1 but I think once you have good regulatory systems
2 available, easy to use, I suspect most people will
3 probably tend to do that.
4 DR. SALOMON: I have no problem with that
5 except carefully looking at what regulatable
6 systems are out there right now and the amount of
7 general concern that these regulatable systems
8 won't function as well as one would hope.
9 I think it is a big issue when one says,
10 look, there is a lot of major questions for gene
11 therapy right now, and then if one predicates a
12 gene therapy on top of that, proving that a
13 regulatable system works, that's, you know, adding
14 a whole another layer of complexity. I mean should
15 you be doing a lentiviral vector gene therapy in
16 the brain where you need to have the regulatable
17 system work as well as the lentiviral system work.
18 DR. SAUSVILLE: Isn't the whole issue
19 related to criteria of proof? I mean, in other
20 words, at one level we have proof, it's up, it's
21 down. I mean if you ask at the level of proof,
22 does this have functional consequences in the long
23 term, I am not aware of any treatment that the FDA
24 regulates that you have to sort of prove that you
25 have an antidote to what you are giving. You may

1 want to comment on this.

2 DR. SALOMON: That would be an argument

3 not to require regulatable promoter.

4 DR. SAUSVILLE: The issue of requiring and

5 saying it's a good idea, and that is I think the

6 distinction.

7 DR. SIEGEL: I am not sure what the list

8 of precedence is, but I think it would be fair to

9 say for most of the products given, if there isn't

10 an antidote, you can just stop giving them, and

11 there are different considerations when you can't

12 do that in terms of the implications.

13 That doesn't necessarily mean that there

14 is a requirement that if you can't do that, you

15 have to have some other mechanism to turn the

16 product off, but it does raise safety concerns that

17 need to be addressed.

18 DR. NOGUCHI: In addition to that, just

19 the ability to turn things on and off is as yet

20 untested in any gene therapies clinically. We

21 don't know if the supposed cure for an

22 inappropriate secretion might even be worse, so

23 there is always that caveat with any of these

24 systems.

25 The more complex you make them, the more

1 opportunity you have for nature to reassert
2 herself.
3 DR. ALLAN: Two things. One is, and I
4 don't remember it, does tetracycline cross the
5 blood-brain barrier? It does, okay.
6 The second thing is in the areas where you
7 injected the virus, and you only saw expression in
8 the cells that you wanted to see, the other
9 cells--it comes actually to another question that
10 someone else had--but in the other cells, are you
11 getting expression, but it is just those cells you
12 get accelerated turnover of the protein? In other
13 words, are you still getting expression by the
14 proteins turning over, so you are not seeing it?
15 DR. KORDOWER: I think it has to do with
16 insensitivity of the antibody to detect it.
17 DR. ALLAN: So, you think it is just low
18 level expression rather than turnover?
19 DR. KORDOWER: Right.
20 DR. RAO: If I can add to that whole idea
21 of regulatable things, even in trophic factor
22 system, it is important to remember, as Dr.
23 Kordower said, that the effect of GDNF is also to
24 cause anatomical changes. There might be neuron
25 outgrowth, there might be better connections that

1 have taken place. Even if you dial off the GDNF at
2 the end, it doesn't mean that you have reverted
3 back to normal.
4 So, it is not necessarily either an
5 absolute requirement or it is not necessarily a
6 cure either way to necessarily say that we have a
7 regulatable system we have changed the underlying
8 situation.
9 DR. SALOMON: Later this afternoon, we are
10 going to talk about animal models, and I think this
11 is very valuable in that you have done some
12 wonderful work in developing an animal model here,
13 so the question I had was I guess it always makes
14 me a little nervous in thinking about validating an
15 animal model when you say, you know, there is
16 absolutely no toxicity.
17 I would almost be happier if you could
18 say, in terms of validating, that there was
19 toxicity and, you know, we did this and that and
20 avoided it. I guess it seems to me that one
21 element in validating an animal model is to
22 demonstrate that you can under some circumstance in
23 that model develop toxicity.
24 DR. KORDOWER: We are interested in doing
25 dose escalation studies, and those will

1 presumptively, some high dose will cause some
2 toxicity, so we might be able to address it in that
3 study.
4 DR. RAO: I guess it is not directly
5 related to the virus, but I was curious that
6 despite having milligram quantities of protein--
7 DR. KORDOWER: Microgram.
8 DR. RAO: --microgram quantities of
9 protein, that the improvement was not back to
10 baseline in terms of behavior improvement. Do you
11 have any--
12 DR. KORDOWER: On the objective hand reach
13 task, if you cull out the animal that didn't
14 recover, the other animals were all back to normal.
15 On the rating scale, difficult. Other people have
16 asked me the same question, I don't really have a
17 good answer for you, but you are right, on the
18 rating scale they weren't back to zero, but they
19 were good.
20 DR. MULLIGAN: How about that one animal?
21 You never told us what happened to it.
22 DR. KORDOWER: Well, what happened was he
23 had complete neuroprotection at the level of the
24 nigra, but trivial neuroprotection at the level of
25 the striatum, and I think as I mentioned earlier,

ajh

1 what happened in that animal, given the lesion and
2 the fibers regressed too quickly for the GDNF to
3 stop it.

4 In fact, there was this sprouting response
5 in the globus pallidus of that animal where we
6 couldn't encourage the regrowth back into the
7 striatum.

8 DR. SALOMON: Go ahead.

9 DR. VERMA: I just want to know when can
10 we have lunch.

11 [Laughter.]

12 DR. SALOMON: You beat me on that one.
13 What I was going to say is I think it is time for
14 lunch. Yesterday, we made it in under 45 minutes,
15 so if we can try and back here in about 35 or 40
16 minutes, we will get started.

17 Thank you.

18 [Whereupon, at 12:45 p.m., the proceedings
19 were recessed, to be resumed at 1:30 p.m.]

1 AFTERNOON PROCEEDINGS

2 [1:40 p.m.]

3 DR. SALOMON: I suppose we have still got
4 one or two people who are out to lunch.

5 [Laughter.]

6 DR. SALOMON: I couldn't resist that,
7 forgive me.

8 There are two announcements. The first is
9 that Marina O'Reilly will be representing OBA in
10 place of Amy Patterson, who had prewarned us that
11 she had an afternoon meeting, and she will join us
12 at the table.

13 The second announcement that I am
14 personally disappointed at is I guess Dr. Cornetta,
15 Ken Cornetta, from Indiana University and Director
16 of the National Gene Vector Laboratory there, was
17 unable apparently because of weather to get out of
18 Indianapolis. I think that is the loss to the
19 Committee.

20 But using the latest in technology, I
21 understand that he has also joined us by telephone,
22 so perhaps just to test that connection, Ken, are
23 you there and can you introduce yourself to us?

24 DR. CORNETTA: This is Ken Cornetta. I
25 can hear you, Dan. Hopefully, you can hear me.

1 DR. SALOMON: Actually, we hear you fine.
2 Everyone at the beginning sort of gave a quick
3 two-sentence thing. Can you introduce yourself?
4 DR. CORNETTA: I am Ken Cornetta from
5 Indiana University. I am a Professor of Medicine,
6 trained in hematology/oncology, and also have been
7 interested in retroviral and now lentiviral vectors
8 and their use clinically. For the past about six
9 years now I have been coordinating the National
10 Gene Vector Lab, which is funded through the NIH,
11 and its goal has been to produce clinical grade
12 vectors for academic investigators performing
13 clinical gene therapy protocols.
14 Indiana has been the center for production
15 of retroviral vectors, so I have been keenly
16 interested in the discussion here today in regards
17 to lentiviral vectors.
18 DR. SALOMON: Thanks for joining us, Ken,
19 and like I said, I only regret you are not here
20 personally along with Dr. Emerman.
21 Dr. Emerman, are you still on?
22 DR. EMERMAN: Yes, I am still here.
23 DR. SALOMON: Okay. One of the things,
24 Dr. Emerman, you could reassure me is, are you
25 comfortable in jumping in, because that same issue

1 is with Dr. Cornetta, it is a little hard, as
2 chair, to stop and ask for the telephone all the
3 time as I forget, but can you jump in, and will I
4 be able to year you?
5 DR. EMERMAN: I don't know, we can try.
6 DR. SALOMON: Don't be inhibited, either
7 of you.
8 DR. EMERMAN: Okay.
9 DR. SALOMON: It is my pleasure to
10 announce that the first talk of the afternoon is
11 from Dr. Susan Kingsman of Oxford BioMedica,
12 Lentiviral Vectors for the Treatment of Cancer,
13 Neurodegenerative Diseases and AIDS.

14 **Lentiviral Vectors for the Treatment of Cancer,**
15 **Neurodegenerative Diseases and AIDS**
16 **Dr. Susan Kingsman**

17 DR. KINGSMAN: Thanks very much for
18 inviting me to come and talk. Oxford BioMedica is
19 a publicly quoted UK company, and we have got a
20 subsidiary in San Diego called BioMedica, Inc.
21 headed up by Doug Jolly, who is in the audience,
22 and is familiar to many of you.
23 This morning, Dr. Salomon said he was
24 hoping to perhaps see if there was any consensus
25 that might emerge within the field, so I am not at

1 all embarrassed that many of the slides and the
2 concepts that I am going to present to you have
3 already been presented by other people very well
4 this morning, but I think part of the process is
5 just to go over it, so I will repeat some things
6 that have been said.
7 [Slide.]
8 The Retroviridae, a nice, ancient
9 classification, lentiviruses fall within that
10 classification, but they are quite distantly
11 related from the type C viruses, but nonetheless, I
12 think it is legitimate to use the experience that
13 stretches back over at least 10 years from the
14 Mammalian C-type viruses to inform the concepts
15 that we are aiming for in trying to develop this
16 set of vectors for the clinic.
17 BioMedica currently has a clinical trial
18 with a retroviral vector in breast cancer, and Doug
19 Jolly has tremendous experience through his work
20 with Biogene and Chiron, so the collective
21 experience in taking retroviral vectors to the
22 clinic is good, and we hope to use that experience
23 to inform the way we move forward with lentiviral
24 vectors.
25 [Slide.]

1 The lentiviruses are themselves a rather
2 diverse group of viruses. This is a dendogram
3 where the pol gene is related. Now, there are
4 functional constraints on that enzyme obviously,
5 and there is some conservation between the
6 different members of the groups, but the HIV-2 up
7 here, for example, bears virtually no sequence
8 relationship outside the pol gene with something
9 like BIV.
10 They divide into two distinct sets, the
11 primate lentiviruses and the non-primate
12 lentiviruses. We have chosen to study HIV-1 as the
13 archival lentivirus, and we also decided to look at
14 a non-primate lentivirus, and we chose equine
15 infectious anemia virus as our non-primate
16 lentivirus to study. This is because it's far
17 apart from HIV, it's a non-primate virus, and this
18 particular virus does not cause an
19 immunodeficiency.
20 I think it is far too soon to say whether
21 any one lentivirus will provide a universal vector
22 for all applications. There are people that are
23 working on FIV, SIV, and I think that is very good,
24 we should explore this group of viruses and see
25 what the range of possibilities is, but you can

1 only do so much, so we have just picked two to work
2 with.
3 [Slide.]
4 Now, the one key fact that we have got to
5 be all very sure of is that there is some reason
6 for developing another type of virus for gene
7 therapy because there are lots of viral vectors out
8 there. We don't want to just go and invent and use
9 one more unless there is some real benefit.
10 I hope I am just simply going to add to
11 what other speakers have said and just reminding
12 you that they have a constellation of properties
13 which, together, make up something which I believe
14 is unique, relatively simple compared to something
15 like herpes and adenoviruses, they can carry up to
16 11 kb, so you can put a nice cargo in these
17 vectors.
18 You have a defined integration of genes.
19 You sweat in the lab to set up precise gene
20 expression configurations in therapeutic genes.
21 You know that they are going to be docked into the
22 chromosome in the same way that you invented them
23 in the laboratory, and that is a very useful,
24 important feature, especially if we are going to go
25 on to look at some more advanced applications where

