 108 nucleotides upstream of U3. Yet, again that happened at this position, which was 58 or some other number of nucleotides upstream of U3.

[Slide.]

The details of that data which I just showed are depicted here, but I won't bother to review it unless somebody has questions.

[Slide.]

So, the question then was if we remove--let me back up a little bit to try to get to the point I was trying to make early, so I should take advantage of that.

All the splits we have made, which are fundamentally accepted as state-of-the-art in vector technology, have these theoretical possibilities associated with them because they don't absolutely eliminate the potential for something, whether it's RNA expression or

pathogenesis or what have you.

With that in mind, our approach was to eliminate the reverse transcriptase and the integrase gene from the packaging construct. The point is without reverse transcriptase and/or integrase, but especially both, even if you had a recombinant that was formed between the packaging construct and the vector, that recombinant itself would have no potential to potentiate the development of RCL in vivo.

Importantly, as Dr. Kingsman pointed out, we can analyze the vector for the absence or the presence of these sequences, either genetically, as she described, or functionally, as I have begun to describe.

[Slide.]

So, now we take a new stock of vector that I call trans-lenti, trans-lenti virus, TLV, generated where reverse transcriptase and integrase are provided in trans. They are separated from the packaging construct, provided in trans via fusion with vpr, and we measure whether that vector can produce resistance colonies, as I have shown earlier, again using the same titer of vector 10^7 particles, we infect this cell line.

Then, we collect the supernatant after introducing VSV-G and tat and rev to test whether this vector contains this genome that can be mobilized, and the answer is no. However, I should point out that if in addition to VSV-G and tat-rev, we also transfect back into this culture, vpr, RT integrase. I don't have that data to show.

Then, we produce positive colonies, and what is the significance of that? It directly tells me direct data, that the block in DNA mobilization that I show here is due to the removal of RT and integrase, where it is provided in trans in the original stock.

[Slide.]

So, to summarize these data, recombination occurs between the lentiviral packaging construct and gene transfer vector. Integrated recombinants express viral proteins. They express, in this case, tat, gag, gag-pol. These recombinants produce progeny virions that are envelope deficient, however, if they are pseudotyped, they can mobilize RNA to naive target cells.

[Slide.]

In particular, I wanted to stress the point I made about recombination within the poly(A)

tract. What does this tell us? It confirms, number one, that genetic recombination has occurred during reverse transcription, and it helps substantiate that this is not an artifact. There are many types of questions and experiments we conducted to try to minimize false positives, if you will, but this is one of the strongest data that we have to indicate that this is actually occurring during reverse transcription as a result of inadvertent incorporation of other messenger RNAs into the very vector you are generating for treatment.

It also suggests that removal homologous sequences from the vector and the packaging construct may not itself be sufficient to prevent mobilization.

In addition, this might represent a mechanism by which genes without homologous sequence can be mobilized, including endogenous genes, and there is actually data published in 1988 using an avian retrovirus to show that oncogenes could be mobilized by a recombination event that occurred in the poly(A) tract of the oncogene message.

[Slide.]

So, that is really old data. The big question for my lab in the more recent future was how about the state-of-the-art lentiviral vectors, so what I compared are those listed here - third generation packaging construct, a SIN vector, and a trans-lenti, and I should point out these don't contain tat or this doesn't contain tat, so we had to devise a new approach, but it is also worth pointing out, not because of the approach, but just because it's different, that the trans-lenti system still contains tat.

[Slide.]

Vector stocks were generated and I want to point out that for third generation vector, we had a titer of 10^8. We also generated a stock with third generation packaging constructs/SIN vector also 10^8 and a transvector at 10^9, not because we can produce more, in fact, just the opposite is true, and we can talk about that later, but because I wanted to understand the differences with respect to the endpoint I will show.

Go back mentally at least to the HeLa puro line. The idea is that since now we don't have tat, we need to rely on mobilization of something else, so that HeLa puro line contained a lentiviral

vector introduced fragment, which contains a packaging signal.

The idea here is when we infect this virus, if a recombinant such as is depicted here was to be produced, when it expressed gag-pol, formed particles, perhaps this message would be encapsidated, and if it was, it could be mobilized and detected in a cell line which constitutively expresses tat under control of CMV, because now we are mobilizing this, remember we need tat still, so once it is in here, once this message is mobilized, reverse transcribed integated, we still need tat for upregulation to confer puro resistance.

[Slide.]

The data for the results are here. For third generation at a titer of 10^8, we detect whatever this is, 50 resistant colonies. For envelope minus virus, it detects zero, and these are really important controls, too, because I think we had to be extremely critical or of ourselves in understanding how these data might not actually represent what I depicted earlier.

The vector minus control is a very important control. The concern was the possibility that even without integration, the trans, the

packaging construct, the third generation packaging construct could be expressed and encapsidate the message which transfer puro resistance, but without vector, it, too, is negative.

The third generation SIN vector is positive, and this is an interesting result perhaps worthy of discussing later, because if you look at the published data, you would expect there to perhaps be a difference of 1,000 or 10,000-fold as compared to a non-SIN vector, but that is not the case.

I have had some ideas as to how that might be explained, and the transvector, even at 10^9 is zero.

[Slide.]

So, in conclusion, at least for this part, the third generation packaging construct and SIN vector generate recombinants with functional gag-pol capable of mobilizing DNA, and when I say "capable," these are envelope minus recombinants, only capable if they have pseudotype properties.

Separating RT and integrase from the packaging construct decreases the frequency by approximately 2 orders of magnitude.

Again something I said earlier, but I

think it is important, since a functional gag-pol genetic structure is absolutely required for the generation of RCL, and this includes not just in vitro, but over the long term in vivo, monitoring vector stocks for the production of envelope minus gag-pol-containing recombinants may serve as an in vitro surrogate marker to control against the generation of RCL in vivo.

The trans-lenti vector design is particularly amenable, but certainly not required for this type of testing.

[Slide.]

I would like to shift gears and show some other data because although I argued against this earlier, most of the data or maybe all of the data is really potential or theoretical based, that is, what value do these envelope minus recombinants really have.

Well, I think with respect to utility, they can serve as a pre-RCR measurement that might have some benefits compared with more conventional RCL assays, but the point I am getting at, are there other issues with respect to these types of recombinants that might be generated when it comes to biological safety, that is, are they

biologically significant.

[Slide.]

There is a lot of data that suggests that envelope is not required for virus transmission, and I didn't say that well, because it is much too strong, but let me walk through this.

It is known for quite some time now that cellular membrane proteins are incorporated into virions during virus binding. It is also known that this initial binding of HIV to its target cell does not require an interaction between the receptor and the envelope glycoprotein.

There are other factors, probably those related to the point here, cell-derived factors that are actually necessary for mediating at least predominantly that initial interaction between the virus particle itself and the surface of the cell.

Interaction between cell-derived membrane proteins and receptors on the cell surface facilitate initial binding. Interaction between cell-derived membrane proteins and cellular receptors can support HIV infection. There are three or four paper published in 1997 and 1998 about this. Let me explain because it is not quite fair I don't think without explaining.

In this case, what was demonstrated is that you could express CD4 in a cell that was producing HIV that did not contain an envelope, and that envelope now budding from the cell and acquiring the cell membrane with it as it buds, is expressing CD4, can interact with the GP120-expressing cell, fuse, and infect.

But the point is that this relationship between whether a specific envelope is required for infection, and simply by removing it, might also not be enough, and, in fact, the most compelling data was published actually this is 2000, in December, by Irving Chen's group, that HIV envelope minus virus can infect CD4-negative cells.

It is on that premise that I set up the experiments which I will show now, which actually shows that is not just true for HIV, but also for HIV-based vectors.

[Slide.]

Also, to reiterate, or maybe to emphasize my point, as it pertains to these experiments, why are we concerned about this structure, this recombinant if it exists?

If it is an envelope minus recombinant that produces envelope minus virions, and it's

mobilized, even independent of specific receptor envelope glycoprotein interactions to another cell, the point is every time it replicates, it potentiates the opportunity for additional recombination especially, if you think about it in vivo over the long term for the possible emergence of RCL.

[Slide.]

So, how do we design these experiments? We took envelope minus HIV-1 vector, real simple, CD4 minus cells, and asked four questions. Do the virions bind, do they synthesize DNA? If they enter, what is the route of entry, and do they actually infect these CD4 minus cells?

[Slide.]

The first question, do they bind? These virions are green fluorescent virions. I won't give you all the background, but using vpr, similar to that which I showed for RT integrase, we could incorporate enough green fluorescence protein into the virus particle by expressing vpr-GFP in trans to HIV-1, or in this case, to the vector. So, GFP is incorporated into the virion and under confocal microscopy analysis, we can actually detect single virions. Those are depicted by the green spots.

So, using this method, what we have demonstrated--I will move directly to the point--is that even envelope minus virus can bind with equal efficiency to both CD4-positive and CD4-minus cells.

[Slide.]

