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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

BIOLOGICAL RESPONSE MODIFIERS
ADVISORY COMMITTEE

MEETING #31 - VOLUME III

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C O N T E N T S

Opening Remarks: Dr. Daniel Salomon

Topic 2: Lentivirus Vector Gene Transfer Product for Treatment of People with HIV

Autologous E-Cells Transduced with VRX496: HIV-1-Based

Lentiviral Vector Treatment for Patient-Subjects Infected with HIV-1:
Boro Dropulic, Ph.D.
VIRxSYS

Open Public Hearing
Susan Kingsman, Ph.D.

Committee Discussion of Questions

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1 P R O C E E D I N G S

2 **Opening Remarks** ,

3 DR. SALOMON: I apologize. I did say we
4 would start at 8:00. Today is the second day of
5 deliberations on the lentiviral class of vectors.
6 I think yesterday set the ground rules for sort of,
7 in general, things about assays, about safety,
8 about mobilization, about transient versus stable
9 production.

10 I think it is a wonderful opportunity as a
11 follow up this morning to begin with Dr. Boro
12 Dropulic from VIRxSYS presenting now what has been
13 presented to the RAC and has been presented to the
14 FDA as what could be the first lentiviral gene-
15 delivery vector trial in patients with HIV entitled
16 Autologous T-cells Transduced with VRX496, HIV-1-
17 Based Lentiviral Vector Treatment of Patient-
18 Subjects Infected with HIV-1.

19 **TOPIC 2: LENTIVIRAL VIRUS GENE TRANSFER PRODUCT FOR**
20 **TREATMENT OF PEOPLE WITH HIV**
21 **Autologous T-Cells Transduced with VRX496**
22 **HIV-1-Based Lentiviral Vector Treatment of**
23 **Patient-Subjects with HIV-1**

24 DR. DROPULIC: First of all, I would like
25 to thank you, the committee members, for the

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1 pportunity to present our work.
2 [Slide. 1
3 First of all, I would like to say that I
4 eally appreciated the discussion that went on
5 esterday regarding HIV vectors, regarding issues
6 f using HIV vectors in the clinic. I wanted to
7 ay that the issues that were discussed yesterday
8 ere really the same issues that we have been
9 rappling with for the last several years, both
10 hen I was at Hopkins and now with my team VIRxSYS.
11 The point that I want to make as a
12 ackdrop for my presentation is that we believe
13 hat this vector system that we are proposing is
14 he safest vector to use in the first instance of
15 linical trial in humans using an HIV vector. That
16 s with two important considerations in mind; one,
17 hat the vector works, that it can actually inhibit
18 IIV in our case and, second of all, that the
19 payload gene is regulatable. And so, in our case,
20 it is tat- and rev-dependent regulation.
21 With that, I will start with the first
22 slide.
23 [Slide.]
24 Just one slide on the company, really,
25 just to tell you who we are. We are established

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1 for three years. We have 40 employees. We are
2 located about three blocks from here. Our mission
3 is to develop gene therapies for serious disease
4 states and our present entire focus is the
5 development of a gene therapy for the treatment of
6 individuals with AIDS. That is all the company is
7 doing at the moment.
8 [Slide.]
9 So target disease AIDS. This is my slide
10 where, every morning, the reason I come to do what
11 we are doing. There are 40 million people infected
12 with the virus worldwide, 1 million in the United
13 States.
14 The drug therapy, HAART therapy, can
15 suppress HIV infection but is not a cure. HAART
16 therapy is toxic. There is a cumulative failure to
17 therapy and, also, resistance to HAART is on the
18 increase. So there is a definite need for new
19 approaches for the treatment of HIV infection.
20 Our approach is to turn the virus against
21 itself, develop and use an HIV vector with anti-HIV
22 payloads to interfere with wild-type HIV
23 replication. We are not saying that this is a
24 cure. Our goal here is not to remove the virus
25 from the patient but basically to interfere with

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1 HIV replication in order to decrease viral loads
2 and postpone the development of AIDS. That is our
3 goal.
4 [Slide.]
5 We believe that HIV infection is the
6 appropriate disease target to first test HIV-based
7 vectors. The reasons for that are as follows:
8 testing of HIV vectors in non-HIV-infected
9 individuals could result in their seroconversion.
10 There is a risk that you could give that patient
11 AIDS. There is that risk.
12 If non-HIV-infected individuals became
13 infected with HIV, there will be ambiguity as to
14 the source of infection. Our target patient
15 population is already laden with wild-type HIV so
16 that is why we really believe that this is the
17 right candidate patient-subject population to
18 deliver an HIV vector and also we are selecting a
19 patient population that has no good treatment
20 options left. They are failing HAART therapy; they
21 have a viral load of greater than 5,000; and they
22 show the X4 strain of HIV.
23 The presence of X4 strain, the T-cell-
24 tropic virus means that the patient is in more
25 advanced disease, they are in the later stages of

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1 disease, rather than treating somebody that is
2 early.
3 [Slide.]
4 We believe that HIV vectors are the
5 appropriate lentiviral vector for clinical testing
6 as the first vector. The biology and pathogenesis
7 of HIV in humans is well understood in contrast to
8 other lentiviruses. We are selecting an HIV
9 population with no good treatment options and this
10 population can be identified. They exist.
11 The introduction of non-HIV vectors into
12 humans, particularly those infected or at risk of
13 being infected with HIV, could result in
14 unpredictable consequences. So that is why we
15 think HIV vectors are appropriate.
16 [Slide.]
17 So this is our proposed clinical protocol.
18 It is an ex vivo. We are not directly injecting
19 the vector so, basically, it is a process where we
20 are taking out cells from the patient, transducing
21 the cells with vector and then expanding the cells
22 and then reintroducing them back into the patient.
23 VRX, the acronym that we are using for our
24 vector is an HIV vector that contains an anti-HIV
25 antisense sequence. The patient populations I have

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1 already described, failing HAART, viral load of
2 greater than 5,000, CD4 counts of 200 to 600
3 because you need at least a certain number of CD4
4 T-cells to be present in order to be able to
5 isolate and amplify them so that you can
6 reintroduce them back into the patient, and the X4
7 strain of HIV.
8 T-cells are isolated, hit with the vector,
9 expanded and then reintroduced back into the
10 patient. That is our proposed protocol.
11 [Slide.]
12 So, a schematic representation of our
13 vector is as follows. Basically, it is derived
14 from pNL4-3, one of the best-studied molecular
15 clones of HIV. These are the fragments in which we
16 have derived our vector. It contains a region from
17 the 5' that contains the packaging sequence. It
18 also contains a region from pol that incorporates a
19 central polypurine track region.
20 The antisense payload is also derived from
21 wild-type HIV. We are not placing in a protein or
22 something that could be potentially antigenic. It
23 is an antisense RNA and it is derived from the
24 envelope region of the wild-type HIV. You can see,
25 it is just flipped and inserted in here.

1 Then what we have is a fragment from the
2 RRE which regulates the messenger RNA expression
3 from the vector. The only heterologous sequence
4 that we have here is a small, non-coding disposable
5 marker sequence for GFP. We have inserted this in
6 there so we can basically track the vector
7 uniquely.
8 What is important is that the antisense
9 payload is tat and rev regulatable. There is a
10 splice, a sector site located just downstream of
11 the RRE region, that basically makes this antisense
12 payload both tat and rev dependent. Tat and rev
13 are needed for genomic messenger-RNA expression
14 from the vector. So it is a highly regulatable
15 system for the expression of the payload sequence.
16 The reason why I have outlined here VRX496
17 and 494 is because, for our laboratory studies, we
18 actually have a vector that expresses GFP. So we
19 can look at marking. We can look at transduction.
20 For the clinical-grade vector, there are no coding
21 regions, no protein-coding regions in this vector.
22 It is a completely gutted vector.
23 [Slide.]
24 So some of the safety features of our
25 vector for our proposed gene transfer in HIV-

1 infected patients; we believe it is the safest
2 approach for gene transfer in HIV-infected
3 individuals because the vector is entirely derived
4 from wild-type HIV and a well-studied wild-type
5 HIV.

6 No sequences other than HIV are introduced
7 into the patient. The patient subjects are laden
8 with the virus and the vector backbone is actually
9 constructed from highly conserved regions of the
10 virus. And NL4-3, the backbone, is derived from
11 strains common to North America.

12 As evidence for safety from nonhuman
13 primates, that also may add weight to the safety of
14 this vector system. Attenuated SIVs that are
15 deleted in accessory genes do not cause disease in
16 nonhuman primates. There are no accessory genes in
17 either our vector or VIRPAC.

18 Now, although attenuated SIVs containing
19 genes such as IL2 can exacerbate the disease, no
20 such reports for attenuated SIVs without genes have
21 been reported and, importantly, our vector does not
22 encode for a gene, any gene, like IL2, for
23 instance.

24 [Slide.]

25 Additional safety features for our vector

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1 are that we have targeted expression of the anti-
2 HIV antisense payload. The antisense payload would
3 only become expressed in cells transduced with the
4 vector that then become infected with wild-type
5 HIV. So it is a highly regulatable way of
6 expression your anti-HIV gene.
7 Another safety is that the antisense
8 payload appears to decrease mobilization of the
9 vector to cells. I am going to show you data for
10 that in a few slides. Expression of the
11 antienvelope antisense results in decreased
12 mobilization of packaged VRX496 genomes, so
13 mobilization is the term where the vector comes out
14 and then goes into another cell.
15 Also, our vector contains a stop codon in
16 gag so that recombination with the helper of the
17 wild type would result in a nonfunctional gag/pol
18 open reading frame if that event should occur.
19 [Slide.]
20 Let me tell you a little bit about this
21 stop codon. The stop codon is located just
22 downstream of the packaging signal. In the helper,
23 what we have engineered is we have basically gotten
24 rid of the packaging signal and degenerated this
25 first region of gag.

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1 So if a recombination event would occur
2 where, for example, the reverse transcriptase comes
3 and jumps over in this region that is homologous
4 between the vector and the helper, it would
5 incorporate this gag/stop signal so the resulting
6 recombinant would not be able to produce gag/pol.
7 [Slide. 1
8 So there was a little bit of a confusion
9 yesterday as a result of the comments that I have
10 made in the document regarding that the result of
11 recombination between the vector and the wild-type
12 HIV would result in either a noninfectious
13 recombinant or wild-type HIV.
14 I wasn't referring to sequence-specific
15 effects. What I was referring to is that if the
16 vector does recombine with wild-type HIV, you can
17 only get either a noninfectious event, a
18 noninfectious recombinant or a wild-type HIV type
19 of virus. It is not meant to be sequence specific,
20 per se.
21 So let me go through some of these
22 possible recombination events that we have modeled
23 here. I have already mentioned the gag stop for
24 the helper. A similar thing would happen if wild-
25 type HIV would be recombining. Let's just look at

1 this event. If the reverse transcriptase comes
2 along here and crosses over in the common region of
3 the cPPT, between the vector and the wild type, you
4 would get the crossover event but the result would
5 be is that you would get a truncated gag/pol.
6 Another event is that if the reverse
7 transcriptase here would cross over in the RRE
8 region, you would have a truncated envelope. This
9 event would probably take two events to occur but
10 you could imagine that if, basically, the reverse
11 transcriptase picks up this antisense payload and
12 then puts it back into the virus, you would still
13 get a wild type. Yes; its phenotype would be
14 changed because now it would contain envelope
15 sequences that could possibly confer an X4
16 phenotype strain to this virus but, nevertheless,
17 it would be a wild-type HIV.
18 [Slide.]
19 But, in order to address the sequence
20 issue of increasing the pathogenicity of the virus
21 through recombination between the vector and the
22 wild type, I just want to make one point--a few
23 points, but one point here. The backbone of the
24 vector contains regions of HIV that are highly
25 conserved; the LTR, this packaging gag, cPPT and

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1 RRE, are all highly conserved regions. I would
2 imagine that a patient infected with HIV at the
3 late stages of disease would contain these
4 sequences.
5 The only region that is actually not
6 highly conserved is a region in the payload
7 sequence, the antisense payload sequence. That
8 confers an X4 tropism. It is in the V3 loop that
9 gives rise to X4 tropism of the GP120 sequences.
10 The way that we address that issue is that we are
11 going to restrict our patient-subject population
12 only to those that demonstrate the X4 strain of
13 HIV. So, if recombination should occur between the
14 vector and the wild type, that patient already has
15 X4.
16 One point that is very important is that
17 we are not claiming that mobilization of our vector
18 is required for anti-HIV efficacy. We have vectors
19 that mobilize well. We have vectors that don't
20 mobilize virtually at all, which is VRX496. We
21 have specifically chosen VRX496 because of its
22 extremely poor mobilization characteristics because
23 of the issue of mobilization for the first
24 lentiviral-vector clinical trial.
25 In the future, mobilization may provide

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1 some additive effect but I doubt very much that
2 VRX, its anti-HIV efficacy is at all contributable
3 to any mobilization effect because we don't really
4 see any significant mobilization. I will show you
5 the data.

