

1                   A F T E R N O O N   S E S S I O N  
2                   DISCUSSION OF HUMAN GENE TRANSFER  
3                   PROTOCOL #01007-488 ENTITLED:  
4                   A PHASE I,   OPEN-LABEL CLINICAL TRIAL  
5                   OF THE SAFETY AND TOLERABILITY OF  
6                   SINGLE ESCALATING DOSES OF  
7                   AUTOLOGOUS CD4 T CELLS TRANSDUCED  
8                   WITH VRX496 IN HIV-POSITIVE SUBJECTS

9                   DR. MICKELSON:  If we could get started then,  
10                  please.

11                  We are now moving towards discussion of  
12                  protocol No. 488, which is a Phase I clinical trial of  
13                  the safety and tolerability of single escalating doses  
14                  of autologous CD4 T cells transduced with VRX496, which  
15                  is a lentiviral based vector in HIV-positive subjects.

16                  The reviewers for the committee were Dr.  
17                  Aguilar-Cordova and Dr. Markert and Nancy King, and we  
18                  have multiple ad hoc reviewers, Dr. Zaia from the  
19                  Beckman Research Institute and City of Hope and Dr. Yee  
20                  from also the Research Institute and City of Hope.

21                  Also, in addition, Dr. John Coffin,  who is  
22                  director of the HIV Drug Resistance Program through NCI  
23                  is present as sort of a special ad hoc member for the  
24                  committee for this afternoon's review.

25                  And we will be following the same format.  Dr.  
26                  Dropulic from VIRxSYS will be doing a 20 to 30 minute  
27                  presentation on the protocol itself and then we will go

1 through the committee reviewer's comments, ad hoc  
2 reviewers and then open the floor for discussion and  
3 comment from the public as well as the discussion with  
4 the investigator, with the result that hopefully what  
5 will come out of this are recommendations from the  
6 committee that will be transmitted to the sponsors and  
7 the investigators as well as their local oversight  
8 committees.

9 So with that, Dr. Dropulic, thank you.

10 DR. DROPULIC: Thank you.

11 First of all, I would like to thank the  
12 reviewers for, you know, reviewing our protocol. I  
13 think that overall it has made it that much a stronger  
14 proposal.

15 What I would like to do today is basically  
16 give an overview presentation of VRX496 and the  
17 transduction procedure for a Phase I clinical trial in  
18 HIV-infected patient subjects.

19 And during the course of the presentation I  
20 would like to touch on basically the questions that  
21 were raised by the reviewers. What I have provided  
22 today is a booklet there which I think you should have.

23 It is basically answering in detail in writing the  
24 reviewer's questions that were not answered in time for  
25 the submission because we had received the reviews  
26 late. Okay.

27 (Slide.)

1           So this is our proposed protocol. It is an ex  
2 vivo gene transfer of VRX496. This is the name of the  
3 HIV-1 based vector that we are proposing for Phase I  
4 clinical trials in HIV-infected patient subjects.

5           VRX496 is an HIV vector that contains an anti-  
6 HIV antisense sequence. This antisense sequence is  
7 about 1 kb. The protocol involves a patient subject  
8 coming in, undergoes leukophoresis, the T cells are  
9 isolated, and then the T cells are exposed to VRX496.  
10 The cells then are expanded at the University of  
11 Pennsylvania cell processing facility and then undergo  
12 rigorous QC testing. Importantly, one of the rigorous  
13 QC testing points is that we would perform a Taqman PCR  
14 assay on VSV-G DNA and show that the final cell product  
15 does not contain any VSV-G DNA present in the product.

16           After QC release the cells are then basically  
17 infused back into the patient.

18           (Slide.)

19           This is the structure of VRX496 and its analog  
20 vector 494. This is the laboratory grade version of  
21 496 and the difference between 496 and 494 is that 494  
22 contains a GFP coding region so that cells transduced  
23 with 494 mark up green and we can use it for laboratory  
24 analysis.

25           In contrast, clinical grade vector does not  
26 encode for any proteins and the only sequence that is  
27 not derived from wild type HIV is a small 186 base pair

1 marker sequence that does not code for anything derived  
2 from GFP that we use to determine cells that are marked  
3 with the vector by Taqman PCR.

4           So the vector, VRX496, is derived from the  
5 prototype molecular clone, pNL4-3, and the components  
6 from which the vector was constructed are shown. We  
7 have a 5' region, a region from the central polypurine  
8 tract. The antisense region is derived from the 5' end  
9 of the env, which is flipped in the reverse orientation  
10 into the vector construct. We also have a region from  
11 the RRE that also contains a splice receptor site. A  
12 disposable GFP marker sequence as well.

13           What is important to note is that the  
14 antisense is expressed only -- it is tat and rev  
15 dependent. That means the genomic RNA is only  
16 expressed in cells that are infected with wild type HIV  
17 because it is located upstream of this major splice  
18 receptor site. This is what we have found that  
19 makes these vectors very, very effective.

20           (Slide.)

21           Okay. Some of the safety features of VRX496  
22 for gene transfer. We believe it is the safest  
23 approach for gene transfer in HIV infected patient  
24 subjects using this type of vector because no new  
25 sequences are introduced into the patient. The vector,  
26 except for that small disposable marking sequence, is  
27 entirely derived from wild type HIV and patients that

1 would be treated during this protocol are laden with  
2 wild type -- with the wild type virus.

3 VRX496 cannot produce a novel pathogenic virus  
4 since the vector is a whole derivative of wild type  
5 HIV. Any recombination event that would occur between  
6 the vector and the wild type could not produce a virus  
7 that is more pathogenic than the wild type virus  
8 itself.

9 What I am talking about here is the vector  
10 itself and I am not discussing about any VSV issues  
11 which I will talk about later. Okay.

12 Also, in addition, our vector antisense  
13 payload is expressed in a targetable manner. The  
14 antisense payload is both tat and rev dependent and  
15 thus is expressed only after wild type HIV infects  
16 vector containing cells.

17 In addition, our vector does not contain any  
18 heterologous *vol* promoter sequences. It is entirely  
19 derived from wild type HIV. The bottom line is that we  
20 are not really introducing any new sequences in the  
21 patient other than what is already there and the vector  
22 is entirely derived from the wild type virus.

23 Also, what we found very recently is that the  
24 antisense payload appears to decrease vector  
25 mobilization to cells and I will show data for that in  
26 a little while.

27 Expression of the anti -- envelope antisense

1 results in decreased infection of mobilized vector  
2 genomes.

3           Also, our vector contains not a triple, a  
4 single stop codon in gag, which basically creates a  
5 friendship mutation and a stop codon downstream. So if  
6 recombination with wild type or helper should occur  
7 downstream from this stop site a nonfunctional gag pol  
8 open reading frame would result.

9           (Slide.)

10           So this is the possible -- we have just  
11 schematically drawn the possible events that could  
12 occur between the vector and the wild type in terms of  
13 recombination. For example, as its well known,  
14 recombination occurs at the RNA level where reverse  
15 transcriptase makes the jump in order to recombine with  
16 a co-packaged strand of RNA.

17           One example of a noninfectious particle that  
18 is produced through recombination between the vector  
19 and the wild type is reverse transcriptase makes the  
20 jump at exactly upstream of that gag stop codon,  
21 resulting basically in a nonfunctional gag pol open  
22 reading frame in the recombinant. That means that this  
23 recombinant would not be infectious.

24           I am not going to take you through all this  
25 but really the overall conclusion here is that  
26 basically the result of recombination between the wild  
27 type and the vector results either in a noninfectious

1 recombinant or wild type HIV. Even if the vector --  
2 even if the wild type virus could somehow pick up the  
3 antisense payload and reverse orient it back in, still  
4 the result is wild type virus. It is not a new virus  
5 of unknown pathogenicity.

6 (Slide.)

7 So let me tell you a little bit about the  
8 packaging construct, which we call VIRPAC. It is also  
9 -- the actual plasmid is called VRX170. We use a two  
10 plasmid system rather than a three plasmid system which  
11 is commonly used in the field. We have found that our  
12 two plasmid system produces three -- at least threefold  
13 higher titers than the commonly used three plasmid  
14 system in 293 cells.

15 This is important because during our  
16 discussions with the FDA the history of the cell line  
17 became very, very important. So we decided that  
18 because the history of 293 cells can be readily  
19 established in contrast to 293 T cells, we have opted  
20 to use a transfection procedure that uses 293 cells and  
21 not 293 T cells. So in that situation we get better  
22 titers with VIRPAC.

23 So instead of physically partitioning the  
24 envelope and gag pol structural open reading frames,  
25 what we have included in VRX170 is a transcriptional  
26 partition of structural envelope genes. What we have  
27 incorporated are core sites both upstream and

1 downstream of the two, you know, determinant open  
2 reading frames for envelope and gag pol. So we feel  
3 that this helps alleviate concerns of safety that one  
4 may expect from a two plasmid system because we are  
5 transcriptionally partitioning the envelope away from  
6 the gag pol open reading frame.

7           Also, what we have done in VRX170 is codon  
8 degenerated various sequences in order to decrease the  
9 likelihood of recombination but what I want to stress  
10 here is what is important in the use of HIV-1 vectors  
11 in HIV-infected individuals, what is really important  
12 is whether VSV-G recombines and forms a VSV-G RCR.

13           This event where gag pol is linked to the ltr  
14 is already amply present in HIV infected individuals.  
15 So the event that we really have to be worried about  
16 and we have focused a lot of our attention is whether  
17 VSV-G basically can become incorporated into RCR and  
18 whether we can in our final preparation show that there  
19 is no VSV-G sequences available for that putative  
20 recombination event to occur.

21           (Slide.)

22           So there was a question by one of the  
23 reviewers is where are the plasmid raw materials  
24 produced. We produce both the plasmid raw materials  
25 and the purified VRX496 vector at VIRxSYS clinical  
26 vector production facility using C-GMP conditions.

27           The cell processing on the other hand is

1 performed at the University of Pennsylvania hospital's  
2 clinical cell processing facility, also using C-GMP  
3 conditions, and that is under the auspices of Dr. Carl  
4 June.

5 (Slide.)

6 When we produce these vectors, and I am not  
7 going to go in -- there is no time to really go into  
8 the production on protocol in the manufacturing process  
9 but basically, in brief, it involves cell factory  
10 production of a bulk harvest that then undergoes  
11 ultrafiltration, difiltration, benzonase treatment and  
12 then final column chromatography before it is  
13 formulated in a bag and it can be stored at -20, for  
14 six months at -20 degrees C.

15 The vector basically that we have used here is  
16 VRX494 and we can show that this vector can transduce  
17 primary human CD4 T cells with very, very high  
18 efficiency. The way that this transduction was  
19 accomplished was a single dose of vector at an MOI of  
20 20 in conjunction with immobilized CD3 and CD28  
21 antibodies. Then once the cells were transduced, they  
22 were cultured in these antibodies and IL-2 and two  
23 weeks later we performed FACS analysis to determine the  
24 percentage of transduced cells. We found an  
25 extraordinarily high level of transduction of these  
26 cells. We can routinely get anywhere between 90 and 98  
27 percent transduction efficiency with this class of

1 vector.

2 (Slide.)

3 So one of the advantages -- there was a  
4 reviewer's comment regarding the use of murine leukemia  
5 viruses instead of HIV vectors. One of the advantages  
6 of using HIV vectors is that with a single dose of  
7 vector, right, at one time you can accomplish this high  
8 level of transduction efficiencies. My understanding  
9 of the literature is that you either have to multiply  
10 transduce or prestimulate the cells extensively to  
11 achieve that level of transduction with an MLV based  
12 system.

13 After transduction of the cells we analyzed  
14 various parameters of the cells to look for stability  
15 of vector transduction and were there any toxic effects  
16 on the cells. This is an arbitrary scale here but it -  
17 - and it designates depending upon what we are looking  
18 at here. Cells transduced at a very high level of  
19 transduction efficiency are EGF positive essentially  
20 almost to the 100 percent level and remain so during  
21 the course of the ex vivo expansion period, which in  
22 this case is 29 days.

23 When we looked at the vector copy number by  
24 Taqman PCR, we found that the copy number in these  
25 cells also remained very, very stable during the  
26 course of the experiment, about nine or so during the  
27 29 day period. And this stability is really

1 remarkable when you think about it because this copy  
2 number is remaining stable even when these cells are  
3 expanding over 1,000-fold in culture. So this is the  
4 fold level of expansion of transduced cells in blue  
5 here compared to untransduced cells in red and you can  
6 see similar levels of expansion occurring and no real  
7 appreciable differences between the expandability of  
8 cells that contain the vector and cells that do not  
9 contain the vector. So the vector does not appear  
10 to be toxic and it can transduce primary human T cells  
11 with very high efficiency.

12 (Slide.)

13 Now we take these cells directly. We do not  
14 select for these cells at all and then simply challenge  
15 them with wild type HIV. In this case we use an L4-3  
16 strain and here we use an MOI of .001 but we used  
17 various MOIs. And as you can see here is that while  
18 control cells that do not contain the vector are not  
19 transduced replicated wild type HIV very well. There  
20 was one, two, three log inhibition of wild type HIV  
21 replication in the cells that were transduced with the  
22 vector. When the cells are transduced to sufficient  
23 levels we do not see any breakthrough occurring.

24 (Slide.)

25 Also, what is very interesting is that when  
26 you look at the frequency of CD4 expressing cells in  
27 these cultures, while cells that do not contain the

1 vector downregulate CD4 expression during the course of  
2 the culture period, this downregulation is a result of  
3 productive HIV replication that results in the  
4 expression of nef, VPU and gp120, resulting in CD4  
5 downregulation. So the frequency of CD4 expressing  
6 cell is a marker, if you like, for the number of cells  
7 that are productively infected with HIV and in this  
8 case more cells here are productively infected with HIV  
9 because they are downregulating CD4 as compared to  
10 cells that were transduced with the vector.

11 As you can see here, there is no significant  
12 decrease in the number of CD4 expressing cells in the  
13 transduced cells compared to the control cells.

14 (Slide.)

15 We have also tested various strains of HIV.  
16 What we have done is transduced primary human T cells  
17 and then challenged them either with prototypic X4 and  
18 X4 strain of HIV, an R5 prototypic strain, and R5  
19 primary stain of HIV, and then looked for the ability  
20 for the virus to replicate during the course of, I  
21 think in this case, about 19 days. These are all the  
22 same time points and the same scales.

23 And so what you can see here is that while the  
24 mock transduced cells that are depicted here in red  
25 replicated the wild type virus to predictable levels,  
26 the vector containing cells strongly inhibited the  
27 replication of the virus no matter whether it was an X4

1 type or strain of virus or an R5. Interestingly, we  
2 did find that protection against an X4 type of strain  
3 was better than R5 strain and this would be predictable  
4 based on the antisense sequence since the antisense  
5 payload that is present in the vector targets an X4  
6 strain and not an R5 strain but as you can see here  
7 still because the antisense is about 1 kb it still  
8 inhibits the R5 strains at least two logs.

9 (Slide.)

10 As we move forward towards the clinical trial  
11 we did a comparability test between VRX494, which is  
12 the laboratory grade vector that expresses GFP, and  
13 also VRX496, which is the vector that basically has  
14 only that small 186 base pair sequence as a marker  
15 sequence, and we transduce the cells of various MOIs,  
16 T1 cells, and then challenged them with wild type HIV.

17 As you can see, the wild type HIV cultures  
18 grew to predictable levels while both the VRX494 and  
19 496 comparably inhibited wild type HIV replication.