So, how about DNA synthesis, what is the consequence after binding to CD4-minus cells? Two types are shown, 293T and HeLa. GC53 is a HeLa cell that contains CD4 and CCR5 that is used as a positive control.

The pluses indicate infection with the vector, and here the pluses indicate 3TC, which is anti-RT inhibitor as a control. We are detecting early. That is strong-stop and full length or nearly full length, what I call R-gag DNA products of reverse transcription in these cells.

What is shown is that the envelope minus vector can not just bind as I show on the previous slide, but synthesize strong-stop and full-length viral DNA in these HeLa, and 293T CD4-minus cells.

In the presence of 3TC, that synthesis is blocked meaning that this is not virus-associated DNA. It requires entry into the cell.

[Slide.]

We were interested in the route of entry, as I mentioned, so we did an experiment, and this certainly isn't a comprehensive analysis, but the experiment involved the treatment of the cells with Bafilomycin A. Bafilomycin A inhibits a proton pump that acidifies endosomes.

The idea is that if the virus, which contains no envelope enters the endosome, which has been shown by others, and synthesizes DNA, we should be able to inhibit that using Bafilomycin. Indeed, that is the case.

With envelope-minus virus in the absence of Bafilomycin, we have DNA synthesis, but in the presence of I think it's 100 nanomolar Bafilomycin, we inhibit by about 5-fold the levels of DNA synthesis.

Importantly, this control isn't affected. This is envelope-positive virus, isn't affected by Bafilomycin because it takes a different route for entry, membrane fusion.

[Slide.]

I don't think I am going to try to describe all this data in detail, but the important take-home message is that in HT1080 cells, which is a tumor line derived from connective tissue, and in

a TU139 cell line, which was reported by Irving Chen, it's a gingival cell line, we find that the envelope-minus virus indeed is infectious, that is, it forms provirus. It's a bona-fide infection.

This infection, very interesting, in the case of HT1080, is not substantially or is inhibited by Bafilomycin as might be suspected from the slide I showed previously where we inhibit DNA synthesis by Bafilomycin, but in the TU139 cells, it is not inhibited, suggestive of two receptor glycoprotein independent pathways for entry, this, through an acidified endosome, and here, independent of the endosome, perhaps through some other membrane interaction.

[Slide.]

I am going to move through these slides very quickly. I wanted to point out that the trans-lenti virus vector, which I have been discussing, has properties very similar to those of lentiviral vectors with respect to gene transduction in targets that I think are of relevance.

Those include hematopoietic stem cells, central nervous system, and the eye.

[Slide.]

In a study done in collaboration with Tim Townes at UAB, we took purified bone marrow cells from mice, we transduced them with both lentiviral--and this data is published--and trans-lenti viral vectors, and grafted them into lethally irradiated mice, and after 16 weeks, analyzed the percentage of those cells which were expressing, in this case, GFP. What we find is the lenti and trans-lenti were very comparable, and importantly, that the bone marrow and this phenotype could be transferred by secondary transplantation, suggesting that we indeed transduced the stem cell.

[Slide.]

That is my depiction of our in vivo data for transduction of neurons.

[Slide.]

Here, the eye. Even after I believe it's 180 days, injection of trans-lentiviral vector into the subretinal space leads to the RPE cell layer being GFP-positive.

[Slide.]

So, in conclusion, the formation of proviral DNA recombinants with a functional gag-pol coding region may increase the risk of RCL. In

vitro monitoring for functional gag-pol-containing recombinants, pre-RCL if you will, may serve as a surrogate marker to control against the emergence of RCL in vivo.

The trans-lentiviral vector design splits the gag-pol function and is therefore particularly well suited for this type of quality assurance.

[Slide.]

I have described this, but I thought I would show it one more time because the transvector design might be better suited for this approach if we find that the lenti design is generating recombinants that contain gag-pol.

[Slide.]

My acknowledgments are to my lab at UAB, the work I mentioned with Tim Townes, Lori McMahon, she was involved in the slide which wasn't shown very well, where we transduced neurons, and Jean Bennett for the work in the eye, and John Wakefield, who is an employee of Tranzyme, who did most of the recombination work.

Thank you.

[Applause.]

DR. SALOMON: I was trying to think of the best way to do this, and I would accept some

feedback here. I thought possibly one way to do it is as the next thing we need to do is get on to the questions, and the first questions are the safety data which makes a nice segue into what Dr. Kappes just presented, would be to take a break now and then 10 minutes, and come back and have Dr. Kappes still here and begin the questions.

I need a break. Okay.

[Break.]

Open Public Hearing

DR. SALOMON: One of my duties is to again reiterate a welcome to anyone from the public to address the Committee at this kind of juncture, which at one of several points where we can hear semiofficial or unofficial open public comment.

I think, as you all know, I have not made any effort to restrict the public from getting involved at any point here, but I just want to invite anyone who would like to. We haven't heard any official requests, and I don't see anyone going to the podium.

[No response.]

Committee Discussion of Questions

DR. SALOMON: It's 4:30, and I am going to do my best in an hour and a half to begin some

discussion on Dr. Kappes' talk and sort of the implications and weave that into Question No. 1, and then if you will forgive me, at some point I will make an artificial distinction because in an hour and a half I want to get through these three questions.

What safety data should be available prior to initial use of HIV-based lentivirus vectors in Phase 1 clinical trials?

They are asking us to consider really the elements that I think have been set up very well up until now by the discussions, and I think highlighted very nicely by Dr. Kappes' talk in which he kind of took a different attitude about, well, maybe replication-competent lentivirus assays isn't exactly the best way to go, and I think we have kind of gone back and forth on that.

So, why don't we start there. I know we had one question waiting at the end. Susan.

DR. KINGSMAN: In my past days as a trustworthy academic, I did a lot of work on the stoichiometry of gag and gag-pol in particles because, of course, the way the virus is set up, is to make 20 gags to every one gag-pol. It is quite a precise stoichiometry, and if you get that wrong,

you tend to get a lot of aberrant particles, tubes, and strange shaped things.

So, I wondered how your system got the stoichiometry right and whether you have done any EM studies to look at the homogeneity of the preparations.

DR. SALOMON: Can I clarify one thing? You are talking about his system?

DR. KINGSMAN: Yes, his very special vpr-gag, vpr-pol system.

DR. KAPPES: It's an important question and fortunately, we began addressing that question far before we had--"we" meaning my lab I suppose--any interest in using HIV as a vector. I have studied virus assemblies since I went to Birmingham 15 years ago, and we were interested in understanding, using vpr, how questions could be asked relating to the fundamentals of virus assembly, that is, what role does gag-pol really play in virus assembly.

So, that from perspective, we were working with HIV and optimizing the system in many regards, which aren't obvious from the data that I showed. But your point is well taken and it is not so simple as depicted where you transfect in whatever

it is, 4 micrograms of this and 3 micrograms of that, and wham, you get a vector which is of sufficient titer.

We can generate titers similar to Lenti, but there is always a difference. We have never been able to optimize the system to generate a titer that is as high as Lenti. If we had done the experiment 100 times, and you took an average, it is probably 3- to 5-fold less because of the point that you make.

There is a stoichiometry. There is even other factors perhaps, and, for example, not to go into--well, to deviate too far from your question, our data would suggest that RT integrase, intermediate, which is produced during maturation of the virus particle, plays an important role in the formation of an infectious nuclear protein complex.

So, there are a lot of factors, and a titer that I showed is the best we can get which compares with Lenti by a 3- to 5-fold reduction.

DR. SALOMON: What I want to do is stay focused. I don't think that the point here, albeit quite interesting, would be the idea that there is maybe yet another delivery vector in development

after what we are doing with Lenti. I don't think that is what we are supposed to be talking about right at this second.

I think that what I would like to do is stay on track with what the implications are of what Dr. Kappes presented, what Dr. Kingsman presented, and the others, in the context of this first question.

Earlier on, I tried to articulate the idea that, well, one of the things that was in front of us was what would be the definition of a replication-competent lentiviral assay, and we made some discussion of that. I am not certain we nailed it, but we made some discussion of it.

But now I guess the question would be, thanks to Dr. Kappes and the discussion that ensued, is a replication-competent lentiviral assay the best sort of assay to hold out, and if it is not, we need to have some discussion about what would be reasonable alternatives to an RCL assay.

Have I got that right? I think that was the strategy here.

Do you want to start with a comment on that specifically now?

DR. KAPPES: I think I will just reiterate

what I presented. I think recombination is likely, I think we will, if you analyzed titers that are likely to be used therapeutically, generate recombinants that contain LTR, albeit perhaps SIN LTR, gag, pol, LTR.

If that is true, you essentially have the essential retrovirus architecture minus envelope. So, I would suggest that measuring for recombinants which contain functions that are absolutely required to perpetuate the risk of generating RCL in vivo would be a feasible and perhaps even reasonable means of trying to get out at safety.

DR. SALOMON: So, following that along, Dr. Kingsman suggested a similar premise, that the gag-pol was absolutely required for a replication-competent lentivirus, and I think that is the same premise you are making.