6 VRX496 is a maximally gutting vector with
7 poor mobilization characteristics but yet it
8 maintains its high transduction and anti-HIV
9 efficiencies and it can still regulate its payload
10 expression through tat and rev dependencies making
11 expression of the payload only occurring in cells
12 containing vector and wild-type HIV.

13 [Slide.]

14 There was also discussion about using
15 potential SIN vectors. But we believe that these
16 are not optimal for AIDS gene therapy. First of
17 all, you would lose the target of expression of tat
18 and rev because, by activating the 3' LTR, all the
19 LTRs, you would have to substitute some other
20 promoter.

21 That other promoter cannot be HIV-LTR
22 because you would create direct repeat sequences
23 making the vector unstable. You would have to use
24 some sort of other promoter. So, likely, you would
25 use a constitutive expressing promoter and, in that

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1 case, you would have constitutive expression of the
2 antisense payload and that may be detrimental to
3 the host cell.
4 We have avoided constitutive promoter
5 elements in our vectors because we wanted to make
6 it highly regulatable. Also, by replacing or
7 modifying the HIV sequences 5′ and 3′ to the
8 antisense, we would have a detrimental effect on
9 HIV efficacy because we believe that these 5′ and
10 3′ sequences allow trafficking of the vector RNA to
11 its target wild-type HIV-RNA. So disrupting this
12 may affect trafficking and the ability of the
13 antisense to accumulate at the epicyte where wild-
14 type HIV is accumulating.
15 Also, we don't believe that the antisense
16 is the only thing having its effect. We believe
17 that, through competition for packaging, and that
18 can be at the level of all these elements that are
19 in the vector, that the anti-HIV effect is
20 cumulative, is due to the vector and the antisense
21 payload because we can see, and others have
22 reported, that just the basic vector alone can have
23 some antisense anti-HIV activity.
24 A SIN vector, by definition, would not be
25 able to compete with wild-type HIV for packaging.

1 Still, you cannot guarantee that a SIN vector will
2 not be able to mobilize.
3 [Slide.]
4 So let me tell you a little bit about the
5 features of our VIRPAC helper construct. It is a
6 two-plasmid system. One of the reasons that we are
7 using a two-plasmid system is because we do get
8 higher levels of production. That may not be
9 important for academic-scale production of the
10 vector but it is very important for scale-up
11 manufacturing of the vector.
12 Every complication, every additional thing
13 that you add to the manufacturing process, results
14 in a decreasing yield. So that is one of the
15 reasons. But what we have done is we have some
16 features in our VIRPAC construct that we believe
17 make it safe.
18 There are no accessory genes in VIRPAC.
19 We have done degeneration of several regions of the
20 helper in order to decrease the likelihood of
21 recombination with the vector, as I have already
22 mentioned to you, the gag region. Also, we have
23 four strong poly-As and two transcriptional pause
24 sites to partition the structural envelope genes.
25 So, let me tell you, we have here a CMV

1 promoter that drives gag/pol. And we have an RRE
2 element that is degenerated that basically then
3 allows tat and rev here to be--tat and rev is first
4 expressed. When there is an accumulation of tat
5 and rev, then that allows the gag/pol to be
6 expressed.
7 Then what we have between this open
8 reading frame and the VSV-G here are two poly-A
9 sites, the bovine growth-hormone poly-A site and
10 then, tandem to it, is the alphaglobin poly-A site.
11 Then, in addition to that, there is a pause site to
12 decrease the chance of transcriptional read through
13 between the gag/pol tat rev and the VSV-G.
14 In addition, there is a poly-A, SE40 poly-
15 A here. There is a synthetic poly-A and another
16 pause site located here to prevent the
17 transcriptional read-through going in the other
18 direction. So we have taken a different strategy
19 to make our helper safe.
20 [Slide.]
21 Some of the safety features of our VIRPAC.
22 First of all, I want to say because we are using
23 the VSV-G and HIV vectors, there is always a
24 theoretical possibility that they can recombine.
25 But what we can do is minimize the potential for

1 vector and helper recombination by intelligent
2 construct design. What that means is that we try
3 to force the putative RCL to go through multiple
4 events. Multiple events would be needed to obtain
5 a putative RCL. That is how you minimize the
6 potential for recombination.
7 You have to do that, minimize the
8 potential for homologous DNA recombination and also
9 reverse-transcriptase-mediated RNA recombination.
10 So there are strategies other than simply splitting
11 plasmids that are available to minimize the
12 potential for recombination.
13 [Slide.]
14 Let's look at DNA recombination and the
15 events. So this is a depiction of the vector here.
16 And I am showing the plasmid backbone here. This
17 is a depiction of the helper. So let me go through
18 it again. CMV promotor driving the gag, which is
19 degenerate. There is no packaging sequence here.
20 pol. Degenerate RRE.
21 Then we have rev IRES tat. Then we have
22 two poly-A sites, a transcriptional pause site.
23 Then we have the promoter that drives the VSV-G,
24 the poly-A and then the backbone of the plasmid.
25 These are the regions of homology. There is

1 homology between the backbones of the plasmid.
2 There is a region of homology between the cPPT
3 regions that are common in both the vector and the
4 helper, and there is a very small region here in
5 gag, just before the stop site, that is common to
6 both the gag and vector--the helper and the vector.
7 [Slide.]
8 So can homologous recombination produce a
9 putative RCL in one step? No. The answer is no.
10 So let's look at this. Two classes of events. The
11 first event here is that you get recombination,
12 DNA, homologous DNA recombination, occurring
13 between two sites; here the pol and this small gag
14 region.
15 So what would be the resulting virus, the
16 resulting recombinant? It would contain the
17 vector. It would contain gag/pol and it also would
18 contain the LTR here. The other class of event is
19 that you get recombination in the backbone and,
20 say, in one of these two sites, for instance. I
21 haven't shown all the three sites, just to make it
22 simple.
23 So you get recombination here and
24 recombination here. What would be the resultant
25 recombinant? Well, you would have the backbone.

1 You would have the poly-A, the VSV-G. You go
2 through all these elements, and then you pick out
3 the gag and that is what you have. You would have
4 the LTR.
5 So the first event is not an RCL because
6 it still doesn't contain any envelope. There is no
7 VSV-G. The second event basically contains no 3'
8 LTR that is necessary for reverse transcription.
9 So one event does not produce an RCL.
10 [Slide.]
11 So do two events produce an RCL? Let's
12 look at that. We have now this gag/pol recombinant
13 that is formed. Let's look at, then, DNA
14 recombination between the gag/pol and the backbone
15 of the plasmid. What is the recombinant? The
16 recombinant has to go this way. It then picks up
17 the VSV-G, the rev and the tat and then recombines
18 that way.
19 So it now does contain VSV-G, tat, rev,
20 gag/pol, but it doesn't contain and 3' LTR.
21 [Slide.]
22 So what does this mean? This is what you
23 would have. This thing would still have a
24 difficult time in being replication competent.
25 First, in the center of its genome, there are two

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1 strong polyadenylation sites, a bovine growth-
2 hormone poly-A and an alphaglobin poly-A. There
3 is, in addition, a transcriptional pause site
4 located between the gag/pol and the VSV-G.
5 To get rid of this event, the virus would
6 have to be able to synthesize this read-through
7 transcript and then delete the pause site, the two
8 poly-As, without removing the promoter element
9 because they would need the promoter element to
10 express the VSV.
11 In addition, it still has this stop site.
12 So if this transcript is made, you cannot produce
13 gag/pol because you have the gag stop here. Quite
14 frankly, it is difficult to see how additional
15 events would produce an RCL beyond that which would
16 be common to any production system, and what I mean
17 is nonhomologous-type events. And, in addition,
18 there is no 3′ LTR.
19 We are not trying to say this is better.
20 We are saying this is comparable to the other types
21 of production systems that are available.
22 [Slide.]
23 Now let's look at RNA recombination. RNA
24 recombination requires RNA not DNA, so let's look
25 first at the transcripts that are produced. There

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1 are three transcripts that are produced from the
2 helper. The first transcript is the gag/pol open-
3 reading frame. And then, also, the second
4 transcript is the rev IRES tat that is needed to
5 further express the gag/pol.
6 The third transcript is the VSV-G. Now,
7 all these transcripts don't contain a packaging
8 site and it must be remembered that these RNAs
9 would have to be copackaged with the vector RNA in
10 order to mediate an RNA recombination event. So
11 already the event is fairly low because you would
12 require copackaging of these RNAs with the vector
13 RNA in order for reverse transcriptase to mediate
14 the crossover event.
15 So let's look at some of these events.
16 [Slide.]
17 Basically here is the reverse-
18 transcriptase molecules using the poly-A--first,
19 let me answer the question. RNA recombination does
20 not produce an RCL in a single event. So let's
21 look at this. This is that the reverse
22 transcriptase takes the poly-A, binds to the helper
23 and then basically crosses over again in order to
24 pick up the packaging sequence.
25 This results in no envelope. There is no

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1 VSV envelope incorporated into this RNA. So it
2 does not produce and RCL in a single event.
3 [Slide.]
4 Does the RNA recombination produce an RCL
5 in two events? Well, in contrast to DNA, the next
6 event--so you have this gag/pol now. The next
7 event would mean that it would have to come up here
8 with the poly-A, bind to the poly-A VSV-G and then
9 pick up the VSV-G sequence. There is no other
10 homologous region to cross over so the next step
11 would have to occur by nonhomologous recombination.
12 I will address that event in another
13 slide, but you can see, in two events, through
14 homology, you cannot get beyond an RCL in two
15 events.
16 [Slide.]
17 What would happen if the read-through
18 transcript would get packaged? Again, I want to
19 caution you that the read-through transcript does
20 not contain any packaging sequence so, again, it
21 has lowered the chance for it to be copackaged.
22 Then it would have to read through these two poly-A
23 sites and the pause site in order to create this
24 read-through message.
25 Let's say that that does occur. If this

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1 occurs and then gets packaged, and then
2 recombination occurs, this is the event that would
3 occur. The reverse transcriptase would take the
4 poly-A, here, pick up the VSV-G, pick up all these
5 sequences and then come back here.
6 So the next slide shows the event.
7 [Slide.]
8 This is what you would have. But this
9 thing still has problems--it is not an RCL. It
10 still would have problems to replicate. Again,
11 there are two polyadenylation sites that would be
12 located within its genome and a pause site. A stop
13 site would be located to prevent gag/pol
14 translation and, again, it would be difficult to
15 see how an RCL would be produced beyond that which
16 would be common to any production system.
17 [Slide.]
18 So, in summary, comparison of VIRPAC
19 between other production systems, including a
20 stable producer cell line, currently there is no
21 producer cell line that has been described, at
22 least to my knowledge, that can produce vector
23 titers to at least 10^7 transducing units of cGMP-
24 grade HIV vector in what we think for the proposed
25 clinical trials in order to get a sufficient dose

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1 to get the transduction that you need to get the
2 vector in.
3 VIRPAC offers advantages. You can produce
4 sufficient amounts of clinical-grade vector from a
5 scale-up manufacturing process. Multiple
6 recombination events would be required to generate
7 a putative RCL. And we believe that VIRPAC
8 contains safety features that are comparable to
9 other transient production systems and produce a
10 cell line.
11 [Slide.]
12 So let's move on from the constructs to
13 the data. These vectors can transduce primary
14 human T-cells and many other primary human cells
15 with very high efficiency. This is just to show
16 you what we can robustly produce with the VRX494
17 vector that expressions GFP.
18 This is done in the multiplicity infection
19 of 20. Control cells. And essentially greater
20 than 99 percent transduction with the vector. Very
21 high transduction efficiency.
22 [Slide.]
23 One of the things that we looked at was
24 how stable was this transduction. We looked at it
25 by a number of means and we also wanted to look at