20 (Slide.)

21 The next thing that we did was in  
22 collaboration with Carl June and Bruce Levine at the  
23 University of Pennsylvania, is that we produced the  
24 vector at patient scale at the level that would be used  
25 for one whole leukaphoresis transduction procedure.

26 So we made patient scale vector and transduced  
27 the cells and then looked for various parameters of

1 toxicity that may indicate that the vector was toxic to  
2 the cells. In red in each of the slides those are the  
3 mock cultures. These are cells that do not contain the  
4 vector. While the blue squares are cells that contain  
5 the vector.

6 As you can see, when the mock and the  
7 transduced cultures were compared for doubling time,  
8 there were no appreciable differences. When the  
9 viability was compared during the course of expansion  
10 there were no appreciable differences.

11 When we looked at the cell size difference  
12 between the mock or the transduced cells during the  
13 course of expansion there was no significant  
14 difference.

15 Also we looked at various cell surface markers  
16 and what you have to do here is you have to compare the  
17 first blue bar with the first red bar that would be day  
18 seven transduced compared to day seven mock and then  
19 you compare the day 11 transduced compared to the day  
20 11 mock.

21 And if you look at each doublet you will  
22 notice that there is no real significant difference  
23 between the expression of these surface markers when  
24 you compare mock transduced cells or cells transduced  
25 with VRX496 at the clinical scale.

26 (Slide.)

27 What we then also did is took this clinical

1 level scale transduction and then took a sample of it  
2 and then challenged it with wild type HIV to see if the  
3 cells could resist wild type -- productive wild type  
4 HIV replication. And as you can see here, all the  
5 control cells replicated wild type HIV very well. Over  
6 two logs of inhibition of wild type HIV was seen with  
7 the vector transduced cells. We were rather happy with  
8 that because when you look at the copy number of the  
9 vector in these cells it was an average copy of about  
10 six per cell, which falls within our specs. Our specs  
11 are between one to ten.

12 (Slide.)

13 So what we have now done more recently is  
14 taken CD4 T cells from HIV infected donor, transduced  
15 the cells with VRX496, and looked for various  
16 parameters. In this case we are looking for toxic  
17 effects and toxic effects is measured by the level of  
18 cumulative cell expansion when we compare mock  
19 transduced cells compared to VRX496 transduced cells.

20 As you can see during the period of expansion  
21 here there was no significant difference between mock  
22 and vector transduced cells. There was a question by  
23 one of the reviewers asking about the relative  
24 transduction efficiencies of normal CD4 cells compared  
25 to HIV infected CD4 cells. We have a very small n here  
26 but we have seen an average three copies versus two  
27 copies, which we do not think is an appreciable

1 difference given the very small n size that we have.

2 (Slide.)

3 Okay. Now what we did is took that cell that  
4 were -- these cells that were transduced -- those cells  
5 from an HIV infected donor that were transduced with  
6 VRX496, expanded and then all frozen down, right, just  
7 like in a procedure that would occur into the clinic.  
8 Then we thawed the cells and grew out the cells in  
9 immobilized CD3, CD28 and IL-2 and looking at it for  
10 the endogenous virus to replicate in these cells.  
11 Right?

12 And what we found is that while the mock  
13 cells, the virus -- you know, this is the endogenous  
14 virus from the patient -- grew extremely well after the  
15 four and the growth in vitro there was a two log  
16 inhibition of virus replication from the vector  
17 containing cells. We do see this bump occurring, this  
18 breakthrough occurring, and I will describe that just  
19 in a moment.

20 We generally see this bump when we basically  
21 have copy numbers in the one to two range. When you  
22 have copy number -- an average copy number in the whole  
23 range, four to six or so, you do not see this  
24 breakthrough effect.

25 (Slide.)

26 What we also did on these cells that were  
27 transduced with the vector derived from the single HIV

1 infected individual, basically we looked for CD4  
2 expression on the cells. And while in the nontreated  
3 control cells we saw 40 percent of the cells expressing  
4 CD4, almost twice as many cells were expressing CD4 in  
5 the cells that were treated with VRX496, indicating a  
6 selective resistance to productive HIV infection by  
7 cells that are transduced with the vector. So we were  
8 rather excited about this.

9 (Slide.)

10 Okay. So now we wanted to look at more  
11 closely about this breakthrough phenomenon and the way  
12 that we analyzed this is by taking the supernatant from  
13 various time points from both the transduced cell  
14 cultures and the mock cultures and then looked for  
15 duplex RT/PCR for the types of RNA that were present in  
16 the supernatant.

17 As you can see here, for example, this is the  
18 mock of day one and this is the vector containing cells  
19 of day one, right, mock vector, mock vector, mock  
20 vector all the way through until you increase to day  
21 16.

22 Now the duplex PCR involves two sets of  
23 primers. One set of primers specifically detects wild  
24 type HIV, right, and the other specific set of primers  
25 detects vector, right. And as you can see in the mock  
26 cultures during the course of virus replication we are  
27 detecting wild type HIV. But what is interesting is

1 that during this viral breakthrough we are seeing the  
2 selective packaging of vector in these cells.

3 So what we are seeing, in fact, is that  
4 qualitatively most of this p24 that is coming out here  
5 is vector genomes being packaged into the supernatant.

6 Okay. Now the question is does this vector --  
7 mobilizable vector -- does this packaged vector, does  
8 it mobilize into cells? Can it effectively transduce  
9 naive T cells? And we have found that it is very  
10 difficult. It is very inefficient to transduce T  
11 cells. I will show you the next slide.

12 (Slide.)

13 We have done similar RT -- DNA PCR now looking  
14 at cells that were transduced with the vector. So  
15 these are the cells that have HIV and vector, right,  
16 and these are the mock HIV cells and this is a DNA PCR  
17 of the cells that were transduced with a representative  
18 of that supernatant. And while you can detect wild  
19 type HIV, you can see that by this assay we could not  
20 detect the vector.

21 So what we did is by this gel PCR method  
22 because we could not detect vector, we undertook to  
23 take Taqman DNA PCR on these cells and what we found  
24 was is that, in fact, there was a very low level of  
25 mobilization and it revealed that 40 copies of the  
26 vector mobilized into primary CD4 T cells per 10,000  
27 cells analyzed.

1           So the conclusion is from these experiments is  
2 that this vector VRX496 can mobilize but very, very  
3 poorly. Okay.

4           (Slide.)

5           We looked more extensively about the  
6 mobilization of this vector into a more sensitive cell  
7 type, MT4 cells, and what we did here is we took either  
8 primary CD4 T cells or T1 cells and took cells that  
9 were either untransduced, right, or transduced with a  
10 vector that did not contain the antisense payload or  
11 cells that contained VRX494, which is the same vector  
12 that contains the described antisense payload. We  
13 challenged those cells at an MOI of .2 and then took  
14 the supernatant and assayed them on MT4 cells and, as I  
15 said, it is a very sensitive cell to pick up HIV  
16 replication.

17           And what we found is that the control shows  
18 there was no mobilization events. The -- while the  
19 VRX430 that did not contain payload, you could barely  
20 detect some level of mobilization. What was  
21 interesting was that when the vector did contain the  
22 antisense payload, the mobilization level went down  
23 and we have done this experiment many, many times, and  
24 this data remains very consistent.

25           (Slide.)

26           Okay. We further looked at the mobilization  
27 events in vivo and this was in negotiation with the FDA

1 to look at two questions, whether mobilization occurs  
2 and what type of mobilization occurs. Are there any  
3 adverse mobilization events occurring? Right? For  
4 example, if the vector mobilizes weakly, does it  
5 mobilize just the CD4 cell or can it now infect another  
6 cell type?

7 So what we did is we undertook this  
8 experiment. We isolated human CD4 T cells and divided  
9 it into two lots. The first lot we transduced with  
10 VRX494, which is the vector that expresses EGFP. Then  
11 we constructed another vector which we swapped out the  
12 EGFP for EYFP. So we could discriminate between cells  
13 transduced between the yellow fluorescent protein  
14 vector and the GFP vector. And then mixed in back CD -  
15 4 PBMCs, which include B cells as well. And then  
16 injected these cells intraperitoneal back into mice.

17 Now if a mobilization event occurred from CD4  
18 to CD4 cells then you should see doubly positive  
19 stained cells. Right? However, if an adverse event  
20 occurred, say mobilization from a CD4 cell of a green  
21 or yellow vector to CD19 cells, for example, these  
22 cells, then you would see either green or yellow  
23 fluorescence in these cells which you could  
24 discriminate by FACS.

25 (Slide.)

26 So had groups of five animals and this is a  
27 representative of the data. And we showed that

1 basically in vivo in this mouse model that VRX poorly  
2 mobilizes from CD4 cell to CD4 cells. So these are  
3 cells that are expressing GFP or YFP but are not  
4 challenged with wild type HIV. Right? And as you can  
5 see there are no significant double positive events.  
6 This is the level of background that we typically see.  
7 Right? While in the cells that are challenged with  
8 wild type HIV at a high MOI of .2, we find that we can  
9 detect some mobilization events, double positive cells,  
10 indicative of some mobilization is occurring.

11 (Slide.)

12 However, when we looked at CD18 cells, right,  
13 whether there was an adverse mobilization event  
14 occurring, in the noninfected cells, this is the  
15 background here, we do not see any double positive  
16 events. And what we are looking for here is CD19 and  
17 EG -- or EGFP or EGYFP double positive cells. No  
18 events here and no events here. It is actually lower  
19 than the background.

20 So what we can clearly say is that VRX496 is a  
21 vector that mobilizes poorly but it does not mobilize  
22 adversely.

23 (Slide.)

24 Again in negotiation with the FDA we have  
25 performed some safety and biodistribution animal  
26 studies using SCID-hu mice. We think that the SCID-hu  
27 mouse system is really the best animal system to look

1 for potential adverse safety events and the reason for  
2 that is that you have the ability to inject human cells  
3 that contain your candidate vector into a mouse that is  
4 not immunocompetent and these cells then can survive  
5 for a long period of time and die off naturally, right,  
6 giving ample time for any adverse event to occur.

7 Now the adverse event that we are really  
8 looking for here is RCR autonomous mobilization into  
9 mouse tissue. That means is that if there is some sort  
10 of strange event that would occur between the VSVG and  
11 the vector to give mobilization events into mouse  
12 tissue, this assay would pick it up. So let me  
13 describe to you the assay.

14 We isolate human T cells and then we transduce  
15 those cells with our candidate vector. These cells are  
16 then injected i.v. at very high dose into the mouse.  
17 The cells then distribute throughout the animal. We  
18 then kill mice and isolate over 10 organs and then look  
19 at those organs at day two, day 30 and day 91 for the  
20 presence of vector in the various tissues.

21 Now at day two obviously you would expect that  
22 all the tissues would contain vector because the cells  
23 are there as well but during the course of time these  
24 cells die off, right, and so if you see the presence of  
25 a vector sequence -- so the adverse event would be is  
26 if you would see the presence of a vector signal and  
27 not the presence of a signal to a human gene. That

1 means that the vector has mobilized into the mouse  
2 tissue so let me repeat that again. The RCR event  
3 would be indicative if you would see a positive vector  
4 signal in the mouse tissue and not a positive human  
5 signal, which would be indicative of residue of human  
6 cells in that animal.

7           So to detect human cells we are using PCR  
8 primer specific to the gene. It is a homeodomain type  
9 of gene. It is a housekeeping gene present on  
10 chromosome 12. The reason why we chose this gene over  
11 beta actin is the homology of beta actin between mouse  
12 and human is 100 percent. You need to have something  
13 to discriminate between mouse and human cells and so  
14 this -- the primers to this gene that would effectively  
15 discriminate between mouse and human.

16           (Slide.)

17           So the first thing that we did is we wanted to  
18 see whether mouse cells to the point of the reviewer's  
19 comment could be transduced with our vector because if  
20 the mouse cells could not be transduced with the vector  
21 there is no point in doing this assay. So what we did  
22 is we took murine hemopoietic cells and basically  
23 transduced with our vector. These are the controls and  
24 these are the cells transduced with the vector and we  
25 analyzed them 13 days after transduction, with  
26 incidentally a very low MOI of 2 and we find a very  
27 high level of transduction, over 70 percent of the

1 cells.

2           There is no question that murine cells can be  
3 transduced with these class of vectors and so if an RCR  
4 is present it should have the potential to infect mouse  
5 cells.

6           (Slide.)

7           So what I am going to do now is I am going to  
8 show you representatives of the data. First for day  
9 two and then I am going to show you a summary table and  
10 for day 30 a summary table and then day 90 in a summary  
11 table.

12           But first I am going to tell you about -- a  
13 little bit about the assay. The assay is a DNA PCR.  
14 It specifically identifies the G tag sequence, that 186  
15 base pair sequence that I mentioned previously, and  
16 basically the sensitivity of this assay is 50 copies  
17 per microgram of DNA. So, for example, in this animal  
18 here we took the spleen and we have three reactions  
19 here of one microgram each. And in this third sample  
20 here we spiked in 50 copies of our control DNA into the  
21 sample and the same here.

22           (Slide.)

23           So what we can show is that we can amplify 50  
24 copies. This validates the sensitivity of our assay.  
25 However, in this group of mice, these are the control  
26 CD4 T cells, that means that these are mice that are  
27 injected with cells without the vector, right. There

1 is no signal present in the unspiked samples. This is  
2 our positive control for our positive PCR control right  
3 here and these are the markers. Okay. So in the  
4 control group animals basically they do not contain --  
5 the cells that do not contain the vector, we do not see  
6 any positive vector sequence.

7 (Slide.)

8 However, when you analyze the mice day two  
9 post-injection of cells that were transduced with the  
10 vector, the mice injected with VRX transgene CD4 T  
11 cells, we find that, you know, a great majority of the  
12 samples light up so these are the spiked controls.  
13 These are the no spiked samples and you can see a very  
14 strong positive signal for vector. This is the  
15 control.

16 Now whenever we see a positive signal for  
17 vector, we then look for huCART expression to see  
18 whether that signal is due from vector that is  
19 mobilized or is a signal due to just the T cells that  
20 contain the vector, human T cells.

21 (Slide.)

22 So again we have a huCART PCR primer set that  
23 effectively discriminates between mouse CART and human,  
24 right, and when we take those samples we get a positive  
25 band. That is the sensitivity of the assay.

26 (Slide.)

27 So now a summary of the day two data is as

1 follows: We have four groups of animals. Group one  
2 animals contain -- are injected with medium only.  
3 Group two animals were injected with cells that did not  
4 contain the vector. Group three animals were injected  
5 with a low dose of VRX496 transduced cells. And group  
6 four were injected with a high dose of VRX496 cells.

7 As you can see in the various tissues that we  
8 tested, we have here a panel of ten tissues, heart,  
9 testes, ovary, liver, lymph node, blood, tail, spleen,  
10 lung, you can read that for yourself. Basically you  
11 can see in every case we saw a positive signal for  
12 vector and you would always find a positive for huCART,  
13 demonstrating that that signal that we see there is due  
14 from the human cells and not due from an adverse event.

15 In some cases we find that PCR from blood was  
16 a little bit problematic mainly because of tissue  
17 sample size.

18 (Slide.)

19 Now the next set of animals are the mice taken  
20 at day 30 post injection. So these human cells were  
21 injected and 30 days later we then killed the animals.

22 And a pattern is starting to show. We are starting to  
23 see that, in fact, the cells -- the human cells that  
24 are in the animals are starting to die off. So you do  
25 not detect vector signal anymore in some of the  
26 animals. So in this animal, this tissue sample of the  
27 tail you can see that this is the spike control but

1 there is no vector signal. The human cells have died  
2 off and with it the vector signal.