1 we might need regulation.
2 This, they share with the retroviruses,
3 but here we are starting to see some differences.
4 This long-term expression increasingly we are
5 finding that we can see gene expression from
6 lentiviral vectors for much longer periods than
7 retroviruses, and then this key feature,
8 transduction of non-dividing cells, postmitotic
9 cells, and very important, the transduction of
10 slowly dividing cells. The retrovirus is going to
11 hang around in the cytoplasm waiting for the
12 nuclear membrane to break down before it can get in
13 there. Chances are it is going to get trashed, and
14 this is what happens. Lentivirus can just go
15 straight into the nucleus and deliver its cargo
16 even if the cell cycle is 48 hours, 72 hours.
17 So, I believe that these vectors do have
18 some unique advantages for long-term, stable
19 therapy of chronic diseases, and they will be
20 vectors for delivering treatments for unmet medical
21 needs.
22 [Slide.]
23 We heard this wonderful talk by Dr.
24 Kordower this morning, and I can't hope to emulate
25 it, but just briefly, if we take EIAV vectors, and

1 that is really what I am going to focus on because
2 we have had a lot on HIV this morning, you can do
3 experiments in the rat where you deliver by precise
4 stereotactic injection to particular sites in the
5 brain, and you can show that you get very effective
6 gene transfer in some important regions of the
7 brain.
8 I am not a neurobiologist, but here we
9 have regions that are important in Alzheimer's,
10 regions that are important in Parkinson's disease,
11 a region that is important in Huntington's disease,
12 a region here that is important in addictions.
13 These are data where we have pseudotyped
14 the EIAV with VSV-G, and what happens there is you
15 get a local gene expression out the site of
16 injection, and you get the gene product will
17 disseminate through the projections.
18 If, on the other hand, you pseudotype with
19 another envelope, which is from the rabies virus,
20 you find that you get gene transfer to sites that
21 are distal from the site of injection, and this is
22 where axons are projecting into the place where you
23 injected, but the cell bodies are out there, but
24 the vector goes in and travels up by what is called
25 retrograde transport and lodges into a distant

1 part, communicating part of the brain.
2 You can harness that property, for
3 example, here you can inject an EIAV vector
4 pseudotypes with rabies into the muscle where the
5 nerve endings are, and the nerve connects with the
6 spinal cord, and you can access spinal motor
7 neurons by peripheral administration.
8 Here, then, you have got an opportunity of
9 accessing these neurons, and the primary target
10 there are diseases of motor neuron degeneration.
11 So, there is clearly great potential of
12 these vectors to access particular neuronal sites,
13 and by changing the envelope, you can do different
14 things. I think another point I might make is we
15 shouldn't get too focused on VSV-G. It is what we
16 have got at the moment, most of our advances, it is
17 terribly useful, but there are other envelopes
18 coming along that may have other issues and other
19 uses.
20 [Slide.]
21 Long term gene expression, in our
22 experience also, you can see gene expression after
23 eight days, and you can see gene expression after
24 six months. We have been following animals for
25 eight, nine months now, and this gene expression

1 persists. So, that is a very good feature because
2 we can have sustained expression, minimizing the
3 need for invasive delivery.
4 [Slide.]
5 Now, we try and do some type of toxicology
6 and biodistribution in all our animals that we set
7 up as models, but, of course, ultimately, there
8 will be a proper systematic approach, but you can
9 see that we have looked at a lot of animals
10 particularly in the brain.
11 In the previous report that we heard this
12 morning, I can't say there is no inflammation, we
13 do see mild acute inflammation. It has resolved by
14 35 days, so you can't tell the difference between a
15 PBS control and the vector-injected control, but we
16 see perivascular cuffing and all the signs of mild
17 acute inflammation that you would expect.
18 We don't see histological abnormalities,
19 we don't see any overt clinical signs of
20 abnormality. We are looking at lentivectors for
21 congestive heart failure. We have done lots of
22 intramyocardial injections, again, no overt signs
23 of toxicity.
24 We have done worst case where we have put
25 vector into the tail vein, we have looked for liver

1 toxicity and damage by histology. At the moment,
2 we have not found any significant amounts of vector
3 in the liver or lung.
4 Now, the problem with these studies, as we
5 have heard before, is this is maximum feasible dose
6 at the moment, and as our production systems
7 improve, when we generate more material, we are
8 obviously going to escalate the dose and have a
9 look and see what happens, but so far, so good.
10 [Slide.]
11 This is not a theoretical exercise that
12 Oxford BioMedica is going through. We are
13 intending to develop product based on lentivectors.
14 One of them is a treatment for Parkinson's disease.
15 We have already heard that the provision of
16 dopamine can have therapeutic benefit, and this is
17 the basis for current treatments where you provide
18 the patients with L-Dopa.
19 What we have done is to configure the
20 dopamine pathway into a single lentiviral vector,
21 so we are making the three key enzymes that are
22 limiting for the production of dopamine. Here, we
23 have been staining for dopamine in the side of the
24 brain that we have lesioned with a chemical lesion
25 to abolish dopamine production, and you can see

1 that we have established a depot of dopamine.
2 We are clearly very interested to learn if
3 this will translate into behavioral correction, and
4 because the established animal model for
5 Parkinson's disease is the monkey, then, we will
6 naturally move on and look at efficacy studies in
7 the monkey.
8 So, where we need to go to a higher
9 primate for efficacy, then, we clearly will, and we
10 can do the toxicity studies, as well.
11 [Slide.]
12 The other product that we are developing
13 is a treatment for late-stage prostate cancer.
14 This is a rather conservative approach. We are
15 using a promoter that has been in the clinic
16 before. We are using a therapeutic gene, TK. We
17 have tweaked it a bit to improve its activity, and
18 we are going to go into late-stage prostate cancer
19 patients.
20 This is a very slow growing tumor, so it's
21 a good target for lentivector. Prodrug strategy
22 will allow us to terminate the therapy easily. The
23 therapy itself is selective for dividing cells, so
24 we have a differential there against normal cells,
25 it is recurrent intractable malignant disease, and

1 we are going to do local delivery.

2 We have had preliminary discussions with
3 our Medicine Control Agency about the general field
4 of lentivectors. We intend to go to our gene
5 therapy advisory committee, which is the RAC
6 equivalent, and the MCA with a protocol for using a
7 lentiviral vector to treat late-stage prostate
8 cancer sometime next year, and we are obviously
9 doing the gene transfer and the efficacy studies to
10 underpin that.

11 [Slide.]

12 Now, our view is that lentivector
13 specification is an ongoing process as with any
14 other drug development process, and there will be a
15 point where we have achieved a basic design and
16 production that we believe to have a good level of
17 safety. There will be a point that we can get to
18 in the future where we have added the endless bells
19 and whistles, and really honed this to perfection,
20 but we would argue it is not absolutely necessary
21 to get to this point for some scenarios. There may
22 be certain constellations of genes or diseases
23 where it is ethical to test the basic design.

24 So, where you are using prodrug-activating
25 enzymes, which are not toxic per se, where you are

1 looking at terminal disease in adults, then, it may
2 be ethical to proceed with a basic system.
3 Where you are looking at growth factors,
4 cell death regulators, chronic disease, and
5 children, perhaps you may wish to advance your
6 vector design, but I think if the field is pushed
7 into making this type of vector too early, then, we
8 are never going to get to this point, we are never
9 going to see the full clinical benefits of these
10 vectors if we don't begin to get some information
11 early on, because we all have to do animal studies,
12 absolutely critical, but they do have their
13 limitations, and clinical evaluation will give us
14 extra information.
15 So, we see specification as an ongoing
16 process, and we would like to promote the notion of
17 doing clinical evaluation where it is ethical early
18 on, and this is where bodies, such as GTAC and RAC,
19 help us to inform those ethical decisions.
20 [Slide.]
21 I have considered two major
22 vector-specific safety issues--obviously, each
23 transgene will have its own safety
24 issue--replication-competent lentiviruses and
25 mobilization of the transfer vector in the target

cell. We want to minimize the generation and the impact of RCLs, we wouldn't be worrying about RCLs if we thought that they had no impact at all, and "minimize the inappropriate dissemination of the transgene" is the way I phrased it.

[Slide.]

We have identified six possible ways of dealing with the issue of RCLs, which I am going to go through each of these in turn, and then top this off by saying that trust me, I am a molecular biologist, doesn't actually work. You can design these, but ultimately, we have to have a way of testing them, and I will address those issues.

[Slide.]

So, if possible, use a non-pathogenic virus, and the consequences of any RCL might be minimized.

[Slide.]

If we just look at the features of HIV and EIAV, our two chosen viruses, EIAV, equine infectious anemia virus, there is not a huge body of research that has been done on that although it is catching up, so in that respect, HIV was a great one to start with, so much information out there.

EIAV is somewhat more simple. It has

1 three accessory genes compared with six. It's an
2 equine pathogen. It doesn't replicate in human
3 cells. It causes a self-limiting anemia in the
4 horses, so there is a chronic carrier viremic
5 state. That is not to say that some horses don't
6 die, but it is really a chronic or a self-limiting
7 disease with a carrier status.
8 It is endemic in horses in the Tropics,
9 and there are rare outbreaks in stables worldwide.
10 Compare this with HIV, it is a human
11 pathogen, it does replicate in human cells. We
12 know it causes a fatal immunodeficiency. There is
13 a global pandemic, and there are 30 million AIDS or
14 HIV-positive people worldwide.
15 So, there are different profiles,
16 obviously safety profiles between those two vectors
17 at the start. One thing to say is 30 million
18 primates with HIV, and I phrase it like that, not
19 to be inflammatory, but to say that there is a
20 study there of natural infection by HIV in human
21 beings where there is every opportunity to pick up
22 endogenous retroviruses, there is an opportunity to
23 interact with other infections, and at the moment,
24 the course of the disease does not reflect the
25 emergence of any super pathogenic strain that has

1 picked up additional retroviral-like sequences or
2 any other properties through replicating in
3 patients.
4 So, we have a different profile. There is
5 no a priori expectation that EIAV would be a human
6 pathogen, but we all know that if put it in by a
7 different route, and modify a virus, we can't
8 absolutely say that.
9 There is a very low probability of any
10 patient ever encountering EIAV.
11 So, if you could show that your
12 non-primate lentivirus did all the things you
13 wanted it to do, had good efficacy, then, our view
14 would be we would choose to use EIAV rather than
15 HIV, but I think it is far from clear whether EIAV
16 would be able to fulfill every potential of
17 lentivectors.
18 [Slide.]
19 Split the vector production system into at
20 least three components.
21 [Slide.]
22 We have heard about this. Really, this
23 was established very well for retroviral vectors in
24 endless studies, that if you do split the vector
25 components up, the chance of generating an RCL is

1 much reduced and we can't really see why we
2 shouldn't just follow those concepts in generating
3 lentiviral vectors, because there is a lot of data
4 out there from MLV, so the basic system is the
5 therapeutic gene, gag-pol, and to my knowledge,
6 everybody is using a heterologous env in their
7 system.

8 [Slide.]

9 If possible, use a stable producer cell.
10 I really just echo in Dr. Verma's comments that if
11 you can, an idea situation is to use a cell line
12 that you can build up years of knowledge about.
13 DNA recombination is unlikely. We have had a
14 debate as to whether we think that is relevant.

15 You can show that it is genetically
16 stable. There are conventional manufacturing
17 parameters established, and there are well
18 characterized starting materials.

19 So, if you can use a stable producer, this
20 would be good, but I think there are arguments for
21 using transient systems, and this is obviously a
22 major point for debate. In my view, the transient
23 system should conform to the split vector paradigm.

24 [Slide.]

25 Eliminate all non-essential coding and

1 cis-acting sequences. This is just to reduce the
2 recombination and pathogenic potential.
3 [Slide.]
4 This is just a busy slide, just shows you
5 that the basic structure of the retrovirus is
6 there. With EIAV, we have got tat, rev, and this
7 other coding lesion called S2. These are the
8 accessory genes.
9 With HIV, we have got six accessory genes.
10 We have also got cis-active sites, we have got the
11 packaging sequence, the cPPT, which is involved in
12 reverse, cycle reverse transcription, the rev
13 response element, which is required for
14 orchestrating the transport and/or splicing of
15 messages, and the polypurine tract, which is also
16 important in replication.
17 So, transfer vector construction is
18 complicated. It is an easy thing to say we should
19 reduce the virus, get rid of everything, but there
20 are so many introns, spliced donors and acceptors,
21 accessory proteins, that is it not obvious, which
22 is why people started talking about developing HIV
23 vectors early in the nineties, and we are now
24 nearly 10 years on coming to the clinic.
25 [Slide.]

