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FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

BIOLOGICAL RESPONSE MODIFIERS

ADVISORY COMMITTEE

MEETING #31 - VOLUME II

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C O N T E N T S

P R O C E E D I N G S

Opening Remarks

DR. SALOMON: Good morning to Meeting No. 31. We still haven't got titles. One of my big disappointments with the FDA is that we have never had any kind of sexy titles for these meetings, and I am not in a position to make them up on the fly, so I apologize.

So, this is Meeting No. 31 of the Biological Response Modifiers Advisory Committee. Today is I think the beginning of a very important two days in which discussions of a new vector class for gene transfer and gene delivery, that of lentiviral vectors will be discussed.

There is just a number of organizational things. There is a lot of new people around the table and I welcome everyone from yesterday, the table has expanded somewhat.

One thing for those of you who have not been at these conferences, but this button in front of you, if you speak, you push down, the button turns on red, and when you are done speaking--that way you don't get pickup from everyone, and the transcribers and the audience will be a lot happier, so I appreciate that.

There will be two days here. Today, we are going to be talking and being educated in some context by some experts in the field along the lines of lentiviral vectors, and there are a series of questions that the FDA staff has generated. That doesn't mean that we can't generate other questions.

I think this is really, particularly in looking a new gene delivery class, is an excellent opportunity for everyone to interact in the context of trying to identify what sorts of issues are important in FDA's approach to developing, regulating, and providing appropriate IND guidance to sponsors in this new field.

So, this is our chance to input this kind of data. With that said, a couple quick things. I am going to try, one of my jobs is to come up with consensus. That doesn't mean that, number one, consensus is always possible or even appropriate, so there will be times when the committee has every right to say no, I don't agree with that, that is not consensus.

There will be other times in which consensus might come in terms of, you know, majority opinion, but I would very strongly

encourage anyone with a well articulated and defended minority position to take it, and that is I think very appropriate and not to feel any pressure from me to be in consensus. If we can obtain consensus, that is excellent, however.

Today, we are also going to hear from a series of sponsors who have special expertise and experience in developing lentiviral vectors for gene delivery. Today, I think it is very important to point out a distinction here. These sponsors are here at the request of the FDA, and they have stepped forward voluntarily to provide us with information that gives us specifics and gives us a chance to look at their experience and sharing their experience.

We are not here to judge their protocols. Many of them are not ready to put them forward for formal INDs. So, it is very different than what is going to go on tomorrow where we have a sponsor who has very seriously stepped up to the plate and proposed a real clinical study now.

I think there, then, the committee has a different charge. So, I just want to explain to everyone that these sponsors are coming up and we really, really appreciate their participation. It

is very important just to cut them some slack.

The idea here is to share their experience and none of them are saying they are ready for a clinical trial tomorrow. They all realize that every strategy has some limitations and some future for it.

So, with that, I think we are going to read the Conflict of Interest Statement or at least an abbreviated form of it, and then we will go around and introduce everybody, and then we will get started.

Thank you.

Conflict of Interest Statement

MS. DAPOLITO: A Conflict of Interest Statement was read for the record yesterday. I don't need to go through the whole entire thing again. I will just repeat that the FDA has appointed Ms. Katherine Knowles, Dr. Gaylor, Drs. Allan, Cornetta, Emerman, Kordower, Lane, Torbett, and Zaia as Temporary Voting Members for the Committee discussions today and tomorrow.

The following participants were issued waivers to participate in the meeting: Drs. Champlin, High, Mulligan, Lane, and Kordower.

I think that is all that needs to be said

today.

DR. SALOMON: Again, just because I don't even know everybody on the Committee, I would like to go around, starting with Dr. Zaia, and introduce yourself, where you are from, and a brief idea of where your areas of interest and expertise are.

Introduction of Committee

DR. ZAIA: My name is John Zaia. I am the Director of Virology at the Beckman Research Institute at City of Hope. I am also interested in clinical research, and I am the Director of the General Clinical Research Center and have an interest in gene transfer studies.

DR. TORBETT: I am Bruce Torbett from the Scripps Research Institute, Department of Molecular and Experimental Medicine. I am interested in gene delivery, myeloid development, and protection from HIV via gene delivery.

DR. HIGH: I am Kathy High. I am the Director of Research in the Hematology Division at the Children's Hospital of Philadelphia, and I have an interest in gene transfer for hemophilia.

DR. ALLAN: I am Jon Allan from Southwest Foundation in San Antonio. My area of study is natural host resistance to SIV, so I study

pathogenesis of AIDS viruses.

DR. GAYLOR: I am David Gaylor of Sciences International. My area is biostatistics and risk assessment.

DR. CHAMPLIN: I am Richard Champlin from the M.D. Anderson Cancer Center. I am the Chairman of the Department of Blood and Marrow Transplantation.

DR. SAUSVILLE: I am Ed Sausville from National Cancer Institute. I am from the Developmental Therapeutics Program, which evaluates and manufactures drugs and biologicals for cancer and AIDS.

MS. LAWTON: I am Alison Lawton. I am the industry rep on the Committee. I chair the Cell and Gene Therapy Committee for the Pharmaceutical Association, PhRMA, and I work for Genzyme.

DR. SALOMON: I am Dan Salomon. I am at the Scripps Research Institute in Molecular and Experimental Medicine. My interests are in cell and organ transplantation, tissue engineering, and gene delivery.

Before we go further, we have through the miracles of modern technology Dr. Michael Emerman, who we are going to have him introduce himself and

test this whole system out.

Dr. Emerman?

DR. EMERMAN: My name is Mike Emerman. I am at the Fred Hutchinson Cancer Research Center. My expertise is in HIV, molecular biology, and replication.

DR. SALOMON: Thank you.

MS. DAPOLITO: Gail Dapolito, Executive Secretary.

DR. RAO: Mahendra Rao. I am the Stem Cell Chief in the Laboratory of Neurosciences at the National Institute on Aging.

MS. KNOWLES: I am Kathy Knowles. I am the consumer representative on the Blood Products Advisory Committee and I am serving in the consumer role here today at this committee.

DR. DELPH: Good morning. I am Yvette Delph. I am with the Treatment Action Group, which is an HIV/AIDS treatment activist organization.

DR. VERMA: I am Inder Verma from the Salk Institute in La Jolla. I am interested in signal transduction and also vectors for gene delivery.

DR. PATTERSON: I am Amy Patterson, Director of the Office of Biotechnology Activities in the Office of Science Policy at NIH.

DR. TAKEFMAN: Dan Takefman. I am a product reviewer in the Division of Cellular and Gene Therapies at the FDA.

DR. WILSON: Carolyn Wilson. I am also a member of the Division of Cellular and Gene Therapies, FDA/CBER.

DR. NOGUCHI: I am Phil Noguchi, Director, Cellular and Gene Therapies at FDA.

DR. SIEGEL: Jay Siegel, Director, Office of Therapeutics Research and Review, FDA/CBER.

DR. SALOMON: I welcome everyone and we might as well just go and get started.

Dr. Verma.

TOPIC 1: LENTIVIRUS VECTORS IN GENE TRANSFER CLINICAL TRIALS

Lentiviral Vectors

DR. VERMA: Thank you very much. Thank you very much for the invitation and to the members of the committee and the audience.

This morning, when I came in, I ran into Luigi Naldini and suddenly realized it was only about five years ago that it was an academic exercise we had whether we can convert HIV into a useful vector system. I am delighted to see that today we are here discussing the possibility that

it might actually have an application in the clinic.

[Slide.]

So my job is really today to give you a general introduction of the vectors. I realize there are many, many experts here in the field. In fact, some of the founders, some of the people who discovered the original. So I apologize to them if this is something very simple, but I would like to bring everybody to the same level so that the rest of the day will be easy for you.

Gene therapy is a form of molecular medicine which will have a major effect on human health in the coming centuries. I think the concept of gene therapy is disarmingly simple; introduce the gene, and its product should have the ability--I am a little confused because a guy is going like this all the time. Okay; I am going to igonre the guy. In any event, I was telling you about gene therapy. It is is a relatively simple concept.

But the fact of the matter is it hasn't been really as successful as we had anticipated and part of it has to do with the fact that the matters of delivery haven't been really quite as exquisite

as we would have liked to have them.

In fact, there are many, many ways to introduce the gene. What I would like to do is really give you one. But just to give you the background of it, as I said, there are many different ways to introduce the genes. Some have generally divided the physical method by which you can directly introduce the gene and there are wonderful ways of introducing the gene but it depends on what you want to do.

The bottom line for all the vectors is whether it is physical or biological, it really depends what you want to do. If you simply wanted to make a vaccine against a small amount of the protein, sufficient amounts of this can be done by direct DNA injection.

[Slide.]

But if you wanted to make sustained amounts of a foreign protein for a sustained period of time, by and large, most of us have concentrated on the biological method. Again, the important point here is not to be exhaustive about the number of the vectors because there are many missing. The important point is that each one of them has a major limitation.

So, again it comes back to what we want to do. For the two people in this audience who don't think about these vector systems, the principle of making these vectors is again disarmingly simple. All the viruses do, in their life, is to replicate. They have really no interest to kill you. Their main job is to simply replicate.

But, occasionally, they do acquire sequences which have the ability to cause disease. So all that we are trying to do, everyone who makes vectors, is to eliminate the disease-causing component, substitute with the therapeutic gene of interest, reconstitute the virus which is no different than the starting one essentially.

So the idea is simply to replace the therapeutic component, in this case removing the pathological sequences and recreate the virus which, hopefully, will have the same sets of function, by and large, that you started out with. So that really is the principle of making most of the biological viral vector systems.

So what we would like to do, really, is to create a vector--as I said, there is no ideal vector, but we would like to set up some parameters which we believe will be useful in the long run for

making, or at least thinking about vectors which can perform many of the functions we are desirous of.

What we would like to do is a vector which we can generate at fairly substantially high amounts. Again, this is for the aficionados in the field, 10^8, 10^9. It depends upon how many virus particles you can introduce, so you need to make substantial amounts.