3 In some animals you still see the vector  
4 signal and so what we do in this circumstance is  
5 analyze these samples for huCART to see whether that  
6 signal is due from the human cells containing the  
7 vector or an adverse RCR type mobilizable event.

8 (Slide.)

9 And again in every case that we find our  
10 vector signal we find that the tissue sample always  
11 amplifies the huCART human cell band.

12 (Slide.)

13 And the summary of the day 30 data is as  
14 follows: Now you are seeing many more cells are  
15 negative for the vector, right. Some cells are still  
16 positive for the vector. But in every case where the  
17 vector signal was seen the huCART signal was seen as  
18 well. Again we have problems with blood in terms of  
19 sample size but the results are, you know, very, very  
20 consistent.

21 (Slide.)

22 And, finally, now the day 91 data and  
23 basically by this time most of the human cells have  
24 died within the animal and so this is a typical result  
25 that we see here. These are the spiked controls again.

26 These are the samples and you see no signal present.

27 (Slide.)

1           And the summary of the data is that everything  
2 was negative except for four independent tissues in  
3 four different animals that were positive and these  
4 bands were extremely light. What we did then was again  
5 we took those and performed huCART analysis and again  
6 we could detect the huCART gene.

7           So what we have seen is that we have seen no  
8 adverse mobilizable events occurring in all the animals  
9 studied to date from the day two to day 90. That is  
10 the summary of the data.

11           (Slide.)

12           So now a brief summary of the proposed  
13 clinical trial. Our proposed clinical trial is now  
14 that we are selecting patients that are failing or  
15 discontinued HAART therapy and we -- what we have now  
16 done is to say that if a patient is showing virologic  
17 failure and can enroll into the study, he can enroll --  
18 he or she can enroll into the study and keep on the  
19 same regimen that that patient subject is on as long as  
20 they do not change their regimen. So to avoid any  
21 ethical issues.

22           So the patient has no opportunistic  
23 infections. We have now at the suggestions of the  
24 reviewers narrowed down the CD4 count range from 200 to  
25 600 but we have still maintained that viral load of  
26 greater than 500, which is demonstrating virologic  
27 failure.

1           We are going to enroll up to 24 patients, 12  
2 will be, you know, in the actual study. The patients  
3 will come in. Their cells will be isolated. And then  
4 the cells will be exposed to a vector. The vector has  
5 been previously produced by the methods that I have  
6 described incorporating benzonase and chromatographic  
7 and ultra filtrating/difiltration schemes, which will  
8 then be QC'd prior to transduction of the cells.

9           And we have an extensive panel of QC tests  
10 both on the vector and on the cells.

11           After the cells are expanded and they are  
12 released by QC, they will then be introduced into the  
13 patient in a dose escalating manner. The trial is  
14 divided into four escalation doses,  $10^9$ , three by  $10^9$   
15 and  $10^{10}$  and three by  $10^{10}$ . And what we would like to  
16 do is to start off at the lowest dose with a single  
17 patient, run that patient all the way through the 28  
18 day cycle, and then if there is no adverse, everything  
19 looks fine, then we would enroll the next two patients.

20           And then after that we will enroll concurrent three  
21 patients at each dose.

22           Both the vector and the cells undergo  
23 extensive QC testing but the only one that I really  
24 want to describe today is the testing for RCR, for VSV,  
25 because I think that is the pertinent issue.

26           (Slide.)

27           So here is RCR testing that we will perform

1 and we have performed on transfected 293 cells and our  
2 vector product. This is before transduction of the  
3 cells. So we would test both the end of production 293  
4 cells, that means the cells that were transfected with  
5 the vector and the helper, and the bulk harvest. That  
6 is the supernatant that is taken from the cells after  
7 transfection.

8           The RCR assay is that we would take, let's say  
9 the bulk harvest here, infect H9 cells, 300 mls of  
10 vector of supernatant will be tested, and then we would  
11 passage those cells for six passages and then in the  
12 final sixth passage we will use Taqman PCR on the  
13 supernatant to detect for any potential RCR using HIV  
14 gag and VSV-G primers.

15           Okay. And what we have found during  
16 validation of this assay is that we can detect by  
17 Taqman PCR a wild type HIV that is 100-fold less fit  
18 than wild type. So what does that mean? We can take  
19 one infectious unit of HIV, take it through three  
20 passages and detect it by Taqman PCR.

21           We will not only do three passages. We will  
22 do six passages and from that final amplification  
23 passage then use Taqman PCR to detect where there is  
24 any virus present in the supernatant. The sensitivity  
25 of our assays for HIV gag is one copy per 10,000 cells  
26 or one copy per reaction, which is generally 10,000  
27 cells. For VSV-G it is ten copies per 10,000 cells.

1           We also at the same time test the end of  
2 production 293 cells and again we are co-cultivating on  
3 H9 cells because there is no wild type HIV here. That  
4 is why we are using H9 cells. And we will take  $10^8$   
5 cells and basically co-cultivate it with H9 for the  
6 first passage and then take the H9 cells through six  
7 passages before also taking that supernatant and then  
8 assaying it by Taqman PCR for gag or VSV-G.

9           So if the results are negative there are no  
10 VSV or gag detection, we will release the vector for  
11 transduction pending other QC tests. There is a whole  
12 battery of them. Although if there is a positive  
13 result, obviously we would not release it. We would go  
14 ahead and characterize what is going on.

15           (Slide.)

16           The cell processing will be performed at the  
17 University of Pennsylvania and a rough scheme is  
18 depicted here. Basically the patient subject comes  
19 into the clinic and undergoes leukaphoresis and then T  
20 cell selection. The cells are then transduced with the  
21 vector in the presence of immobilized CD3 and CD28  
22 beads. The beads are removed by a magnet. The cells  
23 then are washed and concentrated, formulated in a bag  
24 containing DMSO and frozen.

25           During the period of freezing the cells  
26 undergo QC testing. If the cells pass QC testing with  
27 the cell tests then they can be released for infusion

1 into the patient.

2 (Slide.)

3 And so this is the RCR testing for the  
4 transduced T cell product. What we will do is we will  
5 take our ex vivo transduced and expanded T cells, take  
6 the supernatant and then do basically two assays. The  
7 first assay will be a biologically RCR assay where we  
8 now take the cells and infect 293 cells. We do not  
9 infect H9 cells because these T cells are already  
10 infected with HIV, right, so we are infecting a cell  
11 line that is not permissive for wild type HIV but would  
12 be permissive for VSV pseudotype version of HIV because  
13 of the broadly tropic nature of the VSV envelope  
14 protein.

15 So we would take the required amount, infect  
16 the cells, passage it for six passages, and then  
17 perform Tagman RT/PCR on the supernatant. We know that  
18 the 293 cells are readily infectable with the vector.  
19 We think -- we have chosen 293 cells because we know  
20 that these cells are readily transduced with a vector.

21 We know that we can produce the vector from these  
22 cells so the entire cycle of viral replication can be  
23 accounted for with 293 cells. That is why we use them.

24 Okay. In addition to the biological RCR  
25 tests, we will also take the supernatant directly from  
26 the expanded cells and then directly do RT/PCR, right,  
27 and looking for VSV-RNA. If there is any residue of

1 VSV-RNA that is present in that supernatant, if we  
2 detect it, we would not release that vector product.

3 Now for the transduced cells we would take the  
4 transduced cells, co-cultivate it with 293 cells, and  
5 then again for six passages, then look by Taqman PCR on  
6 the amplified supernatant by VSV and gag primers. We  
7 will also at the same time take the transduced cells  
8 and then do a DNA PCR using VSV-G primers, right. If  
9 we detect any signal here, our sensitivity here is very  
10 sensitive, one copy per 10,000 cells, we would not  
11 release that product for clinical trial.

12 So the way that we address the VSV issue is  
13 that our final product will not contain any VSV  
14 sequences that will be capable of recombining either  
15 with the vector or with the wild type virus.

16 (Slide.)

17 Okay. So patient monitoring. In your booklet  
18 you have got the updated protocol. I am just giving  
19 you a snapshot here. Basically at day 28, which is the  
20 important date for dose escalation, we will do such  
21 studies as T cell counts, differential viral load.  
22 What that means is looking at the plasma for both  
23 vector and wild type HIV genomes. We will do  
24 immunological assays. We will look in the RNA for VSV-  
25 G RNA in the plasma. We will for VSV-G antibody  
26 response. At the advice of Dr. Markert we will  
27 perform also a TCRV-beta diversity analysis to look for

1 the repertoire diversity. And also we will do various  
2 hematological and chemistry assays.

3 The dose escalation scheme is as follows, and  
4 one reviewer asked about the difference between 28 days  
5 and six weeks. What we will do is the patient will be  
6 monitored periodically during this 28 day period and  
7 then when these samples are obtained they will be  
8 assayed and then reviewed by the data safety monitoring  
9 board in a 14 day period and then they will decide if  
10 they will authorize dose escalation.

11 The reason why we chose 28 days, and this is  
12 referring to another question, is because CD4 T cells -  
13 - there are two types of T cells, long-lived and short-  
14 lived. The ones that are short-lived are the activated  
15 cells and they generally survive for 14 days. We would  
16 predict that if there was any real adverse event that  
17 would occur as a result of infusion of the vector  
18 containing cells that, you know, it would occur sooner  
19 rather than later, and that is why we chose this type  
20 of dose escalation scheme.

21 (Slide.)

22 Okay. So for patient subject monitoring  
23 looking for the potential adverse events and  
24 toxicities, these are some of the points to keep in  
25 mind. We will -- it would trigger an event if a  
26 patient subject experiences a precipitous increase in  
27 viral load of .5 logs or greater. If this occurs, the

1 viral load will be followed for up to seven days to  
2 determine if the increase is a sustained result. If it  
3 is, then we will enroll another -- we will expand that  
4 dose level to see whether it can be seen in two of the  
5 patients.

6 The same thing for CD4 T cell count. If the  
7 patient experiences a 50 percent or greater decrease in  
8 CD4 count we will again follow it and then this will be  
9 reported to the DSMB and then they will decide whether  
10 to stop the trial or to expand the dose.

11 However, in the case of VSV-G RNA, if we  
12 detect sustained detection of VSV-G RNA, we will then  
13 stop that patient. The patient will undergo apheresis  
14 and then we will look for that patient, whether there  
15 is a virological RCR present. If there is a single  
16 biological RCR depicted in any patient, we will  
17 immediately stop the trial.

18 Also, here this is about the grade 3 greater  
19 or toxicities and we will monitor for those and again  
20 everything will go through the DSMB which we are  
21 presently instituting.

22 (Slide.)

23 Again this is a little bit about the dose  
24 escalation scheme. You have a patient. If there is  
25 one patient that demonstrates toxicity in the group of  
26 three then we will treat another -- not another, three  
27 patients. If there is toxicity in two or more of those

1 patients then we will stop the trial. If there is not,  
2 then we will proceed to the next dose level and that is  
3 how we will proceed through the trial.

4 (Slide.)

5 So, in summary, we have shown that our vector  
6 can attain very high transduction efficiency in primary  
7 CD4 T cells. The vector transduced cells can  
8 significantly inhibit HIV replication in these  
9 cells. We believe it is the safest approach for the  
10 use of HIV vectors since the patients are already  
11 laden with wild type virus.

12 Importantly, we will have very stringent  
13 release testing criteria. No VSV-G sequences will be  
14 present in the cell product that could recombine to  
15 form some sort of RCR.

16 We have found that our VRX vector weakly  
17 mobilizes to CD4 T cells in vitro in a SCID-hu mouse  
18 model but it does not mobilize adversely. It mobilizes  
19 from CD4 to CD4 and not to another type of cell.

20 We have seen no adverse events in our safety  
21 and biodistribution studies in our SCID mouse models.  
22 Our clinical protocol is targeted to HIV patient  
23 subjects that are failing HAART. And our clinical  
24 trial is a Phase I clinical trial. Safety is the  
25 endpoint here and it will be complete when we  
26 demonstrate no adverse events. No precipitous  
27 sustained increase in viral load, no precipitous

1 sustained decrease in CD4 T cell count, no RCR or other  
2 significant toxicity associated with the vector.

3 (Slide.)

4 So I would like to thank my collaborators.  
5 First of all, I would really like to thank all the team  
6 at VIRxSYS. They really are a bunch of talented  
7 people. They have really pushed a lot of this research  
8 within a very, very short period of time and I feel  
9 very grateful to have them on board.

10 Particularly I would like to thank Yung Chang  
11 who has been with me from day one and also Tony  
12 Pascarelli, our CEO.

13 Also, I would like to thank our collaborators  
14 at the University of Pennsylvania, Rob Roy MacGregor,  
15 who is the PI; Carl June, who is also a co-sponsor of  
16 this protocol; and I would like to mention Bruce  
17 Levine, who has been great in terms of cell  
18 processing; Richard Carroll who has helped us with the  
19 primary challenge experiments; and also Peggy Bennett  
20 who has been interacting with TheraSolutions, which is  
21 a company located in Rockville, which is helping us  
22 coordinate the clinical trial.

23 So that is it. Thank you.

24 DR. MICKELSON: Thank you, Dr. Dropulic.

25 While everybody is readjusting to the light,  
26 Dr. Aguilar, would you like to start with your  
27 comments, please?

1                   Thank you very much, Dr. Dropulic.   Just take  
2 notes.

3                   DR. AGUILAR-CORDOVA:    So I guess I would like  
4 to start by commending the investigators for taking the  
5 plunge and obviously going through a tremendous amount  
6 of work in developing this new vector platform.

7                   I will limit my comments or concentrate my  
8 comments primarily on the product.   That was the major  
9 reason that I thought that this was novel enough to  
10 warrant full discussion even though the investigators  
11 have come previously to get some ideas on this  
12 forthcoming study.

13                  And the -- some of the issues that I will  
14 bring up just highlight the difficulties that come  
15 about in following with this particular lentiviral  
16 product.   I would, first of all, caution the  
17 investigators that with the full statement that no new  
18 sequences are included into this study since not all  
19 HIVs are identical.   Clearly there are some that are  
20 macrophage trophic, some that are lymphocyte trophic,  
21 and even within those caveats there are differences  
22 between species, thousands that have been demonstrated  
23 throughout the country.   NL4-3 is particularly virulent  
24 in vitro although we do not really know what its in  
25 vivo phenotype might be.

26                  And certainly recombinants in the envelope  
27 section as you mentioned as a possibility would not

1 necessarily generate the same phenotype of virus as had  
2 been previously found in whatever patient might be  
3 enrolled in this protocol. And one cannot predict  
4 what the addition of a novel viral phenotype in a  
5 particular patient will bring.

6 Thus one can also, only with great  
7 difficulty, say things that one can produce virus that  
8 is more pathogenic than the wild type found in that  
9 patient. In fact, one can produce a virus that is more  
10 pathogenic in a particular patient by recombining with  
11 another virus.

12 Now the mobilization studies and the  
13 difficulty with this whole process is that one can  
14 easily assess by doing the RCR assays a full VSV  
15 pseudotype lentiviral construct. What is more  
16 difficult to assess is a partial chimeric vector, one  
17 that would have only the VSV envelope but not the gag  
18 pol and thus not be mobilized by itself but may be  
19 carried through and then mobilized in vivo and  
20 recombination in retroviruses has been well documented  
21 at the RNA level like you said especially since they  
22 are deployed inside the virion. I think Dr. Howard  
23 Temin had showed that there is as high as say 10  
24 percent recombination frequency inside the virion.

