seroconverted, and if so, how should those be addressed.

DR. TAKEFMAN: So, you are trying to say what if an HIV--oh, I see--so, you gave them the gene transfer vector and then they acquired wild-type. I am not too sure how to answer that one.

DR. VERMA: That would be no different than if the vector doesn't mobilize, it should be the same reason as the one who has the other way around.

DR. TAKEFMAN: Yes, it should make no difference.

DR. ALLAN: I don't really think that that will ever happen, because I don't know that you will be doing gene therapy on infected populations is all I would think, at least with HIV.

DR. VERMA: Well, I think that's not fair.

DR. SAUSVILLE: That depends on the nature of the therapeutic intent. I mean I could certainly imagine issues where--I mean we saw examples of potentially replacing a defective gene that, you know, might have anything to do with HIV, but if the nuts and bolts of getting it there were HIV derived, and that subject were to become

infected with HIV, then, that is an issue.

DR. DELPH: I think one of the reasons I asked this question is that very early in HIV infection, you probably get the highest HIV viral loads that you see at any other time in the disease, and I don't know that we really even know the details of exactly how that differs from and the consequences of that.

DR. SALZMAN: Rachel Salzman from the Stop ALD Foundation. I just want to comment that we definitely are interested in using lentiviral vectors in patients that don't have HIV, and that is why we attend these kind of meetings, to be concerned that it is a safe vector and that it can be useful.

I also do happen to be a veterinarian and from my experience with VSV and learning about zoonotic diseases, there is a population of people, maybe not so much in the United States, but maybe more in Third World countries that have antibodies to VSV, have been exposed to it, their cattle get VSV, and they probably also have AIDS in the same population.

So, there is sort of like kind of this in vivo real model, and I think that they haven't been

optimized and we are just dealing with natural infections, but there are places in the world where animals have VSV, humans have VSV, and humans are HIV-positive, and I don't know if that can be used or not.

DR. SAUSVILLE: My comment on that is, you know, one recognizes that exists, but to me it seems like that is a different situation than where one consciously restitches the hardwaring, as it were, so that the part of one is now intimately related to the part of the other, so I am not sure that that natural history would necessarily be relevant to a new construct base.

DR. SALOMON: That is very interesting. I would comment that from our experience with dealing in xenotransplantation, one of the interesting things was a comment like that in one of our advisory committees led to a worldwide study of patients who had been exposed to pig tissues in this case, looking for porcine endogenous retrovirus, and that came up with the very surprising group of several hundred patients in the former Soviet Union that had gotten pig spleen perfusion.

So, it wouldn't be crazy for someone to go

to these countries and see whether or not you could track a group of HIV-positive patients who got VSV-G. I mean to the extent I have no idea what the incidence of the zoonosis is in that population.

DR. SALZMAN: It's fairly high, in some places, it is fairly high. I know as veterinarians, they teach us sort of not to worry about getting VSV no matter what other diseases we may or may not have.

DR. ALLAN: That also comes back to the point of cell tropism, and even though the VSV-G has a wide tropism, I don't know whether or not regulation of expression is limited to epithelial cells or other cell types, or whether if you took an intact VSV, whether it would replicate in lymphocytes or not. I don't know that answer, so the question is whether or not you get both viruses in the same cell. I think that is something that I am sure it is in the literature, so it is just a question of looking.

DR. SALOMON: I would say one of the interesting questions that just came up this morning, and I would like to return to this afternoon, is this sort of conflict of are

lentiviral vectors more appropriate to test in HIV patients or in non-HIV patients, and I think there are strong feelings on both sides.

I mean some people clearly feel that there is a safety issue and it should be tested in HIV patients, and others, I think who feel equally that there is a safety issue and it shouldn't be tested in HIV patients.

So, I think that will be an interesting discussion to enter in this afternoon. Certainly if it fits into the thread of any of our conversations this morning, I would encourage you to bring it up.

Well, if there is no driving thing, I think this a great time for a break, 15 minutes.

[Break.]

DR. SALOMON: Now, the next part of the session will be two presentations from Cell Genesys. The first will be presented by Dr. Gabor Veres on LentiKat Vectors Overview.

**LentiKat Vectors Overview**

**Dr. Gabor Veres**

DR. VERES: Thank you very much for the opportunity to present some of the data that we generated with lentiviral vectors at Cell Genesys.

You will see that obviously, some of my presentation and even some of the slides will be kind of redundant after the two excellent introduction by Dr. Verma and the FDA representative, but I hope that I can provide you some additional data particularly related to testing strategies and even some data which might be helpful to have a discussion how we want to go forward and what assay sensitivities we have to achieve for the different applications.

[Slide.]

Just to go back a little back, as you all know by now, we are using HIV as a basis for the vector system, and the work was all done in Dr. Verma's lab by Luigi Naldini originally to generate these vector systems.

So, contrary to the fairly broadly used murine retroviruses, HIV is a fairly complex retrovirus, so on top of the basic structure of proteins gag, pol, and envelope, we are dealing with the regulatory proteins rev and tat and quite a few accessory genes which are very important for the in vivo infectivity and the in vivo pathogenesis.

[Slide.]

So, what is the advantage of the Lenti over the existing retrosystem concerning all the issues and probably safety concerns, why won't we use a lentiviral system? It is clearly the transduction efficiency is substantially better than the existing retrovectors. In particular, it transduces non-dividing cells, which is retroviral vectors murine, retroviral vectors are not really capable of.

It can provide the same long-term expression than the other retroviral system. It is also highly efficient for in vivo delivery particularly when it is pseudotyped with VSV envelope, and to make a qualifying statement here, as far as we know, the majority of the population doesn't have preexisting antibody against VSV envelope or the major HIV proteins.

The recent progress which has been reported from several places are the improved biosafety, which means that we try to minimize the HIV sequence in a vector, development of stable producer lines, which is also reported from multiple labs, and a couple of places has now large-scale production capability and also some level of purification.

[Slide.]

Our lentiviral system originally is from a parental HIV isolate LN4-3, and there was a significant departure from the original organization of the virus. We split the HIV genome into four components which I will show you in the next slide, and we tried to minimize the HIV sequence on the vector, so the transfer vector itself has approximately 10 percent of the HIV sequences, removed all the accessory genes, which I mentioned is important for in vivo infectivity and probably plays a substantial role in pathogenicity, so nef, vif, vpu, or vpr are all removed and also the system doesn't require tat for efficient transcription.

We split the rev on a separate construct and this provided in trans [?] to regulate the gag-pol gene expression, and finally, the LTR is deleted, so this is a so-called self-inactivating vector.

[Slide.]

Dr. Verma showed almost the same slide. Again, third generation, put it this way, this is the current vector, what we are using, so these are the four components which consist of the third

generation vector, the helper plasmid codon for the gag-pol.

The rev is on a separate construct and this is absolutely essential to provide high level of gag-pol expression. VSV-G is a heterologous envelope, and then the transfer vector with appropriate promoter and the transgene of interest.

[Slide.]

So, again, this is a summary of the kind of evolution of the vector system. The very first generation had all the HIV sequences except the envelope, and that was pseudotyped with VSV envelope.

The second generation has the minimal packaging construct, but all the accessory genes were deleted, and the gag sequence was minimized on the transfer vector to prevent potential homologous recombination.

Finally, the last generation vector is tat-less. It has the same phenotype and certain other modification has been made to further reduce the overhead between the helper construct and the vector construct.

[Slide.]

As it also has been shown, one of the

major advantage of the vector system, that it can transduce non-dividing cells. I think one of the primary interest in several laboratories to use it for hematopoietic stem cell transduction.

Retroviral vectors would transduce unstimulated CD34 cells very, very poorly. This example shows immobilized peripheral blood CD34 cell population, which was transduced overnight without any cytokine stimulation with lentiviral vector construct expressing GFP, and if you look at the total 34 population, there is a substantial high level of the cell population is transfused, and even the subset, which sometimes it is claimed that it represents the more primitive subset of the CD34 cells, even that cell population has a substantially high transduction rate.

[Slide.]

For in vivo application, obviously, the central nervous system is a very obvious target. In this experiment, we used the SIN vector expressing the luciferase gene under the CMV promoter, and the vector was $10^8$ infectious unit in a volume of 100 microliter.

This vector was injected into the vertebrae, somewhere here, into the mice, so it's

interatrial injection, and you see a luciferase expression in the spinal cord and also in the brain.

This is our imaging system from xenogene is being used. The substrate is injected IP, and about 30 minutes later, the mice are imaged with the CCB camera, and you can see a very high expression in different parts of the central nervous system. The expression is fairly stable, and these animals are still alive, and we haven't seen any particular adverse effect.

[Slide.]

The other major target if you think about in vivo delivery of the vector system, is the liver. Again, luciferase vector was injected in this case directly through the portal vein, and as you see, there is a fairly stable expression and long-term expression in these animals, and the expression is confined pretty much into the liver of these animals.

[Slide.]

Obviously, in this case, one would want to look at the potential toxicity of the vector. We repeated these experiments at different vector doses up to $10^9$ infectious particles per animal,

and we look at one of the most characteristic liver enzymes, and independent of the route of delivery, so either it was injected through the portal vein or into the tail vein, PBS control, we haven't seen any substantial elevation of the major liver enzymes.

This vector does in this case represent approximately to 2 x $10^8$ infectious units.

[Slide.]