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1 whether the vector was toxic. The way that we are
2 measuring vector toxicity is by the effects of the
3 vector on the cells during expansion of the cells
4 in vitro.
5 So what we have here is an arbitrary
6 scale, depending upon what you are really looking
7 at. This is the data culture of the transduction.
8 So we are looking at EGFP. You can see that it is
9 very high transduction and it remains stable during
10 the course of this experiment.
11 Actually, we have shown that these
12 vectors, because the GFP is expressed from the LTR
13 through the spliced message, it is stable for
14 months. If you look at the copy number of the
15 vector per cell by TaqMan PCR, it is also very
16 stable during the course of the experiment.
17 This is all occurring, and this is what is
18 very interesting, during over 1000-fold expansion
19 of the cells. So, if you look at the fold
20 expansion of the untransduced cells in red and when
21 you compare them to the transduced cells, you can
22 see that there is no real significant difference
23 between the level of expansion. This level of
24 variation you would see in any two particular
25 cultures of expanded T-cells.

at

1 So we get very high and stable
2 transduction efficiency with this class of vector.
3 [Slide.]
4 And the vectors inhibit wild-type HIV
5 extremely well. The cells were transduced. We
6 don't select the cells. We just directly then
7 challenge them with wild-type HIV. This is the
8 p24. This is a log scale. This is the date and
9 culture and infection.
10 In this particular case, we are using a
11 multiplicity infection of 0.001 really because we
12 are trying to mimic low amounts of virus that could
13 be seen in a patient, but I am going to show you
14 data that we have done it for higher MOIs, 0.01,
15 0.1, and we get similar effects.
16 Basically, you can see that, while control
17 cells replicate wild-type HIV extremely well, there
18 is three logs of inhibition of wild-type-HIV
19 replication by vector-containing cells. This is
20 really extraordinary.
21 [Slide.]
22 Showing it at higher MOIs. So what we are
23 doing here is we are varying the dose of challenge
24 virus that is inputted into the challenge culture.
25 So these are transduced cells that were challenged

at

1 either with an MOI of 0.001, 0.01 and 0.1. You can
2 still see that, when you compare them to the
3 untransduced cells, that you still are getting very
4 effective inhibition of wild-type HIV replication.
5 [Slide.]
6 What is also interesting is that it seems
7 that the T-cells are also resistant to productive
8 HIV infection. How do we look at productive HIV
9 infection? Well, when HIV infects a cell and
10 productively infects the cell, it expresses GP120
11 VPU amongst other proteins. These proteins,
12 besides their other effects, can also downregulate
13 CD4 expression. They bind for CD4 and downregulate
14 the expression.
15 So we are using downregulation of CDR as a
16 means of looking into whether the cells are
17 productively infected with wild-type HIV. As you
18 can see, while control cells decrease their
19 frequency of CD4-expressing cells during the period
20 of the culture, transduced cells do not.
21 So it suggests that the cells have a
22 resistance, a selective resistance, to productive
23 HIV infection.
24 [Slide.]
25 Also, what we have looked at is inhibition

at

1 of vector-containing cells with different strains
2 of HIV. We have chosen two viruses that are X4-
3 tropic HIVs and two strains of virus that are R4.
4 NL4-3 is virus derived from the prototypic
5 molecular clone. BK132 is a primary isolate that
6 has X4 tropism that is the only passage through
7 once in tissue culture. This was derived from an
8 HIV-infected patient from an associate of Carl June
9 at the University of Pennsylvania.
10 Ba-L and US1 are R5 tropic strains of HIV.
11 As you can see here--maybe you can't see this
12 obviously at the back; I can hardly see it here--
13 but this is Day 23 out after an infection. You can
14 see that both X4 and R5 strains of HIV are
15 controlled fairly well by vector-containing cells,
16 by the vector.
17 You can make an argument that perhaps the
18 antisense payload is having an additional
19 inhibitory effect on the X4 rather than the R5 but
20 with our later data showing that it is really
21 inhibiting well at later stages, I would say the
22 conclusions of this data are that the payload is
23 effectively inhibiting both X4 and R5 strains of
24 HIV.
25 [Slide.]

1 What is also interesting is that cells
2 containing the VRX494 show, again, selective
3 resistance to CD4 downregulation in a mixed culture
4 that contains transduced cells and untransduced
5 cells. So what we are trying to do here is move to
6 situations that more mimic what it would be like in
7 the body or in an HIV-infected individual.
8 So what we did is we transduced the cells
9 so that we would get roughly half of them to
10 basically contain the vector, and that means that
11 they are EGFP positive, and half the cells don't
12 contain the vector. And then we challenged them
13 with different strains of HIV in the MOI, as you
14 can see right there.
15 This is actually data from 36 days after
16 infection, but you can see that, while cells that
17 don't contain the vector downregulate CD4, cells
18 that contain the vector have a selective resistance
19 to this CD4 downregulation indicating that they
20 have a selective resistance to productive HIV
21 infection.
22 [Slide.]
23 So, a summary of the in vitro transduction
24 and challenge data is as follows. We can see high
25 transduction efficiencies in primary human T-cells

1 with this class of vector. When you challenge
2 these cells with wild-type HIV, over 99 percent of
3 the wild-type HIV can be inhibited upon challenge
4 with a variety of MOIs. Both X4 and R5 strains of
5 HIV can be effectively inhibited by the antisense
6 payload which is almost 1 kb in length targeted to
7 HIV envelope.
8 Cells transduced with the vector show a
9 selective resistance to CD4 downregulation and,
10 hence, to productive wild-type HIV replication.
11 [Slide.]
12 As we are moving now towards the clinic,
13 we wanted to do a comparative study between our
14 laboratory-grade vector which expresses EGFP and
15 our candidate clinical vector which only has that
16 GFP marker fragment, no proteins expressed. So we
17 did a transduction in Sup-T1 cells at various
18 transduction MOIs and then challenged with wild-
19 type HIV at an MOI of 0.01.
20 As you can see, while control cells
21 replicated the virus very well, the cells
22 containing the vector inhibited extraordinarily the
23 replication of wild-type HIV, no matter, really,
24 the transducing MOI here. So this shows that our
25 laboratory-grade vector and our clinical candidate

at

1 vector are comparable.

2 [Slide.]

3 So the next stage is that we wanted to see

4 if would can transduce CD4 T-cells at the patient

5 scale. So we basically asked Bruce, who is sitting

6 in the audience here--we sent him up some vector.

7 We made it at the right amounts. He got a whole

8 leukopheresis product and transduced it with our

9 vector.

10 Then he looked at some interesting

11 toxicity endpoints to see whether the vector is

12 toxic to cells during our mock transduction that

13 would be very, very similar to the transduction

14 procedure that would go on in a clinical trial. So

15 the toxicity parameters, we looked at the doubling

16 level, the population doubling level during the

17 culture.

18 In blue, these are the transduced cells

19 and, in red, these are the mock cells. As you can

20 see, there is no appreciable difference between

21 mock and transduced cells. If you look at the cell

22 size during the expansion period, it is eleven days

23 in culture, no significant difference between

24 vector transduced and mock cells.

25 If you look at the viability of the cells

at

1 during the expansion period, again, no significant
2 difference between mock and transduced cells. If
3 you look at a cell-surface profile between mock and
4 transduced cells, you will see no significant
5 difference. The way that you read this is that the
6 first two blue and red bars are blue is transduced
7 and red is mock at Day 7 while blue transduced and
8 mock at Day 11.
9 But if you look at these doublets--and you
10 can look at that in the handouts; we have submitted
11 this data before--there is no significant
12 difference between the surface expression of these
13 markers on these cells.
14 DR. SALOMON: Dr. Dropulic, I will resist
15 the temptation to interrupt you too often, but I am
16 a little stuck here. What exactly did you do here?
17 DR. DROPULIC: What we did here was we
18 made a preparation of vector--
19 DR. SALOMON: What does a "preparation of
20 vector" mean?
21 DR. DROPULIC: We made a pilot lot of
22 vector using our manufacturing procedure. So that
23 was, then, put into bags, sent up to the University
24 of Pennsylvania. The whole leukopheresed product
25 was isolated and then transduced--the cells were

1 isolated, the T-cells were isolated and then
2 transduced with the vector.
3 DR. SALOMON: So it was all T-cells.
4 There was no CD4 purification.
5 DR. DROPULIC: Not in this particular
6 case.
7 DR. SALOMON: There was a monocyte
8 depletion?
9 DR. DROPULIC: There was a monocyte
10 depletion. Bruce, could you comment on that?
11 DR. LEVINE: The pheresis unit that we
12 obtain is first washed in a Code 2991 cell
13 processor and some of that was removed. It is
14 washed to remove the platelets. And then we do a
15 monocyte depletion by adherence that takes about an
16 hour. The cells that we--
17 DR. SALOMON: Can I ask you a question?
18 How many cells did you eventually take--when you
19 say a monocyte depletion by adherence. To what?
20 To large plastic bags? Or did you just take a
21 couple hundred million cells and put them down on
22 plastic Petri dishes?
23 DR. LEVINE: They adhere to magnetic
24 beads. It provides a much larger surface area in a
25 smaller bag than you could do with a T175 flask.

at

1 Basically, the entire pheresis unit can be
2 monocyte-depleted in two 150 ml bags. From that
3 preparation, we take approximately a billion cells,
4 stimulate them with other magnetic beads that have
5 conjugated to them anti-CD3 and anti-CD28
6 antibodies that we have used in all our trials.
7 The vector is added pretty much as a media
8 supplement just as you would add glutamine to the
9 media, we just add vector at the appropriate
10 dilution. Then those cells are grown in gas-
11 permeable culture bags for the duration of the
12 culture that you see up there.
13 DR. SALOMON: No interleukin 2.
14 DR. LEVINE: Well, we don't have to add
15 interleukin 2 but we add a low level of interleukin
16 2, approximately 100 units per ml.
17 DR. SALOMON: That is low? If you gave
18 100 units per ml of interleukin 2 to a human being,
19 they would die.
20 DR. LEVINE: 100 units of--
21 DR. SALOMON: If you gave 100 units to the
22 whole patient, no. But I mean if you tried to
23 achieve a level of 100 units per ml in a patient,
24 that would be very high.
25 DR. LEVINE: What I mean by low is when

at

1 previous investigators have cultured what have been
2 called the lac cells, they use very high amounts of
3 IL2, 800 units per ml or higher. What that tends
4 to do is make the CD8 cells grow out in the
5 culture.
6 What we have found is if we add 20 to 100
7 units per ml, that we maintain the CD4 to CD8
8 ratio. The second point is that that is low enough
9 that you are not conditioning the cells to be
10 cytokine dependent. We believe that when you
11 culture the cells with very high amounts of
12 interleukin 2, 800 units, 1000 units per ml, when
13 you would infuse those cells, they would be
14 dependent on that high level of interleukin 2 in
15 vivo. That is obviously not present.
16 DR. SALOMON: Your evidence that 100 units
17 per ml of IL2 does not condition the cells is--
18 DR. LEVINE: Well, I would say in vivo we
19 don't have evidence of that. But if we do grow the
20 cells without IL2, for most of the normal donors we
21 are growing, they grow just as well. We think of
22 it with some of the HIV cells that we get, it helps
23 maintain an adequate level of expansion.
24 DR. TORBETT: Can I ask a question. Can
25 you tell me, just a little bit, do you add the

1 virus during the full stimulation period? Do you
2 add your vector preparation to the bag during the
3 full preparation?
4 DR. LEVINE: Yes.
5 DR. TORBETT: Could you go over a little
6 bit how you actually transduce the cells during
7 this time on volume?
8 DR. LEVINE: As I said, you just add it as
9 a media supplement depending on the MOI that you
10 would like to achieve and the titer of the virus,
11 so it is a very small amount of the vector added to
12 the culture media. It is added on Day 0 and then
13 basically diluted out as you add media to expand
14 the cells. Then, at the time of harvest, the cells
15 are washed, completely washed, three or four times
16 with volumes of Plasmalyte prior freezing.
17 DR. SALOMON: Last question. What was the
18 MOI in these experiments?
19 DR. LEVINE: Was it 40, I believe?
20 Vladimir?
21 DR. SLEPUSHKIN: I think, in this
22 experiment, this was a clinical experiment and it
23 was an MOI of 200.
24 DR. SALOMON: 100 did you say?
25 DR. SLEPUSHKIN: 200.

at

1 DR. SALOMON: 200 MOI?

2 DR. DROPULIC: Right; by copy number,

3 because you don't have GFP.