So, the assay that she and her colleagues are proposing is one where you amplify it in a target cell line. For right now I don't want to go into the morass of whether the target cell line should be horse or whatever. I think that is an interesting discussion, but maybe not right this second.

You amplify it in a target cell line, an

appropriate target cell line, and then you use a PERT, product amplified reverse transcriptase assay, versus your strategy, which is I guess you would put it into this tat, puro, HeLa cell transfectant, right? And then look for puromycin resistance.

Now, there, your amplification, instead of being PERT, is the selection on puromycin, correct?

DR. KAPPES: Right, and also you are analyzing for a functional gag-pol, wherein Dr. Kingsman's approach, just the presence of reverse transcriptase or whatever was being monitored, that would indicate a recombinant, would be enough to reject the stock perhaps.

DR. SALOMON: Right. So, the RT activity in the viral particle itself could give a false positive in the PERT assay. She gets around that by amplifying it in the target cell line.

DR. KAPPES: Another difference is that I think you can imagine all kinds of recombinants, and I could be completely wrong, especially from packaging cell line won't be recombinants, but if there are, the recombinant itself doesn't pose a problem, so detecting the presence of the recombinant, if it's not functional, perhaps isn't

a biological concern.

DR. SALOMON: So, if I have stated it right, we have got RCL assays, we have a PERT assay after amplification in a target cell line, and we have a gag-pol assay that is based on an amplification on the puro resistance in this tat-HeLa model.

Any comments about relative values? Dr. Rao.

DR. RAO: I actually just wanted to check, and maybe I have missed something here, but let's take the cell genesis system where they are looking at an RCL assay, and they tried to do one additional assay where they used a VSV-G envelope.

I am trying to see whether they were actually now trying to assay the gag-pol, the combination events that have occurred, and whether they could just use that as a modification or an additional part of the RCL assay to pick up those recombination events, and whether that would be a simple test in their mind rather than having to do a separate test at all, and whether there would be any relevance.

DR. VERES: Yes, I had those backup slides. We have a 293G cell, which expresses the

VSV envelope, but under tetracycline control. So, theoretically, if we generate sort of partial recombinant, which is basically a function of the core without an envelope, that could be transfected into the cells and further passage and basically amplified.

So, if it's a fully functional core with full gag-pol, rev, and everything in it, that could be passaged and basically using p24 assay, it will be amplified and detected after that.

DR. RAO: Do you think that that would be a reasonable assay in terms of detecting intact gag-pol?

DR. KAPPES: I think it could be, however, an important difference, as Gabor just pointed out, it would require additional recombinations, that is, by adding envelope alone, you wouldn't detect the recombinant unless the other genetic components of the original vector were also present, that is, that they also recombined.

In my approach, remember--maybe I walked through it too quickly--I provided additional elements in trans, such as envelope, such as tat, such as rev.

DR. VERES: Can I just reply to that? I

mean I do understand because you detect every sort of recombination whether that is recombinant is being functional or not. I mean the question is whether this is really important if we consider this is a product, do we want to detect which potentially will be dangerous, or just do we want to detect a recombinant which is there, but it is actually not going to go any further, and there is no potential that it is going to be dangerous.

DR. SALOMON: That is exactly what we are supposed to be talking about, exactly, and that is, is detecting that a safety factor.

DR. KAPPES: Well, let's walk through it carefully and see. Again, we get into the theoretical how, but we have a recombinant that is LTR, even SIN LTR, packaging signal, gag, pol, LTR. So, you have no rev, so you could make the argument that that transcript will never be expressed, but you can look at the literature and you find that that is not true.

There is a huge difference in rev minus and rev positive expression, but it is not absolute. You look at the effect of the deletion in U3 on expression of the recombinants message. Again, you have a huge difference in the total

amount, but it is not absolute. You still have expression.

So, if you are looking at a measure--and I know I am going to extreme ends--but if you are looking for a measure where I think you can tangibly say that it is or is isn't, I think I would look at an LTR, gag-pol LTR recombinant even if it contains a SIN deletion, even if there is no rev, as a potential risk to safety in vivo over the long term.

DR. RAO: I just want to continue with this thought just for one more second. If we assume that you can do a test in 293G line, then, one difference between the assay as Dr. Kingsman suggested, and what you guys have suggested, is really a matter of which method of amplification you are using and how much time the assay takes.

In your mind, maybe I missed something, is there a difference other than those two things that I mentioned as being, you think one is more sensitive than the other necessarily, or one is intrinsically better than the other in your opinion?

DR. KAPPES: I think one other important difference is the functionality of the gag-pol. In

a biological assay, of course, you are going to require that reading frame to be fully functional.

DR. RAO: Which the RCL in the 293G would require.

DR. KAPPES: Either that or the assay I described, yes.

DR. RAO: But not the assay that Dr. Kingsman described.

DR. KAPPES: Not per se.

DR. SALOMON: But the assays are dramatically different because, in the one assay, you have to get infection of the target line. That is what got into the discussion of you should use a horse cell line or should you use the 293, or should you use some other line.

Once you get infection to that line, the efficiency of amplification of that line is an issue. In Dr. Kappes' assay, you basically are going into the HeLa, and you are amplifying on the basis of the presence of just having that gag-pol with a packaging sequence, because the rest he is providing, and then you are selecting for puro, so it is not based on the infection of the HeLa line, right?

DR. RAO: I understand that. I was just

trying to say that if you have two assays and you have to choose between them, do you have to say that one is necessarily a shorter time period, but equally effective, or is it both are equally sensitive, one takes much longer to do because you have to do an amplification and selection for five weeks because that would be a concentration in any kind of test that you select.

I understand that there are differences, but the goal of those tests remains the same.

DR. KAPPES: In response to that, I will point out the assay that I described needs to be highly controlled and is difficult. It is very labor intensive.

DR. MULLIGAN: I think to try to get done by 6:00, I would very simply separate a real garden grade variety replication-competent virus assay from these other things and see if we can move ahead from the first one.

I would say that from what we heard, Cell Genesys, or Sue's, they are perfectly okay. They are just like MLV. I wanted waste a lot of time with details of which is a better assay. That is, we want real garden grade variety replication-competent virus tested, and it appears

that there are several different ways to skin the cat.

Then, I think I wouldn't get too complicated about which one of these other assays, I think that is a significant issue relative to the MLV field, which is do we care that we are transferring HIV DNA sequences to recipient cells, and I wouldn't make the distinction whether they are gag coding or whatever. They are not replication-competent. I would lump them basically as they are HIV sequences and do we care.

Here is where this dovetails with the different packaging systems, the transient systems, the 2-plasmid, 3-plasmid, 4-plasmid, and I don't think we are going to resolve anything more than there are theoretical different values to these different systems.

I would argue that I would want to know what is transferred in terms of HIV sequences to recipient cells. I don't know whether I would make that a release criteria for the testing assay, but I think it is very important to have that kind of info, but I would not support institutionalizing the need for a gag-pol assay, you know, that transfers gag-pol.

So, I would be very conservative and say we are in good shape with the good old-fashioned assays, and I would leave it to people that want to look for these other things and maybe encourage people to look for these other things, but not to go past that point.

DR. SALOMON: So, that is an important answer. That is exactly the kind of answer that I was looking for, and that was do we abandon an RCL assay, which was the premise of some, and you are saying no, you don't abandon an RCL assay, in fact, if anything, you allow these other assays to be developed and validated, but you stick with good, old basic RCL assays.

Frankly, I am comfortable with that.

DR. BORELLINI: So am I. Based on what we know, the point where the highest likelihood of an RCL to be generated is probably not in vivo, it is in the fermenter where you have 40 liters of cells that are spewing out, the viral components.

So, this is where I think we need to test. At that point, you need to test what represent the biologically active threat, which is the RCL, not the partial. The partial, I think would be very important to test if we had a lot of data

indicating that once in vivo, there is partial, may go and do all sorts of things, but I don't know, from the HIV field, I am not sure the data has been seen in patients that have HIV, picking up of envelope sequences here and there, or endogenous sequences here and there, I am not quite sure the hazard has been seen.

I am looking at the HIV person.

DR. SALOMON: Well, there is certainly data that HIV, had different strains of HIV move around elements, envelope proteins, and LTRs in patients with more than one species of HIV, unless I am totally getting that wrong.

DR. BORELLINI: As has been seen, did they pick up envelopes from, for example, the endogenous retrovirus sequences, or other sequences that share poly(A) or something? I am not aware that that has been shown so far.

DR. SALOMON: Any other comments on this question of RCL? Dr. Torbett.

DR. TORBETT: I guess I would agree with Dr. Mulligan. I don't think we have to go to the extra point of going way beyond what is already standard. I think that the current assays are appropriate. Again, I would agree that going

beyond in experimental systems to validate would be useful, but it seems putting onerous and very serious consideration to how to move forward will slow down the field. The question is do we need those. My answer I personally believe is no.