The biodistribution is something which is probably related to what we discussed previously in the VSV. Depending on the route of delivery of the vector, if it is delivered directly through the portal vein, as you can see the great majority of the vector ends up in the liver.

This is a DNA real time PCR analysis of the animal after approximately 30 days, so the majority is in the liver, but quite substantial part of the vector is actually transfusing the spleen.

In case of the tail vein delivery, the vector distributed approximately 50 between liver and the spleen, and one can see a trace level of the vector, integrated vector DNA in a couple of tissues, lymph nodes in particular, and a little

bit in the lung.

[Slide.]

The production of the vector obviously is an important issue. One is clearly a safety concern, the other issue from a more practical point, how much vector one can make transiently or in the stable producer cell line.

Currently, for most of the application we use a transient production system. The explanation for this, we are still testing different vector construct, so establishing a producer cell line for each of the vectors is very time-consuming, but obviously, a final vector will be put in the packaging cell and that system will be also tested.

But for convenience sake and also for certain application, the transient production might be quite suitable. So, we co-transfect the four plasmids at a certain ratio into 293 cells using so-called cell factory, and after three days, we collect the supernatant treated with benzonase to remove the DNA, and the vector undergoes a purification step, which provides us also concentration, but also removal of the cellular protein, protein from the tissue culture media and also substantial portion of the plasmid DNA.

[Slide.]

Obviously, one of the major issues is biosafety concerning these vectors. The vector system, what we are using obviously is replication-defective, and since we removed almost everything from the vector, as I said, only 10 percent of the HIV sequence is still present, we are not going to transfer any viral genes into the target cells.

The vector is pseudotyped with an unrelated envelope, so wild-type virus cannot be generated, but, of course, someone cannot exclude the possibility that a non-homologous recombination happened and this heterologous envelope might be incorporated into the vector.

The safety concerns are the generation of replication-competent virus, insertion of mutagenesis into the chromosome, and in the case of population which already has HIV, remobilized the vector.

[Slide.]

With this vector system, we tried to look what would be required to generate the replication-competent virus. Based on the characteristic of the system, we believe that at

least four steps are required to restore a fully functional virus.

One is to restore a functional LTR, which would mean that the sequence should be acquired from the chromosome nearby, which has the promoter and enhancer potential to be able to generate the full length transcript from the LTR.

Then, a homologous recombination potentially could happen between the vector sequence and the helper plasmid coding for the gag-pol. Fortunately, this wouldn't be still sufficient because that construct still would require rev to generate the gag-pol sequence, and the rev has no overlay with this part of the vector construct, so that has to be an non-homologous recombination or a plain insertion, and then this construct has to acquire an envelope from the cell to generate a fully functional replication-competent virus.

[Slide.]

So, this is the schematic. If you look at the vector design, what we are currently using, there is an overlap, which is really a few base pair between the 5-prime and the helper gag construct and the vector. This is needed for the

efficient packaging of the vector.

There is a direct overlap in the center of polypurine tract, which again is an important component of the vector system for efficient transduction and also for efficient vector generation.

There is no direct overlap between the RRE sequence because we use it from HIV-2, and this is an HIV-1 RRE sequence, so this has only a 60 percent homology. The rev, as I mentioned, has no overlap with the vector whatsoever, and the same is true for the envelope construct.

[Slide.]

So, what are the criterias when we think about developing an RCL assay? You have to appreciate that we are trying to develop an assay for a potential vector which actually doesn't exist.

It has been pointed out that under the best circumstances, the optimal control would be an HIV which carries a fully functional VSV envelope, and that could have been a positive control. Clearly, we have no wish to generate this and use it in an assay what we are developing, so what are the alternatives?

A pseudotyped vector, which would be sufficient to control the initial infection, but then if you do further amplification, that vector wouldn't be amplified further on because it has no envelope.

We can use attenuated HIV. Attenuated HIV is lacking all the accessory genes, but otherwise, has all the function, envelope, gag-rev and gag-pol. This would be limited to cells which could be infected with HIV.

Finally, we can use an HIV pseudotyped with the VSV-G envelope, which is probably the closest one to the real life situation.

The amplification system, it should be a cell line which is highly susceptible to HIV infection, so we need very few particles to start the initial infection, and that infection could be amplified with the further passage of the cells.

Finally, what is the endpoint? I mean the most obvious one is to follow p24 production because that is a fairly well established assay to detect the progression of HIV infection.

[Slide.]

So, what we are using as a positive control, as I mentioned, HIV without the accessory

genes, it has a full envelope, and generally particles where we add also the VSV envelope in trans, so actually, this generates a chimeric envelope for the first round of infection.

We look at this, the wild-type HIV in different cell lines to see which is really susceptible for the infection of either this control construct wild-type HIV or just the plain attenuated HIV which doesn't have accessory genes.

If you look at the numbers here how many the $TCID_{50}$ needed to establish infection, you should appreciate that this is required on primary human CD4 cells to start HIV replication. Actually, with this construct using the C8166 cells, we could lower the threshold, so we are at the range of one $TCID_{50}$ to start efficient HIV replication.

C8166 is a lymphoblastoid cell line which is available from ATCC commercially.

[Slide.]

So, the test system could look the following. We put the viral stock or the producer cell line on the detector cells, so in this case it's infection or co-cultured with the producer cells, detector cells, the C8166, then, passaged five times to further amplify a potential RCL in

the supernatant, so if there is one replication-competent particle in the system at the beginning, that should be greatly amplified at the end, and the endpoint is p24 ELISA.

[Slide.]

We started to test the system to establish the sensitivity, so in this table we summarize one of the experiments that we conducted lately using the attenuated HIV which, as I said, was pseudotyped also with VSV-G for the first round of infection.

We know that the physical to infectious particle in this particular preparation was approximately 100. So, if you look, we put a different number of particles in the system, 1,600, 160, 16, and, of course, zero, and what you can see here that we could detect approximately 100 particles throughout the infection.

What this represents here, that obviously, in this case, that is probably one viral particle which started the initial infection, in this scenario, probably more than one particle was in the system. So, that is why we estimated, it is probably one $TCID_{50}$, what started the infection.

We are going to do further analysis in

this range to completely establish the final sensitivity.

[Slide.]

In summary, the detector cells, T-lymphoblastoid C8166 cells, the score replication-competent recombinants, detects recombinants also with the heterologous envelope, and we can measure 10 fg p24 of attenuated HIV-1.

When we spiked this positive control into production lot, currently, we have a detection limit of 1 $TCID_{50}$ in 100 ng of p24, which represents approximately $1.2 \times 10^9$ physical viral particles.

[Slide.]

So, what we would suggest at least for an ex vivo application as a testing strategy is the following. In the vector production, and whether that is transient or a producer cell, we know that we can achieve even currently approximately $8 \times 10^{12}$ viral particle as a total, and we can test 5 percent of that.

That corresponds to the requirement that is currently being used for retroviral testing. That means that we would test $5 \times 10^{11}$ physical viral particle. On the other hand, for ex vivo cell therapy, which Dr. Ando will give you the

details of the protocol, we estimated we would use approximately 4 x $10^7$ cells as a starting material.

If we transduce this with the MOI of 5, that represents 6 x $10^{10}$ viral particles, and we suggest to test 1 percent of these transduced cells as a final product, which is approximately 4 x $10^5$.

So, if you look at these numbers, testing 5 percent of the final production lot, it would mean that we are testing 8-fold of the clinical dose which one would use in a clinical protocol.

So, even if the detection limit is not just a single particle, but let's say a particle between 1 to 5, using the multiple of a single clinical dose would allow us to detect the replication-competent viral particles in this scenario.

Finally, I would like to acknowledge my co-workers and also people who contributed previously to all of this work, in particular Luigi, Dr. Verma, Didier Trono, Anatoly Bukovsky, and my current co-workers who work on both vector construction, packaging line construction, some of the in vivo studies, large-scale production and purification, and RCL assay in particular.

Thank you very much.

[Applause.]

DR. SALOMON: The second part of this will be by Dr. Dale Ando, Cell Genesys, on Lentiviral Gene Therapy.

**Lentiviral Gene Therapy**

**Dr. Dale Ando**

[Slide.]

DR. ANDO: What I wanted to emphasize was that actually none of us have worked in lentivirus in the clinic, but a lot of us have worked on retrovirus for about the last 10 years, and I think a lot of the clinical systems and regulations In terms of testing and evaluating patients, I think we can benefit a lot from the previous decade with respect to that, in the same way that the construction of the vector has benefited from the previous experience.

Again, I think a lot of us who have worked in this area are familiar with some of the common themes of germline transmission, insertional mutagenesis, and the strategies for testing the virus in manufacturing and in the clinical trial subjects.

[Slide.]

With respect to the unique lentiviral

clinical issues, there are several, and what we would like to do in our first approach is to really focus on the issue of replication competent and lentivirus and recombination.

This has been approached as we have seen with respect to the design of the vectors to minimize that, and then, which we will get into a little bit more, is the testing strategy, but the idea here is that we would like to test with an assay that we feel can give us limit detection of hopefully at least one particle, completely the vector, and then a portion of the ex vivo product.

Obviously, we can't test the complete product prior to infusion in the patient, but there may be a way actually in what are called qualification lots or practice lots prior to the study to really evaluate whether or not you have RCL in a total ex vivo product.