4 DR. SALOMON: Okay; I'm sorry for

5 interrupting.

6 DR. DROPULIC: No; my pleasure.

7 DR. SALOMON: I was just--there are some

8 details here.

9 DR. DROPULIC: Please interrupt.

10 [Slide.]

11 So then we took this preparation and

12 basically they are normal human--patients that are

13 not infected with HIV--and basically challenged

14 them with NL4-3, and basically looked at the

15 inhibitory effects of the vector-containing cells.

16 As you can see, it inhibited about 2 logs of virus

17 here when you compare the controls to the vector.

18 [Slide.]

19 Now we have moved on with additional

20 studies to take CD4 T-cells from HIV-infected

21 donors. Basically, this was a patient that had a

22 viral load of about 92,000 and a CD4 count in about

23 the 600 range. What we are looking for here is are

24 there any effects of the vector specifically on

25 cells derived from HIV-infected individuals.

at

1 So what we are looking for here is for
2 cumulative cell expansion. In red are the mock
3 cells. In blue are the transduced cells. You can
4 see no appreciable difference. If we look at copy
5 number per cells with this preparation, we can find
6 that normal human T-cells can be transduced at
7 about three copies per cell while HIV-infected CD4
8 T-cells are in the same range, about two copies per
9 cell.
10 [Slide.]
11 So these cells were first expanded, then
12 frozen down and then we thawed them so that they
13 would be frozen down as they would have been done
14 in a clinical trial. And then we thawed them and
15 then grew them out to look at whether the virus
16 would come back and replicate. So this is virus
17 that is endogenous to the patient. We are not now
18 infecting with another virus.
19 So when we cultured them--so we expanded
20 for eight days, froze the cells down, thawed them
21 and then grew them up again and then looked for p24
22 after expansion. So what we have here is that the
23 mock-containing cells, you can see that the virus
24 were all back and replicated while, in the vector-
25 containing cells, it controlled HIV replication for

1 about two logs for about ten days and then you see
2 this bump or breakthrough effect.
3 [Slide.]
4 I will show you what the breakthrough
5 effect is-- we have done analysis on that--in just
6 a moment. But, at the same time, what we had done
7 is we looked at the presence of CD4 on these cells.
8 So this is looking at downregulation of CD4 from
9 cells that are transduced from an HIV-infected
10 donor.
11 So here are the non-treated cells. You
12 can see the amount of CD4-expressing cells is about
13 40 percent while almost twice as many cells were
14 CD4-positive when treated with the vector.
15 DR. SALOMON: Marvin?
16 DR. REITZ: Excuse me. The question I had
17 was this an HIV-infected donor that was under
18 treatment or a treatment-naive patient?
19 DR. DROPULIC: No; I think that patient
20 was on treatment but failing. I can't exactly
21 remember. I have to go back to the people at
22 Hopkins, but I believe it is a patient that was
23 failing therapy.
24 So that is that. Twice as many cells
25 appear to be less--twice as many cells are CD4-

at

1 positive in cells treated with the vector compared
2 to nontreated controls.
3 [Slide.]
4 So, getting back to this issue of what is
5 this bump, we did RT PCR analysis where we looked
6 at the types of viral RNAs that were present in
7 this culture. So what we have here is we do RT PCR
8 where we have two sets of primers. One is for wild-
9 type HIV. That is this band that goes across here-
10 -and one that specifically detects the vector.
11 Each of these is days after infection, so
12 what we are doing is we are comparing mock--this is
13 the transduced cells. As you can see, M is mock.
14 V, here, is the vector-containing cells. You can
15 see early in an infection that the wild-type virus,
16 we can detect the wild-type virus. In the
17 transduced cells, you don't see that packaged
18 vector until very late in infection, in this
19 infection process.
20 And then you can see that the vector is
21 coming up. So the vector is copackaging, or being
22 packaged. In this case, it is being selectively
23 packaged into progeny virions because there is more
24 of the vector being present. So this bump is not
25 entirely wild-type HIV. It is some wild-type HIV

at

1 but the vector is being packaged.

2 Now packaged vector doesn't mean it is

3 mobilized. It is going out there. Mobilization

4 means that it has to go in to a neighboring cell.

5 So we looked at that question by taking samples and

6 then infecting them on naive CD4 cells. That is

7 represented by the next slide.

8 [Slide.]

9 So here you have mock HIV. These are the

10 two primers here. These are mock HIV cells. These

11 are just mock controls. This is a vector and no

12 HIV. And these are the cells, vector, that are

13 infected with HIV.

14 Although you can see the wild-type HIV

15 band, you don't see the vector, suggesting that,

16 actually, the copackaged virus is having a very

17 difficult time mobilizing, getting to the

18 neighboring cell. In fact, you can't really detect

19 the band and we have to resort to TaqMan PCR to see

20 whether there was any mobilized vector at all.

21 What we found, by TaqMan PCR, was that, in

22 these samples, 30 copies of vectors were mobilized

23 into CD4 T-cells per 10,000 cells analyzed. So an

24 extremely low frequency.

25 DR. MULLIGAN: May I interrupt for just a

at

1 second. First of all, I would dispute the--you can
2 call it mobilization of whatever you want to call
3 it, but I would say that what you are demonstrating
4 would be what I would call mobilization. That is
5 mobilization out. There is a component. And
6 mobilization into cells.
7 DR. DROPULIC: I call it packaging,
8 actually. I call it co-packaging and mobilization
9 is as it is actually going into the cell.
10 DR. MULLIGAN: Okay. I wouldn't call it
11 that. But if you quantify the mobilization event,
12 or packaging event, if you look at the efficiency
13 of that event relative to the packaging in wild
14 type in this experiment here, can you give us a
15 sense of how--well, not this one, but the one you
16 just showed--how efficient is that?
17 DR. DROPULIC: How efficient is what?
18 DR. MULLIGAN: How efficient is the
19 generation of packagable vector in this system?
20 DR. DROPULIC: It only occurs at low copy
21 numbers of cells and, on the packagable vector, it
22 is not very efficient.
23 DR. MULLIGAN: Maybe go back a couple to
24 where--
25 DR. DROPULIC: Sure. That is a problem

at

1 with this. Let me just try that. There we go.

2 [Slide.]

3 DR. MULLIGAN: So it is a little busy for
4 me to tell what bands we are looking at here, but--

5 DR. DROPULIC: These are the bands that
6 you are thinking about.

7 DR. MULLIGAN: So you are looking at the
8 ratio of those versus what represents the--

9 DR. DROPULIC: So this is the vector here
10 and this is the wild type here. All I am saying is
11 that we can see this. This is happening. We
12 didn't do any studies in terms of ratios or
13 anything. But when you take this type of soup and
14 passage it, it doesn't go into the cells very
15 efficiently.

16 DR. MULLIGAN: I just want to make sure I
17 have got this right, though. So if you look at the
18 ratio of those two, you are saying, of the virus
19 particles that you are looking at, there is
20 significantly more of the vector than there is of
21 the "helper," the wild type; is that right?

22 DR. DROPULIC: I wouldn't say
23 significantly more. I would say marginally more;
24 yes.

25 DR. MULLIGAN: You would say that that is

at

1 a marginal difference between the--

2 DR. DROPULIC: I don't know what is

3 significant? What is it, maybe a five-fold effect?

4 DR. MULLIGAN: That is a marginal

5 difference?

6 DR. DROPULIC: A five-fold effect.

7 DR. MULLIGAN: I would not call that a

8 marginal difference. But I guess my point is that

9 is suggesting that is a very, very efficient

10 packaging of a vector. So you call it packaging.

11 I will call it mobilization. But there is a very

12 significant amount of vector that has been out in

13 the soup.

14 DR. DROPULIC: Okay. I can tell you that-

15 -do you want to say? Go ahead.

16 DR. LI: My name is Yuexia Li. I work for

17 VIRxSYS. First of all, I want you to know there is

18 a duplex RT PCR. The lower band is a smaller piece

19 so you have a more efficient--when you do the PCR,

20 it is more efficient. So you may have more signal

21 here amplified. Also, this is a qualitative assay.

22 It is not a quantitation so you can't just see the

23 intensity of the band and say, okay, you have much

24 more vector here than the wild type.

25 If you repeat it exactly, you may get a

at

1 slightly different result. What we want to show
2 here is in the p24 peak, we want to characterize
3 just by the nature of that peak, that the p24 value
4 was contributed by the wild type and some vectors.
5 It is not a qualitative assay. You can't just
6 stick in that band and say, okay, you have much
7 more vector than the wild type. We can't draw that
8 conclusion.
9 DR. MULLIGAN: I think I was just trying
10 to raise is that this is am important clue. This
11 is what we have been looking for to see whether
12 there is, indeed, packaging. I think the next step
13 is, of course, why you don't detect it as being
14 capable of infecting.
15 DR. DROPULIC: Right.
16 DR. MULLIGAN: And, in fact, what,
17 exactly, is that species.
18 DR. DROPULIC: Right. We haven't looked
19 into what is the event. But what is likely is
20 happening is that you are getting copackaging of
21 wild-type HIV and vector. You have got two
22 different genomes. If that occurs, you have got
23 the antisense binding by hybridization and that
24 would make it very difficult for that vector, for
25 that packaged recombinant, in order to reverse

at

1 transcribe and integrate because there is 1 kb of
2 antisense that is binding to the envelope region of
3 the wild-type virus.
4 So that is our explanation of why we are
5 seeing it.
6 DR. SALOMON: Have you characterized this
7 at all, then? That was actually in your clinical
8 protocol, I believe so, if this was found during
9 the trial.
10 DR. DROPULIC: Yes.
11 DR. SALOMON: You say you will stop and
12 characterize this.
13 DR. DROPULIC: No; if we see an RCL, we
14 will stop and characterize it or if we see
15 packaging of a VSV signal, we will stop and
16 characterize it. But I don't think if we see this
17 event, we would stop and characterize it. We will
18 monitor for whether VRX is mobilized or not, but it
19 is not a stopping event presently.
20 DR. SALOMON: It would just be interesting
21 after all the very elegant sort of molecular
22 strategy you showed us earlier about this
23 recombination could occur and this recombination
24 could occur to actually look at what got packaged
25 here.

at

1 DR. DROPULIC: I agree.

2 DR. SALOMON: Just to finish this. Then
3 the idea here is--I guess the problem I am having
4 just a little bit here is with adjectives; low,
5 nothing, minimal. So 30 copies in 10,000 cells, I
6 calculate to 3,000 copies in a million cells or
7 about 1 million PBL per ml. So we are talking
8 about 3,000 copies of packaged vector per ml of
9 blood, basically.

10 DR. LI: Actually, that 30 copies is the
11 30,000 cells because when you run the TaqMan, you
12 run triplicate in all three wells together, they
13 only find 30 copies. Each well, you have it.

14 DR. SALOMON: Oh; okay. It says 30 copies
15 per 10,000. So, 1000 copies per ml of blood.
16 Okay.

17 DR. DROPULIC: Next slide.

18 [Slide.]

19 So we looked at mobilization, what I
20 define to be mobilization, by taking basically
21 cells, either primary human CD4 T-cells or Sup-T1
22 cells, and then transducing them with vector and
23 then taking the supernatants, transducing the cells
24 with the vector and then basically challenging them
25 with a very high MOI of 0.2 with wild-type virus,

at

1 and then taking the supernatants of those cells and
2 then infecting MT4 cells, which is a very sensitive
3 cell for HIV infection.
4 So what we have here is we have our
5 untransduced controls. We have here VRX430 which
6 does not contain the antisense payload. It is
7 analogous to VRX494 that does contain the antisense
8 payload. So when we look at the MT4 cells, we can
9 see that, with the vector that contains no
10 antisense payload, you see a very small amount of
11 mobilization. I am just going to use my adjective.
12 You can correct me later--because I like them.
13 While, when we looked at the cells that
14 were infected with the supernatants from VRX494-
15 challenged cells, we saw no mobilization events.
16 In Sup-T1 cells, which are more permissive
17 to HIV infection, we saw low levels of mobilization
18 with the vector that did not contain the antisense
19 payload. But, again, it decreased, significantly
20 decreased, when the vector contained the antisense
21 payload.
22 So the conclusions are that there is a
23 very low level of mobilization that is occurring
24 and, in addition, the antisense payload decreases
25 mobilization. So it is an additional safety

at

1 feature of the vector system.