25 So in your original proposal you had shown  
26 that in 32,000 cells you had been able to detect 27  
27 copies of VSV-G given the detection limits of your

1 assay. And that was dismissed because it was not  
2 detected in bioassay and thus believed not to be an  
3 RCR. However, the bioassay is less sensitive and, as I  
4 mentioned before, one may have partial recombination  
5 without having full RCRs in your product but that would  
6 still potentially generate a de novo recombinant in  
7 vivo.

8           The mobilization studies in the SCID mice are  
9 also not necessarily at the same level of sensitivity  
10 as your PCR so when one says that you can detect 50  
11 copies and you can standardize that to the human DNA  
12 that you have, even though you can transduce the mouse  
13 cells, even a full gag pol VSV vector or virus in a  
14 mouse cell may not necessarily replicate because it is  
15 not just the entry that gets inhibited in the rodent  
16 cells for the replication of the gag pol portion of it  
17 and the ability to form a full virus inside the mouse  
18 cell that may be inhibited as well. So the  
19 sensitivity of using the SCID mouse model may not be  
20 sufficient.

21           I noticed, also, in your Taqman PCR, for  
22 example, in table 3, when you were detecting number of  
23 copies of plasmid per bacterium, you detected only 65  
24 or 133 copies per bacterium, which seems rather low for  
25 plasmid copies inside each bacterium, and I was  
26 wondering whether that would correlate with your yield  
27 from those bacterium or if that implies something about

1 your sensitivity of the Tagman assay.

2 The degenerate gag, rev and tat, which might  
3 ultimately also end up in your -- in any potential  
4 recombinant vector, it was not quite clear to me  
5 whether your PCR detection systems that you were  
6 proposing for release criterion and for assays, whether  
7 they would be at all affected by the degenerate  
8 sequences of that gag, pol and rev PCR.

9 And even though you do give some justification  
10 as to why you are using the only two plasmid vector  
11 system rather than the multiple plasmid vector system  
12 that is currently used with various other studies, it  
13 appeared to me that you could have cross over between  
14 the pol sequence and that only one illegitimate cross  
15 over at the 3' end of the VSV with the consequent  
16 flanking of the ITR would yield to a virion that would  
17 have a full context of a degenerate gag, pol, rev and  
18 the VSV construct in it with an internal promoter.

19 Most of your challenge experiments that I saw  
20 were using very, very low MOIs, which are called MOI,  
21 and I am not sure what volume you are using, et cetera,  
22 but the MOIs of your HIV challenge is in the level of  
23 .001, whereas your vector was we are saying about 9  
24 copies per cell.

25 I was wondering if you had done those same  
26 challenge experiments at higher concentrations of HIV  
27 and also how does that concentration of HIV relate to

1 the -- to what one might expect in vivo not only in the  
2 serum of the patient but also in the reservoirs that  
3 are found in lymph nodes and other sites.

4 In figure 13 you show no dose response in that  
5 situation so do you see a two dose response if the  
6 challenge concentration is greater? And in -- and I  
7 will just mention the figures and perhaps you can  
8 follow that.

9 In figure 19 you show that there was no  
10 detection of the RCRs but what is the level of  
11 detection because there were no positive controls and I  
12 realize that there is a very -- that is one of the  
13 difficulties of this whole system is what is your  
14 positive control and one will not easily go and make --  
15 purposefully make a lentivirus with a VSV pseudotype as  
16 a positive control but then that just raises the bar of  
17 how to set up how many controls you must set up into  
18 this and I am not sure that going into a SCID mouse,  
19 that really increases your detection limits.

20 And in the detection of your RCRs in figure  
21 22, as well as those before, you show that in -- with  
22 the definition of one that you get for your positive  
23 control, and that is based on the TCID50 of wherever it  
24 is that you purchase that virus, the NL4-3 from. With  
25 that definition of one you were able to detect it after  
26 three cycles of cell passages and you say that you are  
27 increasing your detection limit by going an additional

1 three cycles.

2 But I would caution you that really the  
3 limiting factor there is the ability to have infected a  
4 cell in that first passage because if you did not  
5 infect the cell in that first passage you can passage  
6 20 times and you will still not detect it.

7 So the conclusions from figure 26 which was no  
8 detection in the mice is not a strong conclusion based  
9 on the fact that you have not shown that replication  
10 can actually occur within mouse cells. You have only  
11 shown that it is able to transduce it.

12 In the ones that you have shown -- like, for  
13 example, in figure 37 you showed that there is some  
14 mobilization. I think it will be of great importance  
15 to know what do those mobilized genomes look like. So  
16 do they contain exactly what the vector was originally  
17 or do the mobilized genomes show some rearrangements  
18 that would be perhaps not expected.

19 And that might give you some idea of what is  
20 happening in vivo since within the patient one of the  
21 strengths or one of the justifications of -- one of the  
22 few justifications perhaps of using an HIV lentiviral  
23 vector would be you would have some mobilization that  
24 would give you greater efficacy since the high level of  
25 transduction that you are seeing in your CD4 cells is  
26 actually in the pseudotype VSV-G vector and you have  
27 not -- or I have not seen the comparison that you might

1 have if you were to use VSV pseudotype C type vectors.

2 And you showed us right now some data with  
3 fairly low mobilization using your env antisense but in  
4 figure 39 I see that you had as much as 1.89 percent  
5 with a double color mobilization after HIV challenge.  
6 And that is quite significant, I would think, and  
7 certainly sufficient to perhaps evaluate what kind of  
8 genomes are in there.

9 DR. MICKELSON: Dr. Markert?

10 DR. MARKERT: I would like to commend the  
11 investigators for making a number of changes subsequent  
12 to the submission of our comments to right now. There  
13 have been many changes in the protocol. I will go  
14 through a variety of my comments where I would like to  
15 have them in the record.

16 On the preclinical data I really did enjoy  
17 seeing all the animal data included, in particular the  
18 mice data, and I had a few questions that I still am  
19 not clear as to the answers. And just the one for a  
20 little bit of humor, I do not understand -- I  
21 understand the animals who were weighed on day two all  
22 weighed less and these, of course, were all the animals  
23 that were sacrificed. So I do not know if there was a  
24 sign over their cage that they were going to be  
25 sacrificed and, therefore, they weighed less. I do not  
26 know why. I do not know why all the animals that were  
27 looked at weighed less than everybody else.

1           But the data -- it will be very nice -- I  
2 mean, seeing as safety is so much of what one wonders  
3 about is based on preclinical models, there are a lot  
4 of holes in the animal data and it does say that more  
5 data is being accumulated, whatever, but there were a  
6 variety of liver enzymes and other studies that were  
7 sort of rather fluctuating in this. So it will be nice  
8 to get the complete or for the investigators to look  
9 over all the data when it is all available because that  
10 can give clues as to what to look for in the patients.

11           With respect to those white focal splenic  
12 lesions and the pulmonary lesions that were judged to  
13 be incidental, it would be nice if the -- I do not  
14 think one needs to put anything in the consent right  
15 now because what would one put in the consent but it  
16 would be nice to know what those incidental findings  
17 were under the microscope. I mean, are they T cells or  
18 what? It would just be nice to know as opposed to  
19 someone just saying, "Oh, they are incidental and I  
20 did not look under the microscope at them."

21           Under protocol design and methods, with -- so  
22 there has been quite a bit of change here. The -- with  
23 respect to my concern about T cell diversity in these  
24 patients, one -- and I note in your responses that have  
25 come in the table that this method of expanding cells  
26 should not decrease the T cell diversity. Therefore,  
27 the patient should not or the research subject should

1 not be put at risk by losing their diversity.

2 But in just looking through now there is not  
3 an evaluation of the T cell diversity prior to entry of  
4 the research subjects into the protocol. These are  
5 patients who have been on HAART and have "failed."  
6 They may have a very limited T cell receptor diversity  
7 and I might suggest -- what I had suggested in my  
8 comments was doing a study prior to the research  
9 subject receiving this gene transfer and then about six  
10 months later.

11 The way the protocol has been revised now is  
12 the only testing of the T cell receptor diversity is at  
13 day 28 and I do not know that the single time point  
14 will reveal anything. I would think it would be nice  
15 to have -- for safety, to have the research subjects  
16 have some reasonable diversity by immunoscope prior to  
17 entry and then make sure it has not decreased through  
18 the study.

19 Another issue with the research subjects -- I  
20 have seen the addition of the proliferative responses  
21 to tetanus just as an example and if one has a choice  
22 it could be nice to use research subjects who have a  
23 proliferative response to tetanus just to show that  
24 proliferative response remains after the gene transfer  
25 through the next six months or so as opposed to it  
26 disappearing at any rate.

27 So that would be moving it to being a

1 screening test as opposed to just prior dosing. I am  
2 not so stuck on either of those but I do think both the  
3 T cell receptor diversity should be done prior to the  
4 gene transfer.

5 Now with respect to the adverse events, the --  
6 you had a description of what would happen, and I was  
7 so glad that that was included, if a research subject  
8 has an increase in the plasma HIV RNA or a drop in the  
9 CD4 cell count but the description up on the board was  
10 that it would be checked -- the lab would be repeated  
11 and see if it goes on for another seven days or  
12 something along that line.

13 It would -- I would like to have the Data and  
14 Safety Monitoring Board look at that. In the protocol  
15 it says that the Data and Safety Monitoring Board will  
16 meet after the first patient 28 days and then after the  
17 first cohort is finished, second cohort is finished,  
18 third cohort is finished, and I would feel better if  
19 the -- and it does say that for other things the DSMB  
20 may meet but these are -- that is the sort of other  
21 thing I worry about, would be changes in the plasma  
22 HIV RNA and the CD4 count that I would want them to be  
23 meeting about.

24 Sort of continuing along on some of these  
25 issues, with respect to lot release there is the LAL  
26 testing is -- let's see. It is not clear. Is it done?  
27 Is the result -- does the result come back prior to

1 giving the cells? And the -- I appreciate the response  
2 that gave the EUs per ml. Of course, the dose allowed  
3 to a patient depends on how many EUs per kilogram of  
4 the patient but in calculating out what a typical  
5 patient would be, you come well within what would be  
6 allowed if that is your typical response. I was just  
7 wondering if that was a lot release criteria.

8 Let's see. Then -- okay. Now with respect to  
9 the issue of failing HAART, it would seem that it might  
10 need to be a little tighter in the protocol about what  
11 is -- is there some other physician, for instance, who  
12 looks at the patient and says, "This patient really has  
13 failed and there really is not -- there is not some set  
14 of medications I would like to switch this patient to  
15 right away and that my first choice definitely is to go  
16 with treating this standard way because they failed  
17 this HAART regimen and I want to switch to this one."

18 Because the way it is written, it would seem  
19 that a patient being followed in some clinic might fail  
20 the first regimen and then be told, "Oh, we should go  
21 directly to this research protocol," whereas all the  
22 rest of the HIV doctors in the country would have said,  
23 "No, the standard of care would be to do something  
24 else." I would like some comment on how do you decide  
25 that you are not just going to switch to another  
26 standard regimen as opposed to coming on this protocol

27 There are issues of the risk to the research

1 subject of just holding on to a protocol that is not  
2 working very well but the way you have worked your  
3 protocol now that you are going to be looking more  
4 closely if the plasma RNA goes up or the CD4 count goes  
5 down, but I like standard therapy to be protecting the  
6 thymus and, you know, my favorite organ, if at all  
7 possible.

8           Let's see. And I think -- oh. With respect  
9 to the safety of infusing these numbers of activated T  
10 cells into humans, and I do understand that activated T  
11 cells have been infused into humans in other protocols,  
12 I would wonder are they -- have they been activated in  
13 the same way and then this is dose escalation so we  
14 will see what the adverse events are as we go along.

15           I made the comment about IL-6 and not -- it is  
16 not necessary that this be done real time but it might  
17 be nice to save some samples. You never know what  
18 later on could be helpful in trying to determine what  
19 went on in an adverse event.

20           Okay. So with -- I guess the -- so that --  
21 those really are my comments and the issue that I had  
22 initially was, just so that people know where I was  
23 coming from initially, was that could we be letting  
24 the virus just be out of control and destroying great T  
25 cells -- the T cells they have and you put in one  
26 little population. If it is oligoclonal that would be  
27 a problem, which actually brings up the other comment.

1           You might want to check as much as other  
2 people have done this, although maybe it is the same  
3 group, done this amplification of the T cells in  
4 culture and not seen a diminution of the repertoire.  
5 You might want to just check with what you are doing  
6 just to be sure that again -- that the repertoire stays  
7 fairly robust because it would do -- be a very great  
8 disservice to the research subjects if they had virus  
9 sort of go off up -- knock of their own -- the T cells  
10 that were not protected and you put in T cells that  
11 have a limited repertoire. But if they have a good --  
12 if the research subjects come with a good T cell  
13 receptor repertoire and you can maintain that then the  
14 risk is less in my opinion.

15           I thank the investigator team for making lots  
16 of the changes that were discussed in my comments.  
17 Thank you.

18           DR. MICKELSON: Thank you, Dr. Markert.

19           Ms. King?

20           MS. KING: Well, I also want to thank the  
21 investigators for doing so much work with -- between  
22 the time that they received our comments and the  
23 meetings. It is really great to see a lot of positive  
24 changes and I guess most of my questions and comments  
25 have been pretty well addressed. I think I have got  
26 two left.

27           One, I think, I would like to echo Dr.

1 Markert's comment about ensuring that there is some  
2 kind of independent assessment of the potential  
3 subjects not having reasonable standard alternatives  
4 that either are likely to have a good effect or that  
5 are acceptable to them given that some people might be  
6 failing on their regimen but also might be finding the  
7 side effects unacceptable and that sort of thing.

8           So it would be good to have an independent  
9 determination of that and related to that there needs  
10 to be more discussion in the consent form. Right now  
11 the consent form sort of reads like this is standard  
12 treatment but there are other standard treatments  
13 available to you so the alternative section needs  
14 additional work.

15           All right. I still have some lingering  
16 concerns about the -- in the consent form again because  
17 these are sort of key to potential subjects  
18 understanding of the study, the purpose section and the  
19 benefits section, but I do not -- you know, I have a  
20 fairly conservative perspective on what should be  
21 described in the consent forms and I do not want to  
22 micromanage it at all but I do have one suggestion.

23           In your revised consent form, on the first  
24 page of it you have got three paragraphs in the  
25 purpose section. That middle paragraph is really your  
26 benefits section and that should be lifted out of the  
27 purpose section and labeled "possible benefits" rather

1 than benefits and just placed in the appropriate place  
2 on the consent form.

3 That is it.

4 DR. MICKELSON: Thank you.

5 Dr. Coffin, did you want to make a few  
6 comments and then we will open it up?

7 DR. COFFIN: Yes. I have a number of  
8 comments. I would like to go back a little bit to the  
9 -- although this does not directly perhaps affect the  
10 safety of this particular product. I would like to go  
11 back a little bit to the basis, the rationale for -- do  
12 you want to go to somebody else first, Claudia?

13 DR. MICKELSON: I apologize. Yes.

14 Dr. Yee and then Dr. Zaia and then John. I  
15 apologize.

16 Yes, Dr. Yee?

17 DR. YEE: I probably overlap a little bit  
18 several other reviewers comments. First, I am a little  
19 concerned with the vector production system using two  
20 plasmid. I think that severely compromises the safety  
21 issue.