So, there are strategies of trying to approach this, so that we can get some data to see whether or not our systems are working. So, for this particular application, we are sort of not addressing the issue of mobilization because we are using a SIN vector and going into a situation of patients who do not have HIV.

[Slide.]

After sort of extensive looking and discussing with several investigators and internally, we feel like we would like to move ahead in adenosine deaminase deficiency, and I would like to go through some of the rationales for that.

Again, this is a proposal, we haven't finalized this. I know there are a lot of limitations in addition to benefits of this indication.

As you know, this is a severe combined immunodeficiency with a fairly marked loss of T, B and NK cell function, high mortality without treatment, and 20 percent of the cases are related to a specific genetic deficiency in adenosine deaminase gene.

This has been defined genetically in 1972, the gene has been cloned, and actually, there is enzyme replacement therapy available. There has been a gene therapy trial, and actually one of the first gene therapy trials in genetic disease occurred in 1990.

So, again, there has been a lot of clinical experience in gene transfer in this area.

[Slide.]

Wide ranges of ADA expression levels are tolerated and modest levels are needed for replacement. Again, for the eventual efficacy, there is a selective advantage for ADA expressing cells in patients, and to be able to functionally and clinically benefit the patient, the selective advantage is very important.

The other important factor is that enzyme replacement therapy is available, so we are not basically limiting the patient with respect to any maximal clinical benefit, that can come later, so it really allows a stepwise evaluation of this setting with the first step being safety and understanding gene transfer in the periphery, because there a lot of preclinical studies you can do to see whether or not a particular gene transduction and marrow culture procedure works, but you never really know actually until you get into the clinic.

So, again, the studies first will be safety in gene transfer and then if we can achieve an adequate level of gene expansion, then, the PEG-ADA can then be actually decreased in the second portion and the efficacy and T cell

immunologic endpoints can be pursued.

So, in some cases, to us, it represents a "best case scenario" for the general area of gene therapy targeting hematopoietic stem cells.

[Slide.]

Three clinical trials of Moloney retroviral gene transfer to hematopoietic stem cells in bone marrow and cord blood have been done. Actually, that number may be five, and there are some unpublished reports of possibly two patients who have been successfully reconstituted using Moloney vector.

Frequency, however, of gene corrected cells in most of these studies was very low and little evidence of gene expression.

So, really, the efficacy that may have been seen in those two patients probably depends mostly on the fact that there is a selective advantage of the T cells, so that is key.

What would turn this area actually into a fairly uniform or fairly efficacious clinical trial would be to get good levels of gene transfer. That is the real key I think to moving this ahead and then to moving it into other areas of stem cell therapy.

[Slide.]

Again, the rationale, I think it has been described fairly clearly in what Dr. Verma and Dr. Veres have shown in terms of the hematopoietic progenitor cells, and we still have some work to do with respect to figuring out a minimal gene transfer system between hematopoietic stem cells to preserve function and increase transduction efficiency.

Again, the key question is whether there would be greater benefit with increased levels of gene transfer in the study.

If we can achieve low levels of gene transfer, even on the level of 1 percent, this most likely will result in fairly significant clinical benefit in the setting of ADA deficiency and help us in the future in development of in vivo methodologies for human stem cell therapy, gene therapy.

[Slide.]

So, the proposed trial's evaluation of safety and administration of autologous CD34 cells transduced with a lentiviral vector carrying a normal human ADA cDNA in children with ADA deficiency and SCID.

We are using an investigator who is very experienced in this area, Don Kohn, and his group at Children's Hospital in L.A. The objectives are the standard clinical and laboratory safety, and gene delivery to hematopoietic cells and gene expression.

The patients will be infants and children with ADA-deficient SCID, less than 1 percent ADA enzyme activity in peripheral blood, laboratory documentation of impaired T and B cell functions, and subjects basically who are not eligible for HLA-matched sibling transplants, and again negative for HIV.

[Slide.]

The basic trial will be screening to determine eligibility, and this is actually a fairly complex process at Children's Hospital.

Treatment, to remind you, includes taking bone marrow out from the patient, isolation of the cells, and then a manufacturing process at the site with transduction of CD34 cells and infusion.

So, really, there are two manufacturing processes, the manufacture of the lentivirus at the company and then the gene transduction at the site.

Then, the observation period, looking at

safety, gene expression, immune function, RCL testing, and then long-term follow up.

In general, these patients are followed fairly closely by these types of specialists throughout their life.

[Slide.]

The lentiviral manufacturing, as we have previously discussed, will be the transient viral production using DNA transfection in 293 cells, and replication-competent lentiviral testing and release. So, basically, we feel that our current paradigm will allow us to test and have less than one copy per lot in a lot that is probably 8-fold higher than the clinical release.

So, we would feel fairly confident that we have the best sensitivity achieved in the viral testing. Then, this virus will be released, then used in the clinical site for transduction of the CD34 cells, at which point we will be testing 1 percent of the cells.

[Slide.]

So, in summary, we are planning on using our latest generation ADA SIN lentiviral vector. This is a 4-plasmid system without accessory genes. We will transduce CD34 cells with ADA lentivector

infused patients.

RCL testing, as we have noted, in the virus will be extremely complete, and 1 percent of the cell product, and clinical evaluation to follow up the patient according to current guidelines for retrovirus.

Thanks.

[Applause.]

DR. SALOMON: That was excellent.

Again, I just want to remind everyone that questions to these sponsors are very appropriate, but they are not here today to tell you that they are getting ready tomorrow to do a gene therapy trial, so I think we need to just temper the kinds of questions that we ask.

**Questions & Answers**

DR. SALOMON: One of the issues that I think is kind of coming here as a theme that I want to raise just for discussion, clearly from a scientific point of view, a strategy that everyone in the field seems to be using is designing their different plasmid vectors with reduced homology to prevent these potential events of homologous recombination, RT strand transfer, et cetera.

That is very molecularly appropriate, yet,

the weakness, it seems to me, is that if as little as 10 to 25 base pair homologies are adequate for homologous recombination, and certainly in the work we are doing with DNA arrays and things very accurate, and then when you say there is 60 percent homology in basically 100 or more base pair crossover, et cetera, it raises a question.

So, the fallback position seems to be, well, you know what, if we do it and can't demonstrate replication-competent lentivirus, then, what's the problem.

So, the question I have is can we have some discussion about the concept of how much do you have to prove in terms of all this homology or, in the end, is that really just a good way to start and it's all based on proving replication-competent lentivirus doesn't exist.

DR. ANDO: My comment on that would be that if you look at what happened in the Moloney retrovirus, we went through a number of generations of the Moloney, but really the mainstay, and this took several years and actually Richard was involved with this, was really coming up a very sensitive mouse study co-culture assay, of which we based our release specifications.

Actually, there are a lot of different cell lines, so the tack that we are taking now is I guess parallel to that. We have designed scientifically a very nice system, minimized homology, but the real key now is to get unit one viral particle sensitivity and be able to test that one viral particle in a signal-to-noise ratio in something that would be a clinical lot.

We are producing 40 liter scale or 14 liter scale 1 x $10^{11}$ virus. We would like to detect, be able to have a sensitivity to detect one viral particle in that, and that has been a paradigm that has been safe at least in the Moloney area. At least for us, that is a starting point for discussion.

DR. KAPPES: I would like to follow up on your comment. I think it is, for me at least, one of the central issues, but I would like to raise the question, maybe perhaps to a more defined level as it relates to at least what I understood that you said, and that is whether or not any system, no matter how sensitive it is, that we use in vitro for detecting RCL is really an adequate predictor of the outcome of treatment.

I could discuss this more, but I think

perhaps I will wait until after my presentation today, because this is the very issue which I will try to address.

DR. SALOMON: Dr. High.

DR. HIGH: I have a question related to your comment. In your lot release criteria, do you look at contaminating plasmid DNA or mammalian cell DNA in the vector, because, you know, I guess benzonase digestion can't really get to plasmid that may be sort of stuck near the capsid, this sort of thing?

DR. VERES: No. I mean the purification procedure, we really look at the residual protein residue of DNA, also PCR specific to 293 cells, so actually, it is a quite complex assay event, so it is not just a crude DNA's digest, but actually we look at the final product, and I think some specifications are there defining how much DNA is really allowed in a certain product.

DR. MULLIGAN: One of the issues that we may get into at some point is whether any packaging-derived sequences are shown to transfer, and obviously, there is assays for replication-competent retroviruses, assays for tat function, other things, but in principle, there is

assays for any HIV-derived sequences being transferred, if you have ever looked or developed an assay where you would simply, for instance, with PCR, move all the way down, gag involved with little primer sets, and asked the question do you detect any transfer of HIV-derived sequence in recipient cell.

DR. VERES: So far we haven't done any of this, and I think it would be very, very difficult to perform these assays because I mean we would have to assay for multiple components both for rev and also gag-pol, and all of the different--helper constructs are slightly different, I mean not everybody is using the same helper constructs, so we can detect the conserve [?] sequence, for example, in the gag, but that would be only just one part.

DR. MULLIGAN: I missed why that would be difficult. If you just take your helper construct and you ask the question whether any of those sequences transfer to recipient cells, why would that be difficult?

DR. VERES: I mean we can do that. I mean the question is where are we going to draw the line, what is the minimal sequence we are looking

for, are we looking for the whole gag or just part of the whole gag.

DR. MULLIGAN: That is a different issue. I just raise it as a question that may surface in terms of is there any difference between our concerns about MLV versus HIV in terms of the notion of transferring any HIV sequences.