2 [Slide.]

3 So we did this in vitro and now we want to

4 do it in an animal model. This is very difficult

5 to do in an animal model so we kind of made our

6 best stab at it. This is what we came up with

7 looking at in vivo mobilization in SCID mice. SCID

8 mice are nice because, as you all know, you can

9 transplant them with human cells. What we are

10 doing here is we are injecting human CD4 T-cells IP

11 into the animal so you have a small local

12 environment to look for mobilization events.

13 So you isolate human CD4 T-cells. Then we

14 divided the T-cells into two lots. One lot of

15 cells received a vector that expresses EGFP. The

16 other lot of cells were transduced with a vector

17 that expressed EYFP. The next thing about EGFP and

18 EYFP is you can discriminate by FACS. So you can

19 look for dual events. That is what we are trying

20 to look for here.

21 So we have CD4 cells transduced with

22 either vector, mixed together, challenged with

23 wild-type HIV. Then we mix that back with CD4-

24 negative PBMCs and then we injected those cells IP

25 back into the animal, you know, just into the

at

1 animal because they are human cells.

2 The types of events that we were looking

3 for is whether the vector mobilized authentically

4 from CD4 cells to CD4 cells. The way that you

5 would see that event is by looking for double-

6 positive cells. If this green vector mobilized to

7 a yellow vector-containing cell, that would be

8 authentic mobilization.

9 It is mobilization that is restricted to

10 the target tissue. While an adverse, if you like,

11 mobilization event would be if the vector, either

12 green or yellow, would mobilize to a marker CD4-

13 negative cell. We have used B-cells because B-

14 cells are lymphocytes closely related and they are

15 definitely CD4-negative.

16 [Slide.]

17 So VRX mobilizes poorly between primary

18 CD4 cells in vivo. So what we have here is these

19 are the cells that were inserted into the animal.

20 The only difference between this and this is that

21 these cells were not challenged with wild-type HIV

22 and these cells were challenged with wild-type HIV

23 at an MOI of 0.2.

24 This is the background of the events, so

25 you can see here that this is the background of

at

1 double-positive events. You can see some
2 mobilization occurring above the background. So
3 some low level of mobilization is occurring between
4 CD4 T-cells because you are seeing these double-
5 positive events.
6 [Slide.]
7 However, the vector does not mobilize
8 adversely. It does not mobilize to CD19 cells.
9 Again, now we are looking for either GFP or YFP
10 expression and then looking for whether we can see
11 that expression on CD19 cells. These are the
12 noninfected background controls and these are the
13 cells from animals that were infected, the cells
14 that were infected with wild-type HIV.
15 You can see no significant events over the
16 background events.
17 [Slide.]
18 So, a summary of the in vitro and in vivo
19 mobilization data is as follows. Mobilization, we
20 believe, is only a safety concern when the vector
21 spreads beyond the intended target tissue. That
22 is, in our case, CD4 T-cells. Our in vitro and in
23 vivo data show that VRX496 mobilizes poorly between
24 primary CD4 T-cells and our in vivo data shows that
25 our vector does not mobilize beyond CD4 T-cells,

at

1 the intended target tissue. No mobilization was
2 seen into CD19 cells, B cells, which is our CD4-
3 negative marker cell.
4 [Slide.]
5 So we also wanted to look at when
6 mobilization occurs between CD4 and CD4 T-cells, is
7 the vector structure or sequence somehow affected.
8 So we did this analysis where we basically produced
9 our vector in our 293 cells by cotransfection and
10 then transduced primary CD4 cells with the vector
11 preparation.
12 Then what we did is we did PCR sequencing.
13 We PCRed out the vector and then looked for were
14 there any deletions or mutations present in the
15 vector genome. We found none, no deletions, no
16 mutations, no insertions. We sequenced the PCR
17 product. That is what I want you to understand.
18 Then what we did is we challenged with
19 wild-type HIV and then infected--and this was very
20 difficult to do because there wasn't a lot of
21 vector sequence in these cells, but we were able to
22 get out a signal. Again, we PCRed out a product
23 and that product, again, when mobilized with wild-
24 type HIV, again showed no deletions, no mutations,
25 no insertions. It didn't pick anything up. So

at

1 those are the studies that we have just completed,
2 actually.
3 DR. ALLAN: Can I just ask a question
4 right there?
5 DR. DROPULIC: Sure.
6 DR. ALLAN: Did you look at any for
7 recombination? I mean, you are just looking at
8 deletions, mutations?
9 DR. DROPULIC: Anything. Absolutely. I
10 should have added recombination. What we did is we
11 PCRed out the vector sequence. So we used primary
12 in the LTR and one primary in G-tag, the G-tag
13 sequence, PCRed that one fragment out, sequenced
14 it.
15 And then the other side, PCRed that
16 sequence. And there were no changes from the
17 original.
18 DR. ALLAN: But it depends on which
19 primers you are using because you can get
20 recombination and the one set of primers may not
21 function because you do have recombination between
22 wild type virus.
23 DR. DROPULIC: We tried to choose a primer
24 set that would uniquely pull out our vector.
25 DR. ZAIA: I have a question, also. Are

at

1 you saying that--you are looking at virus now two
2 weeks into the infection. This is the virus that
3 is growing out in the presence of transduced cells.
4 DR. DROPULIC: What are you specifically
5 talking about, this last slide?
6 DR. ALLAN: That last slide. When you
7 looked at the--
8 DR. DROPULIC: This was short-term. This
9 wasn't long-term. I am not making a statement
10 about it being long-term. I am just simply saying
11 that when you did this experiment--
12 DR. ALLAN: I guess my question is are you
13 characterizing the virus that is being selected by
14 the transduced--
15 DR. DROPULIC: No; this is not selection.
16 This is just simply transduction, challenge, hit as
17 many cells as you can with that supernatant and try
18 to PCR it out. When you PCR it out, that is what
19 we got. This is no long-term selection. But we
20 have done selection experiments. I am going to go
21 into that in just a moment.
22 DR. ALLAN: But you showed us that it
23 takes about two weeks to see a virus grow out in
24 the system.
25 DR. DROPULIC: This is a separate

1 experiment. Xiaobin?

2 DR. LU: This is Xiaobin Lu from VIRxSYS.

3 This experiment actually characterized the vector

4 cells, not the escape. The escape comes later.

5 DR. ALLAN: I see. Okay.

6 DR. DROPULIC: Next slide.

7 [Slide.]

8 We have shown this data before to the FDA.

9 What we have seen is that when you transduce Sup-T1

10 cells--this particular event doesn't occur in

11 primary human CD4 cells. When you transduce at a

12 relatively low MOI of 5 and then only when you

13 challenge with a high dose of wild-type HIV that

14 you see these effects.

15 So this is p24. This is days after

16 infection. You can see that the control cells

17 replicate wild-type HIV very nicely. Now, cells

18 that contain the vector that does not contain the

19 antisense has an effect. It delays it but it still

20 comes up and goes back down.

21 However, when you challenge the cells

22 containing the vector with the antisense payload,

23 you see strong inhibition early but then you see

24 this breakthrough effect. What we wanted to do is

25 really look into this, what this breakthrough virus

at

1 could be and try to understand these events more
2 clearly.
3 [Slide.]
4 So what we did is we did an experiment
5 where we did high-dose challenge of wild-type HIV
6 at 0.1 in Sup-T1 cells and we did a range of
7 transduction doses with the vector. So these cells
8 were transduced with the vector. Now, this is GFP
9 transduction at an MOI of 20.
10 This is with a transduction MOI of 10.
11 This is a transduction MOI of 5. So, clearly,
12 suboptimal doses of vector can give rise to a
13 breakthrough. But it is related to dose. It is
14 not an escape, per se, in this experiment. It is
15 dose-related.
16 However, that still begs the question can
17 you get an escaped variant that is resistant to the
18 vector.
19 DR. SALOMON: Can I just interpose again?
20 It is this adjective thing. So high MOIs, low
21 doses of vector. So the MOI is--
22 DR. DROPULIC: 0.1 is considered a pretty
23 high challenge MOI. I don't know if anybody would
24 disagree with that.
25 DR. SALOMON: So that is high.

at

1 DR. DROPULIC: Yes.

2 DR. SALOMON: And that would be high

3 relative to what is going on in the patient.

4 DR. DROPULIC: No; it is convention in the

5 field, I think, pretty much, an in vitro challenge

6 dose. I don't think anybody has really looked at

7 MOIs in a patient, per se. I think that is very

8 difficult to study. Low transduction is just by

9 experience, that the MOI of 5 is relatively low

10 compared to an MOI of 20 where we get very good

11 levels of transduction with the vector.

12 So we wanted to look at whether, really, a

13 resistant variant to the payload can occur.

14 [Slide.]

15 So what we did was we took these VRX Sup-

16 T1 cells that were transduced with a lot MOI and we

17 took control cells, and then we passaged the

18 breakthrough virus, as shown here. If you passage

19 this any time on a transduced cell that is higher

20 than an MOI of 5, a transduced MOI of 5, you don't

21 see breakthrough.

22 You have to keep on reinfecting at this

23 low suboptimal dose of vector transduction in the

24 cells in order to carry this infection forward.

25 After three passages, we basically took that,

at

1 basically the sample, and then did PCR sequencing.
2 So the question is do variants develop
3 that, escape the antisense action.
4 [Slide.]
5 So this is the original passaged--this is
6 the original breakthrough. Then, after three
7 passages, what we found is that it seems like there
8 is something that basically is more resistant to
9 the antisense payload effect because the peak of
10 titer here is at Day 12 compared to Day 20.
11 However, it appears that its fitness, compared to
12 wild type, is significantly impaired.
13 So that is what we found. We found a
14 shift but the peak was significantly lower.
15 [Slide.]
16 So then what we wanted to do is take--
17 DR. TORBETT: Excuse me for just a second.
18 Could you back up to the slide. I didn't quite
19 understand what you were doing. I apologize.
20 DR. DROPULIC: All right.
21 [Previous slide.]
22 DR. TORBETT: Could you go over in detail
23 what you did, explain it to us?
24 DR. DROPULIC: Okay. This is the Sup-T1
25 cells containing the vector. We challenged with

1 wild-type HIV. We got the breakthrough. We
2 passaged once, twice and I believe at this time we
3 took the cells out and then did PCR sequence
4 analysis on these cells, I believe.
5 DR. TORBETT: So if spread was occurring
6 during these successive passages--
7 DR. DROPULIC: Yes; we could see that--
8 DR. TORBETT: Is that why it is suppressed
9 then at the end because what happened was the low
10 number of copies that you got into the original in
11 the MOI-5 gradually was increasing because of
12 mobilization of the vector, so that, by the time
13 you got to the third or fourth passage, it was
14 equivalent to a starting T-cell population that had
15 an MOI of 10 or higher?
16 DR. DROPULIC: No; we didn't look into
17 that. All we were looking for in this experiment
18 was purely the resistance issue. That is all I can
19 tell you.
20 Xiaobin, did you want to mention
21 something?
22 DR. LU: I think what we have done is,
23 after the third passage, we take the soup and
24 transduce, infect Sup-t1 cells. Then we extract
25 the DNA and do the PCR and clone the corresponding

at

1 sequence in the plasmid. And we sequence.

2 DR. DROPULIC: Right. That is the next

3 slide, actually.

4 DR. SALOMON: How many clones did you

5 sequence?

6 DR. DROPULIC: I will show you that right

7 now.

8 [Slide.]

9 So here we are basically. We took wild-

10 type HIV-specific primers here. And then we looked

11 at a comparison between the number of deletions in

12 the antisense. We compared the wild-type cultures,

13 and what that means is wild-type HIV just passaged

14 on Sup-T1 cells, compared to the breakthrough virus

15 being passaged on the vector-containing cells.

16 The frequency of deletions in the

17 breakthrough virus was extraordinarily high, 91

18 percent. We did 290 clones, to your question, and

19 264 of those contained some form of deletion. We

20 did 40 control clones and 11 of those were deleted

21 so a frequency of 27 percent.