Particularly for this audience, it is important to have reproducibility and the convenience to make them. We would like to introduce the gene in any cell type regardless of the fact that it is the tumor cell which is dividing or it is a brain cell which is not dividing, we would like to have the ability to introduce genes in a wide variety of cell types.

Since many of the vectors we discuss have the ability to become part and parcel of your chromosome, it would be nice to know where they went. It would be really nice to know with the 3.2 billion basis of the genome where did the vector actually go so we have some idea. That will be a very desirable property.

It would be nice to control the amount of

the protein or the gene transcription from outside; that is to say, you can regulate how much protein when and where you want to make, a sort of a review of turning on and off.

It would be nice to infect any cell type, hopefully liver, lung, brain, kidney, all the cell types. And, of course, we want to have no undesirable immunological consequences. So we are asking a lot. We are asking it to behave like a typical retroviral vector to integrate and yet have the ability to infect nondividing cells. At the same time, we are asking it to behave like an adenovirus, to behave like an episome, and yet have not immunological consequences.

[Slide.]

So we are asking a lot. But, fortunately for us, a completely unexpected ally came over at this time in the form of the HIV. HIV, as many of you know, is a member of the Retroviridae and has a number of properties similar to them but has also the unusual property of the ability to introduce itself into nondividing cells.

Some of the reasons that we got started on was the idea that they can infect neurons, that they can infect nondividing resting T-lymphocytes.

They can infect monocytes. So we were interested to see if that property of HIV could be utilized and we could convert them into a useful vector by which they can introduce their gene, integrate in the chromosome in a manner analogous to the typical Moloney leukemia virus which is a prototype of other retroviral vectors and yet have the ability to be able to produce the foreign protein.

[Slide.]

With that in mind, then, we constituted a team at the Salk Institute with the following idea behind it. This is an idea which really came from the work of Michael Emerman and colleagues, Mario Stevenson and colleagues and also Didier Trono who has been at the Salk Institute, now is in Geneva.

The idea was the following, that a typical retrovirus, when it makes its DNA, is much too large to be able to cross the nuclear membrane and, therefore, the cells have to divide. The nuclear membrane has to break down. The chromosome has to become available. Only then the viral DNA can become part and parcel of the chromosome.

Therefore, retroviruses only infect cells which are dividing because they need for them to go through the nuclear membrane whereas the

lentiviruses, of which HIV is the prime example, have this unusual property and we really don't know the precise mechanism and we can talk about it sometime that they have this karyophilic properties; that is to say, their viral DNA can cross the nuclear membrane and thereby integrate into the chromosome and thereby relieving itself of the restriction that the cells must divide.

So that is the principle on which we based our basic idea and began to ask the question, can we convert an HIV into a vector.

[Slide.]

I apologize for the number of colors and perhaps you can't see from the back, but you will see many renditions of this slide today, I am sure, through the rest of the day. But the bottom line is the following. A typical HIV virus, in addition to getting the prototypic three proteins, the gag, reverse transcriptase and envelope, which is necessary to make the virus which is really common to all prototypic retroviruses of the Lentiviridae or Retroviridae family.

The argument was the following. First and foremost, we want to avoid the envelope of HIV because it has a restriction to a very specific

receptor, the CD4 receptor. We had no intention to limit our vector strictly for those cells which have only that type of receptor.

So the first modification that we performed was to take this envelope gene and separate it out and substitute it from a vesicular stomatitis glycoprotein which is really a cattle virus but it has a glycoprotein which has been shown almost 30 years ago by Alice Wong and Ian Sabosa and Robin Weiss that it can actually phenotypically exchange itself for the envelope of a retrovirus.

Now, the VSAG protein, of course, allowed it to be pantropic meaning it has now the ability to infect a wide variety of cell types and, therefore, eliminate the restriction so restrictive to the CD4-positive cells because of the HIV envelope protein.

In addition, we began to manipulate sequences around it and that will be much of the emphasis today to begin to make this vector such that it has the least amount of dependence on its own sequences but, in fact, uses autologous sequences.

Just to cite an example, the LTR, which is

very common to all retroviruses, can be replaced by other promotors so as to eliminate or reduce the chances of recombination. We will talk about this as we go along.

So, the idea was, then, to make a vector where some of these genes are eliminated, glycoproteins to substitute for the envelope and ask the question can we now create a virus which has the formal ability to infect nondividing cells by virtue of the fact it has acquired that property of HIV which allows it to infect nondividing cells and yet has no ability to make an infectious virus particle.

[Slide.]

This is, again, an old experiment done by Luigi Naldini when he was in the lab along with Didier Trono and Rusty Gage. The very first experiments, we asked the question, A, can you make high titers. The answer is yes, you can easily make 10^6, 10^7 virus particles which, by virtue of the fact it is a glycoprotein from G as shown by Ted Friedman and colleagues, can be concentrated which means, again, for the cognoscenti of the field, that we can make up to 10^9 to 10^{10} virus particles per ml which, of course, is an enormous

titer for these kind of viruses.

Again, for those who do not think about it, meaning you can take a billion cells, put a ml of this virus and all those cells should now be transduced to the foreign gene product.

This experiment simply shows that at least we have with these vectors to infect macrophages. There are our typical Moloney leukemia viral vectors, the vectors we are traditionally using as retrovirus vectors, do not have the ability to make the foreign protein.

So that was the first evidence we had formally that we had a vector which has the formal ability to at least infect cells in vitro which are not normally dividing and, as such, can be transduced.

Encouraged by this, they began to ask the question, what happens if we use these genes in vivo. What I will do today is to give you a bunch of those examples and then tell you a little bit more about the safety modification.

[Slide.]

I know you can't see it, but this is an experiment where we directly injected the virus into the brain of a rat. We asked the formal

question, can you have the production of the foreign protein in the brain and how long we can have the production.

So here is an injection, one side with the HIV vector making brown GFP protein, which is a green fluorescent protein and, on this side, we have the Moloney leukemia viral vector. Again, after about six months period of time, infection of the brain. You have the expression of the protein here and none in the case of MLV.

I can't escape but to again tell you how sad this slide makes me because fifteen years of my career were made on MLV vector and all it can now do is really a control. But that's the way it is.

[Slide.]

But more important, really, is to ask the question how efficient is actually transduction. Here, again, is a single injection, 2 microliters of the virus, about 30 million virus particles directly injected into the hippocampus. Again, you can't see it, but 90 percent of the cells at the site of injection--that is to say, within 2 to 2.5 millimeters at the site of injection, 90 percent of the cells are not transduced. This is about eleven months period of time.

So three things happened for us. One; we can make a vector which can infect nondividing cells. Two; it can be directly introduced in vivo into nondividing cells. Three; there was a substantially efficient transduction at the site of injection and there was a sustained production of the foreign proteins.

So, armed with this, we began to ask the question, what other tissues where we can introduce the gene.

[Slide.]

Muscle. Muscle is a very interesting tissue because 40 percent of the body weight is muscle and it is a good system to secret the protein. For example, hemophilia, the proteins can be secreted if you can introduce the gene in the muscle.

Shown here is again direct injection into the muscle. These are the long fibers which are not dividing. Again, you can see the production of the foreign protein for eight months period of time and nothing in the case of the Moloney leukemia virus, our traditional vectors which do not infect nondividing cells.

[Slide.]

Another example I give you is the eye. A number of diseases are involved in the deficiency of genes in the vision. A lot of our work in the lab is concentrated in the areas which are largely animal-model systems, in a number of mice-model systems in which there is a defect in the vision system, either of the rhodopsin or of the different kinds of other proteins.

So we asked the question, can we directly introduce the gene in the subretinal pigmented epithelium to the specific example of retinitis pigmentosa which is a blindness due to the deficiency of many genes, one of them including the phosphodiasphase gene.

So we asked the question, can you introduce the gene. The answer is yes. If you use our traditional CMV promoter--CMV is a promoter which sort of expresses in every cell type. It allows the expression at the site of injection whereas if we now introduce the gene with the rhodopsin promotor, a promotor especially for the rod cells and the cone cells, now you see the expression largely in the rod cells and the cone cells.

[Slide.]

More importantly, if you now take a mouse which has a deficiency of phosphodiasphase beta, which is required for dephosphorylation of CGMP, removal of which causes blindness in these mice, and, in fact, if you make a section of the eye, it has all the right components except it is missing all the rod cells and the cone cells by virtue of the fact that they have not this enzyme. Thereby, there is apoptosis and thereby there is blindness.

So we argued, can you introduce the gene phosphodiasphase beta and restore at least some of the retinal cells. There are about eight or nine layers and the have none. Can we restore some of those layers.

[Slide.]

The answer is at least--this is difficult from the back--but at least four new layers of opsin which are now found in these animals which contain the phosphodiasphase gene introduced by direct injection in the eye and none in the case of the controls.

These mice haven't really lost their blindness. They are still partly blind, but it at least gives you the hope that you can begin to ask the question to directly introduce these genes in

retinal cells where there is a deficiency of a certain gene.

[Slide.]

Let me give you one other example which will be talked about here quite extensively, and also has the dream of most gene-therapy folks, to be able to infect hematopoietic stem cells because then you will have a continuous production of the foreign protein because these cells continuously produce the foreign protein for the rest of our lives.

So, in collaboration with Bruce Talbert, who is here today, and Hiro Mioshi from the lab in Bruce's lab, we asked a very simple question; can we take human cord blood cells and purify the stem cells from them. This experiment has also been done with many other viruses, particularly the retroviruses.

The difficulty is the following. The stem cells are very few. If I take 1 million bone-marrow cells at any given time from anyone, there may be 100 to 1,000 of these guys. They are not easy to find. The worst of their life is that they don't divide.

Therefore, most traditional vectors have

been difficult to be used although people have very cleverly manipulated them now by using appropriate growth factors. But, by and large, they are difficult to introduce foreign genes because they are not dividing.

Lentiviruses, because of their opportunity to infect nondividing cells, have this unique ability, then, the hope, that they will infect these nondividing, noncycling stem cells and, therefore, allow you the opportunity to have much better transfection than with the traditional vectors.