1 It is so difficult, why bother? I think
2 the reason is that we either don't know what these
3 accessory proteins do, and when you are doing a
4 risk assessment for your recombinant DNA, an
5 unknown is about as bad as you can get, or we do
6 have an inkling that they are doing something, and
7 mostly it is something that you are not very happy
8 about, possible growth factor interferes with some
9 cellular function, cell cycle arrest, and
10 obviously, in hearts is pathogenicity.
11 With EIAV, we have tried all sorts of
12 functional genomics analyses on S2 to try and find
13 out what it does in vitro, and we can't find it
14 doing anything, but it is absolutely clear that if
15 you delete that protein from the virus, it won't
16 cause any disease in horses, so it is a classic
17 pathogenicity factor, and that is one of the
18 problems in analyzing these accessory proteins, the
19 in vitro assays may not define the full range of
20 their properties.
21 So, we would say, if possible, get rid of
22 them.
23 [Slide.]
24 I am not immune from this generation thing
25 because it is a useful concept to show that we have

1 all been working hard to understand our system and
2 to develop it. What you can see is that we have
3 working with John Olsen, who we discovered was also
4 working on EIAV, and it seemed little point to
5 compete, we joined forces, and to try and develop a
6 vector producer cell, a basic cell, a thing we
7 called 8Z-20.
8 Really, I just want to show you the
9 evolution of our thinking. Here is our vector
10 genome, and we stripped out most of gag-pol. We
11 mutated the coding region, but were left to make
12 sure that we couldn't get any proteins produced,
13 but there is still an awful lot of material there.
14 We provided gag-pol, took away the
15 upstream sequences in the leader, which are
16 presumed to be important in packaging to make sure
17 this wasn't packaged, but we still had a lot of
18 sequence down the end.
19 We provided VSV-G with a tet regulation
20 system because if you overexpress VSV-G, it upsets
21 the production system. The problem then, of
22 course, is there is a region of homology between
23 the packaging site and the gag-pol, and there is a
24 region of homology between the RRE.
25 So, here we have got a potential for

1 recombination and also we are obviously still
2 expressing S2 and rev in this system.
3 [Slide.]
4 Nonetheless, we made a producer, stable
5 producer cell, and this has allowed us to scale in
6 roller bottles and we are producing a reasonable
7 titer, 10^6 transducing units per mL for five days.
8 This means we have a benchmark vector on which to
9 monitor our improvements.
10 It is no good making a vector system which
11 is perceived to be safer, and you are only making
12 100 particles per mL. That is the point in this
13 exercise, so we figured we would start off with a
14 benchmark and work up from there. So, this has
15 been useful.
16 [Slide.]
17 But clearly, we would not be able to go
18 clinical with that, so the next thing we did was to
19 look at the transfer vector and strip out as much
20 as we could. This we have done, so we have just
21 retained the packaging sites, and we can dock in
22 two extra sequences that we want.
23 One is the RRE, because for some
24 unexplained reason if you provide rev and RRE to
25 the vector genome, you get improved titers. So,

1 that is an option, and then this cCPT for as yet an
2 unknown reason in some cell types, the cCPT can
3 optimize gene transfer and expression, but again
4 this is optional, not obligatory, we have a site
5 there.

6 Then, the polyadenylation of this internal
7 transcript can often do with being improved. There
8 is an element that a lot of people use, woodchuck
9 hepatitis element, and this can be docked in here.

10 Now, if we use this minimal vector, it is
11 not expressing EIAV proteins, it has got greater
12 than 8 kb insert capacity, but we have still got
13 homology in this region, and obviously, if we
14 include the PPT, we have got homology here, and if
15 we include the RRE, we have got homology there, so
16 we still need to do something.

17 [Slide.]

18 We have got to minimize the potential for
19 recombination, and this really means removing all
20 homologous sequences from the gal-pol packaging
21 plasmid, but we have to do that without
22 compromising the expression of gag-pol, and at the
23 moment, at this point, the dogma was that the
24 RRE-rev interaction was important for gag-pol
25 expression, so we need to examine the requirements

1 for that.

2 One way of changing the sequence is to

3 alter the nucleotide sequence, but retain the

4 precise meosic [ph] sequence because of the

5 redundancy of the genetic code, but it will be

6 pointless to do that if you would compromise the

7 expression efficiency.

8 [Slide.]

9 We have biology on our side, however,

10 because lentiviruses are very peculiar in

11 maintaining a most abnormal codon usage. If you

12 were to look at the codons that were commonly used

13 in mammalian cells, for example, for alanine, 53

14 percent of the time, this one is used, whereas, in

15 a wild-type lentivirus, it is only 19 percent of

16 the time, and we obviously don't have time to go

17 through this chart, but you can find numerous

18 occasions where a lentivirus will choose to use the

19 rarest TRNA in a cell.

20 What we decided to do is to codon-optimize

21 the gag-pol, so that we changed all the codons.

22 Not only would this alter the nucleotide sequence,

23 but it should give us preferred codons to ensure

24 good expression.

25 [Slide.]

1 So, we have made an entirely synthetic
2 gag-pol where the entire sequence of the gag-pol
3 has been changed with the exception of a small
4 region at the gag-pol overlap because there is a
5 frame-shifting event occurs to fuse gag and pol,
6 and that has a requirement for a particular
7 sequence.
8 There are no sequences flanking this
9 gag-pol cassette, that have been anywhere near a
10 lentivirus, totally unrelated. So, the codons have
11 been changed across gag-pol. It removes all blocks
12 of sequence homology, and a really added bonus is
13 this thing is now rev-independent, so that we could
14 get rid of the rev response, so that we have been
15 able to get all that junk down the end, and we have
16 done that for both EIAV and HIV.
17 [Slide.]
18 This allows us to create what we call--we
19 have given up with the generations now--what we
20 call a minimal EIAV vector system, where we have
21 the stripped-out transfer vector, completely
22 synthetic gag-pol, and we have our envelope. That
23 is no obligate requirement for any accessory gene,
24 but rev-RRE can improve the yield.
25 There are no functional viral proteins or

1 significant coding regions in the transfer vector.
2 The transfer vector contains only 1,100 nucleotides
3 of the original EIAV. There is no significant
4 homology between components. What I mean by that,
5 if there is no stretch of longer than 6 nucleotides
6 that is shared between any of these components.
7 It has been approved in the UK by our
8 health and safety executive for containment level 1
9 use depending on the transgene, so if we are using
10 something like feta-gal, this is basic laboratory,
11 good laboratory practice, the lowest containment,
12 and we have a similar system for HIV.
13 [Slide.]
14 We have now gone on to create a second
15 generation packaging cell, and this is work in
16 progress. We don't know if we are going to be able
17 to achieve this. In the transient systems, yields
18 are fine, but with these three plasmids, we have
19 every expectation that we will be able to generate
20 a production system.
21 The packaging system will have the
22 synthetic gag-pol, it will have VSV-G, and then we
23 will make a version that will optionally have rev,
24 and we have code and optimized that, so again,
25 there is no sequence homology with the original

1 vector, and we can optimize expression.

2 We aim to meet the 8Z-20 characteristics,
3 so with this improved system, we can't hit the
4 level that we got with 8Z-20, then, we will have to
5 have a decision as to whether these improvements
6 really were worth the tradeoff in manufacturing
7 efficiency.

8 [Slide.]

9 Reduce the packaging of vector helper
10 components. Well, we are going to learn our lessons
11 from retroviral vectors and we are going to
12 eliminate packaging and dimerization signals.

13 [Slide.]

14 We have done a lot of studies which I can
15 only briefly go into. Clearly, when a construct
16 expresses gag-pol, it can package the RNA that went
17 on to express it, that is the problem. Some
18 lentiviruses do package co-translationally, things
19 like HIV-2, so maybe you would steer clear of those
20 for developing vector systems.

21 We have made all sorts of mutations and
22 changes in the leader sequence, various deletions
23 in the presumptive packaging site. We have a
24 completely synthetic gene which should have no
25 packaging site, and we have made a version that has

1 a two-stop code, so we are only making RNA, RNA
2 control.
3 [Slide.]
4 These experiments are all quantified by
5 quantifying the particles using an assay called
6 PERT, which I will talk about, by quantifying the
7 RNA using real-time PCR, by internally controlling
8 with actin RNA, so that you are always trying to
9 compare a packaging situation where you have got
10 similar amounts of RNA, similar yields of protein,
11 so that you can make some comparisons.
12 The type of data that we have, there is a
13 baseline. You will never get rid of background
14 noise in biology. I really believe that that is
15 not possible. So, we have set our baseline as the
16 highest amount of actin that we found in any one
17 sample.
18 Then, we asked how much RNA did we find in
19 the particles compared to how much of that RNA did
20 we find in the cell. The better a particle is at
21 packaging, the more of the RNA it will have picked
22 up from the cell. That is our feeling.
23 So the wild-type gag-pol with the
24 wild-type packaging site packages itself. The
25 deletion really doesn't package itself, and the

1 synthetic gag-pol doesn't package itself. So, we
2 have knocked down the packaging to background level
3 by altering the packaging site.
4 It is important to do these studies in the
5 context of a transfer vector because another way of
6 getting your RNA into the cell is if you have got
7 any region of homology, you may get dimerization
8 and piggy backing. In fact, we didn't see this in
9 this particular EIAV vector. We have seen it in
10 some mutations that we have made in HIV.
11 The bottom line is the wild-type gag
12 packages itself and the vector. The packaging site
13 mutant gag does not package itself, but it does
14 package the vector, and the synthetic gag-pol does
15 not package itself, but it does package the vector.
16 So, there we have packaging constructs
17 which package the vector, which is good, that is
18 what we need, but which don't package themselves
19 above background. So, partial or complete removal
20 reduces packaging to background levels, and we have
21 had no evidence of piggybacking by dimerization
22 with the transfer vector.
23 [Slide.]
24 So, we have addressed a number of issues
25 for minimizing RCLs, now we need to do some assays,

1 and we want to use sensitive, calibrated assays,
2 and we want the assays to relate to the properties
3 of any RCL, and this actually is the conundrum
4 which we have touched on in the morning as to what
5 is the nature of the RCL.
6 [Slide.]
7 Well, what I have done is suggested that
8 the only way that RCLs could arise in the EIAV
9 vector system is now by non-homologous
10 recombination because we have removed the potential
11 homologous recombination and by background
12 packaging in the particle.
13 By definition, we can't predict the
14 arrangement of genes and the recombination events
15 that would arise from non-homologous recombination.
16 There is no way of doing that.
17 So, we have tried to take a generic view
18 and we have said that all RCLs must have gag-pol.
19 By definition, this is the transferring entity.
20 The most likely gag-pol is vector derived, the one
21 that we put in. If there is any other gag-pol that
22 we have somehow inadvertently empowered from the
23 cell, it is only rendered transmissible with the
24 vector-derived env.
25 So, all our constructs are going to have

1 the vector-derived gag-pol and the vector-derived
2 env, vector-derived gag-pol and some other env that
3 we can't predict, but we can't say that it could
4 never be there, a gag-pol that is endogenous that
5 we can't predict what it is, but we don't want to
6 ignore the fact that it might be there, and the
7 vector env.
8 The universal feature is a gag-pol, so you
9 can screen for transmissible reverse transcriptase,
10 all of these will have that. You can then qualify
11 any ambiguous results by a second screen for
12 transmissible gag and pol, and by screening for
13 transmissible env.
14 So, we have taken a theoretical RCL
15 structure, developed a generic view, and we don't
16 want to restrict our view by adjusting the amount
17 of VSV-G. We don't want to design VSV-G-specific
18 assays because we may not use VSV-G for all our
19 applications.
20 [Slide.]
21 We propose to test vector preparations and
22 propose production cells following the current CBER
23 guidelines for MLV-derived vectors. We are going
24 to use two assays, F-PERT, which is
25 fluorescence-based product enhanced reverse

1 transcriptase assay, as our primary assay tool, and
2 we are going to use a PCR assay to resolve
3 ambiguous PERT assay results. This is a specific
4 assay for hypothetical recombinant molecular
5 structures.
6 We have developed and strategies in
7 collaboration with relevant UK Government agencies,
8 and these are the Laboratory of the Government
9 Chemist, the National Institute for Biological
10 Standards and Control, and also we have had
11 discussions with a contract manufacturer, Q-One
12 Biotech, because it is important that anything we
13 develop can be transferred to a manufacturer for
14 small companies or groups who are not going to
15 develop their own in-house manufacturing
16 capability.
17 [Slide.]
18 F-PERT assay has an advantage. There are
19 many groups working on this. It is being refined
20 all the time. It was originally developed for
21 looking for HIV in plasma. It can detect a single
22 particle. Obviously, one has to qualify that
23 mixing it up with whatever brew you are trying to
24 find the particle in.
25 Basically, you collect particles, you

1 disrupt them, liberating the pol. You then provide
2 an RNA. This is the MS2 phage RNA and a specific
3 primer. Any pol that is there will then make a
4 cDNA.