22 Then, also, we looked at the mutation rate

23 and compared it to the wild-type passage cultures

24 compared to the breakthrough passage cultures. We

25 saw there are mutations here. But what was

at

1 particularly interesting was this one clone here
2 that had 109 mutations which reflected 12 percent
3 of this antisense binding region contained
4 mutations that would try to convert resistance
5 against the antisense payload.
6 That is the next slide. So I hope
7 everybody understands that. Basically, it is PCR
8 out to just wild-type-specific primers and then we
9 do subcloning into plasmids and then we PCR
10 sequence that as plasmids.
11 DR. ALLAN: What is wild-type virus that
12 you are using?
13 DR. DROPULIC: NL4-3.
14 DR. ALLAN: It is?
15 DR. DROPULIC: Yes.
16 [Slide.]
17 So this one mutant I am calling BTP1
18 mutant displayed a mutation frequency of 12 percent
19 in the envelope region that binds to antisense. It
20 is really interesting because this is the region
21 that binds to the antisense. What we did is when
22 we PCRed our cloning, we PCRed a 2 kb fragment.
23 So 1 kb would be reflective inside the
24 region that the vector antisense would bind and the
25 other 1 kb reflects outside that region. It was

at

1 interesting that this 12 percent mutation frequency
2 occurred exclusively in this region and not outside
3 the region, if you can sort of think of it as a
4 control.
5 What also was interesting was that
6 essentially all the changes were A to G base-pair
7 substitutions that we saw in this mutant clone, all
8 along the fragment here. This is just a very small
9 region of it. You can just see an example right
10 there.
11 [Slide.]
12 This is consistent with the known action
13 of antisense. So the deletions and the mutation
14 data strongly suggests that our antisense payload
15 is behaving by the mechanisms of known antisense
16 action.
17 So let me tell you about how antisense
18 works, what are the mechanisms. Adosine deaminases
19 act to convert adenosines to inosines in double-
20 stranded RNA. This conversion leads to an unstable
21 base pair which leads to partial or complete
22 unwinding of the region and degradation or nuclear
23 retention of the RNA.
24 The mutations that we are seeing would
25 hamper ADA conversion of adenosines and then make

1 resistant to the effects of the antisense sequence.
2 [Slide.]
3 So what we did now was is we took this
4 fragment and we cloned it back into wild-type HIV
5 to see whether it could replicate. So this is the
6 data. What we did is we made both of the plasmids,
7 the mutant and the wild type, transfected it, I
8 believe, into 293 cells--I think it was 293 cells
9 because we are just looking at one round here for
10 the first round.
11 Then, basically, we find that, in the
12 first round, you could produce p24 from both the
13 wild-type HIV and the mutant. However, when you
14 take that supernatant and passage it on Sup-T1
15 cells, CD4 cell line, to look for replication,
16 while the wild-type HIV can replicate--this is the
17 second round--the mutant does not appear to
18 replicate. It has very low fitness, if anything,
19 below detection.
20 When you do a TCRD50, you can see that the
21 wild-type HIV can replicate nicely while the
22 mutant, it was below detection. So this suggests
23 that the virus is trying to create mutations
24 against the antisense payload but it pays a price
25 in terms of its own replicated fitness.

at

1 DR. SALOMON: That was the one mutant.
2 DR. DROPULIC: Just the one mutant, but a
3 deletion would decrease the replicative fitness as
4 well, if you know you haven't got N.
5 DR. MULLIGAN: Have you ever associated
6 that mutation with the rest of the proviral
7 sequences? Often, there are compensatory mutations
8 at other locations.
9 DR. DROPULIC: We haven't looked. I mean,
10 the only thing we have done is what I have shown.
11 We did 1 kb downstream of the site. We didn't look
12 at the whole genome, if that is the question. No.
13 DR. MULLIGAN: The question is whether or
14 not that, indeed, this is a fair test in the sense
15 that you have never asked the question whether the
16 entire sequence--
17 DR. DROPULIC: True. We have not done
18 that. But this is what we have done.
19 DR. TORBETT: I have a question real
20 quickly. You flashed by pretty quickly on your
21 envelope sequence.
22 DR. DROPULIC: Oh; I'm sorry. Do you want
23 me to go back?
24 DR. TORBETT: No; that's okay. I just
25 have a quick question. You are going back in to

at

1 Sup-T1s. Your area you are targeting I believe is
2 the V3 area; is that correct, on your antisense?
3 DR. DROPULIC: It is not just specifically
4 V3. It is a 1 kb stretch that is basically most of
5 the 5' end of the envelope.
6 DR. TORBETT: Is there any chance, and I
7 am sure you have done that, looked on CCR5 using
8 cell line to find out if the tropism is changing.
9 You are going back into Sup-T1s which restricted to
10 X4 using viruses. Is there any chance that it
11 switched over to an R5 during these kinds of tests
12 and you would miss it in your biological assays?
13 DR. DROPULIC: We haven't done that. We
14 haven't done that experiment. We certainly can do
15 that, but we haven't done it.
16 DR. TORBETT: Have you run your sequence
17 through any type of blast search to see homologies
18 with other types of envelopes?
19 DR. DROPULIC: No; we have not. We can do
20 that.
21 DR. TORBETT: Thank you.
22 DR. DROPULIC: We can go back. I wanted
23 to make one last point. It is a speculative point.
24 The mutation severely restricts virus replication.
25 And we believe that, and this is speculation, that

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1 this mutant may persist by pseudotyping wild-type
2 HIV. It can persist and survive. It can't
3 replicate on its own but it can survive by having
4 some wild type around to complement it and drive it
5 forward, but it cannot replicate on its own. Mere
6 speculation, but that is our hypothesis of why we
7 think that this thing can be picked up after
8 multiple passages.
9 [Slide.]
10 So a summary of the breakthrough data.
11 The initial breakthrough virus is due to high MOI
12 of wild-type HIV overcoming suboptimal transduction
13 levels in Sup-T1 cells. No breakthrough is seen
14 when sufficient doses of vector are used.
15 A variant HIV can be selected that shows
16 increased resistance to vector inhibition.
17 However, the consequence of this resistance appears
18 to be a decreased fitness for replication.
19 We have seen a very high deletion and
20 mutation frequencies in the target env region of
21 wild-type HIV which strongly indicates that the
22 vector is acting upon wild-type HIV by the known
23 antisense base-pairing mechanism.
24 [Slide.]
25 Now we move on to in vivo by distribution

at

1 and safety studies. There has been a lot of talk
2 about animal models to test the safety of these
3 vectors. We believe that, given everything, this
4 is the best model that we can have. It may not be
5 an ideal model, but it at least has features that
6 basically uses human cells in a xenotransplantation
7 model.

8 So let me tell you a little bit about how
9 these studies were performed. What we have here is
10 we have human cells that are then transduced with
11 the vector and then we inject the cells IV into the
12 mice, and then they distribute throughout the
13 animal. The human cells distribute throughout the
14 organs of the animal.

15 Then, at various time points, we are
16 harvesting the organs and then undergoing PCR to
17 detect for the presence of vector. So the days
18 that we are looking at here is Day 2, immediately
19 after infusion where that is your positive control,
20 if you like, where you would see a lot of vector.
21 You would see the distribution of your vector-
22 containing cells in the animal.

23 Then we looked at Day 30, Day 90 and Day
24 131. Over this period of time, the human cells are
25 dying in the animal. That is the useful nature of

at

1 this model is because, as the cells die, you can
2 look for events of autonomous vector mobilization
3 into mouse tissue.
4 So what we are doing here--
5 DR. SALOMON: Boro, may I just ask one
6 quick question.
7 DR. DROPULIC: Please.
8 DR. SALOMON: These cells, now; were these
9 activated with--
10 DR. DROPULIC: This are from the pilot
11 lot. These are exactly those cells.
12 DR. SALOMON: So these got the 100 units
13 per ml of IL2 and the whole shtick.
14 DR. DROPULIC: These are exactly that lot.
15 DR. SALOMON: Okay. Fine.
16 DR. DROPULIC: So what we are trying to
17 analyze is for the presence of vector in murine
18 tissue by DNA PCR. So the murine tissue contains
19 human cells and then the vector signal will either
20 be due to the vector being present in the human
21 cells or, if some adverse mobilization event is
22 occurring, the vector would mobilize and then go
23 into the mouse tissue. That is the adverse event.
24 We are only looking for a single event.
25 We know that HIVs cannot replicate in mouse tissue.

1 The application of this model system occurs totally
2 within the human cells, the infused cell product.
3 What we are looking for is a single event of
4 mobilization, adverse mobilization, into the mouse
5 tissue.
6 So, if an RCL-like event of the vector is
7 detected, that means if you detect vector in the
8 absence of a signal to a human marker gene, that
9 would be an adverse event. Now, the reason why we
10 used human CART is because--you can't use actin
11 because there is 100 percent homology between human
12 and mouse actin. You have to use something where
13 you can find some primers to be able to
14 distinguish.
15 Hu CART has these regions of this homology
16 so you can specifically amplify whether you have
17 got human cells left in the mouse tissue.
18 [Slide.]
19 So what are the advantages of using this
20 mouse for biodistribution and toxicity studies?
21 First of all, we are introducing human cells which
22 is what we will be doing in the clinic containing
23 HIV vector into an animal model. It is difficult
24 to conceive of other animal models. This is nice
25 because it is immunodeficient and allows for the

at

1 persistence of these human cells in an animal
2 setting.
3 The injected human cells--this is a nice
4 feature. The injected human cells survive for long
5 periods of time in the animal permitting
6 amplification of an adverse event in the human cell
7 population that is resident in the animal.
8 We have got two windows of amplification
9 of an RCR event here. One window is when the cells
10 are amplified ex vivo in Bruce's facility during
11 the ex vivo expansion process. Once you inject the
12 cells, the cells persist. Again, if there is an
13 RCR event that allows for that amplification step
14 to take place within the human cells.
15 Then our final readout is if there is any
16 one single event that just integrates into the
17 mouse tissue. That is what we are looking for.
18 Another feature is that the human cells do
19 eventually die, permitting visualization of adverse
20 events in whole tissues by PCR. So if the human
21 cells would survive indefinitely, you would never
22 be able to discriminate between a signal that was
23 in the human cell compared to a signal that was
24 then mobilized into the mouse tissue.
25 The fact that they die means that you can

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1 look for those events. The HIV vectors can
2 transduce murine cells efficiently, one event,
3 which is likely sufficient for the detection of an
4 overt adverse event. Sensitivity of this assay may
5 be an issue. We acknowledge that. What we are
6 looking for is an overt adverse event in mouse
7 tissues despite the lack of productive HIV
8 infection in the animal cells per se.
9 [Slide.]
10 This is just to show you that murine
11 hemopoietic cells are efficiently transduced by HIV
12 vectors. These are human bone-marrow cells
13 transduced with an HIV vector with a very low MOI
14 of 2 and than analyzed 13 days later. We are
15 getting a 73 percent transduction efficiency.
16 So it validates the fact that, if that one
17 event would occur, you could pick it up. It does
18 transduce the cell, murine cell.
19 [Slide.]
20 This shows you a little bit about our
21 study design. Basically, these are the animal
22 groups. These are the days that we killed the
23 animals and isolated the organs. The first group
24 is just an infusion media control. The second
25 group is a mock transduced control cells. These

1 are cells that do not contain the vector.

2 The third group is vectors, transduced

3 cells, at low dose, 3 by 10^5 cells per mouse. Then

4 the fourth is vector-transduced T-cells at high

5 dose, 2 by 10^7 cells per mouse. These are the

6 number of mice that we used.

7 [Slide.]

8 So what I would like to do is just to show

9 you example PCR data and then a summary slide of

10 the data. What we are looking at here first is two

11 days post-injection of the control cells. So these

12 are cells that don't contain the vector. What we

13 are looking for here specifically is the G-tag

14 sequence, this unique sequence that is present in

15 our vector by PCR.

16 This is the G-tag sequence, this unique

17 sequence that is present in our vector by PCR. So

18 this is the G-tag. This is the positive controls

19 here. Then what we have is this is an example of a

20 spleen, DNA from the spleen from two animals. Then

21 what we have here is we have two test articles

22 without a spike, and then the third one is spiked

23 with 50 copies of DNA.

24 As you can see, we can detect, by the

25 spiked control, validating the sensitivity of the

at

1 assay and, in these control cells, we don't see any
2 signal, specific signal for the G-tag sequence.
3 Obviously, there is no vector in the cells so you
4 would not expect to see the G-tag sequence in these
5 cells.

6 [Slide.]