So, with that aspect and that hope, we took the CD34 cells, transduced them in the virus. Transduction, in this case, you take the cells, put the virus, no growth factors, no lymphokines, no cytokines, and simply introduce directly into the tail vein of the SCID/NOD mouse--we need SCID/NOD because are using human cells--and ask the question do they now make the foreign protein in the peripheral blood, spleen and the bone marrow.

So a simple experiment; take the stem cells, infect them with the virus, put them back into the animal and hope for the best.

[Slide.]

This, I think, is probably what I find one of the most exciting experiments in our lab and that is that you can now produce the foreign protein in the peripheral blood lymphocytes. You can see the peripheral blood lymphocytes now producing the foreign protein, in this case, 18 weeks were infected for the rest of the life of this mouse.

About 15 percent of the cells are producing the foreign protein. You and I make 10 billion of these cells a day. A billion of them now can make the foreign protein for the rest of your life which really encourages and gives you great hope in terms of proteins that you want to produce when there is a deficiency of a given product.

[Slide.]

More importantly, nearly all myeloid colonies--remember, these don't have T-cells because these are SCID mouse, the myeloid colonies are positive for ritchard colonies, venocytic macrophage colonies and even early progenitor cells. So this we think is really one of the most interesting aspects of lentivectors is their formal ability to infect nondividing cells, in this case

the stem cells and, as shown by their virtues, and repopulate, nearly all kinds of the foreign cell types, at least in the myeloid lineage.

[Slide.]

Again, for the true cognoscenti in the field, they only believe these stem cells are really transduced if they can do a second retransplant meaning that if you now take the bone marrow of the first mouse that you transduced, can you take their bone marrow and put in the secondary mouse, and that done again by Bruce Talbert and Hiro Mioshi. You can see, even in the second recipient, nearly all cells are positive what they started out once again suggesting that it is very likely we truly transduced the stem cells.

[Slide.]

So I think I have given you a number of examples of the generality and the wide spectrum which could be utilized by these vectors for a wide variety of different tissues. The question really now is how useful these vectors are in terms of the formal clinical setting and that will really call for how safe are these vectors.

[Slide.]

So what are the HIV vectors? Well, we all

know the HIV has this unique structure of inhibition to these three replication-competent necessary proteins, the gag, pol and envelope. It has this array of six additional genes which are referred to as the vif, vpu, vpr, net, tat and rev. These are all essential for the replication of the pathogenicity of the HIV which are not present in a traditional Moloney leukemia viral vector or the other kind of retroviral vectors.

So the argument was very simple. All we are really interested is to have these vectors introduce their gene in nondividing cells. We have no interest in any of these genes if they do not contribute to that function. So a number of people, Luigi Naldini's lab, Didier Trono's lab, my own lab, they come to a cell genesis. A number of other people have started to ask the question, can we begin to eliminate these genes and asked the question, do we still have a structural prototype which will introduce the gene into a nondividing cell without the baggage of these unwanted genes.

So a number of these have been eliminated. Unfortunately, I don't have the next slide. Can you just put that on for me for a moment? It is left in the United Airlines, that slide.

[Overhead.]

Again, you will see many renditions of this. This is now a vector which is a composite of vectors for a number of labs, our lab, other labs here, just to give you an idea what is now currently considered a third-generation vector from the HIV.

There are other vectors you will hear from, I am sure, Dr. Kingsman and other people later, different species of lentiviral vectors but we are concentrating only on the HIV here today.

The vector is the following. We have essentially eliminated all the fixed genes, the vif, vpr, vpu, tet and rev as well as the envelope. VSV-G is provided separately and the rev is provided separately. In addition, the long-term repeats which are necessary for the replication of this virus for integration have been deleted to what is called SIN vectors, meaning only those residues are kept which are necessary for integration. All the other components which are involved in its ability to cause the transcription, the transcription element and enhancement element in the LTR, have been deleted.

So the vector now constitutes a cell which

will eventually integrate is basically rev-responsive element deleted LTRs and--may I have the next slide. You can shut that off.

[Slide.]

So what we have now in this vector is the following. Altogether, now, only about 10 percent of the viral genome is left in this vector. So this is the starting HIV. This is the vector we have. And these that I have listed here, the base fields that are left from the main genome. They are about 10 percent of the genome left.

So, of the 9,000 or 10,000 nucleotides, there are about 900 to 1000 that are left at various junctures. In fact, most of these genes are gone. Some of these genes are gone. The reason I am showing this to you is to show, first, how debilitated it is, and, second, that the probability that you have recombination with a full-length HIV either following infection or trying to coinfect with it is not zero but it is extremely low because you have at least six or seven new genes to introduce, LTR to introduce and many other sequences, a number of replications, a number of recombination events.

So I think that is currently the favorite

one where almost all genes are deleted and there are a few other bells and whistles over here. But, by and large, this is the vector I think what you will hear a lot today discussed in terms of the utility for introducing in the clinic.

[Slide.]

How good is this vector? Well, it is true we can eliminate everything but does it work? I wouldn't be showing you all this if it didn't work. The answer is yes, it works just as efficiently as the first generation of HIV vectors where we simply eliminated the envelope gene and some other small things. But, by and large, other genes are still present.

It infects, for example, HIV, the stem cells, just as efficiently as we had our first generation vector. Again, in our hands, the Moloney does not do so.

[Slide.]

It can infect even the peripheral blood lymphocytes which have been mobilized with GCSF which I think will be eventually how, in the clinic, a lot of things will be used. They will also transduce, albeit only for a six-week period of time. That is the time point, but they can

actually be transduced with these vectors.

[Slide.]

What about bone-marrow transplantation from the mouse? That does just as well again. You can get the bone-marrow transduction, peripheral blood lymphocyte transduction, just equally well with these third-generation vectors meaning that, regardless of the effect whether we have eliminated all these genes or not, the basic ability of these viruses to transduce foreign cells is still intact.

[Slide.]

Just to expand to it a little bit more, if you now take, in collaboration with Marcus Grompi at the University of Oregon, we have taken--so, if you now have a mouse, which we have fanconi-C and fanconi-A-deficient mice. If you now introduce their stem cells, bone-marrow cells, directly infect them with the virus, in this case the HIV containing the fanconi-C or the fanconi-A, put them back into the animal, they are all phenotypically recovered.

We make the FANCC, if we make the FANCA. But, more importantly--this is a slide given to me by Minoxchi Nole from Marcus Grompi's lab--normally, what happens to them, if you give

them cytotoxin, they become extremely sensitive to them but these mice now all behave like the normal mice.

The yellow here shows you that a mouse which is deficient eventually will die. The heterozygous here shows they all survived. Those which got the bone-marrow transduced with the FANCC or FANCA, in this case, completely behave like the normal. So, for all practical purposes, these mice have now phenotypically the same characteristics as if they had a bone-marrow transplant from a sibling or, in this case, the heterozygous bone marrow.

[Slide.]

What about liver? If we use these third-generation vectors, directly introducing them into the liver, I can show you here is the direct introduction into the liver. We can use the CCD camera to take a light imaging of this liver to ask the question if the cells are transduced.

[Slide.]

The answer, again, is here is directly the liver and here is the autopsy of the liver. You can see lots of cells are transduced. About 4 to 10 percent of the hepatocytes are transduced in these transductions.

[Slide.]

We don't see any liver toxicity, because there was some question whether lentiviral vectors with VSV-G cause liver toxicity. We do not see any liver toxicity regardless of the fact that we have TDS or lentiviruses. So we think these vectors have the added ability to introduce genes not only to the tissue as I have described before but also, again, to the hepatocytes and they do not need to be dividing.

[Slide.]

Let me give you now a few examples of how we have also used these vectors, not just for gene therapy because much of the interest in my lab really is gene-transfer vectors for many biological basic questions. So I want to give you a few examples, just to give you the breadth of these vectors in addition to the safety issues that we shall discuss.

[Slide.]

In an experiment done by Yoshika Azawa in the lab, he basically asks the question which many people are asking, can you convert certain cells, stem cells, into different types of a cell.

He here took bone marrow from a male

mouse, transduced them with the lenti-GRP. Again, the lenti can transduce bone-marrow cells, in this case the stem cells, but them back into a female mouse and asked the question, can he convert some of these bone-marrow cells into hepatocytes because we cause injury in the liver by using the anti-FAS antibody which causes damage to the liver. The argument is can these blood cells now be transduced into the liver cells, and can be they be transdifferentiated.

[Slide.]

I don't know if you can see, again, in the front. About 1 percent of the cells are non-liver cells which we started out--these are the green cells which were marked and they were put back into the animal, so we can actually begin to ask the question, can you do transdifferentiation by introducing genes into non-dividing cells.

This will be particularly useful when you begin to ask if you have pancreatic-specific genes, or liver-specific genes, that you can convert any cell directly into a transdifferentiated cell type.

[Slide.]

Let me give you another example. Many of us in biology these days are very interested in

making knock-out animals. Many of us are interested in making knock-out animals which are conditional, meaning that the animals are born, but the gene can only be deleted post-birth. Otherwise, these genes are lethal to the animals.

So, what we do now is we have a system which is used--to use specific sequences called lac sequences which block the transcription of the gene until you remove these lac sequences from there, which can be done by an enzyme called CRE. Normally, you cross these animals, which is a long process. But now these vectors, lentivectors, can be directly introduced into the tissue where we are interested to remove the gene.

So you make a mouse with these specific sequences, introduce the gene CRE directly by lentiviruses and you can begin to see, at least this is now in vitro, these cells have no expression following the introduction of the CRE by lenti, you have the cell all blue.

[Slide.]

Can you do that in vivo? Here is an example. If you take, now, a gene where it is blocked by lac sites to make the foreign gene in the liver, so this is conditional for the

production of this gene in the liver. If you now introduce directly into this the lentiviruses, you can--now the cell becomes blue within the range the gene was introduced.

So, again, a very useful utility of these vectors is that you can introduce the gene directly in vivo.

[Slide.]