DR. KAPPES: I would like to follow up with a point. If we ignore the recombinants which contain functional gag-pol, we are assuming that those recombinants have no possible harmful effects in the patient who is being treated with the vector.

Now, I would also like to compare that scenario with what we have learned or haven't learned for MLV, which highlights my concern for these recombinants.

I showed data which suggests that even though they are envelope-minus, they might mobilize. In the case of MLV, in our 10 years or whatever of experience, it would suggest to us that if these recombinants existed in that system, that they never grew into some monster, but keep in mind, MLV doesn't infect non-dividing cells, so I think we are comparing apples and oranges, at least with respect to the point I am trying to make.

These HIV recombinants will have a

property unlike MLV in that they will be able, if expressed, to mobilize their recombinant genome to other cells, again perpetuating the opportunity at least for additional recombination.

So, I am not quite so quick to give up the notion at least that detecting these recombinants don't have value for predicting the safety of your vector.

DR. ALLAN: It just seems to me that the issue of recombinant gag-pol, to me would have more interest in (b) rather than (a) because of mobilization with the wild-type HIV. I just have one question.

In your assay system, where you are using puromycin to select your recombinants, those are actual cells that you are looking at, so some of those, is that an amplification of a single--so if you count 400 cells, could that one recombinant cell that has grown out to 400?

DR. KAPPES: Very astute observation, that is correct. That is exactly right. It could be one recombinant-containing cell that mobilized 400 puromycin markers.

DR. ALLAN: I was suggesting that it was one puromycin, it was one introduction into a cell

that was puromycin resistant, and then that cell grew out.

DR. KAPPES: Each colony was a cluster of cells.

DR. ALLAN: Okay, fine.

DR. SALOMON: So, recombination between vector and wild-type HIV, just to sort of continue on this roll, again, we have touched on this several times, is there more to say about that?

DR. MULLIGAN: What is the difference between (b) and (c)?

DR. SALOMON: They are related, but I assume the concept in (b) is not mobilizing it, but basically just recombining.

DR. ALLAN: It should be (c), (b) then, because mobilization leads to recombination.

DR. MULLIGAN: I don't understand. What is the context where we would be looking at--

DR. SALOMON: I am thinking of the transgene could be mobilized, right, in the HIV vector, just packaged as an RNA transcript, whereas, recombination would actually create a de novo lentivirus.

DR. MULLIGAN: I guess I would agree with the gentleman who said (C), then (B) maybe.

DR. SALOMON: I don't have any problem with the order here.

John.

DR. ZAIA: In a way, I think (b) is more important than (c) in the sense that we know already that recombination can occur and make things worse. I mean there are model systems for that. The question is will that ever occur. So, that probably means, who knows, but we should set up the human experiment, so you monitor for that.

So, up to now we have not monitored with MLV, who we probably should be. Had we been doing that for the last 10 years, we would have a database now. I know I am not doing that, maybe some other people have been doing it.

So, as we go to these newer vectors, I think it is going to be important to give more than lip service to 1(b) because we will at least learn how to progress.

DR. SALOMON: I agree with that, and I think one of the suggestions that came up on some of the discussion was that periodically, we ought to be taking blood from patients in these studies as part of this evaluation, and sequencing some of the species specifically for recombinations between

the packaging vector--no, I am saying the wrong thing actually--the lentiviral vector, the transgene, delivery vector.

DR. MULLIGAN: So, you are talking about (b) is in the context of an in vivo case where there is wild-type, but still I think we ought to address (c) first, because the issue is whether or not, I think fundamentally, how high the bar we ought to set for allowing mobilization to occur, you know, a situation where recombination can occur.

DR. SALOMON: I am just trying to be practical. My feeling here with respect to the recombination between the vector and the wild-type is we have got to watch for it, and the only other question that one would follow here, and I am not comfortable with it, but let me just pose it, would be saying you can't use a certain vector class in patients that have wild-type HIV because of the higher risk of recombination between, let's say, an HIV lentiviral vector, a human lentiviral vector, and a simian or a non-primate.

I am not sure that anyone wanted to go there yet.

DR. MULLIGAN: But I agree that is the

relevant issue here.

DR. SALOMON: Can we have some comments on that? Dr. Allan.

DR. ALLAN: Going back to what John is talking about, I mean to me, the greater issue is, okay, you have got chunks that are not replication-competent, and they go into the patient, and then the wild-type rescues it, that is where the issue is. To me, that is a major, major issue here, it is not a small issue. I think it is a major issue.

DR. SALOMON: I am sorry, I didn't mean to say it was a small issue, it's a big issue.

DR. EMERMAN: Can I agree with Dr. Allan there? I think the major issue with going into HIV-infected patients is generating new viruses within those people. Even though the vectors are derived from HIV, if they have LTRs, they will recombine and make new LTRs, that weren't in the patients originally, which is I think an argument for using the SIN vectors in HIV-infected patients.

DR. SALOMON: Just for the transcriber, that is Dr. Emerman, correct, that just commented?

DR. EMERMAN: Yes, that's correct.

DR. MULLIGAN: So, if we trace how that

might occur, basically, you will have an HIV infected cell, that will be like your packaging, then, you infect with your vector, and you would have something that would have greater or lesser capacity to be packaged, RNA to be packaged, and then whether or not it has 3-prime end sequences to be then at some frequency turned into a two LTR mixed-up something.

DR. EMERMAN: The vector has two LTRs, and it gets into an HIV-infected cell. They get co-packaged at an incredibly high frequency, the recombination occurs. That is not a rare event. They get co-packaged, the recombination occurs just as a normal process of reverse transcription in the next cycle.

DR. MULLIGAN: But several of the vectors, people are going to test, are going to be vectors that specifically should not have mobilization capacity, so they would be SIN vectors, that wouldn't make a lot of RNA. They may be--Sue didn't toot her horn about some new vectors that truncate the RNA transcript in the center of the transcription.

So, I think this now brings us directly to relating vectors, specific kinds of vectors to the

issue of mobilization, and if we don't want mobilization, if we think that is an undesirable feature, then, we have to actually begin to set a high bar for the characteristics of the vector as it pertains to mobilization.

DR. SALOMON: Earlier, I had commented that, you know, just as a premise for discussion, I don't think that the first clinical trials of lentiviral vector therapy in HIV-infected patients should have any mobilization. I am not saying that it never should, I am saying I don't think the first trial should.

DR. CHAMPLIN: I guess some analysis of what components of the vector would, in fact, be harmful should go into this. I am just thinking theoretically if antisense to HIV is the sole gene you are delivering, and there is no new product that would make the HIV more pathogenic, would that, in fact, be a risk if it was, in fact, mobilized.

In fact, you could argue that that was the way to deliver your antisense to even more cells and to make it even more effective. So I am just wondering if absolute bans here are appropriate or can there really be a vector analysis of

risk-benefit relationships?

DR. MULLIGAN: I would argue that the risk is the unknown of what you are going to generate. I mean, again, coming back to the question can you make something worse than what is already there. I would argue probably, probably. We can do much more sophisticated--the body can probably do much more sophisticated things than we as vector people might be able to do.

So I would say the theoretical--this would be one case where the theoretical risk of generating something in a patient that is replication-competent, that is not garden-variety HIV is something to be avoided at all cost.

DR. SALOMON: I think if I understand also part of this is it is not so much the concern--not that I am trivializing it--that the RNA ribozyme transgene will get mobilized albeit I think you could talk about that. But the fact is that in systems in which mobilization is going to occur, it implies that there are higher levels of lentiviral vector RNA present and, therefore, recombination with backbone elements of the delivery vector are much more likely to occur and that is more dangerous than any of issues reflecting

specifically to the transgene being delivered.

DR. EMERMAN: I think it is not the vector that we are worried about going about it, the recombination with the wild type is already there making the wild type worse. It is not the vector is making it any worse.

DR. SALOMON: I'm sorry; I was hoping to say that. If I didn't properly, correct it.

DR. ALLAN: I had a question for Mike Emerman. This is John Allan. Where do you see the greatest threat in terms of recombination using this system with the wild-type HIV co-packaged with the vector? Would you see that as any greater threat than wild-type HIV?

DR. EMERMAN: You don't know. If the vector has, for example, sequences in gag that are necessary for the packaging, some of those sequences contain parts of the matrix protein although the matrix is truncated in the vector. The matrix has epitopes for CTL lysis. You have got a recombination. You would generate new epitopes if someone had already had the existing epitopes. It is a mechanism of generating escape mutants, for example.

So, it is the recombination between the

existing elements in the vector and the endogenous HIV, and just making that one a little bit worse.

DR. SALOMON: So, trying again to come to some sort of committee statement to the FDA or agreeing that we can't make the statement, that's okay, too, can we say--I say that you shouldn't do a mobilization protocol in patients with wild-type HIV infection in this first phase of trials.

DR. ZAIA: Because?