5 You then amplify up the cDNA using
6 specific primer pairs and you detect the amplified
7 product using the standard TaqMan technology.

8 This assay is independent of the nature of
9 events that lead to the RCLs. It is broadly
10 applicable and high sensitivity. We have put 10 to
11 100 particles, because I didn't want to be held to
12 a figure. We are obviously going to refine that
13 and come up with a standard window of sensitivity
14 that we deem acceptable for our particular
15 application.

16 This assay has been modified with a series
17 of controls to protect against false positives.

18 [Slide.]

19 We are looking at the sensitivity. It
20 will detect manganese and magnesium-dependent
21 reverse transcriptases. You can find these over a
22 range of dilutions, and we are obviously spiking
23 mixtures and looking at the sensitivity in the
24 context of the soup, the end of production soup.

25 [Slide.]

1 A qualifying assay is a PCR assay, and
2 here we are looking for gag, we are looking for
3 pol, we are looking for env. We are looking for
4 them individually and we are looking for them
5 linked with each other. We are doing that by just
6 making a set of nested primer pairs.
7 These have just been identified by the
8 clever biomathematicians doing blast analyses, and
9 our primers are specific for our vector, and they
10 can, by working in particular pairs, they will
11 amplify a small region or a large region, and we
12 can look to see what sort of things are coming out.
13 [Slide.]
14 Obviously, looking at the sensitivity, and
15 we have defined the sensitivity in the context of
16 the assay cell genomic DNA, and we have set a level
17 that the assay must detect 1 to 10 copies in
18 background of 10^5 genomes.
19 Here, you can see our cutoff points is
20 between 1 and 0.1 for gag, and similarly with pol.
21 In this particular experiment, VSV-G was slightly
22 less sensitive. We obviously can develop these for
23 any envelope that we choose to use.
24 [Slide.]
25 So, our procedure that we are putting to

1 you for discussion, we transduced cells with the
2 test article and we amplified, so we split the
3 cells at an appropriate ratio over an appropriate
4 time, and these are parameters that will be
5 defined.
6 Then, on the supernatant, we do a PERT
7 assay, and then we can qualify that if necessary
8 with a PCR assay.
9 Our amplification is going to be in two
10 types of cell lines - the 293 cells, which support
11 the transduction by a wide variety of vectors and
12 pseudotypes, and they are the production cell line,
13 and also lymphoid cells, and we are currently
14 screening a range of lymphoid cells. This is
15 important because the potential in-patient target
16 for RCLs are hematopoietic cells, and by using two
17 different cell lines, we are sampling a range of
18 viral replication characteristics.
19 As a positive standard, we are using FeLV,
20 because that gives efficient amplification in both
21 these cells. It is a regulatory standard, and we
22 don't get any interference with lentivector.
23 We believe the amplification process is
24 essential, and this is readily adapted for
25 screening producer cells also.

1 [Slide.]

2 So, we would propose that we consider the
3 following points when we are talking about RCLs.
4 If possible, use a non-pathogenic virus, split the
5 vector production system, preferably use a stable
6 cell line, eliminate all non-essential coding and
7 cis-active sequences, minimize the potential for
8 homologous recombination, and we don't want to set
9 an absolute figure on that; reduce the packaging of
10 vector helper components, and use sensitive,
11 calibrated assays for RCLs after amplification in
12 human cells.

13 [Slide.]

14 So, we have gone some way to addressing, I
15 believe, this issue. The next one is mobilization
16 of the transfer vector in target cells.

17 [Slide.]

18 We can see two ways of doing this. First
19 of all, use a transfer vector that isn't very well
20 mobilized, or reduce the level of the mobilizable
21 RNA in the target cell.

22 [Slide.]

23 Well, what do we mean by use a transfer
24 vector that is poorly mobilized, by what? What are
25 we concerned about here? There may be an infinite

1 number of possibilities for concern, but the one
2 that we thought might be of the most concern is
3 HIV. That is the agent of mobilization that is
4 probably of most concern. It is a virus that is
5 around in the human population, and we should
6 probably ask the question is our transfer vector
7 mobilized by HIV.
8 HIV is not the only virus that we might
9 consider, so we decided to look at MLV, as well, as
10 sort of a generic retrovirus, so, yes, they may be
11 endogenous retroviruses, yes, they may be other
12 things out there that we haven't thought of, but if
13 we study mobilization by HIV, as a known human
14 pathogen, and MLV, as a retrovirus that we know a
15 lot about, we thought that might be useful.
16 So, we set up some cross-packaging assays
17 where we take a cell that has a reporter transfer
18 vector, and we put gag-pol and env in this cell.
19 The env is VSV-G, and then we mix the EIAV transfer
20 vector with its own gag-pol.
21 We look by FACS after five days, but also
22 after two serial pathologies, because there is this
23 phenomenon of so-called pseudotransduction where
24 you might score a positive, but you are not sure if
25 that is a genuine integrated event, so you passage

1 the cells to make sure you really are looking for
2 an integration.
3 [Slide.]
4 I am only going to show you some data
5 because I am going to run out of time. These
6 results are quite interesting. What you see is, of
7 course, EIAV mobilizes itself very well, a titer of
8 10^6. It mobilizes HIV at 1,000-fold less.
9 It mobilizes MLV at a little bit lower
10 than that, a couple of hundred. HIV mobilizes
11 itself very effectively, as you would expect, it
12 mobilizes EIAV at 1,000-fold lower level, and MLV,
13 virtually at 1,000-fold level, so the difference
14 between these two is really not significant.
15 So, the ability of EIAV to be mobilized by
16 HIV is just the same as MLV. MLV mobilizes HIV,
17 and it barely mobilizes EIAV. So, there is a
18 little bit of background cross-mobilization, as you
19 would expect, consistent with these particles
20 picking up RNA.
21 The main thing I want to emphasize here is
22 that HIV does not interact with EIAV any
23 differently from MLV. We already have MLV vectors
24 in the clinic in HIV-positive patients. So, I
25 would argue that there is no reason why one

1 shouldn't put EIAV vectors in HIV-positive patients
2 either, but there is the issue, with HIV, that if
3 there is HIV around, it will be mobilized by HIV.
4 [Slide.]
5 So, if you have a situation where you
6 can't address the question by using a poorly
7 mobilized vector, or there is some reason you are
8 expressing a very toxic gene, and even dropping the
9 mobilization by 3 or 4 logs is not adequate, you
10 may want to do something else to the vector.
11 There are a number of ways of reducing the
12 level of the mobilizable RNA in the target cell,
13 and one is by the SIN vectors that we have heard
14 about. I don't think this will be necessarily
15 required for all lentivectors. I wouldn't want to
16 stand up and say I don't think it is important,
17 that is why we haven't done it.
18 We have actually looked at this in EIAV,
19 made a classical SIN vector. It drops the viral
20 genome down to 900 nucleotides. It deletes the
21 LTR. We had a comment that maybe this was useful
22 in reducing the oncogenic potential. There is no
23 evidence for lentiviruses of any oncogenic
24 potential despite massive viremic states. There is
25 no evidence that having a promoter here is a

1 problem.

2 We don't particularly like the SIN vectors
3 because we think there are advantages to
4 introducing the transfer vector into the genome by
5 transduction rather than by transfection, and this
6 is a jolly good site to put tissue-specific or a
7 regulated promoter, so if you decide you are not
8 going to use that site, it restricts your options
9 for making advance vectors.

10 [Slide.]

11 Nonetheless, we have made this.
12 Interestingly, we have compared our SIN version
13 with the basic EIAV LTR, and that is naturally a
14 SIN because it requires tat, EIAV tat doesn't
15 interact with human cycline, so it doesn't work, so
16 it is a sort of a natural SIN, and we have compared
17 it with R8Z-20 line.

18 This is a pure cell line, this is a
19 population, so we have obviously refined the study,
20 and we predicted we would have very, very low
21 amounts of RNA. Of course, this RNA could be
22 read-through, it could be anything, it could be
23 short bits, long bits, we are not quite--you know,
24 one doesn't know what the nature of packageable RNA
25 is, and the CMV RNA likewise could have some

1 upstream transcript.

2 So, the first thing we do is measure
3 packageable RNA using our very sensitive assays
4 that I have described, and we can see that both the
5 SIN vector and the LTR vector give low levels of
6 packageable RNA, which is 3 orders of magnitude
7 lower than the CMV, so we have dropped the
8 packageable RNA by 3 logs.

9 When we look at the titer as to whether
10 that RNA is actually going on to do anything, then,
11 there is a differential of, in this particular
12 experiment, 5 logs. So, you can make an RNA
13 completely, or virtually completely I should say in
14 this audience, unmobilizable because you can drop
15 its levels. We are not convinced that that is
16 important.

17 [Slide.]

18 We have had before us this question of
19 mobilization of HIV vectors by HIV, is this a
20 special case for the treatment of AIDS, so you turn
21 virus escape into an attribute.

22 [Slide.]

23 Yes, there are various versions. You have
24 one before you. We have also been developing one,
25 and there are a number around. The aim here is to

1 put in an inhibitor of HIV replication, preferably
2 an RNA, because I don't think you want to engineer
3 stem cells with any proteins that could be
4 immunogenic. So, a therapeutic RNA, and here you
5 can configure the therapeutic RNA as an internal
6 constitutive transcript or you can configure it as
7 a full-length inducible transcript where when the
8 incoming virus comes in, it switches on gene
9 expression.
10 Now, really, you want your therapeutic, I
11 think, to stop the virus. You don't want to close
12 the stable door. You would like to stop the virus
13 actually getting going. So, you want to knock down
14 chemokine receptors, really stop it getting going,
15 but if it does manage to integrate and start to
16 make more virus, then, by having the vector there,
17 you can not only knock down the level of that
18 virus, but the virus can pick up the vector, and it
19 can pick it up two copies, or in a hybrid, and
20 propagate the seeds of its own destruction.
21 So, mobilization amplifies the therapy if
22 virus escapes the first wave of ribozyme. I think
23 there is a certain amount to be said for that
24 strategy, and I think it is one that really does
25 deserve some debate where you are actually

1 disseminating the therapy through the patient.
2 [Slide.]
3 Whenever you do this in the lab, and you
4 have heard this in VIRxSYS's proposal, you get
5 escape variants, and we all say, oh, in vitro
6 concentration, too many cells, too close, and it
7 won't happen in vivo, but to my knowledge, nobody
8 has actually asked what is the genetic nature of
9 those escapes, and that might be a useful thing.
10 [Slide.]
11 After that brief digression into HIV, and
12 I think we will have an opportunity to talk about
13 that more tomorrow, I hope I have convinced you
14 that we have made some progress in addressing the
15 issues of replication-competent lentiviruses, and
16 some progress in addressing the issues of
17 mobilization of the transfer vector.
18 [Slide.]
19 I have described a specification for a
20 lentivector with a good safety profile, no
21 pathogenic proteins, very poorly mobilized by HIV
22 to the same extent as MLV. No potential for
23 homologous recombination by definition, less than
24 10 nucleotides is regarded as not to be a site for
25 recombination where RCLs are extremely unlikely.

[Slide.]

I would like to suggest that we can begin to look at a generic set of guidelines that will cover all lentivectors, but I think that we will have to embellish them on a case-by-case basis for each particular lentivector for each particular indication.

I think we must show efficacy in a relevant animal model, must be able to produce this vector at GMP or in the spirit of GMP, good manufacturing practice, and it should be configured with the following points in mind.

Eliminate non-essential proteins and sequences. Ensure extremely low, preferably zero, homologous recombination potential between the components. Show a significant differential between self-mobilization and mobilization with HIV, or use a vector that after integration generates a significant reduction in mobilizable RNA, and I suggest we use MLV as a benchmark for mobilization.

Use a minimum of three split components in a stable packaging cell line or in a very low homologous-recombination transient system.