Here is directly in the striatum in the brain. You see the genes are again expressed. So it is really a very useful tool, particularly for those who are interested in tumor genesis. You have a conditional mutant, introduce the gene in the prostate, liver, lung, wherever you want, eliminate the gene and ask the question, what happens to the animal subsequently.

[Slide.]

Let me give you another example. This also refers a little bit to the safety of the issue. We were interested to know can you use lentiviruses for two purposes for transgenesis. So, Matha, in the lab, did the following experiment. He asked the question, what happens if you directly introduce the lentiviruses in the testes and then asked the question, can they be,

then, used to create a transgenic mouse.

His hope was that, by introducing in the testis, he will have the expression eventually in the sperm.

[Slide.]

What he found was that in the control--so here is the lacZ which is nuclear localized and here is the lacZ direct injection into the testis. When he analyzed them, what he found was--this is, again, for those people like me who don't understand too much biology--the point is the following.

You have here the sertoli cells and, eventually, the sperm cells are right in the middle here. All these other cells are really the supporting cells. So the gene was introduced directly here, and we are now asking the question, can you make mature sperm which contain the foreign genes?

[Slide.]

The answer is no. Almost all the expression is in the sertoli cells and none in the case where, in the middle, where the sperm are. So, even if you put billions of virus particles directly into the testis, while you can get the

transduction of all supporting cells, you do not transduce sperm directly.

In fact, if you make pups from these animals which have been directly introduced, you don't see a single pup which is positive for the PCR. The pups are born, but they are not positive for this. So the argument is at least directly injection. We are unable to directly introduce the gene into the mature sperm cells.

[Slide.]

Here is the direct injection again.

[Slide.]

Let me finish my talk by giving you one other very exciting piece. Here, can we actually begin to use these viruses for transgenesis. The standard way of making transgenic animals these days is you take the egg, you introduce the gene directly into the nucleus, fertilize it and then put it back into the animal.

This has been very successful in the case of the mouse. But it has been more difficult in the case of other animals because the nuclease is often pigmented.

So we did a very simple experiment to ask the following question; can we introduce genes

directly into either ES cell by infection with virus of lentiviruses, the formal viruses, the traditional MLV viruses, are unable to do so and can we create a mouse which is transgenic or a rat which is transgenic.

The way to do that is these days you take embryonic stem cells, you infect them with the virus and you get the expression of the foreign genes for over a six-week period of time easily, which is a big distinction from traditional retroviral vectors. They shut off their transcription.

The HIV-based vectors, for some reason, do not shut off the transcription and, therefore, you can have the ES cell. You can also have preimplantation embryo, but you have to remove the zona pellucida. If you don't remove the zona pellucida, you cannot get the infection. But if you remove the zona pellucida, then put the virus, you get the infection.

[Slide.]

The most interesting is here now. This is a litter of four. Many animals are not chimeric for the foreign gene.

[Slide.]

You cannot see this. This is a really beautiful picture. These are live births of animals. You are taking simply ES cells, directly introducing the virus and now you are making--most of these animals are containing the foreign gene.

David Baltimore's lab has also done very similar sorts of experiments. This is another idea to explain to you that the idea of using these lentiviral vectors that, because they have the ability to infect many of these cells, because the transcription is not shut off, they can actually be used for additional purposes like transgenesis. I suspect this will be the method used for making transgenesis from monkeys and many other different kinds of species because you don't have to do nuclear injection, just infect the cells.

But you can't infect the sperm. You can't infect the eggs until you remove the zona pellucida.

[Slide.]

So far, then, all I have told you is the lentiviral vectors can be made easily, large titers. Most of the genes which we think have the pathogenic consequences can be eliminated and it really has a wide utility not only for different

kinds of tissues, for gene therapy, but also for other scientific purposes like transgenesis, knock outs as well as for transdifferentiation of stem cells.

The last part of the few minutes I have, I will tell you a little bit about how we can do regulation. There are number of ways to regulate the transcription of foreign genes. Those of you who are in the field know there is tetracycline, there is the dimerized formation and there are also ectosome receptors.

The first thing we wanted to use is a method which is using tetracycline and, again, no details are necessary except to say this is an antibiotic in the absence of which the gene is turned on in the presence of which the gene is turned off.

The first question we asked was, A, can you use this kind of methodology to make cell lines. That is to say, the way I have described to you so far is we take three or four plasmids, mix them together and we have the virus out. But people who really want to make a very specific gene that they are interested in, they would like to make cell lines where they can produce the protein

continuously--the virus from those cell lines.

[Slide.]

So a post-doc in our lab created the idea that he can actually make a cell line where you can continuously produce the virus rather than having the four plasmids or three plasmids cotransfected into the cell.

What he basically did was to use the tetracycline as a regulatable element. The interesting thing to show you here is that if you now take these viruses which contain the tetracycline-regulatable element, he can generate titers not very different from those vectors where we have cotransfected four plasmids.

In other words, you can make cell lines from these plasmids rather than having always the four plasmids together. It really depends on what you want to do. If you are interested to use different sets of promotors, you may want to do one thing. If you are interested in making only one type of a virus, you might want to make a cell line.

[Slide.]

For the purpose of showing this, these

viruses are equally good in infecting the neuronal cells. In other words, you can make cell lines rather than just using the plasmids.

[Slide.]

These are the third generation which has the same vectors.

[Slide.]

Finally, because we have these vectors where the LTRs have been manipulated and have been deleted, largely, we can now substitute regular TC culture and ask the question can you turn on the gene and turn off the gene at will. So here we introduce tetracycline elements directly inject it in the brain.

In the presence of tetracycline, there is hardly any expression. If you remove tetracycline from the water, there is expression, the work of Karl Kaffree. More importantly, you can turn the gene on, turn the gene off, turn the gene on, turn the gene off, at will for over a six-month period of time.

This is not perfect, but it is a reasonably good way to start thinking that you can actually regulate the sequences just as well.

[Slide.]

So, let me come to the final slides. Where are we now? Remember, we started out by asking what is an ideal vector we would like to have. We would like to have a vector which has the ability to do many of these things. We think--and, again, this is specific for lentis. There are many other vectors which can do many other different things. So this is not a competition or a comparison. It is simply to say what we planned out and this is what we have come out with.

The vectors certainly have the ability to make large amounts of particles. That is not difficult. Convenience of reproduction, at least in the lab, is not a problem. I don't know when you have to make 100,000 liters. That is the business of the people who do it in the biotech companies.

It has the ability to infect nondividing cells and dividing cells. I haven't told you about tumor cells, but you can do that--to integrate in a site-specific manner. We don't know that. We have not learned anything how to control the integration. In fact, that has been a very difficult task. So that part, I don't even know actually how to approach at this point, to have a

site-specific integration.

Fortunately, there is considerable experience in the clinic from the previous vectors, like Moloney leukemia virus, that we have not see any untoward effect of integration. But that is something we have not been able to achieve as yet.

I think we have the rudiments of a regulatory system, that we can turn the gene on or turn the gene off. We can infect a wide variety of cell types. I don't know if every cell type can be but a large number that I have shown you. We have not had any immunological consequences, at least not at the moment, particularly not with the viruses because part of the reason is a lot of the--4 percent of the human genome really is retrotransposon and has sequences much like the gag and the pol kinds of sequences, all the broken ones.

We certainly have antibodies against VSV-G. If you take the dogs, infect them with the virus, we have titers, antibodies; not a surprise, because VSV-G has fallen. But we have not seen any inflammation at the site of injection. But, then, again, we can't compare them with adenoviruses where the titers can be trillions of virus

particles. But, within the constraints of 100 million particles that we can inject, we do not see any inflammation and immunological consequences.

So we believe, at this point, these vectors do offer the opportunity of the ability to infect nondividing cells and a number of people have made strong attempts to try to make them safe, safety in terms of their inability to, perhaps, make a replication-competent virus.

The deletion of LTR offered the opportunity to not allow mobilization of the virus and the fact that we have been able to manipulate the genome such that you have the ability to infect a wide variety of cell types offers many possibilities that these viral vectors have the ability to perform many of the things you would like them to do in terms of the production of the foreign protein and eventually into the patients.

[Slide.]

Finally, I would like to thank a number of individuals. Not all of them are listed here, but I would particularly like to thank Luigi, who happens to be in the audience, who started this along with Didier Trono and Rusty Gage with whose lab I collaborate very extensively. Bruce Talbert

with whom we do a lot of our work on hematopoiesis, and a number of other individuals, and finally a audience for your indulgence.

Thank you very much.

[Applause.]

Questions & Answers

DR. SALOMON: Thank you, Inder.

It is generally our policy to generate some questions and discussion. There is no agenda to this part of the meeting. It is just to get some issues out on the table. So I am very flexible about what kinds of things you want to raise.

Just to start, Inder, one question. You started off by pointing out that one of the first things you established was that you could make 10^9 viral particles per ml. To me, that raises the question, in the context of the FDA thinking about setting some kind of standards for this as a product, exactly what do you think is the best way to express the efficacy of an expression system, transient or stable; in particles per ml? I mean, in retroviruses, that would not necessarily be the best way to describe something; right--that we would talk about infectious titers.

DR. VERMA: I think, first of all, I, perhaps, misspoke if I gave the impression you make 10^9 virus particles per ml. I might have wanted to say that we made about 10^6 or 10^7. Then we can concentrate them by virtue of the fact that the glycoprotein has reached the titers of 10^9, and some people can claim titers of 10^{10}.

So that is the general--now, you are asking what is the way--each lab, I think--there are no standard ways to do that. Most of use p24 as a marker to see how many p24 antigen amount will be equal to infectious units, and use that as a major--that is what we do in our lab.

Some people use reverse transcriptase. Some people actually do the count of the particles. So I think this is something which people who are more familiar with these kinds of things, in terms of measurements, they will have to make a decision what is the best for their cause.

The second question regarding whether you should use plasmids combined together to make the virus or do you make a cell line. That was the implication. I think again it really depends on the individual.

We have used almost always plasmid

transfection so far, three or four plasmids. We have seen, whatever we do in the lab--and, remember, we do these things in 1 liter, 2 liters, 3 liters at best. So you have to take that into constraint that when you go to thousands of liters, what might happen.