Obviously, you can adopt the case that, well, what difference does that make if you don't have a coding sequence, but I am not aware that anyone has really done this in the past, certainly not in the MLV case, but I think it might be a very revealing activity.

The second question was just my eyesight isn't actually so hot, but the biodistributions you showed and the one I looked at, it looked like there is just a tiny, tiny little bar graph bar in the testes, but I couldn't tell whether that was the last thing.

Was there any detectable, after the I.V. or the I.P. inspection, any detectable signal in testes?

DR. VERES: Yes, there is DNA in the testes, but as Dr. Verma showed, I mean it is nothing--I mean we didn't look explicitly which

part of the testes, just took the testes and there is a detectable level by PCR.

DR. SALOMON: Dr. Allan.

DR. ALLAN: I wanted to follow up on his question, which is you are primarily looking at whether you get replication-competent recombinants and also whether you get like tags, pressures, and these other things.

In the case where the patient could be exposed at some point to HIV, which is one of the issues for tomorrow, so even if you transferred a small portion of a gag or pol, or whatever, into the patient, even though it is not replication-competent, and then you come back in with a wild-type HIV, it could rescue partial genes from that patient, and so the issue then is, well, wild-type is worse than--and we will get into that tomorrow--but that possibility still exists, is that even though you don't get replication-competent virus, you may be transferring pieces of genes to the patient, isn't that correct?

DR. VERES: As I said, we don't have any data showing that we would transfer the gag-pol sequence. Obviously, this is something we can look

in the final product, but I am not aware of any data that would really happen, but I cannot exclude it either.

DR. ALLAN: You are not doing PCR for gag and pol in your product, are you?

DR. VERES: No, currently, we haven't done any experiment addressing this.

DR. ALLAN: I have another question that is more general. The SIN vector with the LTR that has basically taken on all promoters and enhancers, do you think you can get recombination in the portion that integrates with wild-type HIV?

DR. VERMA: I think it is a general question. You asking the question of recombination. I think you just have to look at the numbers. If you are asking is there a chance that there could be 1 percent, zero percent, I can't tell you if there is a zero percent chance, but you have to look at the effect.

True, there are 10 nucleotide which overlap, but look at the number of recombination events that must occur in order for it to become a viable particle. It has to have the six genes which are gone, it has to have parts of the LTR, it has to have parts of the gag, it has to have part

of the envelope.

It is not that it is impossible to imagine that can happen one day, but a priori, if you look at it, there are many, many levels of recombination to occur before you can get such a molecule.

So, I agree with the general comments here that the more you assay for it, so there is no reason why one cannot check gag and pol in the final product, yes, it is a perfectly doable thing to reduce the chances, because the fact of the matter is, it is not a MLV. If it was MLV, we will be less concerned it is HIV, so you want to make sure that you go the extra distance.

So, I agree with you, it should be done, more assay, but the probability just by experience of recombination is very low.

DR. SALOMON: I think that is a really important point, and I think to kind of focus what I was asking is, at least my impression now, and again, you know, it is up for discussion, is that from the FDA's point of view, going on to the first clinical trials and think about regulation, it seems to me from everything I have heard, that the type, the definition, the sensitivity, and the confidence we have in the RCL assays is going to go

way beyond any of these theoretical discussions of, you know, we degenerated that and we took out this, and we self-inactivated the 3-prime LTR, et cetera.

If that is true, then, from a safety point of view, a lot of these, you know, very high-level academic discussions of the molecular biology probably ought to be put aside because it is not going to get us to the most important thing. The most important thing is going to be to focus on what is the best RCL assay, what is the attributes of the ideal RCL assay meaning specificity and sensitivity.

Now, that is my premise, and that is certainly open for disagreement or discussion.

DR. DELPH: I may be way off target here because I really don't know enough about molecular biology, but it seems to me that there are two different questions.

On the one hand, can you reconstitute the virus from which you deleted all of these various genes, and on the other hand, if you have already replication-competent HIV present, can that integrate some of these genes, and what is the probability of that latter aspect happening?

DR. SALOMON: Just to put that in the

context of my comments, I agree, and so that is a separate issue, in other words, but very important one. I am asking just should we be focusing on the design and integrity of the RCL assay, and now you have added the next point, and that is, if there is also wild-type HIV, what is the additional risk, and then can we model that.

Dr. Naldini and then Dr. Sausville.

DR. NALDINI: I would like to point out that we do have information, part information in terms of those issues that you are raising, that went into the validation of those generation of the system. This information was acquired by tests made at the experimental level. They may not necessarily have been translated into standard tests to be used as release criteria, but we do have information, for instance, that the level of residual packaging, packaging RNA in the producer cell, which is an important risk factor for recombination, because, of course, recombination not only requires some knowledge, but also requires that two different RNA are packaged.

We have data showing that early generation system allows a certain level of residual packaging which was lost when we went into the advanced

generation system in which we sort of clean the packaging system.

A second type of data is looking for transfer of packaging function like gag and pol, which are expressed, so now are functional to produce particle which would require an envelope to be infectious.

We have looked at that and we have been able to find evidence of that again in early generation system, and not detectable one to certain level of sensitivity in later generation system. All of these data are available in terms of validating the safety of the system. Whether they would be required for release criteria, I think is a matter really for discussion.

DR. SAUSVILLE: I have a question that again may be off base, but I mean it gets to the philosophy of all this. I have no doubt that we can establish a criteria for a release assay, that we will feel confident will yield a low probability of an adverse event, such as the generation or a recombination of either a new virus or an HIV virus.

This is where I turn to our FDA colleagues in terms of guidance. This new type of vector

clearly is different than other vectors that have been conceived in many cases for gene therapy.

Does the Agency have a position with respect to what the toleration is, because if there is any possibility of recombination, then, in a certain sense, a lot of this discussion becomes moot if it renders something as a problem.

So, speak to us on this matter.

DR. NOGUCHI: Specifically, for recombination, I don't think that is limited to lentiviral vectors. That obviously could happen at very many levels in production and in vivo with other retroviral vectors, even with some of the "non-integrating," and we could even envision it for adenovirus as an example.

DR. SAUSVILLE: But you do agree that the pathogenic risks intrinsic or as a result of that, seem to be somewhat different compared to the current circumstance?

DR. NOGUCHI: Well, there is certainly that potential, which is the reason we bring it to discussion, but I would say that we do not have a position set in stone as to something absolutely not.

We have taken that position actually only

in the case of cloning a human being, where we said we have jurisdiction and no, you can't do it. Short of that, we are really looking for the very best advice that we can get.

We are looking for advice on are these safety concerns being addressed in an adequate fashion, if not, what more is needed, are the patient populations the appropriate one for this point of development, or if not, what are the other indications.

But in terms of an absolute yes/no on this particular question of recombination, no, we don't have a set position.

DR. VANIN: Elio [ph] Vanin from St Jude.

People keep on quoting there is 10 base pairs, and I think that that comes from Adolto's [ph] paper, and we have to remember what that was. That was a recombination, that was packaging of two different RNA species into a retrovirus, that then got transferred and recombined to make an RCR. So, that is basically two transcripts and one retrovirus, because that came from a producer line, so it wasn't DNA recombination.

The other thing is, the way the lentiviral vectors are made, you have to package four

different constructs into the same particle and then you have to have the recombination to give you an RCL, and I think we have to remember that.

DR. CHAMPLIN: I think you should be commended for the ability to get a positive control for the RCL assay, but I have some questions about that.

The data shows that this virus, the HIV-A virus, the SDVG suicide, has a lower PCID 50, maybe 100 to 1000 times lower, for certain cell lines. But if you were to then spike your final lentiviral product with this HIV AIDS pseudotype virus, can you still detect it as well or does then it be competed out by the other viruses? Or, more worrisome is that HIV can inhibit other HIVs. Have you looked at that?

DR. VERES: Yes. That is a very good question. Actually, we are testing the sensitivity of the system. That is why I had one sentence down there that currently we are able to detect this one PCID50 of the background of $10^9$ physical particles. We are doing additional experiments to address what is really the limit of this assay and how reproducible this is. That is the goal of continuing this RCL assay.

DR. CHAMPLIN: I had a question on the in vivo tropism of the virus, your biodistribution studies in the mouse suggested a high level liver uptake, but is that in hematopoietic cells or in liver parenchymal cells, is it the spleen and the liver were the only two organs with substantial uptake?

DR. VERES: We didn't look, but Dr. Verma looked I think in the liver, it's both hepatocytes and sinusoidals as transfused, those are Kupffer cells to a certain extent.

DR. SALOMON: Changing the subject just a little bit, in your trial, you know, just rough bones proposal, you do the CD34 purification and the transduction, so I guess one of the things that comes out then is you didn't specify, do you freeze the CD34 cells before you do your testing for RCL?

I know you have thought about it obviously, so how would you do this in terms of product lot test release?

DR. ALDO: That is something we haven't defined. I think there is some controversy now as to whether or not you can freeze these cells and maintain the stem cells viable and gene transduce, and that is actually a major issue to be resolved

hopefully in some of these animal models, but if we are going to test, obviously, these tests take four to six weeks, can't immediately infuse.

You picked up on that, but that will be a major issue with this, with any bone marrow Lenti protocol, and it will be very different from T cell protocols, for example, where you can freeze, and it is fairly well established.