DR. SALOMON: Because of mobilization and because of the danger of creating a novel species of wild-type HIV or I guess now modified HIV, that would have properties that might be more dangerous than the current available crop.

DR. DELPH: I guess my question is would you limit it to patients with wild-type HIV, because as I have been saying earlier, you cannot assume that people who don't have wild-type HIV today won't get it tomorrow.

DR. SALOMON: Fine, that is an interesting point. Why don't we take that as a (b). It was good, I won't leave them one alone, but am I just saying can we agree or disagree that we shouldn't have mobilization?

DR. ALLAN: I guess my question is, is how

can you prevent mobilization? I mean you are going to get mobilization, how do you prevent it?

DR. EMERMAN: The SIN vectors would eliminate the mobilization.

DR. SALOMON: Whatever we have said is basically the self-inactivating vectors, the SIN vectors, or otherwise hobbled vectors that have very little RNA that would get mobilized.

DR. TORBETT: I guess with the SIN vectors, the LTR is upstream of basically the promoter, so unless you inserted somewhere that had an upstream promoter and get a full-length transcript, that would be very difficult because you would get a package of something that would be much smaller.

DR. MULLIGAN: The only thing that is a little complicated is if you look at how people have addressed the question of mobilization in tissue culture, and I think one of them will talk about the VIRxSys. It is not satisfying, I mean it is not a clean picture of things that really should mobilize don't do it efficiently.

So, I think we ought to leave that just as an issue that we are aware of, that the measurements for mobilization, which are

undoubtedly going to be in vitro, not in vivo, will have to be given careful consideration. That is, we need the same sort of talk about mobilization assays as we have had on the helper virus.

DR. SAUSVILLE: I would agree that the information we have been presented today does not present me with confidence that we can pick out ahead of time with confidence the type of assays that would permit mobilization, and I also am drawn to the point of view that one could imagine situations where that is actually a good idea depending on what you are trying to achieve.

Finally, since no one is never without risk of HIV infection, if you make a prohibition, then, we might as well go home, because we are never going to reach--I mean the ultimate never in absolute is I think going to be very difficult to, in practice, realize.

So, I think it needs to be addressed on a case-by-case basis. I think it needs to be tracked. I think it needs to be considered what the goal of the construct is, and beyond that, I just don't think we have enough information.

DR. SALOMON: Is that a position the Committee is comfortable with?

DR. MULLIGAN: I think it would be okay if we did have a disagreement on the committee, and not a consensus.

DR. SALOMON: I agree.

DR. MULLIGAN: We don't have a consensus then, it sounds like.

DR. SALOMON: That is what I am getting at. Let me try and articulate this, and this is going to require some modification from my colleagues.

In terms of Question 1, generally, we feel that we still should put the emphasis as a first cut at safety on more of a classic RCL assay. We didn't quite define the ultimate sensitivity of that, but I thought our discussions that we had when Dr. Kingsman presented her material was pretty good in terms of defining a sensitivity, and the fact that it seemed like nobody disagreed with the concept that sensitivity ranges for detecting RCR in Moloney retroviral vector systems was acceptable to the group unless someone wants to chime in now, in other words, experience, 10 years of experience defining sensitivity of RCR assays seemed to be acceptable to be transferred to assays for RCL.

So, that would deal with that. Now, I

think that the group followed the very interesting molecular biological reasoning for additional kinds of assays, as suggested by the two speakers, but I think that my impression, and again the sense I get from the group, is that none of these newer albeit very interesting assays, are (a) very easy to do perhaps, maybe not that as much as not really validated, and it is really rather far from clear to me at least right now how a positive result in one of these assays would relate to a negative result in an RCL assay.

In other words, I don't think in the end, aside from just a very good scientific line which I follow, that I would be comfortable if I was the FDA saying oh, the RCL assays are consistently negative in this prep, and I think the field is pretty sophisticated now about that, and yet we keep getting a positive in a gag-pol puromycin assay, and therefore, you can't do your trial.

I don't think anything I have heard today would really make me comfortable with that. So, I think there has to be further research and validation of it, which would be a good objective perhaps as follow-on studies for these first trials.

In terms of mobilization, putting (c) before (b), I think that we don't have an agreement, so I think there are some people on the Committee that are saying there are methods like self-inactivating vectors that reduce significantly the amount of mobilization, might eliminate it, might not, and that those would be preferable in the first trials of patients with HIV.

There are others on the Committee that say that's probably not a good idea because there may be situations in which mobilization has a therapeutic benefit, there may be situations in which patients could get infected afterwards or prohibition on mobilizing vectors might lead to a necessary limitation of patient groups that could get these kind of therapies and therefore hold the field back, and that wouldn't be fair because the proof of a detriment of mobilization is far from clear, if it can even be of therapeutic value.

That recombination between vector and wild-type HIV, well, nobody is comfortable with that, but the question is what is it that we could do besides what we have already talked about to reduce the risk of recombination between vector and wild-type HIV.

I guess I don't think that we had any clear answer for you there either, and that may reflect the state of the field.

DR. MULLIGAN: That is coupled to the mobilization question, so I mean I think that the point of view that it is okay to have mobilizable has to be coupled with an articulated point of view about events between wild-type and vector.

DR. SALOMON: So, what Dr. Mulligan is reminding me is that one line of argument is if you allow mobilization to be a part of the therapy, then, you potentially increase the risk of recombination between vector and wild-type HIV, and he is pointing out the logical flaw in that it seems everyone's consensus is that that is not a good thing.

DR. ALLAN: If you put in the SIN vectors to reduce mobilization, you are going to reduce recombination.

DR. SALOMON: So, that would be an argument to favor protocols in this first phase of HIV studies using lentiviral vectors that would reduce mobilization, but there are people on the Committee who are not comfortable with that. That is the cycle here.

DR. ALLAN: But I think the people who aren't comfortable, maybe it is because you are interested in efficacy, and the first studies are safety studies, so I think you have to separate those two.

DR. SALOMON: Dr. Sausville, you were the one who articulated the mobilization argument.

DR. SAUSVILLE: Right, but the limit case of the safe vector will be one that doesn't work when you eventually look for efficacy. There, you have it.

DR. SALOMON: I was pointing out to Dr. Mulligan, just teasing him, that that was one of his comments yesterday, was the safest retroviral delivery system was one that didn't work.

DR. CORDOVA: I would just like to follow up on RCR assays versus this type of vector. The one difference is that this is a human pathogen, and that we can see at least what would be the most likely type of recombinants that are undesirable, and that would be potentially a similar vector or a similar virus that has a broader tropism, for example, so the typical RCR assays would not pick up a partial recombinant, that only takes the VSV gene, for example, into the next step.

Even though we don't want mobilization and we don't want recombination, that may, in fact, occur with the wild-type HIV. Then, if we take the next step where we think that anybody may become an HIV-positive, of course, we can take it to the extreme, but all the same, I think some discussion would be warranted on the possibility of creating a new lentivirus that now does incorporate the VSV as the envelope.

Although it hasn't been detected in vivo, it hasn't really been looked for that hard in vivo either as to natural pseudotypes that then just occur.

DR. NOGUCHI: Dan, if we could pursue a point related to that, the statement has been made that surely with so many HIV-infected individuals, we "haven't seen this sort of recombination that we are worried about."

I would like some sense of the Committee as how valid a statement do you think that really is, has it been looked at, by what means, is it something to give us any comfort at all.

DR. EMERMAN: I think the point that the recombination is going to occur in the fermenters was the relevant point, you know, in populations,

these viruses aren't in the same cell types, and there are not mixed deliberately, so in testing the culture brews is what you are meaning to do.

DR. NOGUCHI: Excuse me, but yes, but in the in vivo experiment, you have everything that you need to package and recombine including non-SIN LTRs, so I agree. I think we all agree we should know as much about the synthetic product that is going in as a therapeutic, potential therapeutic, but the larger question is really whether or not our experience with patients who have HIV infection gives us any reassurance at any point that there is either a lack or that if recombination with endogenous sequences or elements, or even other viruses like herpes, takes place or doesn't take place.

It has been suggested that it's a means of evidence that if recombination of that sort were to occur, we would have seen it, but have we actually looked.

DR. SAUSVILLE: I don't think we have actually looked, but on the other hand, I really wouldn't use the lack of detection or any clinical phenomena that would rise to the fore as being comfort in this regard, because the constructs that

are being talked about, particularly with VSV and the making of these things, are very different than what is running around out there in nature.

So, no, I don't have any comfort that the clinical experience to date has anything to do with this.

DR. CHAMPLIN: I would agree with that. You are putting in artificial genes in viruses now that have been optimized in the vector, and so this really is nothing like what is there in nature, and could obviously result in something very bad.

DR. ALLAN: The other thing is, is that HIV is a highly evolved virus, and it has a selective advantage over almost everything, so you really have to do something or genetically engineer something that is going to have a selective advantage over HIV wild-type.