We have not seen any recombination. We have not seen and PCL-positive tat in the usual tests we do regardless of the fact whether you use plasmids or--so I have really a fairly open mind. I personally think there is no reason if people want to use three, four, plasmids together, that is perfectly fine. So I have really no preconceived notions in my mind on this issue.

DR. ALLAN: Just a point of interest. The VSV-G envelope is very good especially ex vivo where you take the cells out and infect them and get about 90-something percent. What about if you are going to treat someone by injecting the virus. Obviously, you are showing that you can target expression with the tat oppressor genes but I am wondering whether you can do tissue-specific expression and whether you are actually manipulating the envelopes that target specific cell types like, say, hepatocytes.

DR. VERMA: So the question is twofold. One, can you manipulate the glycoprotein so as to allow it to go to a very specific cell. For example, VSV-G being very general, unfortunately we don't know the receptor of the VSV-G, so we it is difficult.

One area we have failed miserably in the lab in the last ten years and that is the area of targeting. If we chose even a single nucleotide in VSV-G, it either refuses to bind and, if it binds, it doesn't fuse. I think the viruses have billions of years of evolution to really make themselves perfect.

There are viruses--Jim Wilson had a paper on philoviruses lately in which he found--now, I am using them philo because if I said they were ebola, it sounds even worse than that. That day he found their G-protein to be very specific for the apical parts of the lungs. So there are specific types of viruses you can use. We haven't really had much success but I am sure those in the audience have done better experiments.

The second is to control it by transcription regulation of a promoter. The only experiments we have really some experience with is

in the case of probasin which is prostate-specific, where, again, you can directly introduce the gene.

We haven't really succeeded much in making intravenous delivery and hope the expression will be only in the tissue where it goes. But we don't have enough virus. There is a lot of biological loss of the virus by the time you go, so we haven't really much experience on that.

DR. RAO: I had a question. Is it clear that when you do lentivirus infections, you have single-site insertion at the concentrations you use it at?

DR. VERMA: So the question is if you want to use a multiplicity infection of 1, 10, 100, 200, 2000. I can't answer the question because we have never systematically done that. But we have rarely seen more than 2 to 3 viral integrations. Rarely. It is not unusual with the retroviruses, very often, that you have very few integrations. The best one I know is the XE cells. We have about twenty integrations when the Rous sarcoma virus was introduced.

But, by and large, we haven't seen many. But, again, I have never really known to do any systematic experiments. Maybe somebody did it. I

just don't know.

DR. DELPH: You showed that when you injected the vector into the testis that there was no transduction of the sperm cells. Have you looked at all to see what has happened to the offspring of transduced animals?

DR. VERMA: Yes. We got perfectly fine animals. There was no a single transgenic-positive animal. That is what I showed in one slide. Perhaps I went too fast. The PCRs are all negative.

DR. DELPH: That is both male and female?

DR. VERMA: Yes. It was like five and five.

DR. SALOMON: Dr. Zaia?

DR. ZAIA: When you are packaging the final virus, I normally think that viruses in nature make mistakes and there are defective particles. In your system that is constrained, is there less likelihood of this or is there more likelihood of having defective or incomplete particles and will that have a biological effect, do you think, when you are injecting vectors into muscle or liver?

DR. VERMA: It is a good question. We

don't really know how many defective interfering parts. I know if you have VSV alone, those kinds of stay in, so there you do make. In cytoplasmic viruses, they often make defective interfering there. I don't know, really. We have looked at the homogeneity of these viruses. By and large, they seem to be the same size, but if there was 1 percent, 10 percent--they might interfere, but it can't be a tremendous interference unless there are a very large number of them to interfere with it. But no systematic study is done.

Incidently, I can't have the opportunity, Dr. Zaia. As I was coming on the plane, I was looking at all the papers of FDA. The guy sitting next to me said, "Ah; that is my sister's husband, Dr. Zaia. Do you know him?" The probability that I should sit next to him in the plane, who knows you so well, I was astounded. So there is always a chance.

DR. SALOMON: I am not sure what chance you are referring to.

DR. VERMA: Very low.

DR. SALOMON: One of the questions I think you are uniquely suited to answer is this terminology of generation, as one of the people who

basically started this. Now, we are talking about first generation and second generation and third generation. So these kind of terminologies tend to become something we are comfortable with.

But, from time to time, as the field evolves, they can also lose their specificity. So one of the things I was struck, and we are going to get into this tomorrow but just to put this into context, was that part of the discussion at the RAC of the VIRxSYS protocol was an argument about whether this was a first generation or a second generation.

When you really looked at the details of what VIRxSYS had done, I am not sure whether it fits your concept of a generation because what you have done in generations here is continually split further apart, whereas VIRxSYS took a very different approach.

So are we kind of done with this generation thing or can you suggest a new way to define generations of lentiviral vectors that will be more useful?

DR. VERMA: I think it is a good question and it really hasn't crossed my mind at all to think about it, really. We do generations that

really actually defines the post-doctoral era of my lab, the first generation post-doc, the second generation. The fact of the matter is the first generation vectors, by and large, are defined by most of us and I think these are may perhaps be different in the system of the simian or the feline ones, as those where really largely the envelopes have changed and some of the long-term repeats have changed except most of the accessory genes would concept. So that is really the first generation of vectors because we really didn't know what genes are required for integration. If you look at the history of what we required for integration in nondividing cells, at least six people will tell you six different genes. So we didn't want to eliminate them.

The second-generation vectors have been defined, at least, and I don't think many people will use that very much, where the tat and nef were still present, or tat and rev were still present where the other genes were eliminated. Again, I haven't read the VIRxSYS thing. It is such a big document to read, but I think they are using the second generation of that type--I think.

But there LTR are not deleted. So you

don't have the SIN vector. So the third generation vectors are the SIN vectors where the LTRs have been truncated, where most of the accessory genes have been eliminated. That is how, really, we are defining them.

But I think in the future, one has to define them as probably fully deleted vectors or something like that. I just haven't thought about how to nomenclature them.

DR. KINGSMAN: Is it okay protocolwise for me to make a comment?

DR. SALOMON: Yes. In fact I would say--you need to identify. I guess I am remiss and I apologize to everyone. I would encourage both the invited speakers, as you are doing, as well as the audience to stand up and come to the mike since there is no attempt to restrict the discussion here.

DR. KINGSMAN: Thank you. I am Sue Kingsman from Oxford BioMedica. As Inder mentioned my name, I felt dutybound to stand up. I don't think that the word "generation" is a useful concept in a regulatory framework. I think it is a laboratory-specific statement to say we are gradually beginning to understand our system and

that people observing our system should realize that we are defining issues, solving them, moving forward.

I think the take-home message is that all of us are making progress towards understanding our system and what we should seek to do is to define some general concepts and parameters that most lentivectors can fit in with because I think the word "generation" will mean different things to different laboratories.

So I think it is the substance of the vectors that we would focus on, not this overall terminology. That would be my viewpoint.

DR. MULLIGAN: Since I think we are going to talk about the relative virtues of the transient transfection versus the packaging cells, I had two issues. The first is, as we have talked in the past, you can make stable packaging cells. Everyone could make these. What seems to be the difficulty is actually transfecting the vector and getting high enough RNAs to make high virus titers.

There are definitely reports by cross infection or reinfection that you can get enough proviral copies to get very good virus titers. But I think the last time we talked there weren't a lot

of people that had actually, with SIN vectors, been able to do transfections and get good virus titers.

So, in your own experience, has that been possible, so when we get to the issue of the relative virtues, if no one can really make good stable producer cells, that will be very important.

The second question just is a more philosophical question which I think we will end up getting to which is the issue of there is a difference between theoretical safety and detectable safety. I want to pin you down on the transient versus stable packaging.

My impression is that whether you use a first-generation, second-generation, third-generation transient-transfection system, people will report that there is no difficulty, there is no helper virus functions, et cetera, et cetera.

We had a meeting here many months ago about good old-fashioned retrovirus packaging cells and the merits of PA317 which you know very well and other more advanced cells. I think the FDA at one point was asking for our guidance as to should they ever legislate against a less sophisticated packaging cell.

The discussion was somewhat controversial and I guess I came down to the fact that, well, if you can't prove, by experimental means, that there is a difficulty, then you have a real difficulty preventing people from moving ahead.

On the other hand, there is no doubt that there are theoretical, good, sound theoretical, reasons to think that the split packaging cells would be a safer product than the transient transfection. So I am curious where you come down on that point.

DR. VERMA: I tried to mention it quickly. What Richard is asking--it is a long question; right? But I think I get the gist of what you are asking. This is also again the question we are often asked in the past. Richard is asking the question--two questions, mainly--if you really can make a cell line from all these systems that you have, you still would like to continue using, for example, the transient transfection.

My experience in the lab largely has been on the transient transfection, so I can only speak very little. The only stable cell line we have is the one that I just described which Karl Kaffree made in the lab prior to his departure. We have

really seen no big difference, again in terms of infectivity, in terms of production of our usual safety efforts of the tat production and so on.

I think it is very hard, really, to say at this point. Theoretically, if you think you might conceive the idea that if you have four plasmids together, that you may be causing real recombination when they are growing up and you are adding to that. It is a theoretical possibility.

I have had no really direct evidence for that. You asked me for a recommendation I will have. I think, personally, if you can make a cell line, and I think we have shown that you can make the third-generation cell line, and the titers are not really compromised because there are ways to do that, I would say that if I were the one doing it, I would take a cell line just because of the convenience of it and that you know the reproducibility of it and you know that you know exactly what you started with.

But I really can't definitively answer your question to say the other is the wrong way of doing it because I really have no experience on that.

DR. MULLIGAN: Can you make one with the

SIN vector?

DR. VERMA: That is the one with the SIN vector. We made it with the SIN vector. That is the paper Karl just published.

DR. SAUSVILLE: You alluded to the karyophilic nature of the virus as being a key advantage. I think that really came through as a real leap with this vector generation. Yet it would seem, from the standpoint of the product definition, that could also be a point in variability in how much expression you get. Could you expand on whether or not there is a concept of how to standardize--is it a function of the gene you are trying to make? Is it a function of sequences that are in the vector that determines that property?