DR. ZAIA: I would also like to change the subject. I would like you to justify your choice of disease that you have chosen to "treat." For the first patient who is non-HIV-positive, who gets an HIV vector, you have chosen a very immunosuppressed patient.

You could argue that it would be better to choose an immunocompetent patient because if there were a problem, there may be less of a problem in that patient, so that it may be a Fanconi's anemia or a hemophiliac, a different patient and a lesser of a problem to confront.

Have you thought about that?

DR. ALDO: We have actually looked at a lot of different genetic diseases. Every one has some problems or issues. Hemophilia is I don't think much simpler. The real positives on this

indication are the fact that there is some effort for the last 10 years on this, and these patients are getting PEG-ADA, so in that sense, their immunodeficiency, although not completely treated, is at least partially treated.

We looked at some of the other SCIDs and say, for example, you may have to do neonates, et cetera, and the other SCIDs do not have any alternative therapy other than, say, transplantation.

There are other biochemical defects that you can look at, but how clearly stem cell transfer would work in those particular diseases isn't as clear, because you have to get something secreted by cells to other tissues and reverse storage problems or specific biochemical defects in certain tissues.

So, given the overall balance, I agree this is not perfect, but we thought this would be a good place to start. I am sure there will be a lot of discussions between ourselves and in public concerning this choice.

DR. SALOMON: It is an interesting issue, immunosuppression and HIV, and it kind of gets back to a question you brought up, and that is the

differences between de novo infection and established infection.

So, in the one piece of one group of data points that has gotten to be quite interesting in organ transplantation has been given successful heart therapy, the HIV community has come to the transplant community and said, you know, we now have long-term lives and we deserve transplanted organs. So, it has become a big issue in the last couple of years. There is an NIH study group now specifically looking at what effects immunosuppression in HIV transplantation has.

One of the things that has come out from the history is that if you take patients who have HIV and get an organ transplant and then are fully immunosuppressed, there is really, interestingly enough, little data suggesting that you enhance or increase the progression of the natural HIV disease in that patient group.

That would be an argument that immunosuppression might not be such a critical issue. There are, however, just to put it in the other light, evidence from a small group of patients who got HIV from the transplanted organ and then were fully immunosuppressed, and there,

there was a really dramatic proliferation of the virus that exceeded the norm, a very rapid, compressed clinical course that led to death in a couple of patients in a couple months, very small numbers of patients, though.

This is by no means I am suggesting, you know, established fact. It is an interesting area, and there will be some more data coming out hopefully from the trials getting set up.

Dr. Kappes.

DR. KAPPES: Your choice of targets is also interesting and I think poses special concerns for recombination. That is, if you do have recombination, and that recombinant is integrated into the pluripotent stem cell, you certain face a situation where you will amplify the presence of that recombinant.

What considerations have you given for this, but with respect perhaps to safety, with respect to monitoring, any comments?

DR. ALDO: Really, just what we have seen there in terms of the testing of the 1 percent, but I think prior to this, we are planning to undergo a fairly extensive in vitro and possibly some of these SCID-reconstituted animals to look at these

issues.

Hopefully, if there is something that comes up there, we should be able to see it. In the end, reducing the probability of this is difficult. I would say the validation of full clinical lots and showing that we didn't see this kind of recombination would be probably the best paradigm that I could think of right now.

I don't know if that answers your question.

DR. MULLIGAN: Could you guys weigh in on the stable packaging cell transient transfection? When the first speaker spoke, I thought the implication was that we are using transient system because it is very easy to use, but--and I forget what the last part of that sentence was--but it was something to the effect, I thought, that, you know, of course, we would move to the stable packaging cells and we have that technology in-house, but then the presentation here was that you would go for the transient transfection.

So, what is your philosophical point of view about the differences between the two systems?

DR. VERES: The philosophy is that the transient transfection system is fairly well

established and we are capable of producing up to 14, 16 liter of material with a particle number of well over $10^{10}$ and $10^{11}$.

We do have a third generation packaging cell line which we actually just started to evaluate, for example, one of the issues, what you raised with the SIN vector, can we establish a clone which will have a high enough titer that it is really practical to use, what are the production criterias, how long we can generate viral supernatants.

I think we hope that in the next three to six months, we can have answers to some of the questions and we can make enough material both from the transient system and the stable system, and we can put them into this RCL assay in different test systems to really establish the safety, and based on that, we can make decision which one we would move to the clinic.

For application like the ADA where the number of patients are fairly limited, the transient production obviously is a possibility because we can easily make enough materials. For other applications, for example, systemic delivery, for example, hemophilia, which probably would

require a much higher particle number, I don't think that a transient production system would be suitable.

DR. MULLIGAN: So, in your hands, have you found difficulty making very high titer, stable producer cells with SIN constructs?

DR. VERES: As I said, we are testing it currently, so I don't really have hard data, and I really cannot comment right now. It is a couple of more months before I can answer this.

DR. SALOMON: That was great. Again, I want to say thank you from all of us on the Committee for your willingness to step up at a preliminary point in your work and share it with us.

The last talk of this morning, certainly not the least and no priority implied, is from Dr. Kordower, on Lentivirally Delivered GDNF for Parkinson's Disease.

It is nice to welcome Dr. Kordower back. He was a valued colleague in the deliberations on neural stem cells about a year or so ago. It is nice to have you back.

**Lentivirally Delivered GDNF for Parkinson's Disease**

**Dr. Jeffrey Kordower**

DR. KORDOWER: Thank you. It is nice to be back. I like gene therapy more than I like stem cells and for my types of applications, closer to the clinic.

What I am going to talk to you about today is all preclinical work using gene therapy in animal models of Parkinson's disease.

[Slide.]

Parkinson's disease, unlike many other neurodegenerative disorders, has a face, a face for America, in fact, multiple faces, and Muhammad Ali and Janet Reno and Michael J. Fox, and the Pope all have Parkinson's disease, as you are all well aware, although what I am going to talk to you about today really doesn't apply to any of these individuals, because these individuals all have advanced Parkinson's disease, and what I want to talk to you about today is using gene therapy which most likely will be most efficacious in patients with early Parkinson's disease, because these patients, because they have advanced Parkinson's disease, their nigrostriatal degeneration is very advanced and there are few nigra neurons left in the midbrain and little dopamine left in the striatum.

[Slide.]

So, these patients require different types of strategies, strategies such as neuronal replacement, which can be accomplished with fetal neurons, possibly stem cells, and a variety of different types of stem cells can be used in this type of application.

[Slide.]

The type of gene therapy that I am interested in, the mechanism is both neuroprotection and neuroregeneration, and that requires having some residual nigrostriatal system left for your compound to work on. Now, the way in which I want to try and neuroprotect and regenerate the nigrostriatal system is with the use of trophic factors.

Now, what I am going to do is spend the first part of my talk, talking about why gene therapy is needed for the delivery of trophic factors, and then the second and third parts of my talk showing why lentiviral delivery is a very potent and promising way to deliver trophic factors to the parkinsonian brain.

The trophic factor I am going to talk about exclusively today is GDNF or glial

cell-derived neurotrophic factor, although it should be noted that there are other trophic factors, such as BDNF and other gene therapy approaches, such as transfecting cells to make certain enzymes that make dopaminergic drugs work better are also in the experimental stage.

[Slide.]

Now, when I mention trophic factors for neurologic diseases, if there are any neurologists in the room, they usually start to roll their eyes at this point, because they say here is another basic scientist with his trophic factor and he has given us NGF for Alzheimer's disease, BDNF for ALS, CNTF for ALS, GDNF for Parkinson's disease, et cetera, et cetera, and the one thing you can say about all these clinical trials, they have all been failures.

Now, have they been failures because the preclinical state doesn't predict clinical outcome? Well, if that is true, we have a lot of problems, but I don't think that is true.

What has happened in all of these clinical trials, that the trophic factor has never been delivered in a way in which the factor ever reached the vulnerable cells that were dying in the

disease, so therefore, there is no reason to suspect or to expect that the trophic factor should have worked in these clinical trials.

[Slide.]

Now, let me give you a little bit of background about GDNF and why we are interested in using this particular trophic factor for Parkinson's disease.

Lin, et al., initially discovered GDNF by its ability to support the viability of midbrain dopaminergic neurons in vitro, and then it was subsequently found that when you give toxins to these cells, which is MPP+, to dopaminergic cells, GDNF also prevented degeneration caused by these toxins.

GDNF has some effects upon normal rats, but the real reason that people got very excited about GDNF in Parkinson's disease is that no matter what animal model you use, whether it's toxins, methamphetamine, age arrest, no matter how you try and destroy dopaminergic cells, GDNF will prevent that degeneration, and if it is applied appropriately, the animals that receive these lesions will not display functional deficits and will have functional benefits from the trophic

factor.

Don Gash and his colleagues then extended these studied to rhesus monkey, and that led Amgen to start a clinical trial which tested the safety and efficacy of GDNF in patients with Parkinson's disease.

[Slide.]

Rush Presbyterian Medical Center, where I work, was one of the centers that participated in this trial, and one of the patients came to autopsy that was in this trial, came to autopsy from events totally unrelated to the GDNF, but it gave us a window to determine whether (a) GDNF was functioning in this patient, and whether anatomically, there was any evidence of regeneration or neuroprotection.