Use sensitive, calibrated assays for RCLs,

1 follow MLV as a guide. There is all that
2 experience out there. Put in an amplification step
3 in an appropriate human cell line.
4 Test for no significant acute toxicity in
5 the relevant animal efficacy model.
6 [Slide.]
7 There is a series of references that you
8 can follow up what I have said if the spirit moves
9 you, and there is a lot of people in the company
10 that have been developing this work, but
11 particularly mentioned are collaborators John Olsen
12 for the production systems, Karen O'Malley for some
13 of the neurobiology, James Uney, where we have been
14 looking at long-term correction of various animal
15 models of disease, and our colleagues in the
16 regulatory agencies and at Q-One Biotech who have
17 been helping us design these systems.
18 Thank you.
19 [Applause.]
20 DR. SALOMON: Thank you very much for that
21 very nice presentation.
22 **Questions & Answers**
23 DR. SALOMON: One of the things I was
24 thinking about when we were going through this is
25 can we begin to articulate what would be the

1 definition of an ideal replication-competent
2 lentiviral assay.
3 Everybody is going to do it a little
4 different, right? I mean we have already heard
5 several different possible cell lines, we know that
6 there are going to be different strategies to
7 engineer the vector. That could have implications
8 into what then would be the target cell line.
9 Can we maybe figure out some elements that
10 if a sponsor brings it forward, you fulfill these
11 elements, it's a good RCL assay?
12 DR. KINGSMAN: Well, my view is the way
13 not to go is to try and design artificial viruses
14 to create some positive control, because you may or
15 may not be right.
16 I think the assay for transmissible
17 reverse transcriptase is a very useful one. It is
18 quantitative, it's reproducible, it is looking for
19 the entity that you are interested in, in terms of
20 an RCL--so this is all, I am restricting my
21 comments to a replication-competent entity--so if
22 there is a reverse transcriptase which is
23 transmissible, then, you need to detect that.
24 That, I think is the important thing, is
25 there a transmissible reverse transcriptase. The

1 PERT assay is a sensitive, reproducible biochemical
2 assay. Now, 10 years ago, these assays weren't
3 available, we used surrogate assays.
4 The mobilization of other vectors, you
5 know, these were indirect assays, and there is a
6 feeling that what is old is good, and we should
7 stick with that, but in actual fact, using these
8 indirect assays, when you have got highly sensitive
9 biochemical assays, may not be the thing to do.
10 With the retroviral field, people were
11 making mouse retroviral vectors in mouse cells, and
12 therefore, there was a whole ethos of looking for
13 these viruses and using mouse assays.
14 We have a horse virus that doesn't
15 replicate in human cells, and doesn't replicate
16 very well in horse cells either. You would have to
17 get primary dermal fibroblasts. So, there is no
18 meaningful assay that you can do.
19 So, the biochemical assay seems to me to
20 have a lot going for it. It is looking for what
21 you are interested in. If you find a transmissible
22 reverse transcriptase, you have got to ask what it
23 is.
24 Then, you go and you can do your PCR
25 analysis to find out what it is, but there is no

1 way of second-guessing from these series of
2 illegitimate recombinations and hypotheticals what
3 you should actually do.
4 DR. SALOMON: If we take that principle
5 then, just to kind of make sure that I understand
6 what you are saying, so you are going to base your
7 assay for RCL on the assumption that an RCL has to
8 be carrying an intact gag-pol.
9 DR. KINGSMAN: Yes.
10 DR. SALOMON: I mean it also has to be
11 carrying a number of other things, but at least it
12 has to have a gag-pol.
13 DR. KINGSMAN: That is the bare minimum,
14 yes.
15 DR. SALOMON: And the pol should encode an
16 RT.
17 DR. KINGSMAN: Yes.
18 DR. SALOMON: So, what would be your assay
19 limit, how do you define the lower limit, any RT?
20 I mean it has got to be zero? We both know that
21 quantitative PCR never gives you a zero result,
22 right, it is all based on a threshold.
23 So, how would one validate a number
24 achieved in this wonderful new quantitative assay?
25 DR. KINGSMAN: You would do it with

1 reference to a standard, a standard that we are
2 going to use is FeLV, and we would do a spike
3 mixture, and we would detect a single RCL, a single
4 virus over our amplification process, so our assay
5 would detect that, and the limits of sensitivity of
6 our assays would allow us to do a plus/minus. I
7 think that is what we would go for, a plus/minus.
8 DR. SALOMON: Okay, so keep going. Now
9 you are just defining the sensitivity of the assay.
10 What I am pushing you to say then is what would be
11 the definition, then, of an appropriate limit for
12 this quantitative RCL assay then, one particle in a
13 background of 100,000 or a million or--I am not
14 talking about detection now, I am talking about now
15 it is safe, you could use this in a clinical trial.
16 DR. KINGSMAN: In the 300 mL's or 1
17 percent of the--so following the guidelines for
18 MLV, so it would be in 100 mL's at 95 percent
19 confidence, and in 1 percent of the post-production
20 cells, if you scored a plus, then, you would sling
21 it out. It is just following the MLV protocol.
22 DR. SALOMON: One last question. Are we
23 saying, then, that we are comfortable with the idea
24 that an RCR limit set out of some empiric
25 experience with MLV is appropriate for lentivirus?

1 DR. KINGSMAN: Yes.
2 DR. MULLIGAN: Just a quick question about
3 the PERT assay. If the principle is you have to
4 have an intact pol, why not go simple and just do a
5 PCR or an RT/PCR for pol sequences? What is the
6 relative sensitivity of those? It has got to be
7 the RT/PCR, doesn't it?
8 DR. KINGSMAN: Yes, I think the RT/PCR
9 relies on having primer pairs, and it relies on
10 knowing something about the sequence. That is
11 really I think why one would go for the biochemical
12 assay of reverse transcriptase, so you are not
13 making any judgments about the sequence in your
14 front line assay. Your front line assay is for any
15 transmissible pol.
16 DR. MULLIGAN: The question is in the
17 normal case where you don't have an unusual pol,
18 what is the relative sensitivity. You would hate
19 to miss in your primary screen something. What are
20 the chances you wouldn't pick up a normal pol
21 sequence via the PERT assay, but you would by the
22 RT assay?
23 DR. KINGSMAN: We need to do repeated
24 studies, but at the moment, the sensitivity, we are
25 saying is that the PERT can detect 10 to 100

1 particles, and the PCR assay can detect 1 to 10
2 genomes in a background of 10^5 genomes.
3 DR. MULLIGAN: So, you would agree that if
4 you moved closer to the PCR, then, well, I guess I
5 would agree that it would make sense if you got
6 closer to the RT/PCR sensitivity, but you may not,
7 right, you may not get to that point?
8 DR. KINGSMAN: Well, the reason I am being
9 conservative in the estimate of the sensitivity of
10 the PERT, other people will say you can detect a
11 single particle, a single virus-like particle, and
12 I think it is perfectly possible to do that, but we
13 don't have a full set of data where we have done
14 mixing experiments and said that in the context of
15 the culture supernatant we can detect a single
16 particle.
17 So, what we are relying on is doing an
18 amplification process where we can--we are not
19 looking for a single particle in the primary
20 harvest, we are looking for the consequence of that
21 single particle amplifying out, so that we can then
22 detect.
23 I would not advocate using either of these
24 assays on the post-production supernatant, that
25 there has to be an amplification step, and then the

1 sensitivity of your assay is kind of balanced with
2 the amplification. If you have a massive
3 amplification, then, your detection, you may have a
4 tradeoff on the sensitivity of your detection.
5 DR. MULLIGAN: I guess the reason why you
6 have to do it that way is obviously you can't do
7 the PERT in the presence of particles that are the
8 pseudotype particles.
9 DR. KINGSMAN: No, no.
10 DR. MULLIGAN: But that is a fundamental
11 difference, so you are not looking, right, you are
12 assaying, you can't look for a rare species in your
13 production of virus by this test, you can only look
14 for a transferrable amplified.
15 DR. KINGSMAN: Yes, and that is what we
16 are defining as the issue. Now, if that is not the
17 issue, then, we obviously need to address the other
18 issues, but what we have said is that what we are
19 concerned about is a replicating entity that could
20 turn a therapeutic vector into something that is
21 detrimental to the patient.
22 So, the thing that we can identify is a
23 transmissible gag-pol that may have some
24 unexplained pathogenic potential. We obviously
25 can't look at our EIAV vector and think of a

1 pathogen just looking at the components that we
2 have got left. We wouldn't say that means it is
3 safe obviously, because nobody has tested a
4 replicating virus with those components injected
5 into a brain under the particular set of
6 circumstances.
7 So, what we are saying is that we are
8 concerned about any replicating entity, but with
9 EIAV, we have not become concerned about whether
10 there is gag-pol there or VSV-G or some other bit
11 of a vector genome. That, we have not regarded as
12 a major issue. The issue for us is whether there
13 is a replication-competent entity, and that is what
14 we focused on.
15 DR. KAPPES: Susan, I thought I had my
16 question together until you made that last
17 statement, but let me say what I was going to
18 anyway.
19 I think I favor the notion or the
20 principle of looking for recombinants that contain
21 reverse transcriptase, and as you pointed out, what
22 it measures is the potential of that recombinant to
23 produce RCR even though it might not itself
24 represent a replication-competent form of vector or
25 virus.

1 Moreover, it is a way of measuring prior
2 to administering that vector or those recombinants
3 if they exist, that is, recombinants which contain
4 functional machinery, such as reverse
5 transcriptase, against the possibility that
6 additional recombination will occur in vivo which
7 can ultimately, although probably unlikely, lead to
8 RCR.
9 DR. KINGSMAN: Yes. I think that is
10 right. We are not making any value judgment as to
11 how these things arose or what their complete
12 nature is. We just think it is not a good idea to
13 have them there, and we are proposing to use
14 biochemical assays to measure these. That is our
15 proposal.
16 DR. BORELLINI: Flavia Borellini from Cell
17 Genesys. In my experience with the PERT assay, you
18 have a problem of a background due to cellular
19 polymerases that can actually score in a PERT
20 assay. So, I would anticipate that you would find
21 yourself in a situation where you have a lot of
22 positives, that then you have to go and verify by
23 your cell PCRs.
24 Then, why not do the PCR right away and
25 skip the PERT?

1 DR. KINGSMAN: Well, we are open to that
2 suggestion of doing both of them. The background
3 with telomerase is well known in PERT, and it can
4 be solved by variously spiking the mixture with
5 calf thymus DNA, and there is a whole series of
6 modifications to PERT that have produced those
7 backgrounds.
8 Obviously, when we do a number of scale
9 runs, if we keep find that we get a level of
10 background positives that require a qualifying
11 test, then, that would inform us to bring the
12 qualifying test up, level with the PERT test, but
13 at the moment, we are going to try and run with the
14 notion of doing a single defining assay and having
15 a secondary backup assay.
16 But clearly, if we find false positives
17 occurring at an irritatingly high rate, then, we
18 will take steps to deal with it, but this issue of
19 telomerase is well known and can be solved by
20 varying the protocol, you can knock it down.
21 DR. BORELLINI: Actually, it is not only
22 telomerase, but it is just DNA polymerases that can
23 cause background.
24 DR. KINGSMAN: Yes, or whatever, I was
25 wrong to imply a mechanism there.

1 DR. NALDINI: Luigi Naldini. I was
2 questioning again the assay for the RCL. I mean in
3 principle, in your amplification step, you would
4 like to use the most permissive system to allow
5 amplification of a recombinant.
6 Wouldn't that be made of a horse cell for
7 an EIAV-derived vector?
8 DR. KINGSMAN: We don't really want to
9 study horse cells when we are interested in human
10 cells, and we know that those cells that we have
11 chosen are incredibly permissive for a whole range
12 of vector configurations with different
13 pseudotypes.
14 DR. NALDINI: But they are not permissive
15 for the EIAV virus.
16 DR. KINGSMAN: Yes, but we are not working
17 with the EIAV, we are working with two components
18 from that virus which there is no envelope.
19 DR. NALDINI: You have an LTR from the
20 EIAV, you have gag-pol from the EIAV. So, if you
21 have a recombinant, to have higher chances of
22 detecting that, you should have a system which is
23 permissive to that.
24 DR. KINGSMAN: I really don't see that one
25 needs to go down studying a horse virus in primary

1 pony dermal fibroblasts in order to find a
2 qualification for a vector system which is so far
3 removed from EIAV you can possibly get it, and you
4 are interested in human cells and what goes on in
5 human cells, and what you want to know is does this
6 thing amplify in human cells, not do we amplify an
7 RCL in h horse cells. It is do we amplify one in
8 human cells.

9 DR. NALDINI: You want a biological
10 barrier when you are using the vector in your
11 application. I don't think you want to have any
12 biological barrier when you are testing your
13 system. You want to have the most amplification
14 possible. I think it is a matter of debate.

15 DR. MULLIGAN: I would say you are both
16 right. I think, if I could reinterpret his point,
17 to make it sound more interesting, it would be that
18 if a horse cell allowed you to amplify something
19 that then was able to be shown to infect human
20 cells, maybe there would be a reason.