DR. VERMA: Implicit in your question is that we understand the mechanism by which the viral DNA actually crosses the nuclear membrane. In fact, that is really, still in my mind a fairly big black box. There have been proteins identified that Didier Trono showed the PL10 protein which binds to it.

At one time, there were different sets of proteins. Once it was the gag protein. Once it

was the VPR protein. They are all involved. We don't know the mechanism of that. So think, at present, to use that any kind of way is probably not a good one. On top of that, you may have seen some of my slides, once again from the French group and from Luigi's group, there are polybrene checks called the cPPT--some call them flaps--which seem to allow a better transduction into the nucleus.

So we don't know if the presence of that will make a difference or not on how efficient is that process. That still remains to be done. So I think that is not going to be a very easy way at this point to use as a mechanism to define that as a late property.

DR. KINGSMAN: I think that question needs to be answered on a case-by-case basis, that when you are doing your efficacy studies, you will design a vector that will transfer genes into the cells that you are targeting and will give the effect that you want. Sometimes, you may have to alter the properties by adding the cPPT in and other times you won't. But you will have defined the potency of your product with the specific endpoint in mind.

So I think you will be able to get a

product definition for your particular product but I agree with Inder. I don't think you will be able to come up with a generic specification for all lentivectors to perform similarly under all circumstances. I think if we try to go down that route, it will be a very long tortuous path.

DR. SAUSVILLE: I certainly agree that that is an area that is of great theoretical interest to figure out and also, in a particular case, to define. Yet, it seems to me, that would ultimately influence the number of particles that would result in an efficacious outcome and, therefore, this issue of background safety issues then becomes potentially influenced by this sufficiency issue.

DR. VERMA: I really can't answer any better. I just don't know enough about the actual mechanism of transfection.

DR. SALOMON: I guess one question I think Inder has already answered it for his experience, but one of the key issues for me when I look at the safety of a transient versus a stable line is the question that I don't know the answer to, so I want to pose it to the group. The answer may be, as Inder has already said, that he doesn't know. But

the question would be if you have a situation in which you have a transient-transfection system in which up to four purified plasmids are transfected at the same time.

So, forgive me, but my image is of all this semipurified DNA in very high concentrations at various points in the cell and in the cell cycle versus a stable cell line, packaging cell. We are thinking about relative safety now, not efficacy or production, not that those aren't very important.

The question would be is there any data out there suggesting that such a multi-transfection system leads to higher rates of recombination? I am not saying that you can't make alterations in the vectors and lack of homology, et cetera, all of which have cleverly been done and proposed by different people, but is there just any evidence that there would be more recombination in such a multi-plasmid system? Does anybody have an answer to that?

DR. VERMA: Theoretically, you might imagine, because there are all these thing and maybe recombination--I think a lot of work has been done in the past on one or two plasmids, particularly with the recombination of the

endogenous genes. That has been extremely low.

But to actually have high amounts, because we have been asking the question what amounts, I think it really comes down in the end to individuals, how they want to proceed with it. I don't believe there is any strong evidence at this point whether three plasmids versus four plasmids versus two plasmids gives you any worse result if you have a cell line, if there is any greater recombination. I don't think there is any direct evidence.

DR. KINGSMAN: Could I just add to that. In the early days of plasmid-based gene transfer, if you go back and read the papers in the early '80's, people addressed those questions about what happened to plasmids when they went into cells. What happens is they do recombine and concatenate and rearrange.

So, a priori, you might expect that there would be some DNA-DNA interactions when you put large amounts of DNA in a cell. But whether anybody has then studied retroviral vectors coming out of that and done some of the studies like Howard Temin did to ask what are the nature of retroviral recombinants, I don't think they have.

But, a priori, there will be DNA-DNA interactions.

DR. VERMA: But it is the final product you are really interested in in the end.

DR. KINGSMAN: Yes.

DR. MULLIGAN: I would echo Sue's point that there is no question that the DNA that is the template for making the RNA in a transiently transfected cell is a very complicated DNA. So there is no doubt that there is recombination at very, very high efficiency, probably near unit efficiency. So I don't know if anyone has actually looked at the RNA transcript in a transiently transfected cell but I would bet you that you would undoubtedly see very funny things.

Now, Inder's point is, all that being said, what gets selected to be packaged and transferred and so forth appears to be no different. What I would think I would really strongly emphasize that this is not the optimal way to generate RNA to be packaged.

If people were to look, if we thought it was important to look at this process, I would think we would undoubtedly see the effects of that. So that is a fundamental difference between having integrated templates for helper functions and

vector functions in the transient system.

DR. SALOMON: Yes. I just wanted to point out that that is sort of the point. My point is that I think one of the questions that the committee has in front of it, and we are not going to answer it immediately, but as we consider safety, if we agree that these are important scientific questions and the data is not out there, it may be important to solve these issues before you say, we are going to defend the use of one or another type of strategy.

If it turns out to be a wash by the time you package the vector, then great. Then you could do it any way you want.

DR. NALDINI: Luigi Naldini from Torino. I apologize for my voice. One point, in terms of the packaging cell line versus transient transfection which has to be made, I think we have to be careful in really using experience with retroviral vector into the lentiviral field.

The lentiviral vector that we have discussing until now uses the VSV envelope making a packaging cell line, the VSV envelope poses challenges not only in terms of regulating that envelope because it is toxic but also because it

allows superinfection of your cells, quite extensively. Even if you have an inducible system, you may not completely suppress that.

I think, overall, that means that, in the long time in which you grow your cell, there is actually more changes for recombination to take place and for recombinants to spread in the system and to accumulate the multiple steps required to build a virus as compared to the short window of time of transient transfection.

So I think it is obvious that a stable cell line has an advantage in terms of manufacturing, standardization. I would doubt that actually, at the moment, we can think it is actually safer. Transient transfection, as long as you use multiple plasmids in a very short window of time, makes it very unlikely, even if there is recombination going on and there is no question, that you rebuild a complete genome.

In a stable cell line which grows for a long time, we may allow a certain level of infection going on even by partial recombinant, this may happen. So I think we have to be very careful with that.

DR. VERMA: You can be careful with that

but the bottom line is, in the end, it is the final product whether you made it with one system or the other system. That is the one we want to really need to know, whether that has recombinants in it or not.

DR. KAPPES: John Kappes. I am from the University of Alabama at Birmingham, UAB. We took a very careful hard look using highly selective pressures to address whether RNAs were incorporated into vector particles that could recombine during reverse transcription.

Specifically, we were looking for recombinants that could generate something that would be produced from the cells. So, minimally, you would have to generate a recombinant which had the capability to produce a retroviral particle because we were providing envelope in trans. So this would be an envelope minus recombinant, to say the least.

But my point is, in that context, in that examination, we did find DNA recombinants that had properties that, when envelope was provided in trans, by transfection of those cells that received supernatants from vector-generated stock which contained DNA, that that, too, could, as our

endpoint, mobilize retroviral DNA or marker genes which we had introduced into the cell.

So the point is, in rare cases, we did identify DNA recombination.

DR. SALOMON: Dr. Mulligan and then Dr. Jolly.

DR. MULLIGAN: Just on Luigi's point, we have actually, with a MLE VSV-G packaging cell, looked at the transient-transfection issue. I think if you were to look in your system, you would see probably that the same thing happens in the transient transfection. It somewhat depends on how you do your harvests, but we have seen with intron-containing constructs that, even in transient transfections into the stable packaging cells, that you can detect intron incision and remobilization suggesting that what you say can occur in both the transient transfection and the stable cells.

DR. JOLLY: My name is Doug Jolly. I work for Biomedica, Incorporated. Just I guess the first thing I would say is there is almost no data about this. It is pretty early to make any choices without the data. I think part of the problem is, drawing on the experience from murine retroviral

vectors, we had a packaging cell line which retained some homology although it was split into three pieces.

Really, the way we gathered data on that was to do 60 200 liter preps. Then three of those had RCR positivity. So that is only assay for the very rare events that we are worrying about is to do something like that. You can't see it often unless it is an acute event in the scale experiments that we are talking about now.

So I think it is too early to close any doors with respect to the lentiviral vectors.

DR. SALOMON: Yes. I agree with that. I guess I would just also point out to broaden the context that this is not--I don't think the only safety issue for any sort of vector delivery is replication-competent lentivirus or replication-competent retrovirus albeit, obviously, that is front and center, particularly with this class.

But it is also if recombinations occur that alter the integrity or the structure of the trans gene could also be very potentially dangerous in terms of autoimmunity and other effects. It certain would affect efficacy.

DR. VERMA: I agree. I think it is a point worth thinking about. I certainly too thinking about how to actually how you can do the experiments. I was thinking about it.

DR. SALOMON: I think that was excellent, Dr. Verma.

We had a discussion yesterday that, having grown up on the East Coast, born in Boston and now have been out in Southern California, I am having this conflict about referring to people by their first name or referring them as Doctor. So I am going to try and go with the East Coast formal until we can finally get the FDA to have one of our meeting out on the West Coast.

DR. NOGUCHI: As long as you host it.

DR. SALOMON: I think I can say that Scripps would be happy to host the next FDA-BRMAC meeting. I don't think that is going to really happen though.

It is my pleasure to announce the second speaker which is Dan Takefman from the Office of Therapeutics Research. He is going to talk specifically about lentiviral vectors and continue our discussion of potential safety issues.

Lentiviral Vectors: Safety Issues

Dr. Daniel Takefman

DR. TAKEFMAN: That was really a great introductory talk by Dr. Verma. I am very encouraged by the excellent discussion thus far.

[Slide.]

Today, I will be speaking about safety issues associated with the use of lentiviral vectors in the clinic. As many of you know, the first patient participating in a gene transfer clinical trial received cells that were exposed to a murine gammaretroviral vector. Since that time, murine gammaretroviral vectors continue to be tested in clinical trials, one long-term gene expression is desired.

[Slide.]