22 In the regular vector production system we  
23 use, in general you have four plasmids instead of two  
24 plasmids. You have a GABA expression plasmid, you have  
25 a VEGF expression plasmid and RIF expression plasmid,  
26 and then the vector. In most of the systems people use  
27 they do not use tat because they use CMV promoter to

1 produce the virus.

2           So with four plasmid vector production systems  
3 it is much safer than the two plasmid in terms of  
4 recombination to generate RCR. I guess the reason you  
5 use two plasmids is because you use 293 cells for  
6 vector production so you can get higher vector titer.  
7 And with four plasmids people use 293 T cells and again  
8 can get a very high vector titer.

9           So I like maybe if you can elaborate a little  
10 bit more about what is the problem with 293 T cells  
11 because this is a cell line everybody uses and that  
12 probably is the cell line people are going to propose  
13 for the next HIV vector clinical trials so that, I  
14 think, is a very important issue.

15           The second problem is the original idea used  
16 the functional ARTI in your vector system because it  
17 can be mobilized by wild type HIV. But since your  
18 preliminary data shows that it cannot be mobilized very  
19 efficiently by while type HIV, my question is can you  
20 go to the third generation HIV vector that is seeing  
21 vector without any functional ARTI so it cannot be  
22 mobilized by wild type HIV. That again increases the  
23 safety of using this vector in this particular clinical  
24 trial.

25           And the third question: I am not particularly  
26 concerned with RCR because with RCR you can detect with  
27 your current system. The p24 assay is a very sensitive

1 assay and it can detect anything above seven picogram  
2 per milliliter of p24. So that is a very sensitive  
3 assay.

4 I am more concerned with the recombination  
5 event that generates a vector containing only the HIV  
6 GAT protein or VSV-G genes. And it is clear from a  
7 publication from University of Alabama that this kind  
8 of recombination happens and happens quite frequently.

9 It depends on what kind of assay you use to detect  
10 this kind of recombination.

11 And I am sure -- unless your transfection  
12 method is different from anybody else, I think you and  
13 everybody else all experiment using a plasmid  
14 cotransfection in 293 T cells probably will generate a  
15 recombination event which generates either VSV-G gene  
16 recombined to a vector or GAT protein recombined into a  
17 vector.

18 And in this case you probably will not be able  
19 to detect those because those viruses are now  
20 replication competent. They can be delivered into  
21 target cells but they cannot spread. So you probably  
22 will not be able to detect by p24 assay or DNA PCR or  
23 RT/PCR assay.

24 You mentioned that if you have a vector with a  
25 VSV-G gene that integrates into the host cells and then  
26 you have an incoming wild type HIV, then the VSV-G gene  
27 will get activated -- the expression VSV-G gene can get

1 activated and pseudotype the wild type HIV. Then you  
2 have a wild type HIV that now can infect not only the  
3 CD4 cell line but any other cell types. So this is a  
4 potential problem.

5 So I think assays should be established to  
6 detect this kind of problem. Again this is the G gene  
7 and if you have a GAT protein this is derived from  
8 NAO4-3. If this GAT protein is delivered into target  
9 cell and then recombined with endogenous wild type HIV  
10 it may generate a different HIV strain which can give  
11 you higher toxicity. So I think it is very important  
12 to establish an assay to detect this kind of  
13 recombination event.

14 And again related to this issue in table 15  
15 you actually can detect VSV-G genes by DNA PCR and you  
16 explained that. That is obviously important. Why you  
17 can detect VSV-G genes even after several passages of  
18 the transduced cells in culture?

19 In terms of animal studies, again I think for  
20 mobilization in vivo it depends on HIV replication,  
21 while type HIV replication. And again we know that  
22 wild type HIV does not replicate very efficiently in  
23 animal -- in mouse cells so I wonder about the  
24 sensitivity of this kind of an in vivo assay in mice.  
25 Is it necessary to have this kind of assay because what  
26 is the sensitivity of this assay?

27 So these are some of my comments.

1 DR. MICKELSON: Thank you, Dr. Yee. I  
2 apologize again.

3 Dr. Zaia?

4 DR. ZAIA: Thank you. I would also like to  
5 congratulate you for bringing this to public  
6 discussion.

7 I want to address a different area that I  
8 think is most important as we begin this kind of a  
9 discussion and that is what is the best design for this  
10 kind of a study. The dose escalation study proposed  
11 here is the kind that Dr. Greenblatt and his colleagues  
12 are so expert at and that is for cancer drugs you want  
13 to protect the patient and you want to make sure that  
14 the dose -- that you know what the toxicity is so that  
15 the dose you give can be observed. And during that  
16 period when you expect to see that toxicity you can  
17 then make an adjustment and you may have to adjust the  
18 dose and de-escalate it.

19 So the question here is what is the toxicity  
20 that we are expecting to see? Well, there are  
21 certainly patient related toxicity but there is also --  
22 let's call it societal safety. That is this talk we  
23 are hearing about -- from the virologists about a  
24 recombinant event really relates to society outside the  
25 patient -- I mean, safety outside the patient.

26 So there could be, in fact, close contacts of  
27 that patient, research participant. People how have

1 intimate relationships with that patient may be part of  
2 that same safety profile. And it may even be larger  
3 than that. I do not want to make it any more complex  
4 but you can imagine. But if you do see that one  
5 patient who does have a recombination that could have  
6 put a new envelope on to the virus, it could infect its  
7 sphere of infectivity.

8           What do you do with that person? I think you  
9 need to be prepared to address that issue.

10           But what I am really driving at is this choice  
11 of a dose escalation after 28 days of observation. You  
12 are -- I know your rationale and it is reasonable but I  
13 do not think it is completely correct when you look at  
14 the broader aspects of safety.

15           Now what would be the best way? Would it be  
16 28 months or would it be 28 years? You know, who  
17 knows? But you could imagine that if there is from  
18 your data in vitro -- you see the spread of virus after  
19 about two weeks and so I think you use that two week  
20 scheme. And in nature you get a new infection with HIV  
21 and probably in two to six weeks you see detectable  
22 HIV. Maybe two to four weeks. But it is possible that  
23 T cell that you have put in there is going to need to  
24 be activated by influenza next winter and once it is  
25 activated, at that point it is going to then allow up  
26 growth of a recombinant virus.

27           So I do not know what the best time is but I

1 do not think it is 28 days but I think my advice to the  
2 committee in terms of the recommendations would be that  
3 you not have a dose escalation scheme but that you have  
4 a scheme that uses a single dose with a period of time  
5 of observation that allows you to capture the data that  
6 you need for safety.

7           Okay. Going to another part of the study  
8 design, study number two is really kind of efficacy  
9 related. You want to see a change in the viral load  
10 and stability of CD4 cells, which I think is, you  
11 know, fantasy that you will ever expect to see with an  
12 infusion of this number of cells an effect on the  
13 virus.

14           Maybe you will see it but you certainly will  
15 not see it. I would not think you would see it in six  
16 months. Maybe you would see it in six months but my  
17 guess is that you are not going to see in a person who  
18 is failing HAART therapy the infusion of these cells  
19 correcting change in the virus load. The CD4 count may  
20 be stable anyway in these patients even with HAART  
21 failure.

22           So the question is what else can you really  
23 do? I think that if there is going to be one thing to  
24 do that is going to help the field, it is to  
25 demonstrate that the cells that you have put in there  
26 that are so-called protected actually survive for a  
27 period of time.

1           And that may require that you go back to the  
2 old scheme that has been shown in the past, namely a  
3 controlled vector. So it does change the equation of  
4 risk because now you are going -- now the possibility  
5 is you will put in cells transfected with two such  
6 vectors so you double the potential problems for the  
7 sponsor of the study and also for the reviewers but  
8 that at least will answer the critical question that  
9 you have posed here in a definitive way.

10           I will just comment briefly on the choice of  
11 vector from my own standpoint. I think that your  
12 rationale is to look at the issues of homologous  
13 sequences in part in the construction of your vector  
14 and yet you leave the LTRs untouched. And I have a  
15 problem with that. I think you should -- if you think  
16 homology is so important, I think you should go the  
17 extra mile and make these LTRs safer for mankind.

18           And then the concept that Dr. Yee referred to.  
19 I think philosophically I like the idea of multiple  
20 site in packaging systems to minimize the possibility  
21 of some kind of a recombination and putting everything  
22 together I would think limits that strategy.

23           In terms of the preclinical data, I just have  
24 one comment that concerns me. I have seen your  
25 comments on the others but one that still concerns me  
26 is this outgrowth of virus after two weeks in vitro at  
27 a time when your cells are showing predominance of the

1 correct phenotype. That is the survivor phenotype.

2           So what is going on in that virus? Has that  
3 virus made an envelope in the presence of the antisense  
4 and outgrown? And maybe that envelope is different.  
5 Have you actually looked at that? And if the envelope  
6 is different, what does that mean? Does that mean the  
7 pathogenesis of the virus is going to be different? A  
8 theoretical possibility.

9           In terms of the protocol itself, I do not  
10 have many comments. I think the pulmonary tox -- I  
11 think the issue of the infusion is minimal in terms of  
12 our concerns of toxicity but pulmonary toxicity I think  
13 is the one thing that may occur -- I mean, fever of  
14 course but there may well be pulmonary toxicity. I am  
15 not sure that was dealt with that thoroughly. I could  
16 be wrong about that.

17           And finally -- oh. I still -- I think there  
18 is the perception of conflict between the person who is  
19 responsible for the quality assurance and release  
20 testing of the cells if that person has a proprietary  
21 interest in the method that is used to expand the  
22 cells. And I notice in your response you said that you  
23 did not think that there was a perception of conflict  
24 there.

25           That is all. Thank you.

26           DR. MICKELSON: Could you just go over that  
27 last point again? You think that if a person who has

1 the authority for lot release criteria --

2 DR. ZAIA: Carl June discovered the method and  
3 he is --

4 DR. MICKELSON: Okay.

5 DR. ZAIA: -- I think expanding the cells at  
6 the University of Pennsylvania. Presumably his lab  
7 will do the release testing.

8 DR. JUNE: That is not true.

9 DR. ZAIA: No, that is not. Okay.

10 DR. JUNE: We have an external --

11 DR. MICKELSON: If you could can come to this  
12 mic here.

13 DR. JUNE: This is Carl June so I would like  
14 to just clarify that. At the University of  
15 Pennsylvania we have established a quality assurance  
16 program that is external to the cell production but we  
17 do GLP based QC release criteria and there are five of  
18 them that will be in place for this protocol with an  
19 external quality assurance that has been established  
20 over the last year.

21 DR. ZAIA: That was not clear. So you are not  
22 responsible for signing off then on the --

23 DR. JUNE: No, we have a quality assurance  
24 that does not report to me.

25 DR. ZAIA: Okay. That is all I wanted to  
26 know. That is all. Thank you.

27 DR. MICKELSON: Thanks. Great. Now, Dr.

1 Coffin, thank you.

2 DR. COFFIN: Okay. I have a number of issues,  
3 many of which echo what some of the previous reviewers  
4 have said but I will repeat them anyway. And, also,  
5 some that get at some of the basic science underpinning  
6 your approach here.

7 The history of retroviral vectors is a history  
8 of argumentation that certain kinds of adverse events  
9 cannot occur and then discovery that in the face of the  
10 right kind of experiment those things, in fact, do  
11 occur and I sense a little bit of that in here so I  
12 would like to be sure that we root out as much of that  
13 as possible.

14 I mean, the first question that occurs to me  
15 is what is, in fact, the mechanism by which this vector  
16 is inhibiting HIV replication. In vitro it certainly  
17 seems to do so. It is quite impressive in that  
18 respect. I do not know what the mechanism of antisense  
19 inhibition is in these cases and I am not sure that  
20 anybody else does and given that one has to be very  
21 cautious about making assumptions about properties of  
22 what is happening.

23 For example, you make a statement that  
24 hundreds of mutations would be required to make the  
25 virus resistant to suppression but I see no  
26 experimentation that supports that and, in fact,  
27 relatively small numbers of mutations between some of

1 these different subtype E sequences do seem to make  
2 some difference and some more experimentation. Perhaps  
3 you have done it but I did not see it in here. Using  
4 more diverse viruses, for example, or subtypes or  
5 things like that would certainly go a long way towards  
6 supporting the -- what appears to be an unsupported  
7 claim to that respect because I am concerned that, in  
8 fact, resistance of the resident virus to this might,  
9 in fact, be able to evolve in some straight forward  
10 way, although perhaps straight forward but  
11 unanticipated way during the course of your treatment.

12 In fact, there may be -- in your figure 15  
13 where there is a little bit of breakthrough virus  
14 coming up -- may, in fact, be exactly that. You do not  
15 carry those experiments out far enough to see the  
16 appearance of breakthrough mutations according to the  
17 standards that people have done when they have put in  
18 specific mutants and looked for reversions and so on  
19 and so forth or odd ball recombinants.

20 So certainly some experimentation along the  
21 line. By the same token, you do not propose in your  
22 follow-up to do any real virology to see if the virus  
23 is changing, if resistant virus is, in fact, emerging  
24 in these patients. I would think it would be a very  
25 important thing to do since you will have created this  
26 base of where you have this virus trying to replicate  
27 against this inhibitory sequence at least in some small

1 fraction of the cells that are in the individual.

2           So I think significant follow-up on the  
3 phenotype of the virus regarding its ability to  
4 replicate on transduced cells and the appearance of odd  
5 ball variance would be called for, I would think.

6           I am not convinced. I am puzzled that the  
7 vector mobilizes poorly when, in fact, it works fine  
8 when you transduce it with the help -- with your helper  
9 construct. You are not in a sense doing anything  
10 different and I -- you do not give the details of the  
11 design of that experiment or at least I do not know  
12 them or I did not see them.

13           This may be somewhat of a -- of really what is  
14 an old phenomenon in virology. What you have done is  
15 created what used to be called "defective interfering"  
16 virus that when you go to low multiplicity rapidly  
17 disappears from the population only because it does  
18 interfere with virus replication and because it is  
19 incapable of mobilizing itself.

20           So at the high multiplicity -- in what are  
21 called high multiplicity double infection conditions  
22 when you have your high levels of transduced cells you  
23 pick up a lot of this and it mobilizes well in the  
24 virions and, in fact, it does get transduced in cells  
25 efficiently but because I might guess not knowing what  
26 your protocol was the virus has been replicating for  
27 some -- is allowed to replicate for some period of time

1 and the wild type virus simply out replicates the  
2 initially transduced virus and it looks like the ratio  
3 has changed a lot. Now I may be misinterpreting the  
4 experiment but that is the way it looks from the way I  
5 saw it presented here.

6 To change the subject a little bit again I  
7 agree strongly with the issue that Dr. Aguilar-Cordova  
8 raised and that is how you define the wild type. You  
9 call this virus wild type but what is in any given  
10 patient might be quite different. A virus that is in  
11 that patient might be of a kind that because we know  
12 the genetics of the virus have a lot to do with the  
13 eventual outcome of infection and how long that patient  
14 lives, by introducing new sequences there is a chance  
15 within that individual patient you will improve the  
16 quality, if you like, of the virus that is there by  
17 inadvertently repairing some defect in the LTR, for  
18 example. And that could have -- at least in a  
19 theoretical sense that could have important negative  
20 consequences for that particular patient.

21 I am not, I must admit, as concerned about  
22 what happens in the context and so on and sort of to  
23 society because you are not creating things that could  
24 not have -- except with the exception of the VSV-G  
25 issue as far as recombination between your vector and  
26 the resident virus, you are not creating things that  
27 could not have happened naturally and probably in a

1 sense may well have in some patient or another  
2 somewhere in the past at some time. NL4-3 after all  
3 did come from -- originally from a combination of  
4 naturally occurring viruses.