21 He is saying there could be a reason that
22 this would be a uniquely sensitive way to amplify
23 something, not to make it into something different,
24 but just so it is detectable, but ultimately, you
25 would have to show that that virus that you

1 amplified on horse cells was capable of having
2 human infectivity.
3 DR. KINGSMAN: And I can't see the point
4 of doing that.
5 DR. ALLAN: Can I follow that same line of
6 questioning? Just in the sense that your vector
7 now, is it a SIN vector now, you are generating a
8 SIN vector?
9 DR. KINGSMAN: You can, but I don't think
10 you need to use a SIN vector.
11 DR. ALLAN: So, you have an intact LTR,
12 EIAV LTR.
13 DR. KINGSMAN: Yes.
14 DR. ALLAN: So, then, the issue then is
15 relevant because you could use a cell line that has
16 tat expressed in it, and then you can look to see
17 whether that LTR is going to function, if you
18 contain the tat in the cell line, so you could
19 actually have a reporter assay.
20 DR. KINGSMAN: Yes, I think you could
21 infinitely manufacture cells with various
22 reporters, you could provide tat, you could
23 provide, you know, EIAV envelope to make sure it
24 maximally picks up the gag-pol, to make sure that
25 you are maximally testing that, but I don't see the

1 advantage of doing that over and above the strategy
2 that we have proposed, particularly as I have shown
3 that the vector is completely stripped out of all
4 EIAV pathogenic entities.
5 There is no tat, there need not be any
6 rev. There is gag-pol and yes, you are right, we
7 don't know if that has a fundamental pathogenic
8 potential, but that may be less likely.
9 So, I am a little bit averse to recreating
10 a horse virus out of this, when we are really
11 trying to look for entities that replicate in human
12 cells, and we have stripped this vector system down
13 to such a point that although we are not going to
14 say there is no possibility because that would be
15 wrong, it is going to be very, very unlikely, and
16 it is very difficult to draw out any entity that
17 you could say would be problematic.
18 So, I think I would try to resist
19 artificially creating viruses to validate these
20 lentiviral vectors, which are different from MLV
21 and that they have heterologous proteins, they are
22 much more stripped down than any MLV vector that
23 has ever gone into the clinic, and it may not be
24 sensible to try and artificially create a positive
25 standard.

1 DR. MULLIGAN: Can I change the topic?
2 There is a mobilization question. We will talk
3 much more about the virtues of mobilization, I
4 guess, but since we have you here and you did give
5 a sales pitch for your approach, one of my concerns
6 would be unless you have a model that would reveal
7 to us convincingly that having mobilization
8 potential would be helpful, I weigh that against
9 the risk of a bad thing happening in vivo, and the
10 question is I can't think of how you would possibly
11 test this.
12 How would you possibly test and make the
13 case that having the capacity for mobilization
14 would be useful?
15 DR. KINGSMAN: I agree with you that the
16 sole advantage in my mind of using an HIV vector to
17 treat AIDS is the potential for mobilization,
18 because if you are just asking for tat regulation,
19 then, there are other vectors that can switch on
20 things with tat. If you are asking for
21 CD34-positive gene transfer, you can use retroviral
22 vector.
23 If you are asking for stem cell gene
24 transfer, maybe you could use something that wasn't
25 HIV, although I have to say HIV is superb at

1 putting genes into stem cells, that is superb. But
2 what only HIV will do is mobilize the therapy.
3 So, I think you would make an intellectual
4 statement, first of all, that there is the
5 potential for mobilization. Then, I think in your
6 lab studies, you would study that and make sure
7 that in a coinfection with HIV, sort of studies
8 that Irving Chen did, you can see your vector being
9 distributed.
10 So, you will know that it would be picked
11 up, but how you validate that your therapy was
12 successful in the patient as a result of having had
13 mobilization, I think that would be difficult.
14 DR. MULLIGAN: I meant whether you think
15 there is any animal model system where you could
16 assess the therapeutic virtues of a mobilizable
17 vector, and I can't see how that would be tested,
18 and I don't buy the intellectual argument that this
19 may be better, and since it may be better because
20 more is better, you know, reinfection is better,
21 doing something where there might be a risk factor
22 associated with it.
23 So, you can't think of--
24 DR. KINGSMAN: No, I mean nobody wants to
25 switch to using higher primates, that would be

1 outrageous, really in my view. The only thing you
2 could do is some sort of SCID mouse, but I don't
3 think that sounds very sensible.
4 I think the standpoint that we have really
5 looked at this from is in terms of long-term
6 reconstitution in pediatric AIDS where you are
7 really trying to give a very limited number of
8 treatments to patients who are going to have this
9 disease for a long, long time, and therefore, the
10 minimum number of manipulations that you can do
11 would be useful. But it is a theoretical argument.
12 DR. MULLIGAN: To pin you down further,
13 sink you deeper in this, mechanistically, even
14 intellectually, what do you think would actually
15 happen, who much mobilization would you possibly
16 need to have happen out of the cells you infect to
17 have it be a therapeutic virtue?
18 That is, you are talking about essentially
19 making a virus-producing cell after you have done
20 your gene transfer, and that the titer coming out
21 of that cell, the mobilized virus, is going to have
22 a therapeutic effect.
23 You must have done the calculations. How
24 could that possibly be a good thing? I mean the
25 amount of virus you would need, the viremia you

1 would need out of those cells would be fantastic,
2 wouldn't it?
3 DR. KINGSMAN: I am going to back down on
4 that really, because I think it is an important
5 question, but it is very difficult to answer. I
6 think you could do studies in vitro where you are
7 looking at the progeny virus that came out of an
8 infectious cycle, and you ask what was the ratio of
9 genomes to transfer vector, and if the ratio of
10 genomes to transfer vector was 100 to 1, you might
11 think the risk wasn't worth it.
12 If, however, the ratio of genomes to
13 transfer vector was 50-50, then, you might think
14 that in half the reinfections, those cells then
15 might be protected and maybe that would be a good
16 thing to do.
17 DR. MULLIGAN: I would argue even worse,
18 that if it was that, I would be more worried. That
19 is, once you get up to a point where you are
20 getting very significant mobilization, and you have
21 significant gene transfer, think of how many
22 absolute virus particles you will then have in the
23 person. I mean do you really think that you want
24 to make a producer cell out of a person.
25 DR. KINGSMAN: This is assuming that the

1 patient has not had their virus load knocked down
2 by anything else. So, yes, you are not--I see
3 where you are coming from--you are not demanding
4 that the patient has a rampant HIV infection in
5 order to make your therapy work. That would be an
6 absolute mistake.
7 DR. MULLIGAN: I will stop after one last
8 point, which is that but then if you go down to the
9 point where you have very little infected cells,
10 therefore, you have very little mobilization, how
11 possibly could that be helpful?
12 DR. KINGSMAN: Well, because you have got
13 to that stage.
14 DR. MULLIGAN: I mean how could it be
15 helpful producing out of the small number of cells
16 that are coinfected--
17 DR. KINGSMAN: At that point, it wouldn't
18 be, because you would have achieved your goal, you
19 would have dropped your viral load. I don't think
20 anybody is talking about a cure here. You are
21 talking about another way of dropping viral load to
22 manageable levels without having to stay on drug
23 regimens for 30, 40 years.
24 DR. SALOMON: You could argue this is a
25 safety factor. What I think you are bringing up

1 here is, is mobilization in a gene therapy protocol
2 now in HIV, is that something we should be saying
3 no, there should be no mobilization, and that is a
4 safety issue, or should we say that if there is a
5 therapeutic argument for it, then, that it might be
6 still something to leave on the table, that
7 mobilization under the right circumstances would be
8 a positive thing.
9 That is what I think is the important
10 issue here.
11 DR. MULLIGAN: But I am questioning how
12 even the theoretical of the mobilization, and I
13 think it sounds very reasonable that your
14 mobilization, more virus particles, but I can't
15 find any context as we are walking through either a
16 large number of infected cells, infected by the
17 vector, or a tiny amount of vector, virus-infected
18 cells, infected by vector, that it would be
19 helpful.
20 In the one case when you have a large
21 number of cells, then, if it works effectively, you
22 are making just a ton, you are mobilizing a ton of
23 lentivirus vectors into the circulation of the
24 patient.
25 If you have a tiny number, then, the

1 amount of mobilization is insignificant.
2 DR. SALOMON: What I see from what has
3 been published in this field is, for example, if
4 you take a whole leukophoresis unit, which is a
5 good target, it is readily obtainable, but it is
6 still a whole lot of cells, 10^{10} or more
7 lymphocytes, you make your transduction on that,
8 infuse it into the patient, people are excited
9 about seeing somewhere between 3 and 10, maybe 15
10 percent maximum of circulating lymphocytes X months
11 later, right?
12 Now, if there was a survival advantage for
13 that set of lymphocytes, then, the first question
14 would be, would having 15 percent of our T cell
15 repertoire, or T cell numbers, I shouldn't use the
16 word repertoire, that that would be adequate, but
17 if you argue that the protection was mobilizable
18 and therefore would spread, so you would have 50,
19 60, 80, 100 percent of your T cells circulating,
20 then, that would be a theoretical argument for
21 arguing for mobilization. I am not, but I am just
22 trying to construct the mobilization argument.
23 DR. MULLIGAN: But just on that point,
24 then, if you said that you had 50 or 60 percent,
25 then, mobilizing your vector, I would think people

1 would have grave concerns. I mean think of the
2 amount of virus production then you are having or
3 vector production you are having, do people really
4 think they want to do that.

5 DR. SALOMON: I don't know. I am
6 perfectly willing to discuss that. I was just
7 trying to follow the argument for mobilization.
8 But I would follow it by saying that if it was
9 true, that mobilization, to the extent that you
10 were effective in reducing HIV replication with
11 your therapy, then, your mobilization would be
12 self-limiting, right?

13 You would stop mobilizing when you were
14 treated, and you could even have fun and argue that
15 if it came back, you know, then, you would actually
16 mobilize again, bring the titer down, and I mean
17 that might be a wonderful way to treat HIV.

18 DR. MULLIGAN: The proof of the pudding
19 would be observing toxicity. If it's a non-toxic
20 event to mobilize virus, so what. On the other
21 hand, if people get immune complications or just
22 complications of the vector load, then, obviously,
23 that would have limitations. So, I mean one would
24 ideally like to model it in some animal system
25 before going forward with humans.

1 DR. DELPH: I guess my other question on
2 that is would the mobilized virus be transmissible?
3 DR. KINGSMAN: Well, you are talking about
4 a transfer vector genome, and then it could be
5 transmissible, so I think that the safety testing
6 of the transgene is absolutely paramount in these
7 type of studies.
8 Actually, we have no intention of putting
9 forward a clinical protocol of this AIDS therapy
10 until there is an awful lot more studies being
11 done, but I think I would only do an RNA
12 therapeutic because the data suggest that there are
13 no significant immune responses against the
14 therapeutic RNA, so the studies that have been done
15 to date suggest that RNA molecules will not create
16 an immune response because the difficulty would be
17 is if in engineering the patient's stem cells you
18 suddenly made them targets for an immune response,
19 you would then create an immune deficiency, which
20 is obviously completely bad news.
21 So, I think these type of mobilization
22 strategies are really useful for RNA therapeutics,
23 but I would myself not campaign a protein
24 therapeutic down this route, but that's just my
25 view.

1 DR. MULLIGAN: One last time on the
2 mobilization. The point is that you are
3 essentially in an in vivo context trying to make
4 producer cells that then transfer this vector.
5 DR. KINGSMAN: Yes, that would be the
6 concept.
7 DR. MULLIGAN: Presumably, for that to be
8 therapeutically useful, that is, the mobilization
9 virus, you are going to have to have efficient
10 infection of the cell population by that mobilized
11 virus.
12 So, that mobilized virus is made in the
13 circulation essentially, so what is the
14 concentration that you would have to have of
15 mobilized virus to have a therapeutic effect in an
16 of itself?
17 DR. KINGSMAN: I wish I hadn't put those
18 two slides in. I thought, well, it probably is
19 helpful for the discussion. I think the only
20 answer I can make is if you can dilute the potency
21 of HIV genomes with therapeutic genomes, that
22 should be a useful thing to do, but your
23 therapeutic strategy cannot be mobilization per se.
24 I don't think anybody is--well, I hope
25 nobody is saying that. Your therapeutic strategy

1 is the ribozyme that you put into the target cell
2 to prevent infection in the first place. All I was
3 suggesting is that sometimes that won't be
4 successful, you will get virus escape, and that if
5 you do get virus escape, it might be a jolly good
6 idea to try and arm some of that virus with
7 therapeutic gene, so you get an added benefit.
8 I think the concept of the therapy being
9 dependent on mobilization is completely erroneous.
10 That is not what people--well, it is certainly not
11 what we are saying--we are just saying it's an
12 adjunct, it's an added benefit.
13 DR. MULLIGAN: What would be the
14 difference between mobilization, just shooting in
15 the virus I.V.?
16 DR. KINGSMAN: The vector.
17 DR. MULLIGAN: The vector I.V.
18 DR. KINGSMAN: I think if you could come
19 up with a protocol for I.V. administration with
20 sufficient amount of vector, I think that's a jolly
21 good approach. It is just that we would have
22 thought that is something that would happen much
23 later on in the development of these vectors, the
24 intravenous administration of vectors, I would have
25 thought would follow on from protocols that did ex

1 vivo administration.