This is a figure you are going to see a number of times today. Lentiviruses, like gammaretroviruses, belong to the Retroviridae family. Gammaretroviruses have encode for three open reading frames - gag, pol, and env. Additionally, the genome is surrounded in both ends by long terminal repeats

Lentiviruses, such as HIV, depicted here, have a more complex genome. In addition to gag, pol, and env, there are two regulatory proteins,

tat and rev, which promote viral gene expression through transcriptional and posttranscriptional mechanisms respectively.

There are also four accessory genes, vif, vpr, vpu, and nef, which are involved in viral replication and pathogenesis.

[Slide.]

The complexity of the lentivirus genome has made adaptation of this virus family to a vector system challenging, but a worthy goal, as Dr. Verma mentioned, a major advantage to the use of lentiviral vectors is that they transduce non-dividing cells.

Interestingly, in lentiviral systems, you see efficient adaption to SIN technology, or self-inactivating technology, and this is in contrast to what you see with gammaretroviral SIN, and I will elaborate on this point later on in my talk.

In both systems, you have the advantage of integration to host chromosome potentially resulting in long term gene expression of the transduced cells and the progeny cells. Additionally, in both systems, there are no viral genes expressed in target cells.

Both systems have the disadvantage for potential of recombination events occurring, resulting in replicating virus with potential pathogenicity.

[Slide.]

There are a number of lentiviral vector systems currently under development, two that are based on primary lentiviruses, such as HIV and simian immunodeficiency virus, and two systems based on non-primate lentiviruses, such as FIV and equine infectious anemia virus.

[Slide.]

So, what are the safety concerns specific to the use of lentiviral vectors?

Recombination during manufacturing may generate a replication-competent lentivirus, an RCL. Of course, I should mention in my talk. I am primarily going to focus on the use of HIV-based vectors.

In terms of generating an RCL, of course, this is of particular concern with HIV-based vectors, since HIV is a known human pathogen. Additionally, since lentiviral vectors are commonly pseudotyped with G glycoprotein, a VSV, a broadened tropism may potentially result in increased

pathogenicity of an RCL.

Additional concerns are associated with the use of HIV-based vectors in HIV-positive subjects. Recombination of vector with wild-type virus in HIV-positive subjects is a concern and has the potential to lead to a more pathogenic wild-type virus.

Additionally, mobilization of vector by wild-type virus is a concern, and I am going to touch upon this point again later on in my talk.

[Slide.]

In terms of recombination events, we certainly have learned a lot from the gammaretroviral vector field as from basic research done in the gammaretroviral basic research areas.

It is known that homologous recombination can occur when two different RNAs are packaged into one virion. This is the result of reverse transcriptase template switching or undergoing a process of strand transfer.

This same mechanism has been shown to occur with HIV RT, as well, in in vitro systems.

[Slide.]

In terms of a recombination event leading to a replication-competent retrovirus, or an RCR,

we know that this is a safety concern from the well-known study in which immune-suppressed rhesus monkeys were exposed to bone marrow cells transduced with a preparation of RCR-positive vector.

In that study, 3 out of the 10 animals treated developed lymphomas and died within 200 days. Follow-up analysis revealed that these animals had sequences identified as recombinants between vector and helper, and vector and endogenous sequences. I should point out that in the system, the investigator was using a murine leukemia virus-based vector and murine cell lines for production.

[Slide.]

So, how do we use these lessons learned for the manufacturing of gammaretroviral vectors? It is known that homologous recombination occurs at a rate approximately 100 to 1,000-fold lower than non-homologous recombination. Therefore, reduction in homology between vector and helper sequences will lower the likelihood of a recombination event occurring.

I should point out that in a study by Otto and co-workers, it was shown that as little as 10

base pairs of nucleotide identity between packaging and vector sequences were sufficient to allow for RCR generation.

[Slide.]

Additionally, splitting helper sequences into more than one plasmid, for example, splitting env and gag-pol open reading frames, is likely to decrease the incidence of RCR generation by increasing the number of recombination events necessary to generate an RCR.

[Slide.]

Vector mobilization. This is an additional concern with the use of lentiviral vectors in HIV-positive subjects. Mobilization occurs when a vector genome is packaged by a wild-type HIV present in the same cell.

Mobilization occurs by the same mechanisms that allow for helper sequences to package vector genomes.

[Slide.]

So, there are potential advantages and disadvantages to vector mobilization. Mobilization of a vector designed to inhibit or prevent HIV replication or pathogenesis has been argued to enhance the therapeutic effect by allowing for

spread of the therapeutic transgene.

In terms of disadvantages, vector spread beyond the intended target tissue may have safety consequences. Additionally, co-packaging of wild-type HIV RNA and vector RNA may result in recombination.

[Slide.]

How to address these safety concerns. I list here four approaches - vector design, safety testing during manufacturing, preclinical safety studies, and clinical monitoring.

In terms of vector design, one can incorporate features intended to decrease the likelihood of recombination and mobilization, and again, lentiviral vectors benefited from the beginning from lessons learned from gammaretroviral vectors.

[Slide.]

I very briefly want to highlight some of the features in what has been called first, second, and third generation vectors with, of course, the caveat that these definitions may be outdated in the future.

Very brief, as an example of producing a first-generation vector, one might perform

transient transfection of three plasmids. Again the packaging plasmid would contain all HIV viral genes except for env. The envelope plasmid contains VSV-G for broadened tropism or your vector, and in the case of a HIV-based vector, the HIV transfer vector, would contain the gene or cDNA of interest and the minimal cis-acting elements of HIV.

[Slide.]

Just a few of the highlights of first-generation vectors include limited homology between vector and helper sequences, separation of helper plasmids. Again, these two are benefited from the use of gammaretroviral vectors. Additionally, in first-generation vectors, we see the retention of all the accessory genes in the packaging plasmid, which is in contrast to second-generation vectors where we see the elimination of accessory genes from the packaging plasmid.

[Slide.]

Interestingly, this seems to have no effect on vector titer. These vectors still retain the property of transduction of many dividing and non-dividing cells, and it could be argued that

there is an increased safety margin with these vectors since there is fewer wild-type HIV genes involved in the manufacturing process.

[Slide.]

Third-generation vectors certainly have a number of interesting features, but I just want to describe in detail the use of a self-inactivating, or SIN, vector.

This involves a deletion in the enhancer region of the 3-prime U3 of the long terminal repeat. During the process of reverse transcription, this 3-prime deletion is transferred to the 5-prime LTR and results in a transcriptionally inactive vector that cannot be converted into a full length RNA in the target cell.

We also see a reduced likelihood of RCL generation and SIN seems to hamper mobilization by wild-type HIV.

Additionally, the use of SINs may reduce the risk of tumorigenesis via promoter insertion.

[Slide.]

There is certainly many other developments, and I just wanted to give a brief outline, but other developments include the use of

a four-plasmid system in which one would split helper sequences into three separate plasmids. As an example, rev can be split on a separate plasmid, or gag-pol coding regions can be split in two separate plasmids.

There has been development of stable packaging cell lines based on third-generation technology, and there has also been development of non-HIV-based vectors, such as the EIAV, SIV, and FIV, which are not known human pathogens.

[Slide.]

So, even with the incorporation of all these safety features, one cannot reduce the risk of a recombination event occurring to zero, and therefore, it is important to have appropriate and sensitive assays in place that will detect a recombination event during the manufacturing process.

[Slide.]

It is certainly going to be very important to have an assay in place that will detect an RCL. RCL assays are typically done by an infectivity type assay which would involve several passages on a permissive cell line or cell lines. Then, one can perform endpoint assay for viral or transgene

sequences by a PCR-based assay.

The use of a positive control might be problematic since the generation of a replication-competent VSV-G pseudotype lentiviral vector may not be desirable.

[Slide.]

One can also detect for helper sequences, and this could be done by functional assay. The transfer assay is an assay that has been used in the HIV field for a number of years. This assay tests for the generation of a recombinant that expresses a functional tat protein. The assay relies on the ability of tat to transactivate an LTR reporter gene construct in the target cell.

In the absence of tat or in the absence of a tat recombinant, no LTR-driven reporter gene expressed in the SIN.

One can also test for recombination intermediates, and we are fortunate to have Dr. Kappes in to talk about this concept in the afternoon session.

[Slide.]

Certainly, one can directly test for helper sequences in a vector production lot or in transduced cells by a PCR-based assay. While this

can be a very sensitive assay, perhaps it is not the most biologically relevant assay to perform especially in terms of detecting an RCL.

Perhaps this assay would have usefulness for VSV-G detection when you are treating HIV-positive subjects with an HIV-based vector since transfer of the VSV-G gene into a HIV-positive subject is highly unwanted.

[Slide.]

Finally, in terms of addressing safety concerns, I wanted to briefly outline how one might go about performing preclinical safety studies and clinical monitoring. I mostly want to emphasize some concerns especially with the use of HIV-based vectors in HIV-positive subjects.

A lot of these concerns are going to be addressed to the Committee in the form of questions, both in this afternoon's session and in tomorrow's session.

[Slide.]

In terms of the use of animal models to assess safety, studies to assess mobilization and recombination with wild-type HIV are difficult. This has been learned in the HIV vaccine field.

It is difficult to find an animal model

that can examine the replication and pathogenicity of HIV. In terms of non-human primates, it is known that HIV replicates, but is non-pathogenic in chimpanzees. Perhaps the macaque model might be appropriate for SIV-based vectors. Unfortunately, the murine model is very limited due to the fact that HIV does not replicate in murine cells.

Along the same lines, a SCID mouse model will also be limited, perhaps can serve as a "in vivo test tube," but any replication of your vector seen will still be limited to the human cells that are added in.

[Slide.]

In terms of clinical monitoring, it certainly will be important to have an assay in place to detect for RCL in gene transfer recipients, and this is analogous to current recommendations with gammaretroviral vectors. How best to perform this assay in an HIV-positive subject is a question. There is certainly a number of ways one can go about this.

Additional concerns again are in terms of recombination events of your HIV-based vector with wild-type HIV. It is difficult to predict the outcome of this recombination event and therefore

consideration should be given to have an appropriate assay in place.