7 Then this is an example of data of murine
8 tissues two days post-injection of cells transduced
9 with our vector. Again, this is a specific band
10 that we are looking for. You can see here that it
11 is positive, the spiked control and the no-spike
12 test articles were positive, showing that the
13 tissues contained vector-containing cells.

14 We know that the tissues from this data--
15 we know the tissues contain vector. What we now
16 have to do is PCR for a human specific sequence to
17 see whether that signal is due to human cells or an
18 adverse mobilization event.

19 [Slide.]

20 This is to show you when you do hu-CART
21 analysis of these samples at Day 2, transduced
22 cells, that they are positive. So this is the hu-
23 CART band, positive control, negative control. And
24 these are those two tissues that you saw that are
25 positive for hu-CART showing that the bands that

at

1 you saw of VRX496, the vector, are due to human
2 cells. That is what you would expect at Day 2,
3 right after injection.
4 DR. MULLIGAN: One question. I am missing
5 how you link this to the--why couldn't both things
6 be occurring? I don't get that.
7 DR. DROPULIC: I'm sorry; say that again.
8 DR. MULLIGAN: Why couldn't both events be
9 occurring; that is, you are getting mobilization in
10 your human cells.
11 DR. DROPULIC: You can't detect that. You
12 can't detect that in this animal. What you are
13 looking for is animals, that when the cells have
14 died off, the human cells have died off, if that
15 event has occurred, it would have integrated into
16 the human cells and then that is when you are
17 looking for it.
18 DR. SALOMON: At Day 2, you cannot make
19 the conclusion you just made. At Day 2, you have
20 surviving human cells in a compartment and you get
21 a PCR signal for the hu-CART. So that tells you
22 you have surviving human cells.
23 DR. DROPULIC: Correct.
24 DR. SALOMON: At Day 2, you also find the
25 VRX496.

at

1 DR. DROPULIC: Right.

2 DR. SALOMON: That doesn't mean it is all
3 in the human cells in that compartment. It could
4 be in mouse cells in that compartment.

5 DR. DROPULIC: All right.

6 DR. LI: In order to answer you and Dr.
7 Mulligan's question, you have to use a specific
8 technology called in situ amplification. So you
9 have to see your human signal coincide with the
10 vector sequence. But that technology has not been
11 developed. In situ PCR does exist, but it is a
12 tremendous problem.

13 DR. SALOMON: There are other ways to do
14 it. I mean, one of the ways to do it is to use
15 ratios like we published and GTI, our collaborators
16 at GTI published, when we did this because we had
17 the same concerns in xenotransplantation infection
18 where you could have a situation where you had pig
19 cells chimeric in compartments that were also
20 expressing porcine endogenous retrovirus. That is
21 all published. We don't want to spend ten minutes
22 talking about that. You can find that in papers in
23 Science and Nature. It would be another strategy
24 here.

25 DR. LI: We know such a problem will be

at

1 existing because this is not in situ technology.
2 You disrupt the tissue. You can see both signals.
3 You cannot say--in vivo location they are together.
4 But, at the current technology we can do, this is
5 the best.
6 DR. DROPULIC: The way the assay is
7 designed is to look for those events later when the
8 human cells die off. Point taken.
9 [Slide.]
10 So this is a summary of the Day 2 data.
11 Basically, you can see that these are the groupings
12 here. These are the tissues that we analyzed,
13 heart, testes, ovary, liver, lymph node, blood,
14 tail, spleen, lung, bone marrow. These are the
15 groupings. These are the animal groupings that
16 received the cells, either low dose or high dose.
17 As you can see, most of the animals
18 contain both the vector signal. Whenever we saw a
19 vector signal, we always saw a concomitant human
20 signal as well. Blood, in certain cases, was
21 difficult. These assays generally fail because of
22 sampling size but we had no problems in terms of
23 all the other organs.
24 [Slide.]
25 SO now we go on to Day 30, thirty days

1 post-injection. What we see here is that the human
2 cells--what we see here is that certain tissues are
3 lighting up positive for vector and certain tissues
4 are not.
5 This is an animal here. This is the
6 spiked control. You can see that, in this
7 particular sample, you can detect the vector
8 sequence. In this particular sample of DNA, the
9 spiked control validates the sensitivity of the
10 assay and there is no presence of vector-containing
11 sequence in this particular sample.
12 [Slide.]
13 So the summary of the data. We took those
14 samples that were positive for the vector and then
15 did Hu-CART analysis on these samples. In every
16 case, whenever there was a positive signal for the
17 vector, we also saw a concomitant positive signal
18 for Hu-CART.
19 [Slide.]
20 This is the summary of the Day-30 data.
21 You can see that basically the numbers of samples
22 that are lighting up positive for the vector is
23 decreasing. But whenever you saw a sample that was
24 positive for the vector, you always saw a
25 concomitant Hu-CART-positive signal as well.

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1 [Slide.]

2 When we look at the Day-91 data, basically
3 most of the tissues are negative for the vector.
4 This is the spike controls. These are the test
5 articles.

6 [Slide.]

7 This is a summary of the data. You can
8 see that everything except four independent tissues
9 from four independent mice were negative for the
10 vector. However, in the case that, again, the
11 tissue was positive for the vector, you saw, again,
12 a concomitant positive Hu-CART signal.

13 [Slide.]

14 This is not showing up well, but this our
15 latest data, Day 123, post-injection. All the
16 tissues are now negative for the vector.

17 [Slide.]

18 Again, that is just the complete data.
19 Everything now has turned negative.

20 [Slide.]

21 So, a summary of the animal by
22 distribution. Toxicity data is that infused human
23 T-cells containing vector could survive for long
24 periods, up to 91 days, in these SCID mice. All
25 the tissues studied with the G-tag vector signal

1 was associated with a concomitant Hu-CART signal
2 for human DNA.
3 So the interpretation is, and this was
4 just discussed, I suppose, that the G-tag signal is
5 due to vector-containing cells. That is the
6 extrapolation I would make.
7 A total of six animals from the study
8 displayed clinical manifestations that were not
9 treatment related. There were some clinical
10 manifestations but they were all, by a certified
11 pathologist, shown to be not as a result of the
12 test article. And no adverse RCL-like events were
13 observed.
14 [Slide.]
15 Now a little bit about the manufacturing
16 process for the vector. We used certified 293
17 cells, transfected in NUNC-cell factories with
18 VRX496 and VIRPAC plasmid DNA constructs using a
19 calcium-phosphate precipitation method. The vector
20 is then purified using ultrafiltration,
21 diafiltration and column chromatography.
22 The plasmid raw materials and the purified
23 vector preparation is prepared at VIRxSYS's
24 manufacturing facility using GMP conditions. We
25 both have BL3 labs and Class 10,000 Labs. The

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1 Class 10,000 Labs are used for manufacturing of the
2 vector.
3 Then the cell processing will be performed
4 at the University of Pennsylvania Hospital's
5 Clinical Cell Production Facility using GMP
6 conditions with Carl June and Bruce Levine
7 [Slide.]
8 So there are two steps; making the raw
9 material, the plasmids, and then using those
10 plasmids, then, to transfect into cells to produce
11 the vector. This is routine. Basically the
12 plasmid manufacturing process; you culture the
13 bacteria. You centrifuge them down.
14 [Slide.]
15 I am not going to belabor on this too
16 long. I just want to give you a feel for it. The
17 cells are lysed. They undergo filtration.
18 [Slide.]
19 Then the plasmid is purified, centrifuged
20 and then filtered, stored and then QC testing is
21 performed on the plasmid.
22 [Slide.]
23 When it passes QC testing, then it can be
24 released for use in vector manufacturing. The
25 plasmids are one of the raw materials for the

1 vector-manufacturing process.

2 [Slide.]

3 The vector-manufacturing process; cells
4 from a 293 master cell bank are thawed and
5 expanded, five passages to 16 cell factories. The
6 293 cells, then, are transfected with vector and
7 helper plasmid DNAs using calcium phosphate
8 precipitation. The bulk harvest, the viral vector
9 harvest, is the medium and they are collected at
10 24, 36 and 48 hours.

11 The vector-containing medium is stored at
12 2 to 8 degrees until 60 hours post-transfection.
13 The vector then undergoes filtration and
14 concentration. Then the product is subsequently
15 concentrated via ultrafiltration.

16 [Slide.]

17 Then the vector undergoes diafiltration
18 and benzonase treatment to remove cellular host DNA
19 and also the plasmid DNA from the transfection
20 process. The vector is then purified using size-
21 exclusion chromatography and stored at -20 pending
22 the results of in-process tests.

23 [Slide.]

24 Then, finally, the vector is formulated to
25 storage. We can store this vector at -20 degrees

1 for over six months and then basically it undergoes
2 a lot of QC testing before it is released by QA for
3 use in the cell-processing part of that process.
4 [Slide.]
5 Just a couple of data slides. Our vector
6 purification; we can purify from our bulk harvest
7 to our chromatography step 1000-fold. It is 1000-
8 fold purification.
9 [Slide.]
10 This is just to show you a gel here. So
11 this is what the vector looks like after the bulk
12 harvest. We are validated by Western blot, but
13 this is a VSV-G band. This is p24. This is p17.
14 This is what it looks like after bulk harvest,
15 after diafiltration. And this is the final
16 product, so it cleans it up very nicely.
17 [Slide.]
18 After the product is made and there are a
19 bunch of QC tests. There is a whole battery of QC
20 tests that really need to be performed but I think,
21 for the purposes of here, I just really want to
22 talk about the RCT assays and the detection of an
23 RCL because I think that is very important. I
24 think that is the critical thing in this protocol,
25 the assays and the detection of a putative RCL.

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1 What we will do for the final--after
2 production of the vector from the 293 cells, we
3 will take both the bulk harvest and the end-of-
4 production 293 cells and run them through and RCL
5 assay.
6 So let's go through the bulk harvest. The
7 bulk harvest will take out, and basically will
8 infect the bulk harvest on H9 cells using the
9 specifications, the guidelines, recommended by the
10 FDA and then passage it for six times to amplify
11 any potential RCL.
12 Then use TaqMan RT PCR on the supernatant
13 to detect HIV gag and VSV-G in that final sixth
14 passaged supernatant. So we will also take the
15 end-of-production 293 cells, co-cultivate them with
16 H9 cells, the correct amounts, passage that for six
17 times and, again, perform RT PCR, TaqMan RT PCR
18 using both gag and VSV-G primers.
19 Negative results will mean that the vector
20 can be released for use pending other QC tests. If
21 it is positive--we haven't seen this to date--but
22 if a lot would become positive, we would obviously
23 not release it and fully characterize it.
24 Our sensitivity of our RT TaqMan PCR is
25 HIV gag, is 10 copies per input volume. For VSV-G,

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1 it is 10 copies per input volume. The overall
2 assay sensitivity is that we believe or have
3 extrapolated that we can detect the HIV with a
4 fitness of 1 percent of NL4-3.
5 The next slide will show you how we came
6 to that calculation.
7 [Slide.]
8 This tells you a little bit about the
9 sensitivity of our assay. What we have done here
10 is we take the cells and then we start either
11 cocultivating or incubating the supernatant in H9
12 cells and then we are passaging, and we are
13 splitting the cells as we are passaging.
14 What we have found out is that if we have
15 taken one infectious unit of wild-type HIV and
16 passaged it, that, after three passages, one
17 particle comes up positive by p24. What we will do
18 is, in addition to that, we are going to
19 additionally passage the culture for another six
20 times.
21 Given that this is 100-fold expansion of
22 the H9 cells, we are extrapolating that this is
23 giving us 100-fold sensitivity over our positive
24 control which, in this case, is wild-type HIV.
25 The problem with positive controls is that