5 I think you need to do -- to repeat -- some  
6 serious experimentation to show that recombination of  
7 wild type HIV does not occur either in your growth  
8 experiments -- both your growth experiments in vitro  
9 and even if that means, for example, infecting cells  
10 with a virus that has been deliberately crippled by  
11 reducing -- by making some mutations in the LTR to help  
12 transcription factor binding sites or something to make  
13 it replicate a little less and then see if you can pick  
14 up that LTR again from -- see if you can pick up that  
15 LTR from your vector or looking directly in patients to  
16 see.

17 The obvious issue that might more seriously  
18 arise is if you can switch the phenotype of what -- of  
19 the -- of an R5 virus in a patient to an X4 virus. And  
20 I do not know whether you have -- whether your envelope  
21 sequence includes everything that you would need to do  
22 that. For example, the V3 loop was unclear what --  
23 most of the envelope sequences, gp120 sequence, is  
24 there although not all. And whether that includes all  
25 the sequences that you need to do that I could not  
26 tell.

27 Getting to the patients, the relationship of

1 your protocol to HAART therapy is still somewhat  
2 unclear to me. I guess from what you said, patients  
3 can be continued on HAART during -- on preexisting  
4 HAART therapy during the time that their cells are  
5 taken and are treated. That would seem to raise a  
6 problem of how you can be sure you wash out all the  
7 drugs from the cells so you can actually effectively  
8 infect them with your vector.

9           And the confusion that could arise during your  
10 analysis if the therapy is changing or if the patient  
11 status is changing would seem to be something you have  
12 to worry about a little more carefully than you do.

13           Also at least in the protocol that I saw  
14 originally was -- there were some discordance between  
15 what kind of sampling you were going to do when. On  
16 one page the virus load assays were being taken on  
17 different days than they were on -- on page 15 there  
18 were different virus load assays than there were taken  
19 on different days than on page 18 and following.

20           You -- I gather from what you said now that  
21 all patients will have been treated, which means  
22 presumably that there will be no patients in this group  
23 who would have been classified as long term  
24 nonprogressors or patients with very low load who have  
25 very low risk of progressing because I would be  
26 extremely concerned about treating a patient where  
27 there was a good reason to believe that his virus was

1 genetically crippled in some way and then inadvertently  
2 improving that virus, that virus' fitness in that  
3 particular patient.

4           In the follow-up for the cells, one of the  
5 things that might be worth looking for or perhaps  
6 should be looked for just on the off chance adverse  
7 things were being done to the cells, is for the  
8 possibility that surviving -- a few surviving clones of  
9 the transduced cells grow out and that can be detected  
10 either by looking at T cell repertoire or actually  
11 better by looking for clonal integration of the vector  
12 -- for the appearance of clonal integration of the  
13 vector.

14           And I think that is just about all of the  
15 specific questions that I had.

16           DR. MICKELSON: I know there are lots of  
17 questions. I hope you have written them down. I have  
18 written some too. If you want to maybe try to group  
19 some of them because a lot of them did overlap in some  
20 ways.

21           DR. DROPULIC: Yes. I was wondering, Carl,  
22 did you want to -- did you want to handle the clinical  
23 questions? Do you want to do that now? First group  
24 that together or shall I just go forth with some of the  
25 virologic questions first.

26           DR. MICKELSON: Yes. If you want to go up  
27 front. Could you just be sure to introduce yourself.

1 DR. MacGREGOR: My name is Rob MacGregor. I  
2 am the PI for the clinical trial in the Infectious  
3 Disease Division at Penn.

4 And as I recall the comments relating directly  
5 to the clinical protocol seemed mainly to be dealing  
6 with the selection of the patients and I would agree  
7 there are ways that we could make it more explicit as  
8 to the kind of patients we are looking for. But to  
9 answer that question, the kind of patient that we want  
10 to offer participation to is the patient who has been  
11 on treatment with several different regimes and has not  
12 been able to maintain or to gain control of their virus  
13 production so that in the face of ongoing treatment  
14 they have had continued virus production and loss of  
15 CD4 cells down to but not below 200.

16 In our clinical group we estimate that we have  
17 15 or 20 patients in that category at our place and if  
18 we think of the whole city of Philadelphia, we think  
19 that there are enough patients who would fit in a  
20 category like that who might be interested.

21 The plan would be to let people know that this  
22 approach for people who have failed treatment in terms  
23 of -- the definition of failure being the ongoing  
24 production of virus despite antiviral treatment.  
25 Patients of that nature who are desirous of trying to  
26 do something more. There are some patients and some  
27 doctors who would say in a setting like that if I am

1 not losing ground I will wait until more antiviral  
2 agents come along and hope I stay alive long enough to  
3 have that happen. That is a reasonable alternative.

4 But there are a number of patients who would  
5 say that I am unhappy that I am continuing to make  
6 virus and that my CD4 count was higher and now is  
7 lower, and I would be interested in participating in  
8 this in hopes that (a) we would learn something more  
9 about this approach and (b) as all patients who  
10 participate in any trial think this might possibly  
11 benefit me as well. Although we would say that we  
12 have no guarantee, of course, that that would happen.

13 DR. MICKELSON: Dr. Markert?

14 DR. MARKERT: I would like just to address a  
15 question here. This is the reason made from the  
16 immunological perspective that I felt patients should  
17 be screened by immunoscope prior to enrollment because  
18 here would be a patient who is failing HAART and they  
19 may be down along to the 200 CD4 cell count. If their  
20 diversity is low then the patient is -- all I see is  
21 increased risk for the research subject participating  
22 because taking T cells with a low diversity and putting  
23 the antisense vector into them and expanding them up,  
24 you give back to the research subject T cells with a  
25 limited diversity, which are not going to be all that  
26 helpful. And if there were that, I just do not see can  
27 -- a limited diversity will not help the research

1 subject and you can end up -- I mean, it just -- I  
2 mean, it just -- so you have a lot of cells of a  
3 limited diversity but it will not help with infections.

4 DR. MacGREGOR: Yes.

5 DR. MARKERT: And so I think that the only way  
6 that this -- I mean, aside from all the virology issues  
7 -- that this can be helpful is if the patient starts  
8 out with a reasonable repertoire because you cannot  
9 generate a repertoire from a very oligoclonal  
10 situation.

11 DR. MacGREGOR: That sounds like a reasonable  
12 thing to do. It would probably limit the number of  
13 patients that we would have who would be eligible and I  
14 guess you would have to balance the problem of not  
15 having patients that we could include versus the  
16 benefit of wider diversity.

17 DR. MARKERT: Yes. But I do believe that  
18 putting patients -- research subjects on who have  
19 limited diversity, what is the point? And that should  
20 -- I mean, if -- I mean, I certainly feel that it would  
21 not make any sense and I would put a whole page in the  
22 consent form that here is a problem. If you have no  
23 repertoire, this cannot possibly make your ability to  
24 respond to a variety of infections any better. And  
25 then who is going to sign that?

26 DR. MacGREGOR: Carl, do you want to comment?

27

1 DR. JUNE: Carl June from the University of  
2 Pennsylvania. My role would be in the cell  
3 manufacturing but I do have experience on that with  
4 some of the trials with Cell Genesis that we have done  
5 with CD4 zeta and in patients similar risk profile that  
6 was outlined.

7 So you can look at it, I think, in two ways  
8 legitimately. One is, yes, you have patients that  
9 are going to have a skewed CD4 beta spectro pipe, you  
10 know, and some of them more so than others in this  
11 failed group and you can say those are cells that are  
12 left overs and not useful and that is why they have not  
13 been killed because they never saw an antigen. Or you  
14 can say that those are cells that are keeping that  
15 patient alive and, in fact, you would like to clonally  
16 expand those clones because they are the ones that  
17 while -- you know, in order to enroll in this protocol  
18 those subjects have to have no OIs. So these are  
19 people failing HAART but that are free of OIs.

20 So you could say that what is left in these  
21 people is a good pot of cells, although with limited  
22 diversity, and why not expand them if they do provide  
23 antiviral. You know, if they render a cell that is  
24 infected with a protease resistant virus if it renders  
25 it resistant to that.

26 So I think there is a rationale both for a  
27 subject who has a gaussian distribution of V beta T

1 cells as well as someone who has a skewed distribution.

2 Maybe it should be separately specified in the  
3 protocol but I think scientifically at least I do not  
4 know the answer to what would happen there.

5 But as long as we evaluate that I think your  
6 comments that we need to evaluate the V beta gaussian  
7 distribution, you know, not just at the end of the  
8 protocol but at baseline and then a real outcome is  
9 comparing within that baseline -- within the intra-  
10 subject comparison of baseline to end of study and that  
11 we should have as you suggested sequential evaluations,  
12 baseline, day 28 and then six months off study.

13 DR. MARKERT: Your point is well taken. The -  
14 - if the initial study is done prior to actually  
15 enrolling the patient in the gene transfer, then  
16 whoever is looking at it at least can look at it  
17 because it is awfully hard just a priori to say this is  
18 what it would have to look like to enroll someone. I  
19 have taken your point here. But at least for someone  
20 to look at it and be thinking about it at the  
21 beginning.

22 DR. MICKELSON: Dr. Ando?

23 DR. ANDO: I would just like to comment since  
24 I have worked in this area with both interleukin 2 and  
25 with the gene therapy but basically if you are failing  
26 HAART and your CD4 count is 50 or 150, you are losing  
27 cells at 50 per year, and 100-150, you are basically at

1 the point -- that is the point where you get the  
2 opportunistic infections, et cetera.

3           Cliff Lane here has done a lot of work and the  
4 beta scope narrowing seems to be associated with a  
5 really clear sharp increase in OIs. And, in fact, what  
6 Cliff has done is been using -- trying to find patients  
7 who are responsive to HAART, low CD4s and using  
8 interleukin-2. Even though they have a limited beta  
9 scope, if you can get their CD4 counts up, they do  
10 better.

11           So that data suggests that it does not  
12 necessarily preclude -- it would not preclude patients  
13 who had a limited beta scope. They are actually better  
14 off at 150 with a limited beta scope than at 50. An  
15 absolute count of 50, you are definitely going to have  
16 a limited beta scope and you are definitely worse just  
17 from the numbers game.           So the reason they choose  
18 200 is that below 200 it is a very narrow slope before  
19 a very aggressive pathway of OIs and, you know, the  
20 actual outcomes.

21           DR. MICKELSON: Go ahead. Yes, Dr. Zaia?

22           DR. ZAIA: Yes. Dr. Carl, this is another  
23 topic that we did not really touch on that I think is  
24 important. We touched on it but we did not go into  
25 detail. That is your sampling from the peripheral  
26 blood, which I think represents about one percent of  
27 the total body CD4s, so that there is 99 percent that

1 are below the surface. And the question then is do you  
2 have -- really have a reasonable chance of affecting  
3 the virus outcome with just modifying that one percent?

4 I realize you cannot predict the future but is  
5 the -- I mean, there is the possibility that the  
6 rationale is flawed unless you can address that  
7 question.

8 DR. JUNE: I think the ways to address that  
9 are, as you know, I mean that there is not -- I mean,  
10 there is a pool of circulating T cells and there is a  
11 pool of secondary lymphoid resident cells but there is  
12 interchange. I mean, maybe 30 seconds is maybe the  
13 average resonance time of a T cell in the peripheral  
14 blood before it goes back in the lymph node.

15 So if we sample at any one time it will  
16 contain a mixture of all the various types, whether  
17 called mucosally derived T cells and lymph node  
18 derived, and memory and so-called naive cells. So  
19 it is the only practical way we can do it outside of  
20 infusing stem cells.

21 There are direct estimates of that and I was  
22 not here this morning to see if Dale Ando showed some  
23 of the studies from even your institution with CD4 zeta  
24 where there have been biopsies of the gene marked cells  
25 in the case of CD4 zeta that I am aware of and also in  
26 a few other gene marking studies where people have  
27 looked at the frequency at least of the cells following

1 infusion and there is a good correlation in both  
2 tonsils and lymph nodes in the CD4 zeta studies that I  
3 saw that were sponsored by cell genesis.

4 I think Dale could probably talk more about  
5 that but I think that is the persistence and the time  
6 of appearance that supports the rationale to do that.

7 DR. DROPULIC: Okay. So I would like to  
8 address some of the questions that were posed. I am  
9 just going to go through the list that I have here.

10 Regarding the X4 strain and potentially  
11 changing the phenotype, antisense is, in fact, derived  
12 from N04-3. It does include the V3 loop and so that is  
13 an X4 strain.

14 What we could do is restrict the patient  
15 population that is more advanced, that is demonstrating  
16 X4 strain, so that there would be no issue regarding  
17 switching a patient from R5 to X4. We have discussed  
18 this previously. We can -- you know, we can do that if  
19 that is what is required.

20 Regarding partial VSV recombinants -- well,  
21 the way that we are handling the whole VSV issue is  
22 that our release testing criteria will demonstrate no  
23 VSV DNA or RNA sequences in the final product. If  
24 there are no VSV sequences in the final product then in  
25 the case of patients that are infected with HIV, we do  
26 not see how we could -- how a detrimental RCR could  
27 evolve with recombination. We have very sensitive

1 assays. We are using Taqman PCR.

2           Regarding the 27 copies of detected VSV-G DNA  
3 in our pilot clinical lot, that was using a  
4 manufacturing protocol that is now being improved. We  
5 can now show that we do not have any DNA or VSV-G DNA  
6 present in the final cell product.

7           And what we found was is that this residue of  
8 VSV DNA, the 27 copies, was present outside the cell.  
9 It was not in the cell because when you wash the cells  
10 you could remove it, okay, by multiple washings. So  
11 it is not some one type of event, one single event,  
12 and it is integrated into the cell. It is residue of  
13 VSV hanging out on the outside.

14           Regarding the mouse SCID studies, and we are  
15 not saying that those studies, the biodistribution  
16 studies amplify any potential RCR. They are only  
17 detecting a single event. Obviously there are issues  
18 of sensitivity but all we can say is that there is no  
19 significant RCR type mobilization in these animals. We  
20 feel it is the best possible animal model used to look  
21 for such adverse events.

22           Regarding a question regarding higher MOIs of  
23 challenge. We have done that and the cells have been  
24 protected. We can provide that data if required. That  
25 is not a problem.

26           The question was regarding low mobilization  
27 and saying that 1.89 percent was relatively high. Our

1 background for those assays can be anything up to one  
2 percent so in reality, you know, it is really in the  
3 .89 percent. It is on the limit of detection by FACS  
4 for us. We can barely detect it.

5           Regarding the question on the mechanism of the  
6 antisense, what we have shown is that when -- first of  
7 all, the reason why you do not affect production is the  
8 antisense is against wild type HIV env, there is no --  
9 there is no wild type HIV env that is targeted during  
10 production because there is no wild type HIV in there.

11           To your point in terms of the mechanism --

12           DR. COFFIN: That was not my question.

13           DR. DROPULIC: That was not the question. I  
14 would like to get at your question.

15           DR. COFFIN: The question was how -- since you  
16 did show that you had a lot of -- you know, at least in  
17 one case you had a lot of pseudotyping of the vector  
18 with virus that was -- with HIV that was in the culture  
19 at the same time and you are highly -- in one  
20 experiment where you had the naturally infected cells  
21 and you transduced those and then you got this  
22 breakthrough virus coming up.