Likewise, one might want to assay for changes in wild-type HIV following administration of a lentiviral vector. For example, if your vector was targeting a specific HIV gene, one might want to assay.

[Slide.]

So, in conclusion, recombination during manufacturing is a safety concern, one that perhaps can be adequately addressed through incorporating safety features in the design of your vector. Additionally, it will be important to have appropriate and sensitive assays in place to monitor for recombination events occurring during the manufacturing process.

In terms of recombination of vector with wild-type virus in each of the positive subjects, it is worth considering having appropriate assays in place to monitor for recombination events in subjects.

[Slide.]

In terms of mobilization by wild-type virus, certainly a lot could be shown through in vitro assays as to the potential for vector to be

mobilized. Unfortunately, preclinical animal models will be difficult and perhaps consideration should be given to having appropriate assays in place to perform clinical monitoring.

I will end there.

DR. SALOMON: Thank you, Dr. Takefman.

[Applause.]

Questions & Answers

DR. SALOMON: So, this discussion is obviously now kind of beginning the FDA staff's leading us towards some questions that we are going to discuss this afternoon, but we already began some of this discussion of safety issues, and I encourage some discussion now. After that, we will take a break, so just to give you kind of an idea how the morning will flow.

One question that I have, again, it may not be totally answerable right now, is we keep talking about the VSV-G protein, and that seems at the moment, I think partly through the first generation of vectors to use this, but is that a safe envelope to be using? Is that an issue that we ought to be dealing with at some point as a direct safety issue?

The molecule itself is toxic, right, when

it is expressed, if it's expressed at high levels, it can even kill the target cells? We don't know its receptor.

Certainly, in vivo we understand that it is targeting, at least brush borders an intestinal epithelium, but the question is, if injected, if it's present and injected in cells, so in terms of in vivo gene therapy, we really have no idea whether it is even functional.

DR. TAKEFMAN: Those are good questions. I would welcome the Committee's opinions. Certainly, in my mind, a major issue is potential transfer of VSV-G gene to an HIV-positive subject and resulting recombinant.

DR. SALOMON: I guess the point that I was making here, though, is given how little we know about VSV-G's function, some of its features would certainly make one think that it was a major safety concern in the sense that it can be toxic.

On the other hand, given that it is unclear to me at least, and again I defer to an expert audience here, about what its function would be in vivo. If it has no or little function in vivo, then, its expression on an HIV particle would be pretty meaningless from a safety point of view.

I guess these unknowns bother me in the context of the safety discussion.

Dr. Sausville.

DR. SAUSVILLE: I was going to say, on the other hand, though, there are certain features of it that could actually be construed as quite beneficial. I mean this field has had a problem with efficiency of transduction in many of its aspects, so to me, the question really comes, I mean as was alluded to, there is a marked problem with recombination with HIV.

We may have to consider different safety issues in a non-HIV infected population, because I think the potential safety ramifications are quite different actually, and you might reach different conclusions.

DR. CHAMPLIN: Of course, the non-HIV population can become HIV-positive two days after the gene therapy is administered, so one has to think of these things and then think of the truly rare event if one in a million event occurs once to develop a highly pathogenic virus, that can obviously have major public health implications.

The preclinical studies in primates, has there been much experience there in terms of

looking at safety and stability in animals?

DR. SALOMON: Dr. Verma.

DR. VERMA: I think there is some experiments, but very little. I think Dalcone is doing some stuff. It is relatively recent. There is not very much known, but nothing untoward that I know of at the moment.

Regarding the VSV-G, it is not that it is not a human pathogen, there have been outbreaks of VSV infection. It is a cattle wild normally. I know it because when I was post doc in David Baltimore's lab, we worked on VSV, and we mouse popped everything in those days. We don't do that, but it's fact we used to do that.

So, it is known to be human pathogen, but it is something in terms of toxicity, there is I think enough data on it, it is a just a matter of somebody to mine it, because there have been periodic epidemics of it, of VSV.

DR. CHAMPLIN: If you put VSV and SIV, would it have any increased pathogenicity in the monkey?

DR. VERMA: I think the argument really there is the testing of it. There is no reason why there should be any VSV gene that should come

through in the mouse, there is no reason for that. So, I think that is a moot point really.

DR. SALOMON: I guess the point I was making was I know we are tending to take this default that everything that we raise as a safety issue means it will make it less safe, and I was actually raising the point that it could cut both ways.

If you could demonstrate that VSV-G, having cut past the mucosal surface, which is its natural target, as you just pointed out, from the known zoonotic disease and from its disease in the cattle, if you could demonstrate that it had very little, if any, targeting effect when released into the circulation, you could then use it to say even if our strategy allowed a VSV-G recombination, it would have little--I mean we are making this assumption that oh, my gosh, if VSV-G got onto the lentiviral vector, we would suddenly have this horrible new pathogen.

I am okay with that concept, but where is the data for it?

DR. KINGSMAN: I am Sue Kingsman. There is some evidence that VSV-G is quite pretty rapidly inactivated by human complement, which may be a

point in its favor.

DR. VERMA: I think the whole work on the VSV, the Moloney vector VSF-G, there is considerable experience on that, that could be used as relevant experience in this case, in terms of at least the G toxicity.

DR. MULLIGAN: I would separate the toxicity from the mobilization question, and we will get into that, I am sure, so I think any mobilization context where you could pick up a gal VM or any other envelope, I think would be a real issue. That is a real safety issue.

I would view the gene no more dangerous and safe than other kinds of envelopes. One argument is that G is toxic and therefore you would maybe be better with that because you will kill the cell effect, and not propagate it, but work with the packaging cells, suggest even at a low level where you don't have toxicity, you can get virus particles that are infectious. So, it suggests there is a potential for a level G that gives you infection without having F.

DR. NOGUCHI: The discussion is superb and we really appreciate it, but there are just a couple of cautions I will continue to try to put

out. One is, for example, the fact that there should be no VSV gene in the final product is, of course, what we expect, but what we expect is not what we always get.

It is sort of going beyond what the data are or have been generated. We need to be able to consider some of the further ramifications of what could happen, so even if there is no data, that doesn't necessarily mean we discard the concern.

Regarding human complement inactivation of the VSV envelope, the same argument had been made for murine retroviruses, as a matter of fact. You may inhibit or you may deactivate a certain number of viruses, but as many virologists have told us, well, it just takes one to get an infection, and you may have 10^7 clearance, but if you are putting in 10^9, it then becomes a moot point as to whether or not it is inactivated by complement to whatever extent.

So, as you are going along, there are certain data-driven declarations that can be made, there are certain speculative things that will be made, but the fact of the matter is in all these things, there really are no advantages or disadvantages, it is just the best we can do at the

time.

DR. SALOMON: Dr. Allan.

DR. ALLAN: One of the points you made was, you know, if you got a recombination event and you had VSV-G on either HIV or SIV, and maybe that's not any worse than anything else.

I graduated from vet school too long ago to remember how VSV is limited in terms of its infection. I mean if you get a limited infection, does that necessitate that it is VSV-G or not, is it the immune response, is the immune response to VSV-G highly protective, in other words, it limits the infection soon after, or is it at a level of the cell tropism? I don't know that in terms of how that particular virus replicates.

But I think those are some of the questions you can ask, too, in terms of if you did get a recombinational event and you get VSV-G expressed with HIV, would it replicate less well than an HIV wild-type virus because of the immune response to the envelope, so that would be something that I would ask, and I don't know the answer to that.

DR. MULLIGAN: I think that is a great question. The tropism issue is obviously much more

than the envelope protein, and I guess, again, I think when we move to the mobilization issues, the issues are can you protect the biological effects of a recombinant HIV that has some different characteristics, and I think that would be a grave concern about whether or not you could possibly model what would be the tropism characteristics of something.

I read somewhere in one of the voluminous IND packages or heard a comment that, well, you can't make anything worse than HIV, you know, the worse that could happen is you will get back what you already have, the patients, I think that is very ridiculous and I think the issue with mobilization is definitely whether or not, not only are you picking up VSV-G, but you are putting it into something, let's say, that has codon-optimized gag-pol sequences or has something, or has non-HIV long terminal repeats, and all of those elements would give you a very good chance of different tropism characteristics.

DR. VERMA: But the VSV biology, by itself, is really well understood. It's a negative stranded virus which replicates in the cytoplasm. So, there is considerable biochemistry and

molecular biology of the gene, if not in terms of now introducing directly what it will do in terms of genicity, that is less known, but the biology of the virus itself is very well established.

DR. ALLAN: So, do you know its cell tropism?

DR. VERMA: In fact, it's all cell types, very broad cell type, but initially, the infection in the mucosa initially, a lot of intestinal infection.

DR. SALOMON: But the one correction, again, if I am wrong, please correct me, but the statement it affects all cell types is largely based on in vitro cell culture infections, not specifically on in vivo infections.

DR. VERMA: Right. In vivo, the only data that I know is really largely in the case of cattle, because that is really the VSV-G is a cattle virus, is largely the infection of the mucosa in the intestine.

DR. SAUSVILLE: But if you were to parenterally introduce it beyond the sanctuary, you would expect replication, correct?

DR. VERMA: By itself, I don't know the answer.

DR. SALOMON: I don't think we know the answer to that question. That was the question I was asking.

I guess all I am trying to do here is play maybe a devil's advocate, but the question is that what we are doing here is taking all these different elements from different viruses and there is very appropriate rationales, we want tropism, we want higher efficiency of integration, et cetera, and I guess I am just asking the questions of what are the sorts of if we now want to go from pioneering molecular studies to clinical trials, I think the job of the Committee is to try and help identify those issues that we should be--you know, there are certain experiments you might not do as an academician trying to develop a new area, that are now critical to go back and do if we are going to go forward safely in a clinical trial, if we can identify those things, that would be a big advantage I think.

DR. DELPH: I just wanted to ask whether there were any different or additional safety concerns between someone who were HIV-positive and given HIV gene vector therapy as against someone who were given HIV gene vector therapy and then