Let me just give you a little bit of information about this patient. He was a 65-year-old male with a long history of PD. Initially, he had a good response to levodopa, and that is critical because you don't want to give a dopaminergic trophic factor if a patient doesn't respond to dopaminergic pharmacology.

He initially entered into a double-blinded trial, and still the blind hasn't been broken to

me, but then he entered an open-label phase in which, through an Elmira reservoir, this patient received monthly interventricular injections of GDNF into the ventricular space in an ascending dosing limit. You can see the doses here.

Following his final dose of 300 micrograms, three weeks later he died at home from a heart attack, and we were able to get the brain from this patient and examine both the behavior and the anatomy in this patient.

Clinically, this patient was evaluated using the UPDRS or Unified Parkinson's Disease Rating Scale. There are two types of scores here. There is a motor score and then the ADL is an Activity or Daily Living Score. "On" means this patient was tested while on levodopa, "off," while off levodopa.

The details of the scale really aren't important right now, but all you have to realize is high scores is bad, low scores are good, and what you can see here at baseline to last visit is no matter how it was measured, the scores continued to rise, and the patient's parkinsonism continued to worsen.

Not only did his parkinsonism continue to

worsen, but there were side effects related to the interventricular injections, loss of appetite, nausea, Lhermitte's sign, which is like an electrical stimulation down the back of the neck, and these are all temporally related to the injection, so as soon as he got the injection, these symptoms were seen.

Then, there were other side effects that were quite serious, that weren't necessarily temporally related to the injection, but we think were involved related to GDNF infusions - hallucinations, this person did not hallucinate prior to the GDNF trial, inappropriate sexual conduct, and depression.

[Slide.]

So, clinically, nothing good happened and some bad things happened. When we got the brain of this patient, we basically saw that the GDNF did not enhance nigrostriatal function. Now, you might see here in the top panel, Panel A, this is tyrosine hydroxylase staining through the forebrain of this patient, and there is some staining here in the caudate nucleus.

However, down in Panel B, this is a patient, Parkinson patient, that did not receive

GDNF, and so this is a typical finding that can be seen in PD patients.

The critical region that must be reinnervated is the putamen, this region here, and you can see in this GDNF-treated patient, and in Panel D at higher magnification, there is virtually no dopamine in the striatum as a result of the interventricular GDNF infusion.

[Slide.]

So, we thought, well, maybe let's look down at where the cell bodies of origin are, the substantia nigra, and basically, we found that there was no effect in the substantia nigra. On the left panel, you see TH staining in the normal patient, the loss of cells in the middle panel of a PD patient without GDNF, and the third panel, the patient that did receive GDNF even had fewer dopamine cells within the nigra.

[Slide.]

So, basically, no clinical efficacy, side effects, and no evidence that there was any kind of regeneration or neuroprotection in the brain.

Now, I just showed you a slide previously where we had all this preclinical data that suggested that it should work, so why didn't it

work in this patient, and, in fact, it didn't work in the clinical trial in general?

[Slide.]

The reason it didn't work is because the GDNF never got to the cells that were vulnerable in Parkinson's disease. On the left here, we have two monkeys that received, not monthly injections of GDNF, but high-dose chronic injections of GDNF into the lateral ventricle, and you can see here in the brains that were stained for GDNF, just trivial amounts of GDNF staining in the caudate nucleus and here in the septum, which is an irrelevant location, and the monkey, too, it all backed up into the singular gyrus.

So, basically, the reason it didn't work is because the GDNF was not delivered in a way in which it could work, and that is why you need to have gene therapy, a site-directed delivery of GDNF if this is ever going to be an efficacious strategy.

Just, if I can step back in general, putting trophic factors in the ventricular system in general is a very bad idea. Lots of things do happen, most of them are bad. So, you want to have site-specific delivery into the parenchyma and gene

therapy allows that.

[Slide.]

Well, the previous speakers did a far better job than I could in describing why we want to use lentivirus for gene therapy, so for time sake, I am not going to go into it, but we are going to use lentivirus GDNF in our animal models.

[Slide.]

Before we go into our monkey models using GDNF, we wanted to do a quick study just to see whether we get any kind of transfection in monkey at all, so we did three consecutive rhesus monkeys and injected with lenti beta-gal or marker gene.

The first two monkeys were sacrificed at a month, the third at three months, and each one of these little dots here represents a successfully transfected cell with lenti- beta-gal.

[Slide.]

Look at just how many cells there are, just really--and I will give you the quantitation of this in just a moment. Just look at Panel C and D here. It is interesting that things change over time. At one month, just the cell bodies seem to be transfected or expressing the marker gene, but at three months, the marker gene is now not only in

the cell body, but expressing both in dendrites and in axons.

[Slide.]

Well, how many cells were transfected? Let's just look at the striatum total. In monkey 1, we had 930,000 cells; monkey 2, a million 2, and this was actually a transposition; monkey 3 was a million 5.

That is a lot of cells, and to just give you some kind of context, back in '95, we published the first report of a postmortem case of a fetal transplant that came to autopsy, and on one side of the brain we had 125,000 cells, and the other side of the brain we had 85,000 cells, and we couldn't be happier.

We were so excited to have so many of these cells surviving and doing what we wanted them to do, and here, we have an order of magnitude greater in terms of the number of cells doing what we wanted them to do. These are direct injections into the striatum.

So, our first study on three consecutive monkeys demonstrated very successful transfection.

[Slide.]

Now, what cells were transfected? Most of

them on neurons. The top panel on each side is the beta-gal. On the left here we have NeuN, a neuronal marker. On the right we have GFAP, an astrocytic marker, and then the merged image where the yellow shows that between 84 and 88 percent of the cells that were transfected were neurons, the rest were astrocytes.

[Slide.]

Now, we have talked a lot today about safety, and safety involves not only just some of the issues that were discussed, but also in vivo toxicology, and I will talk a little bit more about immune status and toxicology a little later in the presentation, but I just want to use this slide to illustrate a couple of points.

Here is a needle track right here, and this is a blow-up of where this needle is right here. This is perivascular cuffing. That was the only vessel I ever saw that had perivascular cuffing in any of these three monkeys, and it was sitting right on top of the needle track, and you can get perivascular cuffing just from putting an injection in the brain.

Panel D, the needle track is right here, and this is a NeuN stain, and I want to illustrate

the fact that there are many, many healthy neurons right adjacent to our injection sites. So, from a toxicological point of view, not only do you have lots of cells being transfected, but the striatum appears to be intact, and not expressing any kind of toxic insult from the injections.

[Slide.]

So, now we are ready to go into our animal models. In our initial studies, we chose what is often an unusual animal model for Parkinson's disease, and we decided to use aged monkeys. I have a large colony of aged monkeys, and I define an aged monkey as 22 years of age or older. Every monkey year is about 3 human years, so it is about 66 to, in this study, 66 to 90 years of human age.

[Slide.]

Now, there are many reasons why it shows aged monkeys as our initial step. One of them is I wanted to make sure that we had somewhat of a present nigrostriatal system there for the GDNF to work on, but there are a number of other advantages to using aged monkeys as a model of PD.

One is that the changes that occur in the nigrostriatal system are slow and occur over decades, much like it does in Parkinson's disease.

Now, many of us who do lesions write our NIH grants, and routinely we get some reviewer who says, "Well, your lesions, and they occur over a week or a month period of time, that doesn't mimic what occurs in the disease state."

It is true, it is also very often a trivial comment, but on the plus side, using aged monkeys does allow us to have the temporal changes occur in a manner that is more analogous to Parkinson's disease.

The reason I chose aged monkeys, though, is point 2. Aged monkeys don't lose nigral neurons, they lose their ability to synthesize dopamine in existing nigral neurons, and that is one of the first things that happens in the nigra of a Parkinson's patient.

A cell doesn't just go into an apoptotic cascade and just die or become necrotic and explode. One of the first things that happens, it shuts down its synthesis of dopamine, and that is what we can model here using aged monkeys.

There are a number of other interesting aspects of using aged monkeys. They are all progressive motor declines that are associated with nigrostriatal degeneration. For other reasons, you

may want to study the cognitive impairment or concomitant age-related problems, so there are a lot of reasons to use aged monkeys.

There is a disadvantage. Aged monkeys do not have Parkinson's disease, and they do not respond to levodopa, so the first study I am going to show you is purely anatomical, and then we will switch model systems and I will show you the functional and anatomical studies using a different model.

[Slide.]

Well, we gave injections of our lenti-GDNF into the caudate nucleus and the putamen and the substantia nigra, and the first inkling that we were on the right track came from our PET scan studies. We used fluorodopa uptake, which is a measure of dopaminergic terminals in these aged monkeys.

This is one monkey at four different levels preoperatively and three months postoperatively. We put the injections on what is your left side. You can see the caudate nucleus and putamen here, and you can see the dramatic increase in fluorodopa uptake in all of these panels on the side of the lenti-GDNF injections.

[Slide.]

Right after that we sacrificed the monkeys. I already showed you this slide showing the lack of GDNF expression in the brain when you infuse it into the ventricle. I want you to compare that to what happens when you give lenti-GDNF right into the striatum, and look at the panel on the right.

[Slide.]

Don't worry about these holes. These were punches taken for postmortem analysis. But here is the caudate nucleus and here is the putamen, and we can virtually cover the entire striatum with GDNF expression. This is three months postoperatively.

Just to show you that it is not due to just putting needles in the brain, when we do lenti-beta-gal, and we stain for GDNF, we don't see anything.