2 But if you are allowed to go straight I.V.
3 or straight into the bone marrow with an injection
4 of a lot high titer of an HIV vector, then, I think
5 that would also achieve protecting a large number
6 of target cells, which is all that we are talking
7 about here, so I can't disagree with you.

8 DR. CHAMPLIN: In vivo production of virus
9 would presumably occur in lymph nodes more so than
10 the blood itself, so you would be adjacent to
11 uninfected lymphocytes, so you might very well have
12 a local concentration that would be adequate even
13 though the systemic concentration would be low.

14 DR. ZAIA: I would like to talk some more
15 about mobilization, but in a different context, and
16 that is the context of whether or not--I can accept
17 an HIV mobilizing in HIV, and thinking the
18 resultant virus is going to be at least on the same
19 genetic part of the ladder that you showed us.

20 But when I see that HIV could mobilize
21 EIAV, then, I worry that maybe that virus is going
22 to be different. So, can we agree that there may
23 be a greater safety issue involved if we are using
24 EIAV that is being mobilized in the context of an
25 HIV infection, or would you disagree with that?

1 DR. KINGSMAN: Well, I will turn it around
2 to how do you feel about MLV? I mean MLV is
3 mobilized to the same extent as EIAV.
4 DR. ZAIA: Not quite as much.
5 DR. KINGSMAN: Well, 500 compared to 800
6 in a series of experiments that when they are done
7 to completion, may actually--you know, it is the
8 same order. I think there is no real difference
9 between them. So, MLV is currently in trial in
10 HIV-positive patients for hemophilia.
11 DR. ZAIA: But still there was a 3 log
12 difference, but it wasn't zero.
13 DR. KINGSMAN: It won't be zero. I mean
14 you have sticky proteins and sticky nucleic acids.
15 You will get nucleic acids picked up and
16 transferred, and there have been studies with HIV,
17 and really, there is always a background level of
18 packaging of things.
19 So, if you are going to try and shoot for
20 absolute zero, then, I really think that we should
21 shut up shop and come back in maybe 50, 60 years
22 time, because I don't think biology gets down to
23 absolute zero.
24 So, there is a level of mobilization, and
25 I think you have to balance the--

1 DR. SALOMON: The implication here, and I
2 thought it was interesting, and I thought of it,
3 too, you know, in a sense this would be an
4 iatrogenically induced zoonosis, but the idea here
5 would be if MLV is mobilized and you have done MLV
6 in an HIV-positive patient--by the way, that is not
7 exactly the best argument in the world, maybe we
8 should stop those trials--
9 DR. KINGSMAN: It is not, but it is an
10 argument, it's not my trial.
11 DR. SALOMON: I just was going to point
12 out that there is a logical flaw there, but let's
13 assume that there is. Is there any difference
14 specifically in mobilizing a class of virus, i.e.,
15 in this case, these are both lentiviral vectors,
16 although they are quite different--
17 DR. KINGSMAN: But what is left there?
18 You know, what are we mobilizing? Are we
19 mobilizing a lac-Z?
20 DR. SALOMON: How do you know you are not
21 mobilizing this incredibly engineered gag-pol from
22 your packaging line?
23 DR. KINGSMAN: Because you have screened
24 for that.
25 DR. SALOMON: And you have screened for

1 that in your third assay.

2 DR. KINGSMAN: You have screened for your

3 mobilized RT, you can screen for mobilizable RT.

4 DR. SALOMON: But there was one

5 replication-competent retrovirus in X number of

6 particles, so those were the couple that carried

7 the--

8 DR. KINGSMAN: Yes, that happened to get

9 into a cell that happened to be coinfected with

10 HIV, that happened to then recombine.

11 DR. SALOMON: But it happened to get into

12 the cell with the HIV is your whole purpose, right?

13 DR. KINGSMAN: Well, I don't want to

14 muddle up the HIV therapy for AIDS, because HIV

15 therapy for AIDS, I think is a special case, and

16 what the bulk of my talk was, and somebody did say

17 to me you should leave the HIV slides out, and I

18 wish I had done--but the bulk of my talk was about

19 EIAV vectors and whether these are safe and whether

20 these can now be used in the clinic.

21 DR. SALOMON: My comments were

22 specifically in the HIV case, it was not the case

23 of the EIAV.

24 DR. NOGUCHI: Dan, I would just remind

25 you, and, Dr. Kingsman, we do appreciate your

1 willingness to come and present, but we were going
2 to be more cordial and not really discuss specific
3 products for our guests who are presenting their
4 future plans.
5 But I do have to say that in terms of just
6 biologics production, going back to the little
7 debate before on sensitivities of assays, really,
8 we are talking about not so much whether you are
9 regenerating a horse virus which has no relevance,
10 we are talking about can you detect something that
11 we want to know about.
12 It may be that a horse cell might be more
13 sensitive for amplification that you are talking
14 about, or it might be something like Moose Dooney,
15 we simply don't know, but the point is if there is
16 an actual recombinant that we want to see, however
17 you amplify it to get to be detectable is
18 appropriate even if it happens to be in horse
19 cells.
20 But in the general presentation, I think
21 if we could focus on the science that you
22 presented, that would probably make everybody a
23 little more comfortable.
24 DR. KINGSMAN: I have to say I am not
25 uncomfortable. I think the debate is really very,

1 very interesting and very useful.

2 DR. SALOMON: I think the principles that
3 I am still trying to struggle with here would be I
4 think we did a pretty good job trying to get a
5 little bit at the definition of an RCL assay, and I
6 think that your approach to it is very reasonable
7 actually.

8 The part that we are talking about now, I
9 guess there is a couple like themes here that I am
10 not sure we have really got a discussion going and
11 completed yet, and that would be, number one, in
12 generating trials for HIV with lentiviral class
13 vectors, one could say you should not have
14 mobilization, it is too early in the field, one
15 should start off with a non-mobilizing strategy,
16 and a principle should be no mobilization, not that
17 you couldn't construct a theoretical argument for
18 mobilization, and I was doing that as a
19 point/counterpoint with Dr. Mulligan.

20 That didn't mean that I was trying to
21 argue that that was appropriate for the first
22 trials. So, I think one issue that I would like to
23 see the Committee discuss, I am not going to try
24 and even achieve a consensus, but just should one
25 in these first trials say just say no to

1 mobilization, not saying the next generation of
2 trials might require mobilization.
3 So, that was one point. The second point,
4 which I was kind of getting at, and I think that
5 was the point Dr. Zaia was getting at, is in these
6 HIV trials, is there an argument, a specific
7 argument to be made for using an HIV-based vector
8 or using an EIAV or an FIV or an SIV vector, which
9 are all possibilities, right?
10 In other words, is there a rationale from
11 a safety point of view--science is not the
12 point--for using a certain class of vectors or not
13 using a certain class of vectors in these trials?
14 So, if we could kind of deal with those
15 two things, mobilization now or never, and class of
16 vector as it relates to the use in a trial.
17 DR. CHAMPLIN: In the absence of any data
18 that mobilization is really toxic, I mean I
19 wouldn't categorically exclude it. I mean it would
20 be the burden of the group bringing forward a
21 project to demonstrate that in the preclinical
22 system that it seemed to be beneficial, at least
23 the overall approach was beneficial, and there
24 wasn't undue toxicity, and that as one went into
25 human trials, when that day would come, that one

1 would select the proper patient group where the
2 risk-benefit relationship would be rational, but I
3 would exclude it completely.
4 I could envision cell-cell interactions in
5 a lymph node where you would actually want to have
6 the vector distributed through the cells and the
7 node to more completely administer the therapeutic
8 effect.
9 So, it very well could be a positive thing
10 if it didn't sort of overshoot and produce toxic
11 effects.
12 DR. EMERMAN: This is Mike Emerman. I
13 just want to talk about the mobilization with HIV
14 patients a little bit. First of all, the principle
15 is if the HIV vector gets mobilized, it means it is
16 not working very well, so presumably the vector is
17 there to inhibit HIV production, and if it is
18 officially mobilized, it means it is not
19 efficacious at all.
20 The major problem with mobilization is
21 that the mechanism of recombination in retroviruses
22 is co-packaging. So, if we are worried about
23 possible recombinants between endogenous or the HIV
24 in the patient and the vector, that is how it is
25 going to be occur, is by co-packaging by the

1 mobilization.

2 So, in both cases, mobilization is a bad
3 thing.

4 DR. SALOMON: That came across.

5 DR. ALLAN: I am not a vectorologist, but
6 can you completely design something that is not
7 going to be mobilized if you are treating a patient
8 that has HIV, and you are using HIV as a vector,
9 can you prevent that? I don't think you can unless
10 you have something that is 100 percent effective
11 like Michael was saying.

12 DR. MULLIGAN: Just to come back to your
13 crystallized issues, on the first point, I would
14 argue very simply that no, not now, because I think
15 that there is no one who has ever made the case yet
16 that mobilization for this therapy would be
17 anything more than a bell and whistle or chrome to
18 the concept.

19 No one has said that this mobilization
20 approach was likely to make it work when it
21 wouldn't work without the mobilization, so I agree
22 with exactly the way you put it, that the issue is
23 not to say this is never a good idea, but I think
24 we are very far from having any compelling, even
25 intellectual arguments, much less really

1 experimental data, which I think would be very hard
2 to get.
3 Then, Michael's point I think is actually
4 very good, that I hadn't even gotten to, which is
5 just as he says, if it allows the virus, if it
6 isn't protecting things, and mobilization
7 increases, things aren't working so hot. I think
8 it's a good point.
9 DR. BORELLINI: When I came up here, the
10 topic was RCL, so I am going to ask my RCL
11 question, and I am picking up again from Dr.
12 Noguchi's comment. I think that it is very
13 important to not have barriers to amplification in
14 the design of your assay because as it is designed
15 now, you could have something amplifying really
16 well, and growing out really well if your
17 amplification system was an equine cell line, and
18 with your system now, you would release this, and
19 based on the fact that it does now replicate in one
20 human cell line, but you don't really know the
21 tropism, what this thing is going to be, and you
22 are putting a lot of weight on that one human cell
23 line to tell you that it is now going to replicate
24 into any other human cell lines.
25 DR. KINGSMAN: Well, we are going to use

1 two, we are going to use lymphoid lines and 293, so
2 we will use two cell lines, and we will put in
3 amplification on those two cell lines, and we have
4 designed the system where the probability of any
5 RCL emerging is lower than for any other retroviral
6 or lentiviral vector system that has been designed,
7 and I think that by using the appropriate controls
8 and making sure that we can detect a single control
9 RCL, and we have chosen to use FeLV, that this will
10 be adequate.

11 By taking horse cells and designing a
12 positive control that is some theoretical virus
13 that might emerge and studying this in horse cells,
14 I cannot see how that is going to be--it might give
15 you a false sense of security, but the type of
16 entities that may come out of this are
17 hypothetical, diverse, random recombinants that
18 will be generated in a human cell, and that we are
19 wanting to know do they amplify in a human cell.
20 That is what we are talking about.

21 DR. KAPPES: I am not sure if I completely
22 agree, and let me explain my vantage point. In a
23 moment, I think, I will show a system that I
24 devised where I have enhanced, and I will show the
25 details, enhanced every opportunity for that

1 recombinant to be detected.

2 So, what did we learn by doing that? We

3 learned, well, first, we derived sufficient

4 recombinant to analyze, and then by analyzing it,

5 we understood its nature in a way that I believe we

6 are able to address the very questions we are

7 trying to get a handle on, and that is how to

8 design a vector or an assay for QA/QC to minimize

9 the risk of the emergence of RCR in vivo.