23           DR. DROPULIC: Right.

24           DR. COFFIN: Most of the genomes in that virus  
25 you showed were vector and not --

26           DR. DROPULIC: Yes.

27           DR. COFFIN: -- and then you claim that

1 despite their -- your claim then was, I think, that  
2 despite the fact that there was a lot of vector in  
3 there, you were getting --

4 DR. DROPULIC: There is not a lot. There is  
5 not a lot of it.

6 DR. COFFIN: There is a lot more -- but there  
7 is more vector than virus.

8 DR. DROPULIC: Yes, but there is a --

9 DR. COFFIN: But you are getting --

10 DR. DROPULIC: It is a small amount.

11 DR. COFFIN: -- but your experiment then  
12 appeared to show much less mobilization of vector of  
13 the genomes -- the majority of the genomes in that  
14 particular --

15 DR. DROPULIC: Right. Those genomes --

16 DR. COFFIN: And then you concluded from that  
17 this was mobilized very poorly.

18 DR. DROPULIC: Yes.

19 DR. COFFIN: Despite the fact that it was  
20 apparently taking the virions reasonably well at least  
21 in that one -- at least in that one setting. My  
22 suggestion was that may have been due to allowing  
23 multiple rounds of replication before you did the  
24 analysis.

25 DR. DROPULIC: I see.

26 DR. COFFIN: Maybe you did not do that but I  
27 could not tell from what you said.

1 DR. DROPULIC: Okay.

2 DR. COFFIN: I understood perfectly why it did  
3 not interfere with the --

4 DR. DROPULIC: Okay. Fine.

5 DR. COFFIN: -- that was not the --

6 DR. DROPULIC: But in terms of -- yes, that is  
7 right. But in terms of the mechanism just as a side  
8 note, we do have a construct that contains an antisense  
9 against gag, right, and env, the titers for production  
10 do go down. So it shows that the antisense is having  
11 an effect because you had a question.

12 But I think that the effects are not purely  
13 just the payload. I think there are competition  
14 effects just to your point regarding defective  
15 interferon particles in competition. That effect is  
16 also occurring.

17 DR. COFFIN: But there are a lot of other  
18 possible ways in which antisense can have these kinds  
19 of effects. They can be double stranded RNA and can be  
20 directly toxic to one mechanism.

21 DR. DROPULIC: Okay.

22 DR. COFFIN: And other kinds of things you can  
23 imagine.

24 DR. DROPULIC: Okay. With regards to Dr.  
25 Zaia's question of measuring the survival of the cells,  
26 I think in this report here I have now said that we  
27 will be looking for direct survival of the cells. I do

1 not know whether it answers your issue regarding  
2 control cells versus antisense containing cells but it  
3 was not in the original protocol and now we have  
4 included using the unique 186 base pair fragment.

5 A lot of questions.

6 DR. MICKELSON: I think there were a number of  
7 general sort of questions on the vector production that  
8 might deserve comment. User of higher numbers of  
9 plasmid systems.

10 DR. DROPULIC: Yes.

11 DR. MICKELSON: 293 versus 293 T.

12 DR. DROPULIC: Right. Yes.

13 DR. MICKELSON: LTRs remaining.

14 DR. DROPULIC: Right. So let me take the  
15 functional LTRs. First of all, I do not understand if  
16 I understood your question but we do not degenerate the  
17 vector. Right. That is not what is being degenerated.

18 And in the helper, right, construct, where we are  
19 doing the degeneration, there are no HIV promoters.  
20 They are heterologous promoters.

21 So in terms of promoters there is no  
22 homologous regions. Right? You cannot really  
23 degenerate the vector at all because you need the  
24 elements in order for it to do its function, transduce  
25 and compete with wild type HIV. So I suppose I really  
26 -- I did not understand exactly that question.

27 But the bottom line is that we are not saying

1 that recombination will not occur between the vector  
2 and the wild type virus, for instance, but the outcome  
3 of that recombination is still that you get wild type.

4 You may change the phenotype but if we restrict the  
5 phenotype to patients that are already demonstrating X4  
6 where you are putting in more of a homologous vector,  
7 if you like, against the endogenous HIV strain.

8 Regarding the trace-ability of 293 --

9 DR. AGUILAR-CORDOVA: Just a second. Just to  
10 follow-up on the degenerate sequence.

11 DR. DROPULIC: Yes.

12 DR. AGUILAR-CORDOVA: The question was that if  
13 you took that degenerate sequence into the partial  
14 recombinant and then it became part of a new  
15 replication competent vector or virus, given the assays  
16 that you were describing, whether your PCR fragments or  
17 anything else, and whether the biology of that vector  
18 would then be changed because you were taking a  
19 degenerate sequence that was no longer the wild type  
20 sequence, it was again addressing the issue of whether  
21 you could bring in new sequence into the vector types.

22 DR. DROPULIC: Okay.

23 DR. AGUILAR-CORDOVA: Even though you do not  
24 have HIV LTR driving it. The sequence is still there  
25 and it could be imported into a retrovirus.

26 DR. DROPULIC: You are talking about which  
27 sequences are still there? I am sorry.

1 DR. AGUILAR-CORDOVA: Your helper vector has  
2 the degenerate sequences.

3 DR. DROPULIC: Yes.

4 DR. AGUILAR-CORDOVA: Those sequences then  
5 could be imported into a partial recombinant.

6 DR. DROPULIC: Yes.

7 DR. AGUILAR-CORDOVA: Would your assays detect  
8 those sequences?

9 DR. DROPULIC: We are focused on now detecting  
10 the VSV-G. That is what you are saying in the final  
11 vector preparation. We have not got an assay to detect  
12 any of the gag pols because -- no, we have not got an  
13 assay, right, to detect those degenerate those  
14 sequences but we could do that. That should not be a  
15 problem.

16 Okay. So let me take on 293T versus 293. It  
17 is -- I think, Dr. Yee, you asked that question in  
18 particular. We have switched to 293 because of trace-  
19 ability issues, right. 293 was derived from Frank  
20 Grimes' laboratory at the University of Toronto and you  
21 can get the 293 cells directly with a sublicense from  
22 Microbix. And so the trace-ability of the cell line,  
23 what serum was used to passage the cells is clearly  
24 established. That is important because you want to use  
25 serum from non-BSC countries. Right? I am not so sure  
26 if that trace-ability you can obtain for 293. We found  
27 it was just easier to go from 293 and so that is what

1 we have been using. It is a trace-ability issue for  
2 the cell line and what serum was it passaged in.

3 My understanding is Michele Kalos from the  
4 West Coast developed the 293 T cell line but I am not  
5 so sure of the trace-ability in terms of what serum was  
6 used to passage it.

7 Okay.

8 And the four plasmid versus three plasmid  
9 versus two plasmid systems. Again I do not know if  
10 there is any direct evidence showing that one is better  
11 than the other. What we have done is we are using a  
12 two plasmid system and we have included other things  
13 into that plasmid. These pore sites to prevent read  
14 through that could give rise to, you know, a gag, pol,  
15 tat, rev, VSV, you know, that would be co-packaged and  
16 recombined.

17 If you have documented evidence to show that  
18 four is better than three or two, you know, I suppose I  
19 would like to see that. I do not know of any and I may  
20 be wrong but I do not know of any.

21 That is all.

22 DR. MICKELSON: Are there other comments here?

23 Dr. Noguchi? I think there are some comments from the  
24 audience as well but, Dr. Noguchi.

25 DR. NOGUCHI: Just a technical thing on the  
26 VSV-G detection. I am not aware of any system that can  
27 detect down to the absolute molecule. I think that the

1 issue being brought forward is that in any production  
2 system there is an inherent limit of sensitivity to  
3 whatever you are trying to assay for and the  
4 possibility, maybe not the probability but the  
5 possibility exists that VSV-G gets through the whole  
6 process.

7           And I think to say that it is not in the final  
8 product is not quite answering the question about the  
9 potential for recombination where VSV-G could then  
10 pseudotype an endogenous -- not an endogenous but a  
11 naturally existing HIV in an HIV infected person.    So  
12 I do not think it is easily addressed because it is a  
13 hypothetical question but I do not think that saying  
14 that it is not there is the right answer.

15           DR. DROPULIC: Yes, I suppose it is not  
16 detectable by the techniques that we are using,  
17 correct.

18           DR. MICKELSON: Are there any comments?

19           Dr. Zaia?

20           DR. ZAIA: What would you do then if a patient  
21 did have VSV-G virus isolated and if there were damages  
22 to that patient or to his environment would you be  
23 prepared to pay for those damages because they are  
24 unknown. I mean, if South Philadelphia has an outbreak  
25 of VSV HIV, VIRxSYS is not going to be able to solve  
26 that problem.

27           DR. DROPULIC: Well, obviously, right.

1 DR. ZAIA: It is kind of a rhetorical  
2 question.

3 DR. MICKELSON: Are there other questions or  
4 comments from the audience?

5 Yes, you have to come to a microphone and  
6 introduce yourself, pleas.

7 DR. HUTCHINS: Hi. I am Beth Hutchins and I  
8 am -- although I am associated with a gene therapy  
9 company, CAN-G, we do not work on retroviral vectors  
10 adenovirus. My question relates to just as a member of  
11 the public.

12 Ms. King raised a question about the  
13 description of the clinical product that you are  
14 testing in your informed consent and you revised that  
15 slightly but although you say it is a gene transfer  
16 product, you do not say it is a viral vector. You do  
17 not say it is a new vector class. And the risks that  
18 you list do not address any of the issues relating to  
19 those factors.

20 And there may be politic reasons why you want  
21 to take that approach but I would think that you risk a  
22 really horrible PR problem once the patient or the  
23 public find out what it is that you are testing and  
24 that you would be better dealing with that up front in  
25 the informed consent and not just the process orally  
26 but the written material, and I would just like to know  
27 your rationale and what you plan to do about that.

1 DR. DROPULIC: No, I think that is a fair  
2 comment. We can put into the consent form that it is a  
3 new class of vector and, if you want HIV vector, we  
4 could do that as well. I would rather have it in -- I  
5 will lean on the side that you are saying. I would be  
6 happy to do that.

7 DR. HUTCHINS: I think you would be heading  
8 off more problems than trying to appear as though you  
9 are hiding something.

10 DR. DROPULIC: Okay. We were not really  
11 trying to hide anything.

12 DR. MICKELSON: Ms. King?

13 MS. KING: I just wanted to say I really thank  
14 you for making that comment and for following up on  
15 that point because I think it is an important one.

16 DR. MICKELSON: I think certainly that members  
17 of the RAC are very willing to help and work with you  
18 on the informed consent document. There is a great  
19 deal of experience here on this committee that at least  
20 those kinds of issues would be something that we would  
21 be anxious to -- we have a lot of people who are not  
22 scientists who can read for clarity kind of thing to  
23 figure out, gee, I did not understand that one at all.  
24 So you might as well take advantage of it.

25 DR. DROPULIC: We would work on that.

26 DR. MICKELSON: I think in the interest of  
27 time I might just go through, and to be quite clear, I

1 am only going to be able to, I think, hit some of the  
2 highlights and these points and recommendations that I  
3 will bring up will -- I might miss a few and sort of  
4 when we come to looking at all of these and coming  
5 forward for recommendations, it is with the  
6 understanding that if other members of the committee  
7 remember things that I have left out that we can notify  
8 Dr. Patterson because the letter that will go to the  
9 investigators will have all of these things and you  
10 will all have seen it so we are sure we have gotten the  
11 intent correctly because I might have gotten -- it is  
12 quite probably I missed a fair amount of this.

13 I think some of the initial points -- they  
14 certainly always focused on the production of the  
15 vector, safety issues as well as then some of the  
16 monitoring that would go on for the particular patient  
17 population, as well as some of the inclusion criteria  
18 and certainly the committee would recommend that it be  
19 a little clearer, at least, that for patients that had  
20 -- it was not just patients that had failed HAART but  
21 that were failing HAART. But that also it was quite  
22 clear that alternative -- that there could be enrolled  
23 -- or that, if possible, there were other alternative  
24 drug regimens available that this be another person or  
25 another party involved in making -- possibly making  
26 that decision or referring people to enrollment in this  
27 trial if they were failing one particular drug regimen

1 to be sure that there was a clear description of  
2 alternatives.

3 I think that the investigators laid out a good  
4 outline of certain clinical adverse events that they  
5 would be monitoring for. Dr. Aguilar outlined a number  
6 of issues with the vector production and that certainly  
7 raised the point that what is novel is certainly almost  
8 an individualistic thing here per patient and that it  
9 is very difficult -- you cannot say that there are no  
10 new sequences present but that there are many HIV  
11 variants and tropisms that could be novel.

12 And that even though -- and I think the  
13 investigators recognize this to a certain extent and  
14 are recommending or saying that they might restrict the  
15 patient population to those that demonstrate a narrow  
16 band variant so that it would be less likely to --  
17 somewhat less likely to develop these -- I guess  
18 genetic drift within the population there.

19 I think there also was a recommendation that  
20 there be development of -- that the investigator  
21 include methods and assays to detect survival of the  
22 transduced cells, that it not just be the -- by unique  
23 -- the unique base sequence in the 186 sequence but  
24 that also you look to develop an assay that also --  
25 within the vector stock and then also possibly within  
26 the patients for the degenerate gag-pol sequences that  
27 you were talking about as well. The investigators

1 agreed to that.

2 I think -- I am not sure that we came to an  
3 agreement on whether the use of higher number of  
4 plasmid packaging systems increased safety or not. I  
5 think the investigators feel that they have  
6 incorporated a number of mutations within their two  
7 plasmid system that might offer some level of safety.  
8 However, there is at least in some of the basic  
9 research laboratories three and four plasmid systems  
10 certainly get much significantly lower levels of  
11 generation of replication competent viral vectors but I  
12 think we should put this in the letter as something  
13 that we discussed.

14 I think you have to understand that each time  
15 -- for each recommendation we make you can certainly --  
16 when you respond to the committee can say that -- no  
17 for whatever reason and certainly lay it out if you  
18 disagree with this point or this recommendation and  
19 that certainly that is your position that you can take  
20 on that but I would certainly think that that would  
21 deserve a response of some sort.

22 I think, also, Dr. Markert raised a number of  
23 very good points on inclusion of the focal lesions that  
24 might be incidental findings but that the fact that  
25 these were found should be put into the informed  
26 consent document.

27 DR. MARKERT: Actually I did not -- well, what

1 I was looking for was just the descriptions so that the  
2 investigators would know and not to put something into  
3 the informed consent because now we would not know what  
4 to put in. It is probably -- I mean, probably not --

5 DR. MICKELSON: Because you do not know the  
6 significance of them.

7 DR. MARKERT: Yes, but it is just to know what  
8 is in these lesions and actually going back to just  
9 your last comment, it seemed to me, although I am not  
10 the virologist here at the table, that maybe there was  
11 not good data out there saying that the two versus the  
12 four plasmids. It was -- I mean, which was the way to  
13 go. It did not seem to me that there was hard and fast  
14 data. Therefore, I would not have that be something in  
15 the letter because all it will do is generate a lot of  
16 confusion at the receiving end about what needs to be  
17 done but it --

18 DR. MICKELSON: Did you want to --

19 DR. YEE: This is so theoretical and there is  
20 no real data suggesting the four plasmid is safer than  
21 two plasmid.