It is possible if you have got a VSV-G envelope in there, maybe it would have a selective advantage over HIV, but you look at patients, even if they were rescuing bits and pieces of viruses, whether herpesviruses or other endogenous viruses, they are certainly selected for because people keep getting HIV-1, they don't get weird recombinants, and they keep transmitting HIV-1.

So, at least in nature, I think HIV-1 has such a selective advantage over anything else. That experiment has been done.

DR. MULLIGAN: I would disagree. I would think that all the other pathogens, herpes, whatever, the route of entry is different than it is going to be via gene transfer. If you compare the difference between a natural VSV infection in someone who works with horses, cows, to having a template in an retroviral RNA that has VSV-G sequences, the chances for an event to occur that could cause HIV to pick up the VSV-G, is far greater than it would be, I think, from a natural infection.

So, I think I would echo several other points, that there is no reason to think that because we haven't seen these things, that we couldn't get these things.

DR. SALOMON: I think just for clarity, I think Dr. Allan and Dr. Mulligan absolutely agree. You disagreement was with what Phil thought.

DR. DELPH: I think that looking at this, 1(b) and (c), since we will be going into Phase I clinical trials, which are as was pointed out earlier, primarily safety trials, what we would

want to go in initially with would be a vector that has potential for efficacy, but has the greatest potential for safety that we can guarantee while, at the same time, having some potential for efficacy.

DR. SALOMON: I just think that as long as we are trying to make sure that the FDA hears all points of view, then, I would, as uncomfortable as I am with this, try and represent the HIV community, you know, as I have experienced them in several different venues, and one of their responses would be, my God, guys, you know, you take a heart, you know, failure HIV patient, they are going to die. These are young people, you know, this is unacceptable to do anything based on such theoretical safety issues, that, you know, you wouldn't move forward in this community, and it is insulting to suggest that you couldn't ask these patients not to use safe sex or to refrain from primary contact and participate in all the safety features.

I think that, you know, it doesn't seem like there is anyone here from Act Up or any of the HIV communities, so I am not trying to represent them here at the table for a second.

DR. SIEGEL: Just a comment about your comment about safe sex, could we not presume that if an HIV type vector wound up getting packaged in VSV, then, its transmission might be other than sexual? VSV is not sexually transmitted.

MS. KNOWLES: I would agree. I think you have to make sure that every safety issue is addressed, so that there is no repercussions down the line.

DR. SALOMON: As long as we realize that that is the ultimate super safety issue might prevent this from ever coming to clinical trial in the next decade, you know, at some point I think the FDA is very well aware of this, that you have to draw a bar somewhere and decide how high that is.

DR. EMERMAN: Isn't the question about the VSV-G recombinant addressed when you are just looking for RCL?

DR. SALOMON: I am sorry, I didn't get that.

DR. EMERMAN: We were talking about VSV-G recombinants, but those are going to be looked at when you look at whether there is replication-competent viruses in the production

stock, whether they are VSV-G recombinants or some recombinant with some endogenous envelope. Either way, those would be picked up.

DR. SALOMON: I guess they would be providing you sequence the RCL that come out of these assays, right?

DR. EMERMAN: I guess that is one argument to use the gag-pol PCR as an endpoint.

DR. CHAMPLIN: The wild-type HIV is not in the production cells. This is an in vivo consideration to mobilization end or interactions with wild-type HIV.

DR. EMERMAN: The VSV-G isn't in the patient, it's just the VSV-G protein. The VSV gene is all gone, and they are testing for the absence of DNA in the production lot.

DR. SALOMON: That is not true if a transient transfection system ends up packaging a certain amount of VSV-G transcripts in the vector that you then deliver to the patient.

DR. MULLIGAN: This comes back to the mobilization business, of course, which is that if you don't test for something that transfers, but is not replication-competent, VSV-G, in a case where you have wild-type HIV, and that is a

mobilizable--packageable VSV-G, then, you might well get something that you wouldn't have been able to detect before because it didn't exist, didn't have the substrate, whereas, in the patient, you might have the other helper sequences.

DR. EMERMAN: Maybe we could design a test for that, though. I mean you could make a test where you use your supernatant to infect HIV-infected cells, and then look for something with an increased tropism to grow out, bypassing 293 cells.

DR. MULLIGAN: In fact, someone raised you could also look in the patient, I think you maybe mentioned you may want to look at samples from the patient to get a sense of what weird things might be happening.

DR. SALOMON: Correct. I had one question that I kind of want to end on, hopefully, we are ending on this one, so we can go on Question 2, that we earlier kind of touched on this and then left it, that there are going to be patients with HIV that are going to be candidates for lentiviral gene transfer vector therapy and patients without HIV.

If you talk about patients who don't have

HIV for a second, that are potential candidates for lentiviral gene transfer vector therapy, would it be appropriate to ever give a vector supernatant that had even one RCR? I mean could you ever be sure that your vector supernatant was absolutely negative?

DR. SAUSVILLE: You are referring to HIV based vectors.

DR. SALOMON: Yes. I could handle giving a 1 to 2 RCR risk to a patient with wild-type HIV. I think we could get our heads around that, but I just wanted, before we ended, to ask the question of the group about to a non-HIV-infected patient.

Dr. Zaia.

DR. ZAIA: Well, there is no certainty in clinical research, and that is the purpose of doing it. So, you would set a bar, and you would see whether your bar had been set high enough by the clinical experience.

DR. SALZMAN: I am speaking I think on behalf of the non-HIV patients, actually children that have fatal disease, that they don't have a long time to live, and what I have learned here and from my own background is nothing is 100 percent, and when you are talking about 1 RCR and whatever

that may be or may not be, because we just don't know, and how long it is going to take to further explore that versus a fatality within 12 months, you definitely have to weigh it out.

So, again, on behalf of the non-HIV pediatric fatal disease community, we see things a little bit differently in terms of our approach towards safety. While we believe it is obviously paramount, you can never be 100 percent, and we don't want to spend the next 10 years getting to 100 percent. It's not worth it in some cases. That's all.

DR. CORNETTA: This is Ken Cornetta.

DR. SALOMON: Yes, Ken, go ahead.

DR. CORNETTA: I think partly just to follow up the last statement, I think you never can get to 100 percent. You would have to test every virus particle that you gave back to a patient. To get to say that this is absolutely free is just a non-attainable goal.

I think the challenge for the recommendation from the Committee, and what the FDA and investigators combined will try to develop is a thing where you are as assured as possible that there is no RCL, but you can never, without going

again through every virus particle, assure that there isn't an RCL in that product.

DR. SALOMON: Fair enough. I just really asked that question to get that on the table as we sort of left this particular section. You could go another step farther just for purpose of discussion. You could say--and that's what I think the person from the audience addressed--you could say, okay, then, you should only do this. Now, getting to this question, should the first trials be done in patients with HIV because we would have no idea about the possibility of an RCL, and that would be so terrible potentially to give it to someone who didn't have HIV.

I am not saying that is my bias. I am just putting it on the table.

DR. CHAMPLIN: You obviously can't answer the question whether it is safe in terms of transmission of HIV. I agree with the comment that in very high-risk patients with end-stage and fatal diseases, where again the risk-benefit relationship would be on the side of going forward with the test, that it would be a place where that could be assessed.

DR. CORNETTA: Dan, this is Ken Cornetta

again. I think when you are talking about initial studies here, you are talking about Phase I studies, and I think the risks and possible side effects that you might see in an HIV-infected individual may well be different from what you would expect in someone either with a normal immune system, or just because of these are lentiviral vectors going into an HIV-infected versus non-infected.

So, the outcomes and the potential risks may well be different, and we may need to think about clinical trials in these populations separately rather than as a single entity.

DR. SALOMON: I like that. That is a good point.

DR. DELPH: Would it be possible or is it more difficult to detect RCL in somebody who has wild-type HIV than in somebody who doesn't?

DR. SALOMON: What you mean is not detect wild-type RCL, but you are talking about vector-derived RCL.

DR. DELPH: Right.

DR. SALOMON: Would it be harder?

DR. SAUSVILLE: This does touch on the issue of what goes into the construction of the

input, as it were, HIV-derived strains. I mean one might imagine that the RCL that would be derived from them might have some marker or might have some tag, might even be constructed with the pol and the protease or with sensitivity to drug.

I mean there is all sorts of ways that one could conceive of following in some way or another the input, and we might encourage, if this is on the table for a particular case, the design, so that we could actually follow them easily.

DR. SALOMON: Fair enough.

DR. KINGSMAN: I apologize if I got confused or lost the plot. I think there are two issues that may be getting pulled into one. One is using a SIN vector to prevent mobilization in the target cell, and the other is using a SIN vector to reduce the amount of LTR that comes through in the components, and if you have got extended bits of LTR flanking VSV-G, that could be bad.

They are two separate issues. You could reduce the amount of LTR, but not have a SIN vector because you could put a tissue-specific promoter in. So, I think I would like to just ask that the word "SIN" is not seen as just another way of naming mobilization. It is one particular type of

vector.