10 I know your comments were slightly

11 different, but in a general sense, I point that

12 out.

13 DR. KINGSMAN: Well, yes, I mean I know

14 what you are going to talk about, and I am looking

15 forward to hearing that, and I hope you can move on

16 it fairly swiftly, but this is the nub of the

17 matter, is to how far we recreate what we feel is a

18 suitable positive control based on our best guess

19 as to the likely entity that might emerge, and if

20 we use as the positive control, are we all going to

21 go away feeling super comfortable that we have

22 second-guessed this thing and we have designed

23 something, where it could take quite a long time to

24 recreate this hypothetical virus in studies in cell

25 lines that we are not really interested in knowing

1 about the pathogenic principle.

2 Really, this is why I go back to one of my
3 early slides, which says that the development of
4 these vectors should be matched with the target
5 population and the disease, and that we can begin
6 to learn about the performance of these vectors in
7 the clinic.

8 Our proposal is to take a vector for
9 prostate cancer into the clinic in late-stage
10 patients who have no other treatment option, a
11 local delivery where we can monitor gene transfer,
12 we can monitor the blood for the presence of
13 sequences, we can monitor the delivery site, we
14 re-biopsy, and we ask about what's actually going
15 on, and we learn in the relevant host with relevant
16 assays what these vectors are doing.

17 I certainly wouldn't advocate taking these
18 vectors at the current stage of development and
19 going intravenous into young children or who can be
20 treated with an enzyme or some other therapy right
21 now. It is clearly an ongoing process.

22 Now, I think we need to be very careful
23 not to inhibit the developments of the field by
24 making too many scenarios for hypothetical events,
25 you know, we could delay this now for a long time,

1 and I think there are vectors. We have heard this
2 morning, we have seen some very carefully crafted
3 vectors. I am biased, but I believe that we have
4 carefully crafted some vectors that have a good
5 safety profile.
6 I think one can assay for the major safety
7 components and by trialing them with a transgene
8 that is per se non-toxic. I would suggest that we
9 are nearly ready to make progress with these
10 systems for certain diseases, for certain
11 endpoints.
12 I think the HIV in HIV-positives is a very
13 special peculiar case, and we should not get
14 sidetracked by the special features of that
15 combination when we have much more global features,
16 and we have diseases that can be treated with these
17 vectors, which there are no other vectors out there
18 that can tackle some of these diseases.
19 So, I think we need to bear that very
20 strongly in mind.
21 DR. DELPH: I have two questions, but I
22 would just like to make a comment on a statement
23 that you just made, and that is, I do
24 disagree--well, I agree that HIV gene vector
25 transfer may be somewhat special in people who are

1 already HIV-positive. I think we cannot ignore the
2 fact that there is a possibility that people who
3 are HIV-negative can become HIV-positive, so I
4 don't think we can behave as if they are mutually
5 exclusive.
6 DR. KINGSMAN: I absolutely agree with
7 you, and I was not intending to imply that, and, in
8 fact, I think any decision that would disadvantage
9 a particular group of patients and exclude them
10 from a therapy is to be guarded against at all
11 cost.
12 This is why I made the comparison between
13 EIAV and MLV, and suggested that the performance
14 characteristics of a vector, such as EIAV, are
15 compatible with using them in any type of patient
16 irrespective of their HIV status, because we don't
17 currently prescreen patients for entry into MLV
18 trials for their HIV status. We don't exclude
19 people on that basis, and I would argue that there
20 is no reason to exclude a group of patients with
21 EIAV vectors.
22 What I mean is the special case is an HIV
23 vector in a patient who has overt levels of virus
24 where I think there must be special consideration
25 of the biology.

1 DR. DELPH: I have two questions, and the
2 first one is are there external factors that
3 increase the probability of the emergence of
4 replication-competent retroviruses or of
5 mobilization?
6 The second one is what are the effects of
7 irradiation on cells that have undergone retroviral
8 gene transfer therapy?
9 DR. KINGSMAN: The first question, are
10 there any special circumstances that impact on RCLs
11 and mobilization, I mean we heard the original MLV
12 study in the immunocompromised primate, so there
13 was the suggestion that if the immune system is
14 compromised and an RCL emerges, then, the
15 consequences would be possibly more drastic.
16 I just don't think I have an answer for
17 the question are there things that you can do to a
18 cell to make it more likely to make RCLs or to make
19 the RNA more likely to be mobilized. I can't quite
20 get a handle on thinking--somebody might want to
21 help me out on that one--I can't think of something
22 we could do to a patient to add to any issues, but
23 that may be a deficiency in my--
24 DR. MULLIGAN: I think there are things,
25 but they are probably not relevant here. There is

1 like demethylation, if you demethylate sequences,
2 viral sequences that are not transcriptionally
3 active, which probably isn't going to be the case
4 here, but that would then lead to mobilization
5 increases.

6 DR. KINGSMAN: Yes, I mean these are
7 things you would do in the laboratory, but I can't
8 think about things in the patient.

9 The other question was irradiation, what
10 would irradiation do to patient cells that contain
11 a gene therapy transfer vector. I have to pass on
12 that one. I haven't got any expectation of any
13 definite.

14 DR. SALOMON: I am pretty sure there is no
15 answer to that one.

16 DR. KINGSMAN: Yes, good, I can't think of
17 one.

18 DR. SALOMON: I would like to move on to
19 Dr. Kappes.

20 If it is brief?

21 DR. CORDOVA: Just very brief. We have
22 heard about the biological barriers, however, this
23 is being produced within the human cell line, and
24 so clearly, you are providing whatever barriers
25 there are, you are overcoming them.

1 What other barriers would there be,
2 biological barriers, would it be the envelope
3 perhaps that lets it only replicate as a wild-type
4 in equine cells?
5 DR. KINGSMAN: Well, the LTR doesn't
6 function in human cells, so that is obviously a
7 barrier, and the envelope, we believe you
8 can't--well, we don't believe, we know--even if you
9 get a vector genome into a human cell, if you are
10 relying on the LTR, tat activity to drive it, it
11 won't work, and I showed you those data. So, there
12 is an entry barrier, there is a gene expression
13 barrier.
14 I don't know if there are any other
15 barriers. Presumably, I mean there are situations
16 with murine retroviruses where the gag-pol is a
17 determinant, but clearly, the gag-pol functions in
18 these vector systems, so one would have to assume
19 that the gag-pol is not a barrier.
20 So, it is envelope and gene expression.
21 DR. CORDOVA: So, within the context of
22 what you created, your vectors, you really have
23 overcome those barriers because you are producing
24 them in a human cell line, correct?
25 DR. KINGSMAN: Yes, be definition, you

1 would have to have done that.

2 DR. SALOMON: Thank you very much. You

3 became, unfortunately, the point person for a

4 couple key parts of the debate, I really don't

5 think they were directed specifically at you,

6 Susan.

7 The last speaker of today is Dr. John

8 Kappes from the University of Alabama at Birmingham

9 on Predicting Lentiviral Vector Safety in Vivo.

10 **Predicting Lentiviral Vector Safety in Vivo**

11 **Dr. John Kappes**

12 DR. KAPPES: I would like to thank the

13 organizing committee for the opportunity to discuss

14 my ideas and also, maybe preemptively, point out

15 that there is nothing absolute about my choice of

16 words here for predicting lentiviral vector safety

17 although I think there is merit, and that is the

18 focus of this talk.

19 [Slide.]

20 This slide, knowing that I was presenting

21 later in the day, was meant to summarize probably

22 what was presented, and in the case truly is what

23 was presented and discussed in great detail, but

24 let me walk through it.

25 The status of the field obviously, I think

1 everyone recognized tremendous progress has been
2 made in vector design without removing the
3 potential of these vectors to effectively transfer
4 genes, not just in vitro, but in vivo.
5 The primary challenges that we face
6 include an approach or approaches for safe
7 administration of the vector, and in particular,
8 for issues related to recombination and then
9 generation of RCL. That, of course, relates to in
10 vitro QA and QC.
11 I would like to pause here for a second to
12 try to take what I have heard this morning and
13 place into context the data that I am going to
14 present and the ideas I am going to suggest.
15 There is no doubt that tremendous progress
16 has been made in the design of these vectors, and
17 there are many ideas discussed particularly that
18 related to the use of RCL assays for quality
19 assuring against the generation of RCL in vivo, but
20 we keep coming back to academic theoretical
21 possibilities of is this true or isn't this true.
22 For example, let me walk through the
23 vector design. The envelope was removed to prevent
24 specific transfer of any progeny by
25 envelope-receptor interactions. The accessory

1 proteins were removed because they play a role in
2 pathogenesis of disease, but does the removal of
3 those genes mean a recombinant won't be pathogenic?
4 I don't think anyone knows, but certainly it
5 wouldn't have the pathogenic properties of the
6 parental virus.
7 Tat is completely gone. Rev, in the case
8 of HIV, is provided in trans, although keep in mind
9 in the absence of rev, again, you don't have an
10 absolute block in the transfer of RNA from the
11 nucleus to the cytoplasm. So, again, you can get
12 into these theoretical arguments.
13 Similarly, with the SIN vectors, while
14 there is a huge decrease in the transcription from
15 the LTR with the deletion in U3, there still is
16 what I will call basal transcription, and in part,
17 this is affected by the position at which the
18 vector integrates.
19 So, again, you come back to arguments what
20 if, and what if, and how about, or whatever, and my
21 point is how about if--maybe I shouldn't ask--I
22 will suggest that if we move away from using or
23 focusing on RCL as an endpoint for what constitutes
24 a safe virus, this is our determination of RCL in
25 vitro for safe vector, and think--I go back to what

1 Susan was talking about-- think about a marker that
2 is absolutely required for the generation of RCL.
3 I will give an example. If you remove U3,
4 you have a SIN vector, yes, you can still get
5 background transcription. That transcripts can be
6 mobilized. We just had a half-hour discussion
7 about it. If you use an assay which ensures your
8 vector prior to administration lacks critical
9 reverse transcriptase function, then, perhaps the
10 assay, although it is not for RCR, maybe we will
11 call it pre-RCR, to me has more definition and
12 perhaps more value in advancing these vectors
13 toward the clinic.
14 So, that is what I hope to do, present
15 some data that might help advance the vectors
16 toward the clinic.
17 [Slide.]
18 Simplistically, we have the packaging
19 construct, the gene transfer vector, and the
20 envelope, and the concern is that through genetic
21 recombination, now, this is in the primary
22 transduced cell, we would generate a structure here
23 depicted as LTR-gag-pol-env-LTR/RCL.
24 I think that is highly unlikely especially
25 now if we separate rev and other components and

1 minimize U3 regulated expression, and, in fact,
2 neither the second nor the third generation vectors
3 have been shown to generate RCL, I don't think it
4 is going to happen, I don't think we are going to
5 find RCL. That is in part why I am stressing the
6 potentially, at least the limited value of its use
7 as a marker for safety.
8 On the other hand, a single recombination
9 event between the packaging construct and the gene
10 transfer vector can generate this kind of
11 structure. That will be the focus of the rest of
12 my talk, but first, hopefully, it is not too
13 laborious, I am going to show five or six slides to
14 try to underpin where I am going with this.
15 [Slide.]
16 First point. Genetic recombination, I
17 would be as bold to say is likely. These
18 retroviruses use genetic recombination as part of
19 their life cycle, part of the reverse transcription
20 process.
21 Our past experience with MLV might also
22 suggest that reverse transcription or through
23 reverse transcription, genetic recombination is
24 likely, not just with components that comprise the
25 vector, but also in certain cases with endogenous

1 genes.

2 This issue of genetic recombination, as we
3 are all acutely aware, underpins our concerns with
4 respect to safety and RCL.

5 [Slide.]

6 My point that I am trying to get at is in
7 vitro measurements of RCL are not predictive of the
8 emergence of RCL in vivo in the long term. In
9 fact, the way we could think about the in vitro
10 assays for the generation of RCL would be for the
11 emergence of a structure which had replication
12 potential, while in vivo, the detection of RCL,
13 whether it's months to years after administration,
14 would be recognized as failure even though at this
15 step, perhaps the vector didn't generate RCL.

16 [Slide.]

17 So, what are the requisite safety
18 measures? I think we have discussed each of these
19 today. There is PCR-based assays, there are
20 RCL-based assays, and what I will describe as a
21 gag-pol recombination assay.

22 [Slide.]

23 I will quickly try to move through the
24 advantages and disadvantages of each of these,
25 again trying to emphasize why I think a gag-pol