1 nobody wants to make a VSV-G recombinant HIV. Even
2 if you would, you don't know whether that would be
3 the event that would ultimately be seen. So our
4 approach has been just to use wild-type HIV and
5 then increase the sensitivity of the assay by
6 passaging for longer to try to pick up the event.
7 And p24 and RT PCR will be used for the virus
8 detection. In this case, we just used p24.
9 [Slide.]
10 Another thing, one of the questions,
11 should an in vitro for the detection of functional
12 gag/pol LTR be used as a lot-release assay. First
13 of all, I would like to say that this assay may
14 have utility for HIV vectors in non-HIV disease
15 applications. However, HIV disease, the final
16 product, already contains these types of events in
17 abundance. There is wild-type HIV there. So we
18 don't necessarily see the relevance for our
19 particular case although it certainly may have
20 relevance for other situations.
21 Also, another thing to consider is that
22 those events that you have seen, we have found that
23 when we construct vectors with those types of
24 events, they are actually more efficient in
25 inhibiting wild-type HIV than our fully gutting

at

1 vector. So the presence of such an event in a
2 final product may not be a detrimental one in AIDS
3 gene transfer.
4 [Slide.]
5 So what is our cell-processing procedure?
6 Again, Bruce and Carl are doing that. We are
7 providing our vector to their facility. They run
8 the cell processing at U. Penn. The patient
9 undergoes leukophereses and T-cell selection. I
10 think Bruce has already described the process but I
11 will just briefly go through here. The cells are
12 transduced with the vector in presence of
13 immobilized CD3, CD28, antibodies. The beads are
14 removed. The cells are washed and concentrated.
15 [Slide.]
16 Then the cells are formulated and then the
17 cells will undergo QT testing. The cells are
18 frozen. That is the nice thing about this whole
19 procedure is that we can freeze the cells, perform
20 the QC testing before releasing the cells to use in
21 the clinic.
22 [Slide.]
23 So, again, what are the important assays
24 for an RCL detection. Obviously, this is now the
25 final product. These are the cells transduced with

1 the vector and this is what we really have to
2 examine very carefully for a putative RCL. So,
3 other than all the other assay, these, we believe,
4 are the critical ones.
5 We look at it at two levels. One, we will
6 perform a biological type RCL assay. The second is
7 a molecular detection type RCL assay to detect for
8 any residue of VSV-G DNA that may be present in the
9 final cell product.
10 So we have our ex vivo transduced and
11 expanded T-cells and we will take both the
12 supernatant and the transduced cells and undergo
13 these tests. So let's look at the transduced cells
14 first.
15 We take the transduced cells and we will
16 run through the biological assay. The biological
17 assay is now we will take the cells, cocultivate
18 them on 293 T-cells because H9 cells would be
19 permissive to wild-type HIV and kill the culture,
20 so we want to use a CD4-negative cell line in this
21 case. We will take the correct amounts, passage it
22 for six times and then look, by TaqMan PCR, on the
23 amplified supernatant using particularly VSV-G
24 primers.
25 The transduced cells we will also take

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1 directly and undergo DNA PRC directly on those
2 cells to look for any residue VSV-G that may be
3 present. If the results are negative, then we will
4 release the cells. However, if the results are
5 positive, we will not release it.
6 Obviously, if there is anything going on
7 here, we will fully characterize the event. We
8 will also take the supernatant from these ex vivo
9 transduced cells. We will infect them onto 293 T-
10 cells, passage them for six times and again, the
11 passage supernatant will undergo TaqMan PCR to
12 detect for any potential event using VSV-G-specific
13 primers.
14 The supernatant we will also directly take
15 and perform RT PCR to look for any VSV-G signal
16 that may be present in that final supernatant.
17 Again, if it is negative, we will release it. If
18 it is positive, we will not release and we will
19 characterize.
20 Our sensitivity assays for VSV-G DNA
21 detection; we can see our assay down to 1 copy
22 sensitivity. But because of the issue of false
23 positives over sampling size, we are now saying
24 that we can definitely detect our detection limit
25 to be 10 copies per 10,000 cells. And I have

at

1 already mentioned that.

2 [Slide.]

3 Just to show you that we can get rid of

4 the VSV-G DNA in our final cell product, what we

5 have taken is basically the cells of the

6 transduction. Before the wash, we do have a very

7 low residue of VSV-G DNA in the cells. However,

8 when the cells are washed, we can get rid of this

9 DNA. At least, it is below the detection limit. I

10 don't want to say that there may not be absolutely

11 no VSV-G DNA present in that preparation, but it is

12 below our detection limit by TaqMan PCR.

13 So this is two independent experiments.

14 Before wash, we do see some residual VSV-G DNA.

15 After wash, it is below the detection limit.

16 [Slide.]

17 So a summary of our proposed clinical

18 trial. We are taking patients that have no good

19 therapeutic options left. They are failing or

20 discontinued therapy. They do not have

21 opportunistic infections. They have a CD4 count of

22 between 200 and 600 and a viral load of greater

23 than 5000 and they demonstrate X4 strain of HIV.

24 That means, they are more advanced in their

25 progress to disease.

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1 The patient comes in. Twelve patients.
2 Basically the T-cells are isolated by a
3 leukopheresis procedure. The vector is produced.
4 When it is released by QC, then it can be
5 transduced onto the cells. It undergoes an ex vivo
6 transduction and expansion process. Then the cells
7 are frozen down.
8 After the frozen-down cells are tested for
9 QC and then released, then the cells can be
10 released for infusion into the patient. Our dose-
11 escalation scheme starts off with a very low dose
12 of cells. The first dose, there will be only one
13 patient at that dose, the first dose escalation.
14 Then the next escalation will be the other
15 two patients at this first 10^9 dose. Then we will
16 dose escalate in patients of three up to 3 by 10^{10}
17 T-cells infused into the patient.
18 DR. SALOMON: Can you make one thing clear
19 to me. You started out--what you called patient
20 scale was 1 times 10^9 which is about a unit of
21 blood. Then you activated and these cells
22 proliferate.
23 DR. DROPULIC: 50-fold or so expansion.
24 DR. SALOMON: But you are only giving 1
25 times 10^9. I guess what confused me is in these

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1 early ones, are you not going to do any activation
2 and proliferation or every time they are going to
3 be activated and proliferated just like they were
4 described and cultured for X number of days? How
5 long? Is it going to be longer if you want more
6 cells?

7 One of the ways to interpret something in
8 the protocol is that you would count every other
9 day or something and stop it when you got to the
10 right number of cells which means that patients
11 later in the trial at higher doses would get cells
12 that had been in culture for longer. That seemed
13 like a very awkward trial.

14 DR. LEVINE: I can tell you the cells will
15 be stimulated and then expanded in the same way for
16 every subject at every dose. From an average
17 leukopheresis, we have 50 to 100-fold times more
18 cells than we would need for the given dose. So we
19 freeze the excess cells at two points; after the
20 monocyte depletion and then, if we have excess
21 cells after the expansion, we freeze those cells.

22 That is useful if something were to go
23 wrong in the culture or the transduction. Then we
24 have these cells frozen. After the monocyte
25 depletion, we can do a second transduction, expand

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1 the cells frozen from post-monocyte depletion.
2 They behave in exactly the same way and then that
3 could be used for infusion as well.
4 DR. SALOMON: So, just as a bottom line,
5 how many days will these cells be in culture with
6 activating antibodies and interleukin 2 before you-
7 -
8 DR. LEVINE: Eight to ten days.
9 DR. TORBETT: I have a naive question.
10 These are from HIV-infected patients. What is
11 just, on the average, the number of T-cells that
12 are infected and, if you activate the cells and it
13 is a low-level infection, would that virus spread?
14 Are you going to include antiretrovirals during the
15 cultures situation?
16 DR. LEVINE: Again, it depends on the
17 viral load. I think on the order of 1 out of
18 10,000, 1 out of 50,000, cells would be infected.
19 In someone with a CCR5 virus, we have shown that
20 CD28 simulation downregulates CCR5 and upregulates
21 the beta chemokines and that there is a diminution
22 to below detection of HIV in CCR5 patient cells.
23 CXCR4; that is not the case. What we have
24 been able to do is to demonstrate that in the
25 CXCR4-positive patient, if we add antiretrovirals

at

1 to the culture after the transduction that we can
2 suppress the virus.
3 DR. TORBETT: Thank you.
4 [Slide.]
5 Next slide.
6 DR. DROPULIC: So patient monitoring; we
7 are going to perform patient monitoring both early
8 and late. Let me just take you through. There
9 will be patient monitoring, samples taken at 24
10 hours, 48 hours, 72 hours, one week, two weeks, 28
11 days, three months, six months, a year and then
12 yearly for life.
13 I am just giving you one example here, the
14 28 day, because that is where we are proposing our
15 dose escalation to occur after the 28-day period
16 sample is processed.
17 I will just go through the ones that are
18 important. Basically, we will perform, obviously,
19 CD4 counts. Basically, we will also perform this
20 differential viral-load assay. This is kind of
21 important because what we will be doing here is
22 looking for mobilization of the vector. We will
23 have a PCR assay where we will be comparing the
24 amount of wild-type HIV RNA compared to, if there
25 are, any vector RNA present in the serum of the

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1 patient.

2 That is what we mean by a differential

3 viral load. We will also perform some

4 immunological assays. This is very important.

5 Basically, we will also have a TaqMan PCR assay too

6 to look for VSV-G RNA in the plasma. If we start

7 detecting that guy, and it is a consistent result,

8 then we will immediately end the trial because this

9 would be like a red flag to say, hey, there is some

10 sort of VSV-containing RNA that is replicating in

11 this patient.

12 In that case, the patient will undergo

13 leukopheresis and then we will fully characterize

14 the event. So that is a red flag there.

15 We will look for the VSV antibody

16 response. We will look for the repertoire, of the

17 T-cell repertoire. We will also monitor for the

18 phenotype and genotype using the drug-resistant

19 profile assay kits that are available to see

20 whether there is any change in the virus phenotype

21 with respect to drug resistance.

22 Then there are other chemical type assays

23 that we will also perform.

24 [Slide.]

25 So, I again reiterate--I should have put

1 this slide before the other slide--patients will be
2 monitored for the short term, 24, 48, 72 hours; 7,
3 14, 28 days and long-term; 3, 6, 12 months and
4 yearly for life.
5 The dose escalation would proceed after 28
6 days. This is our rationale, because most of the
7 short-lived activated T-cells would have died
8 within a few weeks. These are the cells that are
9 most capable to support wild-type HIV replication
10 or the replication of the putative HIV recombinant.
11 Long-lived cells, on the other hand, are
12 normally quiescent and, during their quiescent
13 state, they are not capable of supporting HIV
14 replication. However, they could support HIV
15 replication upon their sporadic activation with
16 antigen. That is why we have a long-term follow-up
17 scheme as well.
18 Since activated T-cells are most abundant
19 immediately after infusion because we are using an
20 immobilized CD3-CD28 approach to activate and
21 expand the cells, the greatest risk for an adverse
22 event, we believe, is short-term. So that is why
23 we have done the dose-escalation scheme that we
24 have proposed.
25 [Slide.]

1 So, in summary, HIV vectors can transduce
2 at greater than 90 percent transduction
3 efficiencies. We can inhibit wild-type HIV
4 replication by over 99 percent and provide CD4 T-
5 cells with what appears to be a selective
6 resistance to productive HIV infection.
7 Our vector, we believe, is the safest
8 vector for this type of trial. It is a fully
9 gutted vector. There are no novel sequences. We
10 are not putting in a CMV promotor or something else
11 into the vector. Even the antisense payload is
12 entirely derived from wild-type HIV. The backbone
13 of the vector is derived from highly conserved
14 sequences.
15 We have shown VRX does not mobilize beyond
16 its target tissue. In vitro and in vivo studies
17 showed poor mobilization occurs only between CD4 T-
18 cells.
19 Our vector-production methods use vector-
20 packaging systems that we believe are comparable to
21 those used in other gene-transfer studies. Our
22 animal by-distribution toxicity studies show the
23 vector to be safe, we believe.
24 [Slide.]
25 Our final cell-product release-testing

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1 criteria are highly stringent; no detection of VSV-
2 G DNA in the final cell product, no detection of
3 VSV-G RNA in the final cell supernatant, no
4 detection of an RCL after biological amplification
5 in a highly permissive human cell line, and
6 detection by TaqMan PCR.
7 Treating HIV individuals with advanced
8 disease, we believe, that have no good treatment
9 options left affords the lowest risk for testing an
10 HIV vector and the highest chance--I am not saying
11 at the low doses anything will happen, but the
12 highest chance for benefit, particularly at the
13 high doses.
14 Drug-therapy failure due to toxicity is
15 common and viral resistance to these drugs is
16 increasing. So there is a real need for new
17 approaches for the treatment of HIV infection. We
18 believe that biological control using HIV against
19 itself may offer new treatment opportunities for
20 individuals with AIDS.
21 [Slide.]
22 Would like to thank particularly the
23 VIRxSYS team. They are really a great bunch of
24 people. The reason why we have been able to
25 progress so rapidly is because of their talent and