So, that is one point. The other point is this VSV-G issue. If we take VSV-G and create it as an issue, then, this applies outside of the lentivector field. It applies to anyone who is trying to make MLV-based vectors, which are packaged with VSV-G, which may have useful properties.

So, I think the VSV-G issue may be get rid of a native type of LTR that could possibly recombine with an HIV LTR and deal with that separately from mobilization in the patient. I just wanted to say that.

DR. SALOMON: That's fine.

Yes, Carolyn.

DR. WILSON: I just wanted to briefly address your last point, which is that up to now there are no clinical trials with MLV-based vectors using a VSV-G glycoprotein, and we do except that the concerns regarding VSV-G would be the same with an MLV vector.

DR. SIEGEL: I just want to put on the table just an issue, not for discussion, but just for background and comment. It has troubled me about some of the recent comments. That is the

notion that although the risk of perhaps a certain toxicity, such as having an RCL in a HIV-negative individual, given that they are very small, they would well be counterbalanced potentially in high-risk patients by the prospects of benefit.

I mean we are talking about the context now is the first introduction to people, and I think any honest view of the first introduction of any therapy to people, including the first introduction of something this highly experimental, would say that minuscule as our concerns are about any particular toxicity, our hopes for benefit of the first patient who will probably receive an extremely low dose as a single time in something that has not been studied, and without any dose or route optimization or vector optimization, are surely minuscule, and the notion that that patient would consent to that therapy thinking that they had a substantial incidence of benefit, suggests that there is a problem with consent.

So, I think as you look at product development in the bigger sense, you could say these small risks are well compensated in a population by the fact that the research may lead to important therapies, but from the perspective of

the individual patient who I guess would probably not be a child, who is going to first get this, I think we realistically have to look at risks and benefits from a little bit different context.

MS. A. SALZMAN: Amber Salzman. Maybe I can give a perspective as a mother of a child who potentially could be saved from this. I sort of feel like why not at least give him a chance to live. I mean I understand that the efficacy may be low, and there is a question about the safety, but if you do due diligence and you think maybe there is a shot, I just would hate for a room of these people to say that you wouldn't give my son a chance to live even though it may be low.

DR. SIEGEL: I appreciate that, and I understand that, and I would hope what your doctor would tell you is that this could be very important in terms of developing a therapy, and it may well be the only chance for your child, but I think if your child is the first person to receive this therapy, it is likely not going to be even given in a way that holds out any substantial hope of being curative. That is just the way new therapies are developed, and I think that is the context we have to consider this in, in these early experiments.

DR. SALOMON: I think that is a good point. I guess my response to it and to the mother is the purpose of the Committee is to define these sort of general safety issues. I think that if a sponsor can find a group of patients, I don't think that the Committee here wants to particularly specify any group is inappropriate. I didn't think you were saying that, Dr. Spiegel.

DR. SPIEGEL: No, let me clarify because this is very important. I am not disagreeing with the prospect that we want to make impossible potentially valuable therapies for serious diseases because of theoretical risks. We don't want to do that.

I understand and I agree and support that principle completely. I just don't want to be--you know, some are suggesting that, well, we will introduce these into high-risk patients because they will stand more to benefit than lose, and you run into a significant concern in those patients if you haven't informed them that their chance of benefit is either zero or extremely close to zero, you haven't gotten appropriate consent probably for the type of research that we are talking about, and when new products are introduced to people, they

can consent and often will consent recognizing the importance of the research and even based on such small chances of benefit.

But, you know, there are important ethical questions in vulnerable populations in particular in getting consent based on the fact that somebody is desperate because they have a seriously ill disease, and have the potential to believe that there is chances for benefit which far exceed those that are there.

MS. A. SALZMAN: I guess I would say I work for a major pharmaceutical, so I am very, very familiar with clinical trials, and that really comes down to a very good consent form, and I know with all of the hoopla over the last year or so, we are getting much better consent forms.

DR. CHAMPLIN: Needless to say, the people in the middle of the Phase I trial, are now up to a meaningful dose, may well benefit, and what you said certainly is true, and consent is a complicated process, and one tries to give just the message that you indicated, but there is hope, hopefully, that when you get into the meaningful doses, that even in the Phase I trial, that there will be some benefit.

So, I think we want to just be sure that the patients selected, where if, in fact, there is an adverse event, we aren't going to make them worse in a much more substantial way that would have been the natural history of their disease.

DR. SAUSVILLE: I certainly agree with those comments and also I agree with Jay, but on the other hand, I think when this has been looked at in study after study, patients go on Phase I trials despite the protestations of lack of benefit because they think they are going to benefit. I mean that is the way it works.

So, I think, as you state, the point of a consent form is to highlight or balance both aspects of the science to be gained, and the theoretical, although perhaps low, notion of benefit.

I can understand the scenario where 1 RCL of HIV in a given population might actually be acceptable. I can certainly imagine populations where it would be absolutely unacceptable, and I think that has to be factored into this.

Also, just as a final point, the notion of dose is applied to biological therapies in general, and in particular, this type of therapy, I don't

know what the middle of a Phase I means, because I think we are definitely treading new ground here.

DR. DELPH: I think as we are talking about safety, in trials, we need to consider that when we are looking at safety for most pharmaceutical agents, you are talking about safety as it relates only to the person who is taking that pharmaceutical agent.

In this instance, where recombination may be a possibility, and you may get replication-competent virus, you may be involving the safety of others, as well.

DR. SALOMON: I think that is a good way to end this. I think if we started with Dr. Cornett's comments and kind of follow the loop that followed, I don't think that the Committee--again, if someone doesn't like this, jump in, but I don't think that the Committee is coming down one side or the other on whether the safety risks to an HIV patient population with a lentiviral vector versus the safety risks to a non-HIV, they are different, as Dr. Cornetta started us off with very clearly, but I am right now fairly neutral.

I mean I think it is going to be a case-by-case basis and I think we would look to the

sponsors and look to the vector and look to the data that they have, and make those decisions, but I don't see any really compelling argument to say no, no, yes, yes. I think it is fairly balanced right now, which is an interesting place to be.

The quiet I assume is that we are okay with this?

DR. HIGH: I wanted to make one other point, and just to echo something that Eduardo said earlier, because I bears emphasis, that although it may be adequate to just leave this at RCL assays, since this is a new therapy, and since there are other methods we have heard about for looking for helper sequences in other ways, I think it would be good to encourage sponsors to incorporate that into their design of the trial.

DR. SALOMON: I agree with that. I have tried to capture that in my statement of saying these would be really valuable follow-on assays to be added on. In fact, that would be something even to lobby the NIH to support these sort of clinical assays added on to clinical trials. I agree.

Question 2. What should be the appropriate species for in vivo, preclinical safety and toxicology evaluation of lentivirus vectors?

Specifically, consider the following:

Wild-type HIV-1 does not infect monocytes, lymphocytes, or other target cells in rodents nor in cynomologous or rhesus macaques and will only poorly infect CD4 T lymphocytes from chimpanzees, so mobilization studies will be complicated.

Lentiviral vectors pseudotyped with different envelopes, VSV-G, but also rabies and flaviviruses, and I guess flaviviruses include the ebola virus, may have expanded cell tropisms, but the infection may be limited, for example, mouse cells have multiple blocks to HIV replication.

DR. ALLAN: I haven't been following the gene therapy vector field very much, but I mean the premise here is that there is no animal model system essentially. That is what this basically says is HIV doesn't infect rodents, it doesn't infect monkeys, so let's go to humans.

I spent the last 14 years working on SIV, and we have good monkey model systems to study recombinant SHIVs that replicate extremely well in monkeys and kill monkeys, and function almost exactly like HIV-1.

You have to reduce and redesign, but it would just be a proof of concept. Everything is

theoretical. I mean basically, what you are saying is here is the needle, close my eyes, hope for okay. I mean that is what you are doing, I mean essentially, because you are betting on what we know scientifically, and you have heard some people that have more concerns than others.

So, if you took and designed these things, you could take a VSV-G recombinant S, whatever the vector is, and pop it into monkeys, you could shoot the virus directly into monkeys. You could do the studies where you took the cells out of monkeys, you could infect them with the SHIV virus, and then put your CD4 cells with the vector in afterwards, beforehand. There is all kinds of studies you could do in monkeys that seems to me have been totally underutilized, but like I said, I haven't been following this field, and maybe some of those studies have been done, but I think that if they haven't been, I think people have missed the boat, because the model system is just sitting there waiting to be used.

You can look for recombinants very easily if you pop a monkey, you have got a whole ecosystem, and whatever pops back out, you may see it. I am sort of perplexed why that hasn't

surfaced.

DR. SAUSVILLE: I guess my concern is that while there is, as you indicate, a whole biology that could be explored, I guess one has to have a balance between closely mirroring the clinical application of a proposed product and the doing of ultimately toxicological research in a very interesting model.

I would come down on the side of recognizing the difficulty here as an intrinsic part of the biology, attempting, how imperfectly it may be, to pick a system that most faithfully could replicate something of the human biology, but really focusing on the safety testing on a close as possible mimic with the product to be used to the proposed clinical study, and let it go at that.