There is one other point I want to make here. Cliff Saper, who is the editor and chief of JCM, one of the best journals, always says you are supposed to present your representative case, but you have got to show your best case because if you show your representative case, people will think that is your best case.

Well, this is both our best case and our representative case, and one thing I want to emphasize about the data that we have collected that is incredible to me is that every single monkey shows virtually the same thing. We have yet to have any monkey fail in having outstanding gene expression, whether it be lenti-beta-gal or lenti-GDNF.

So, all our monkeys look like this three months postinjection.

[Slide.]

I would like to show this slide. Here is the cerebral aqueduct here, and here is the cerebral peduncle down here. This is one, 5-microliter injection of lenti-GDNF, and we can cover virtually the entire hemi-midbrain with this one lenti-GDNF injection.

[Slide.]

Not only that, the lenti-GDNF gets transported throughout the basal ganglia system. Here is an injection in the putamen, and this staining is not from an injection, but an anterograde transport of the GDNF from the putamen to the globus pallidus.

Look how the staining respects the

boundaries of the globus pallidus, outlining the striatal pallidal pathway, and there is also staining down in the substantia nigra, pars reticulata, outlining the striatal nigral pathway.

There is also retrograde transport of the secreted GDNF following the striatal injections.

[Slide.]

Well, how much GDNF is actually being made? This is now a different set of monkeys that were sacrificed 8 months following the injection. Their immunocytic chemistry was identical to what I just showed you from our short-term studies, and the punches we took went through GDNF ELISA.

This is a typo here. This should be nanograms per milligram of protein. But from these punches, we got 2,500 and 3,500 nanograms per milligram of protein.

Each one of those holes I showed you was about a milligram of protein, and so if you examine the type and number of the staining, what I am telling you here is that for a least eight months postinjection, we are getting chronic microgram doses of GDNF being synthesized and secreted from the lenti-GDNF injections. That is an incredibly high dose.

[Slide.]

Biologically, what happened in the aged monkeys? Here is a lenti-beta-gal-treated animal. Here is the low intensity of TH staining that is seen in the striatum of an aged monkey, and here is the site of the injection that received lenti-GDNF, and I think you can appreciate the dramatic increase in TH staining on the side of the lenti-GDNF expression relative to the intact site.

[Slide.]

Both dopamine and HVA levels are dramatically up following lenti-GDNF on the side of the injections both in the caudate nucleus and in the putamen.

[Slide.]

Some of our most dramatic effects were actually seen back in the level of the substantia nigra. Here is the nigra of an aged monkey that received lenti-beta-gal treatment, and here is a monkey that received lenti-GDNF treatment.

There are three things I am going to show you on the next three slides - more cells, bigger cells, more good stuff in the cells.

[Slide.]

In terms of more cells, let's just

concentrate on the right side here, because that is the injection side. There is an 85 percent increase in the number of TH-positive cells on the side of the lenti-GDNF injection. That is an incredible response.

What is interesting are the absolute numbers. We have previously published that aged monkeys have about 60,000 nigral neurons, and young monkeys have about 120,000 nigral neurons. So, basically, what we have done is made an old substantia nigra into a young substantia nigra with lenti-GDNF expression, and we believe this is not due to any neurogenesis, but basically, all those cells that downregulated their expression of tyrosine hydroxylase has now been boosted up and the downregulation has been prevented, so now they can be counted, just what we had hoped for when we designed this study in the beginning.

[Slide.]

The volume of each one of these nigral cells is increased by 35.7 percent, and for those of you who aren't familiar with quantitative morphology, a volumetric increase of 35 percent is a huge increase in cell size.

[Slide.]

Finally, I said there is more good stuff within each cell. This is tyrosine hydroxylase mRNA staining, lenti-beta-gal-treated animal on the left, lenti-GDNF-treated animal on the right.

Obviously, there are more cells here, but I want you to appreciate that each cell is darker due to the fact that there is more TH mRNA within each cell, and when you do the quantitation of the optical density for TH mRNA, there is a 21.4 percent increase in the relative optical density.

So, we have no toxicity, we have consistent and robust gene expression that is long term, and we have robust effects at the level of the striatum and the nigra with lenti-GDNF delivery, but still we are missing one thing, we are missing recovery of function, because that model system, as I mentioned, does not respond to dopaminergic drugs.

[Slide.]

So, now we have to switch model systems. I think most of you may be familiar that the best animal model of Parkinson's disease is the primate model of MPTP.

MPTP was discovered in California as a byproduct of drug abusers making synthetic heroin

in their basement and created this byproduct called MPTP, and they were wheeled into emergency rooms in San Jose with all the symptoms of Parkinson's disease, and were for all intents and purposes were end-stage Parkinson's disease cases with the exception that they were all 20 years old.

Bill Langston and Irwin went on this remarkable detective story, in which they went to their houses and they got the drug, and they found that the offending agent was MPTP. Actually MPTP is a protoxin, the actual toxin is MPP+, and the MPTP is broken down by monoamine oxidase into MPP+. It doesn't work very well in rats, it doesn't work at all in rats, it works somewhat well in mice, works exquisitely well in monkeys.

So, now we are using this model system. What we do is we train these animals on a fine motor task and also we score them on a modified Parkinson's disease rating scale. It is analogous to the UPDRS scale that I talked to you about previously.

Then, all the monkeys get a single injection of MPTP up the carotid artery. Now, there is one problem with this model system, that monkeys don't always get parkinsonian symptoms with

a single injection, so what we have to do is we start out with a large number of animals, in this case it was 20, and we inject them all.

Three or four days later, you go into the room, and I can take any one of you into the room and say which one of these animals are parkinsonian, and you would be able to pick out those that are parkinsonian. They have this crooked arm posture, they drag their leg, and many of them will rotate around in their cage. It is a very obvious, obvious clinical phenomenon.

Then, what we do is we just take those animals because we know from experience that those animals will always be parkinsonian unless you intervene and will never display spontaneous recovery.

So, after we take those animals a week after the MPTP, we then distribute them based upon parkinsonian rating scores into a lenti-beta-gal group and into a lenti-GDNF group. We test them for three months on the same behavioral tasks, we give them a fluorodopa PET scan and then we run them through the same anatomical studies that I just showed you previously.

[Slide.]

Here is a cartoon of the pick-up task. Basically, it's a modified home cage. We put apple in these recessed food wells, and simply just time the animals for how long it takes them to remove the food treats or apple out of the food wells.

[Slide.]

Let's not worry about the red bars. This is for a different talk. A normal animal can perform this task in about eight or nine seconds. You give them MPTP, and then if you look at the yellow diamonds, which are the lenti-beta-gal group, these animals get worse and worse and worse, and the longest we let them go is at 30 seconds. There are no error bars here because all controlled-treated animals cannot perform this task within 30 seconds.

In contrast, animals receiving the same lesion, same lentivirus injections, but now encoding for GDNF, they initially get a little worse, but then they get better and better, and stay stable significantly better for the duration of the study.

You may notice there are pretty big standard error bars here. That is because one animal did not recover, but all the rest recovered

completely, and went back down to normal. I will talk to you in a little bit about why that one animal didn't recover.

[Slide.]

In terms of the parkinsonian rating scale, a normal animal will score a zero. Once they are given MPTP, they score about 11 or 12 on this task. Lenti-beta-gal-treated animals stay stable parkinsonian throughout the duration of the study. Lenti-GDNF-treated animals get better and better and better. It is relatively small, so these changes did not get statistically significant through the last four evaluation points, but still a robust anti-parkinsonian effect.

[Slide.]

We had our first indication again that things were going well anatomically. Certainly, that was excellent news behaviorally when we looked at the fluorodopa uptake. This is the side of the MPTP infusion, the side of the lenti-beta-gal injections, and you see basically you lose all fluorodopa uptake on the side of the MPTP injection.

In contrast, when you give the lenti-GDNF to parkinsonian monkeys, you are able to prevent

the degeneration of the nigrostriatal system completely. In fact, in this monkey, there is more fluorodopa uptake here than here.

[Slide.]

When we looked at the brains of these animals, these are coronal sections through the anterior commissure. Here is the caudate nucleus, here is the putamen. You can see lenti-beta-gal-treated animals lose virtually all their dopamine within the caudate nucleus and the putamen.

In contrast, animals receiving the same lesion, same virus, but now encoding for the trophic factor, we get not only complete preservation of the nigrostriatal system, there is more dopamine here than there is even on the intact side.

[Slide.]

When you do the quantitation, lenti-beta-gal-treated animals lose TH optical density dramatically, and it appears to be a normalization here in lenti-GDNF-treated animals, but if I would have culled out that one animal that didn't recover, there is actually an overshoot and there is more dopamine in the striatum as a group

under those conditions than on the intact side.

[Slide.]

Well, what about the nigra? The same type of phenomenon. At the level of the entopeduncular fossa, here is the intact side, this is the MPTP-treated side, and this is an animal that received the controlled vector lenti-beta-gal, and you see the dramatic loss of TH-positive cells on this side. Same vector, same lesion, just now encoding for GDNF, and there is a complete preservation of the nigrostriatal system.

You can see the gold staining up here. This is regenerating fibers, of sprouting fibers that have resulted from the intranigral injection of the GDNF.

[Slide.]