22 DR. MICKELSON: Okay.

23 DR. YEE: Again, I would argue that it  
24 depends on your assay system. I mean, people use the  
25 same method to -- with different assay they can detect  
26 recombination and we use p24 assay and PCR assays the  
27 same. We do not get RCR detection. But again I would

1 argue strongly that other assays need to be established  
2 to detect the recombination event, which those events  
3 can lead to -- eventually to pseudotype or wild type  
4 HIV.

5 DR. DROPULIC: So what you are saying is if we  
6 have an assay to look for those events that would --

7 DR. YEE: That would be great.

8 DR. DROPULIC: All right.

9 DR. YEE: You can set up a similar assay so  
10 you --

11 DR. DROPULIC: We will do that.

12 DR. YEE: -- and you show an active result,  
13 that will be great. I will be satisfied.

14 DR. MICKELSON: Thank you.

15 Dr. Markert also suggested that prior to and  
16 after enrollment of patients that they be tested for  
17 their T cell receptor diversity and that -- and then  
18 that -- whether there was a limited diversity or not, I  
19 think, was -- and whether that should be an  
20 inclusion/exclusion criteria was not something that we  
21 wanted to make a recommendation on but that would be  
22 just good to know, immunological diversity --  
23 immunological competence of these patients.

24 She also recommended looking for a tetanus  
25 response before and after.

26 DR. \_\_\_\_\_: (Not at microphone.)

27 DR. MICKELSON: Oh, they did that. Oh, I

1 missed that one.

2 Also, she recommended just asking the DSMB  
3 look -- review results if a patient shows a drop in CD4  
4 counts or dramatic increase in plasma viral load just  
5 to be sure that that was clear that that would be  
6 looked at.

7 And archiving some samples from the patients  
8 just in case you may wish to look later on for  
9 different indicators of some adverse response.

10 DR. MARKERT: They did that.

11 DR. MICKELSON: Oh, did you? I am sorry. You  
12 had a question about lot being released.

13 DR. PATTERSON: Lot release criteria, whether  
14 LAL assays would be done prior to patient  
15 administration.

16 DR. MARKERT: That was a question.

17 DR. PATTERSON: Okay. Did you want to --

18 DR. MARKERT: (Not at microphone.)

19 DR. PATTERSON: Okay.

20 DR. JUNE: I can address that. It is fairly  
21 standard that the antitoxin LAL will be done. It is  
22 done at the time of cryopreservation. In our current  
23 negotiations with FDA they prefer that just as we  
24 cryopreserve it to take a sample and it is not -- the  
25 lot would then not be released unless it met the  
26 correct negative result below the specified limits for  
27 LAL. The same thing -- mycoplasma is done at the same

1 time and then, you know, the other lot release  
2 criteria for this would be looking for magnetic beads,  
3 residual in the final product that were used to grow  
4 the cells and to look at p24 levels as well as standard  
5 culture and sensitivity.

6 DR. MICKELSON: Thank you.

7 Ms. King recommended some possible changes to  
8 the informed consent document. Certainly some of the  
9 paragraphs that were initially in the introduction  
10 were, in fact, benefits and might -- the informed  
11 consent document would benefit from shifting those.

12 I think some of the -- Dr. Yee's comments on  
13 the vector production system, plasmids, multiple  
14 plasmids dealt with those, I think. Is that correct --  
15 certainly -- I think the still remaining question that  
16 was brought up and I am not sure what people would like  
17 to say. There certainly was a lot of discussion of  
18 generation of novel recombinants and generation of  
19 potentially pseudotyped virus but that might be  
20 something that if I would -- my guess would be that  
21 given the lot restrictions that you have that that  
22 would be quite unlikely. However, you would probably  
23 be looking -- you will be analyzing viral loads in  
24 patients. Would there be --

25 DR. DROPULIC: Absolutely. We also have an  
26 assay for VSV RNA so we will see if there is anything  
27 there.

1 DR. MICKELSON: Would there be any use for  
2 looking for some of these other unusual recombinants  
3 for possible --

4 DR. AGUILAR-CORDOVA: I would just think that  
5 your sensitivity of the assays need to be -- in  
6 whichever method that you choose need to be better  
7 specified even though you can detect 50 copies per  
8 microgram, for example, in some of those assays.  
9 That is of the human DNA and that is not necessarily  
10 the same as your VSV and you would have to have in that  
11 particular copy 50 RCRs in order to be able to detect  
12 them because it is not going to replicate probably.

13 I am just doing this off the top of my head.  
14 I think the assay development as described was not  
15 really detecting the sensitivity levels that you might  
16 at first glance assume because they were replicating in  
17 the mouse.

18 The other thing that we mentioned, Claudia,  
19 was the issue of going forward to characterize the  
20 mobilized genomes to see what it is that you are  
21 mobilizing.

22 DR. DROPULIC: Yes.

23 DR. MICKELSON: Right.

24 DR. DROPULIC: Yes, we can do that.

25 DR. MICKELSON: Right.

26 DR. DROPULIC: Actually just as a side point  
27 which I forgot to mention, we have actually taken off

1 not the mobilized but the produced vector, transduced  
2 cells, and then did PCR on the DNA in the transduced  
3 cells and shown that it was completely the identical  
4 sequence what was put in so now we will do that for the  
5 mobilizable genomes as you suggest.

6 DR. MICKELSON: Dr. Zaia brought up some very  
7 interesting questions about the design of the trial  
8 which I think certainly we discussed. I am not sure  
9 what we would like to do. Mentioning that the dose  
10 escalation which is standard for drugs may not be the  
11 most appropriate for this initial study. I think that  
12 also the selection of just the dose escalation after 28  
13 days -- he suggested that one might consider using a  
14 single dose and then waiting for a longer period of  
15 observation at least at this initial time.

16 I do not know whether -- how -- Dr. Gordon?

17 DR. GORDON: I think the way that discussion  
18 went I feel most comfortable with the letter mentioning  
19 that these issues were discussed with some uncertainty  
20 about what the best approach might be.

21 DR. MICKELSON: I agree.

22 DR. GORDON: I do not think we decided that  
23 the approach in the protocol is the worse and I do not  
24 think we had a specific suggestion for improving it but  
25 nonetheless it may not be the best so I think we should  
26 just say it was discussed in that context and leave it  
27 at that.

1 DR. MICKELSON: I think the investigators made  
2 it clear that if you chose the 28 day observation  
3 period based on the --

4 DR. DROPULIC: The active --

5 DR. MICKELSON: Yes, the reactivated  
6 mobilizations or the breakthrough seen after 14 --

7 DR. DROPULIC: Will be activated -- that cells  
8 -- activated T cells survive generally for about 14  
9 days and so we said 28 days.

10 DR. MICKELSON: Right.

11 DR. DROPULIC: Because those cells would in  
12 the majority of cases die and, you know, you have the  
13 greatest chance for an adverse event early rather than  
14 late.

15 DR. MICKELSON: Absolutely.

16 DR. DROPULIC: Yes.

17 DR. MICKELSON: Dr. Coffin?

18 DR. COFFIN: No matter the escalation study.  
19 I and maybe a number of others here might think that  
20 the overall six month follow-up time might be kind of  
21 short. If at all possible I would suggest that at some  
22 -- there being some ongoing follow-up beyond that time  
23 frame.

24 DR. DROPULIC: In the new protocol it is  
25 yearly for life.

26 DR. MICKELSON: Yearly. Okay. I think that  
27 Dr. Coffin brought up several interesting points, which

1 may or may not go into the letter at all. Certainly  
2 made it clear that not a -- there are multiple  
3 mechanisms by which antisense vector might inhibit  
4 replication but also recommended that in some instances  
5 where there was breakthrough mutations that these be  
6 looked at and characterized to see what the genome  
7 structure sequence was for some of these breakthrough  
8 genomes?

9 DR. DROPULIC: Yes, we will be happy to do  
10 that.

11 DR. COFFIN: This goes towards eventual  
12 efficacy of the vector. It does not directly address  
13 the safety issues. It would seem also in the patient  
14 samples that you get out to test the virus that is in  
15 those patients for perhaps having acquired some  
16 resistance.

17 DR. DROPULIC: Right. We have done some small  
18 meta-studies.

19 DR. COFFIN: It would be fairly easy to do I  
20 am sure.

21 DR. DROPULIC: We have done some of those  
22 studies but we will have a complete study.

23 DR. MICKELSON: As part of this you would  
24 probably be looking at the genotype of the virus for  
25 variance in the patient before. You might or not?  
26 Would that be helpful to know? Just to know the  
27 sequence of the variants in the patients before they

1 put into the trial.

2 DR. DROPULIC: Yes.

3 DR. MICKELSON: It might help with what you  
4 were talking about, right? No?

5 DR. GORDON: Also what comes out.

6 DR. MICKELSON: Yes. And then what comes out.

7 DR. COFFIN: Also it is present at the end.

8 DR. MICKELSON: Yes.

9 DR. COFFIN: I think looking for some change,  
10 genetic change in the virus.

11 DR. MICKELSON: Right.

12 DR. COFFIN: Would not be --

13 DR. MICKELSON: Right, so you would do a  
14 baseline and then -- right. Okay. I think, also -- I  
15 am sorry. Dr. Noguchi?

16 DR. NOGUCHI: Also Dr. Coffin also brought up  
17 the very interesting observation that the -- one of the  
18 old mechanisms of defective interfering particles may  
19 be a part of what is going on.

20 DR. MICKELSON: Right, exactly.

21 DR. NOGUCHI: And I think that is certainly  
22 worthy of more future discussion. I am not -- it is  
23 not clear to me which way actually would work in this  
24 case but it could be in an adverse way.

25 DR. MICKELSON: Right. But that is certainly  
26 something that would be worthwhile. I think certainly  
27 Dr. Coffin supported the detection or baseline and post

1 assay analysis for the TCR repertoire and possibly  
2 another way to look at that was after the transduction  
3 to look for clonal integration sites if there was a  
4 limited number of T cells and T cell clones that were  
5 grown up and reinfused into the patient.

6 And it was quite clear that the investigators  
7 would not include nonprogressors or patients with very  
8 low viral loads so I think that goes without saying  
9 because we are looking at patients that have failed  
10 HAART.

11 DR. COFFIN: There is one more point related  
12 to that that I forgot to mention that really did not  
13 come up and that is one of your sort of failure  
14 criteria is a half log increase in virus load but you  
15 are not, as far as the protocol goes, collecting any  
16 baseline information on the stability of the virus load  
17 in each individual patient.

18 DR. DROPULIC: We do screen and obtain a  
19 baseline count but not --

20 DR. COFFIN: You have just one.

21 DR. DROPULIC: Yes.

22 DR. COFFIN: And that does not then address  
23 the stability of that. If you have a patient whose  
24 virus load is increasing for other reasons you could  
25 have --

26 DR. MICKELSON: John, we cannot hear you.

27 DR. COFFIN: I am sorry. If you happen to be

1 a patient -- I am trying to --

2 DR. MICKELSON: I know. It is impolite to  
3 have to turn your back.

4 DR. COFFIN: The issue is the protocol just  
5 shows a single virus load time point.

6 DR. MICKELSON: Right.

7 DR. COFFIN: But in some patients the virus  
8 load may, in fact, be on an increase or in some way  
9 unstable, inherently unstable and may report an adverse  
10 event of an increase in virus load when there really is  
11 not one in this because the patient -- this is an  
12 unstable patient and it would seem to their interest to  
13 collect some more to be sure that the virus load in any  
14 individual patient is put on protocol is reasonably  
15 stable at the time that that happens.

16 DR. MacGREGOR: Sure, that sounds very  
17 reasonable. The patients that we are going to be  
18 recruiting are going to be well studied patients  
19 because they will have had to have a history to show  
20 the failure on treatment so what we could do would be  
21 to include a trend over a six month period or even a  
22 year period as far as that goes.

23 And as they enter the study there will be two  
24 so we will have the screening viral load and we will  
25 also have the initiation of viral load so we will have  
26 a mean there, too. I think that is a good idea.

27 DR. COFFIN: Also in patients with very low

1 viral -- your entry criteria goes down to 500 copies,  
2 which is very low, and a half log increase in that is  
3 only up to 1,500 and I do not think many of us would  
4 think that that was a significant difference. I would  
5 worry about entering patients with that low a load.

6 DR. MacGREGOR: Right. I think that is true,  
7 too, and I think most physicians and patients who were  
8 stable above 200 with a viral load of 500 would not  
9 want to be in such a study anyway, nor would I  
10 recommend it to any of my patients but we ought to put  
11 that in more explicitly. Sure.

12 DR. MICKELSON: So now given -- yes, Dr. Zaia?

13 DR. ZAIA: I would also encourage the letter  
14 to at least ask the sponsor and investigator to address  
15 how the -- what measures will be taken to protect the  
16 public health from this experiment in the worse case  
17 scenario because I think that still is going to be the  
18 most limiting question in terms of implementing the  
19 study. I mean, you may, for example, have to isolate a  
20 patient for the first 28 days in order to be sure that  
21 in the case of a VSV recombination it is not going to  
22 get spread before you know about it.

23 I think that is going to be an important issue  
24 to address in the further review of this.

25 DR. MICKELSON: I certainly think that is  
26 something that we should address and comment on public  
27 health issues, whether you agree with what scenarios

1 may or may not be feasible or viable and I think that  
2 certainly this is something that we would not  
3 ordinarily be asking but as the first use of a  
4 lentiviral vector. I think one of the things is  
5 certainly what you come back with as your response is  
6 something that you would have to use your best judgment  
7 on but the committee would look at that.

8 Ms. King?

9 MS. KING: Just one other thing to add to the  
10 list of things that goes in the letter, which is the  
11 expanded discussion description of the vector in the  
12 consent form.

13 DR. MICKELSON: Oh, yes, the expanded  
14 discussion description of vector. Certainly. But  
15 again we would be willing to look at a new informed  
16 consent document if you would like -- if you think that  
17 would be helpful.

18 DR. DROPULIC: Yes.

19 DR. MICKELSON: We would be very happy to try  
20 to do that.

21 DR. DROPULIC: Yes, thank you.

22 DR. MICKELSON: Given all of those waffling  
23 and comments that I think we have probably come to in  
24 recommendations, may I have a motion that we  
25 incorporate what we have just discussed into both the  
26 indications of comments and discussions that occurred  
27 as well as recommendations, as well as the

1 recommendations that the investigators had agreed to  
2 already in the form of a letter that will go to the  
3 investigators?

4 DR. GORDON: I just want to confirm before  
5 moving --

6 DR. MICKELSON: Yes.

7 DR. GORDON: -- that we will be able to see  
8 this draft before it goes out.

9 DR. MICKELSON: Oh, absolutely. Absolutely.  
10 That is always done.

11 DR. GORDON: In that case I so move because we  
12 touched on some pretty sensitive issues here and I want  
13 to make sure that the draft is at least in my --

14 DR. MICKELSON: That is the standard process.

15 DR. GORDON: Makes me comfortable, yes. I  
16 just want to --

17 DR. MICKELSON: It is part of the standard.  
18 Yes. No, it is good for everybody to know that we do  
19 not just get one version of what is going on. All  
20 right.

21 So can I have a motion? Dr. Aguilar. May I  
22 have a second for that?

23 DR. AGUILAR-CORDOVA: I second.

24 DR. MICKELSON: Excuse me. So it is the other  
25 way around.

26 All those in favor, please, raise your hand?

27 (A show of hands was seen.)

1 DR. MICKELSON: All in favor, nine. And there  
2 are no abstentions, no votes. So it is nine in favor.

3 So let's have a ten minute break and then we  
4 will come back for the data management report. Thank  
5 you all very much. It was very, very interesting.

6 (Whereupon, at break was taken.)