I think that to go beyond that, certainly to engineer things that might look for effects, I wouldn't know how to extrapolate them back to the intended clinical use.

DR. MULLIGAN: I would say that you would have to look at this on a case-by-case basis, but Jeff's talk was a revealing talk in terms of the kinds of questions you can ask about, certain tox questions, you know, immune consequences of CNS

gene transfer. I think that is always going to be the case here, you know, you are going to do the best you can. You are going to be able to ask some questions in some reasonable system, and obviously, when people develop their preclinical information, I think there is going to be an expectation by the FDA that they address the obvious things that people would think you could address in that system, but I think the tone of this is, is there any, you know, this is probably back to the old monkey, you know, you have got to do monkeys or something, and clearly, many of the issues we have just been talking about are so complicated that there is no easy answer to look for mobilization in the context of a monkey or something like that.

Just to make it appear that we are moving ahead, on the third point, I do think in vitro that there is a lot of assays, in fact, I think the only thing we can grasp probably over the last couple of hours are some of these mobilization issues could be better addressed in vitro, obviously, the ICR assays, and so I think that there is no reason not to do as much as you can in each of those systems.

DR. SALOMON: It seems to me you could break it down a little bit, right, and I think that

Dr. Sausville kind of pointed that out to us, would be a toxicology or a toxicity, direct toxicity of the gene delivery, you could do in a monkey. You could even do it in a rodent model.

I think as Dr. Kordower showed, I mean if his strategy of putting GDNF in a monkey, you know, and then the monkeys got better and they didn't have strokes and, you know, he followed them for X number of months, and I don't want to say all the obvious things to everyone here.

That's pretty good. I mean I was pretty impressed frankly, almost to the point where my only comment, if you remember, was I find it hard to validate a primate model when the guy is telling me there is no toxicity. I would rather hear that there was some toxicity and you avoided it.

So, I think, to me, it always worries me a little bit when there is absolutely no toxicity and everybody is cured.

But with that said, I think there would be some things then that we could feel comfortable modeling in rodent and non-human primate models. Would we at least buy into that first part?

DR. CHAMPLIN: I think the animal models in general are a useful proof of principle, but

obviously, they are different reagents, they are different drugs, if you will, and different biologics. It is great to do it as a proof of principle, but when you come down to the individual agents being proposed for human trials, and once you have proved the principle, now you are still left with doing it in man.

DR. SALOMON: What he did, he actually used his HIV GDNF vector in the monkeys, so that was his product at least to my understanding.

DR. CHAMPLIN: But as you are looking at recombinant events and those kind of things, which is what we have been talking about, you know, you need to do it in a parallel system using SIV, which obviously hasn't been applied to the HIV vector that is ultimately going to be used in humans.

DR. ALLAN: I think when you go to monkeys and you start talking about SIV, you say, well, gee, I am not going to redesign all these vectors and redo all this stuff, and, you know, we are not using this in humans, so, you know, that seems like a lot of work.

Well, you know, I mean for some of these trials, you know, you can use most of the same vectors. You have already got the same VSV gene,

you have got the same whatever therapy you are trying, I mean you have already seen that it is a human gene, you put it in the monkeys, and you don't have to redesign anything there usually. Sometimes, you know, maybe they are only 95 percent related, but you are talking about if you are doing gene therapy, I mean the monkey is 95 percent or whatever, the chimp is 99 percent, so there is not a lot you have to do.

Even with the difference between HIV and SIV, when you are just looking at gag and pol, the studies that have been done so far, and they haven't really--people haven't spent enough time on this, which is unfortunate, but there is only a small region in the gag gene that doesn't work in terms of packaging between HIV and SIV, it is just a small piece.

So, you are not talking about all these things you have got to redesign, it is just very little, and I am not saying that that has to be done before you go on to humans, I mean I sit on these xenotransplant committees and I know that that is not going to happen anyway, but I think that you have really got these model systems and they are sitting there and people ain't using them,

and those are the model systems that are going to be able to tell you, I think, you know, in terms of safety.

Certainly in toxicity, but I think also in safety and also in terms of recombinational events, I think that is the model system.

DR. NOGUCHI: Jon, would you expand a little bit on that, would you be comfortable with creating a VSV envelope SHIV as an example, to try to get to some of this?

DR. ALLAN: No, I wouldn't do that. What I would do is create the same system.

DR. SIEGEL: Are you suggesting specifically that a SHIV, you could take a VSV product developed, packaged product developed for human use, perhaps with a SIN vector or without a SIN vector, and use a SHIV model as a useful way of exploring mobilization, how much occurs, which vectors mobilize more and which don't, and where and what time course, and things you would want to know for human use, and you could do that with vectors designed for humans in that model, and perhaps that would model behavior of use in HIV-infected humans.

DR. ALLAN: It should especially in

HIV-infected humans, especially in HIV-infected humans in terms of mobilization and recombination, and I think that is really the critical area, but we would have to redesign like gag-pol vector. I am not saying it's a small thing, but--

DR. JOLLY: This is Doug Jolly. Are you saying that you think HIV vectors rescued by SIV would be a suitable model?

DR. ALLAN: What I am saying is depending on what you are looking at, you can use a SHIV virus in the monkey and you can make a SHIV packaging vector, essentially an SIV packaging vector with a SHIV challenge.

If you use SHIV, any kind of study you want to do whether it's an antisense, HIV, envelope or whatever else, you could use a SHIV. You could also use SIV certainly, so it just depends on what you are going to use as a therapy and tested in the monkey.

DR. JOLLY: But the vector itself, the backbone of the vector would have to be SIV, right, so it is a different vector?

DR. ALLAN: Just the gag-pol

DR. JOLLY: So the actual vector genome, the 900 nucleotidase of HIV that are left in the

vector, you would use that vector, not an SIV-based vector.

DR. ALLAN: I don't think it matters. You could just plump in the gag gene and that probably would take care of it. I mean Mike Emerman may have some more insights than that, but I think you could probably do that very easily.

DR. EMERMAN: I don't think you could do it very easily I think you are talking about a much different kind of experiments using SHIV. The kind of recombinations you will get are going to be different, acting vectors are going to have to be much different.

I think it is an interesting exercise, but I don't know that it actually tells you about the product that you are actually going to be using.

DR. ALLAN: I am just talking about proof of concept and trying to examine the issues recombination and mobilization. I think that model will give you that information, but not on a specific product.

DR. EMERMAN: Dr. Allan, it's a five-year grant. It is not a straightforward simple experiment.

DR. SAUSVILLE: I think we have got where

we want to be because while I agree that it would be a very interesting, intellectual, and biological exercise, I think to make that a product-related matter, you know, each product somehow has to go jump through a hoop would be problematic.

DR. SALOMON: I think we all were thinking, you know, you take this and that would be your background and significance for your RO1 or your program project probably would even be more appropriate here, and I think it might enhance the field, but I think we all agree that that wouldn't be advice to the FDA to hold sponsors to that at this point.

But I do think that we have articulated a very important problem in the field and we spent the whole day articulating it. I doubt it's not clear to you by now, right? I am sure it is very clear to you that we are concerned about mobilization and recombination and replication-competent alteration.

This is the kind of thing, you know, to OBA. This is where NIH leadership to gene therapy could come out of these kinds of discussions. I mean these are really important questions and maybe this is the kind of thing there should be for an

RFA.

DR. MULLIGAN: This stuff haunts the gene therapy field, how do you get the basic research that most directly supports these things. We were talking about the assays, our interest in having better assays for gag-pol, and so forth, we ought to really make the case, it is very key.

DR. SALOMON: Any other comments? I think we have answered basically all your questions, but if we haven't, this is a good time to tell us.

DR. WILSON: I think the Committee has done a really commendable job going through some very difficult territory today, and we really want to thank everybody on the committee for their very thoughtful and thorough discussion of all the issues that have been raised today. Thank you.

DR. SALOMON: I also want to thank the committee, this is a lot of hard work, to our speakers, to the audience who actively participated. I think it really contributed to the whole balance of things.

Tomorrow morning we begin at 8 o'clock sharp mainly because it is so important, but a number of us are going to have to make planes, and I don't want to decimate the committee without

really getting to the meat of tomorrow's sessions. So, tomorrow morning we will definitely start at 8 o'clock on the money, although we were pretty good this morning and we did finish at 6:03, so I guess we did pretty well today, as well.

Thank you all very much. See you tomorrow morning.

[Meeting recessed at 6:03 p.m., to reconvene at 8:00 a.m., Friday, October 26, 2001.]

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CERTIFICATE

I, **ALICE TOIGO,** the Official Court Reporter for Miller Reporting Company, Inc., hereby certify that I recorded the foregoing proceedings; that the proceedings have been reduced to typewriting by me, or under my direction and that the foregoing transcript is a correct and accurate record of the proceedings to the best of my knowledge, ability and belief.

Alice Toigo

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