When you do the quantitation, lenti-beta gal-treated animals lose almost 90 percent of their cells, and this is completely prevented with the lenti-GDNF, and, in fact, there are more cells here, and we don't think that this is due to again any neurogenesis.

What we think happens with the MPTP going up the carotid artery, there is a little bit of leakage to the other side, and so basically, we

think we have protected everything on this side of the GDNF, but we didn't protect the small loss that is seen on the opposite side.

[Slide.]

Again, if you look at the volume of the changes, the changes in volume of nigral cells, of the remaining cells in the lenti-beta-gal group, these cells shrink by 32 percent, just like they do in Parkinson's disease. In contrast, not only is that prevented, but these cells hypertrophy, and if you look at the difference here, there is almost a 76 percent difference in the size of these cells.

[Slide.]

Again, if you look at TH mRNA, again, the remaining cells, there is a loss of TH mRNA within the nigral cells, just like it is in Parkinson's disease, and again not only is this prevented, but there is an augmentation of TH mRNA within individual nigral neurons.

[Slide.]

I am going to skip all this.

[Slide.]

Again, so we have all this great stuff. We have got functional recovery, we have got anatomical preservation to the max, just what we

would hope, but nothing is worthwhile if have immune responses and toxicity.

So, we carried out detailed immune studies using CD45, CD8, and CD3 markers, and what I am showing you here is all CD45, which is the most ubiquitous of those stains. What I am showing you in Panel A and Panel B is the worst response we got--oh, excuse me--the most intense staining we got from any of these markers on the worst or most intense section from that animal, and this is all that we have ever seen, just a little bit of staining here, a couple of cells with microglial morphology even in other brains, right through the needle track, and that is an antiimmune response.

We got nervous that maybe we were having a problem with our staining protocol, so we threw in an Alzheimer's piece of tissue that stained up beautifully to illustrate the specificity of this response.

So, there is no immune response following lenti-GDNF injection in these animals.

[Slide.]

Finally, there is one other bit of caution I did want to pass along. Now, we did our injections in the caudate nucleus and the putamen

and the nigra, and why did we choose all three sites? Because we were gutless in the beginning. These are very expensive studies, we wanted to show which sites would be more important, so we figured we are going to inject all of them.

Well, it turns out it is interesting that we injected the nigra, and I showed you all those good things that did happen, but bad things can happen also. This is the lateral septum, and look at this very robust sprouting response seen here in the lateral septum.

From an anatomous point of view, that is pretty cool, that's things we would like to see, but the problem is the cells of origin here are not nigral, they are from the adjacent ventral tegmental area, and when you augment the adjacent ventral tegmental area, that is what in part causes schizophrenia.

So, I think it is very important that we do not put dopaminergic trophic factors down in the midbrain, because you are not going to be able to control them sufficiently to ensure yourself that you are not going to augment an adjacent nucleus that can cause very severe side effects in patients that are taking levodopa and are potentially

teetering on hallucinogenic behavior anyway. So, that is one important point I wanted to make.

[Slide.]

So, in closing, where do we go from here? I think it is absolutely essential that no one goes into a clinical trial with gene therapy, at least the types of trials that I am discussing here, without your ability to control gene expression, and it is not just enough to be able to control gene expression, you have to be able to show that you can shut off your gene, and that shutting off your gene reverses whatever you did, because, for example, too much dopamine can cause abnormal involuntary movements called dyskinesias, and many of you may be aware of the recent report about fetal transplants that cause these runaway dyskinesias in these patients, and they have no way of reversing that.

What we are doing is we have a study ongoing right now in aged monkeys where we are putting the lentigene in with the tet-Off system, and we will do fluorodopa uptake on PET scan. Then, half the animals will get tetracycline, we will attempt to shut off the GF gene, and we will also measure dyskinesias in these animals and see

whether we can reverse the fluorodopa uptake and reverse any changes in dyskinesias.

I think it is absolutely essential that these first two points be met before anyone goes to the clinic with a therapy such as lenti-GDNF.

One of the big questions that will be asked by regulatory agencies is what is the appropriate patient population to go into. Typically, trials start with more advanced patients, especially safety trials, especially in a disease state that has other therapeutic strategies available to them, but this type of strategy, GDNF strategy theoretically should work best in, as I mentioned earlier, the less advanced patient.

So, we are also doing studies to model, instead of modeling early Parkinson's disease, modeling late-stage Parkinson's disease to see whether GDNF will be efficacious in that system, if it is not, that would question whether we should be doing trials from the beginning in earlier patients rather than late stage patients.

Then, just in closing, I showed you a lot of work, and I tend to go around giving the talks while all the people back in the lab are doing all the work. I am very proud of my group who

collected all this data, as well as Patrick Aebischer and Jocelyne Bloch and Nicole Deglon who provided all the vectors, University of Wisconsin group that did all the PET scanning, as well as Philippe Hantraye and Didier Trono who participated in other aspects of the study.

I will stop there. Thank you.

[Applause.]

**Questions & Answers**

DR. SALOMON: So, one of the things you started out by saying is that when any of us went in the room, we would be able to detect the animals, so after you did the gene therapy, would we now have difficulty detecting the animals?

DR. KORDOWER: Yes, you very much would have difficulty detecting the animals. In fact, the fact that these animals have some score on the Parkinson rating scale really attests to the experience of the observers and the trained observers who do this all the time.

If just someone who didn't do this for a living went in there, you would have a hard time detecting which animals were parkinsonian and which were untreated, which were GDNF treated and which were untreated. I am sorry.

DR. SALOMON: Is that what you meant to say?

DR. KORDOWER: Excuse me - which are normal and which are GDNF treated.

DR. VERMA: Were you not surprised that if you are using it for eight months uncontrolled expression of GDNF, the monkeys, that there was nothing bad that happened to them by and large?

DR. KORDOWER: There was nothing bad that happened at all. In fact, all the caveats I brought up are theoretical, there is no empirical data at all to suggest that bad things will happen, but there is one big caveat, and I think this is the caveat that the Freed [?] people ran into. No one ever did fetal transplants in monkeys into levodopa-prime Downs, and it is undoubtedly initial clinical trials with gene therapy will go into patients that have been on levodopa, and that could be a major variable.

So, monkeys are not parkinsonian patients, and that is a key parameter that needs to be tested, and we are testing that currently.

DR. MULLIGAN: This is kind of an irrelevant question vis-a-vis the meeting, but it is interesting one I think. The lac-Z infections,

you showed a time course and you showed that the cell bodies looked like they were making some lac-Z, but then over time you saw protections.

Do you have any idea what that is, what accounts for that, and have you looked ever in these to see whether directly there is integrated sequences, on integrated sequences, is there a transition from unintegrated to integrated sequences?

DR. KORDOWER: We haven't looked at that. What I think is basically happening is that the gene product, both lac-Z and GDNF, is being made and is just being integratedly transported down axons to normal target cells.

DR. GROSSBARD: Elliott Grossbard, Amgen.

Would I be correct in inferring that some of the preclinical studies with proteins were done in MPTP primates?

DR. KORDOWER: That is correct.

DR. GROSSBARD: So, you haven't really explained the inconsistency because you suggested they were trivial delivery of the neurotrophic factor even in the primates, and yet they had a clinical response.

DR. KORDOWER: If you read those papers

carefully, some of those clinical responses are pretty trivial.

DR. GROSSBARD: Oh, okay.

DR. ALLAN: How long can you wait after you induce Parkinson's in the monkeys before you won't have an effect?

DR. KORDOWER: Well, we are not sure. We think we were right on the bubble. I mentioned there was one animal that didn't recover. That animal had great gene expression. We think what we ran into with this particular animal is that there is some variability in the speed at which the fibers regress, and that particular animal may have had quicker fiber degeneration than the others, and the gene that was not able to capture that.

It is interesting that that animal had complete protection at the level of the nigra, but did not have protection at the level of the striatum, and that animal did not recover.

I don't want to appear too flippant about my response to the previous questioner. A lot of those MPTP studies involved interparenchymal injections. The trivial response that I was referring to were studies that used interventricular administration of the protein.

DR. HIGH: You described adverse events in the patient that you took care of who had GDNF protein therapy, and I was wondering if any of those adverse events were accurately modeled in the primates.

DR. KORDOWER: No, we didn't see any adverse--what we would call an adverse event in the monkeys, and that is quite typical. You know, with other trophic factor deliveries, when we did studies with NGF, and we put NGF secreting cells in the ventricle of monkeys, they did very badly and they had significant side effects. You do those same studies and put them in parenchyma, and you don't see the side effects. It gets back to the point I made earlier, I don't think trophic factors should be put in the ventricle.

DR. RAO: It seemed implicit in your statement that GDNF is not causing sprouting, if you think that the failure to see response was because you couldn't reverse the regression?

DR. KORDOWER: No, there is evidence for sprouting, certainly at the level of the nigra, but to get the sprouting, the trophic factor has to get to those fibers that have the receptors on them, and the distance may have been too great for that

to occur.

I didn't have a chance to go into it, there is other evidence, and we are presenting some of that in the Science paper, I believe, to suggest that there is both protection and sprouting.

DR. SALOMON: Was this a VSV-G pseudotyped?

DR. KORDOWER: Yes.

DR. SALOMON: So, at least we could say that in vivo injections into the brain, VSV-G was an effective delivery system.

DR. KORDOWER: Correct.

DR. SALOMON: Did you ever take any of these tissue biopsies at, let's say, a month after delivery, take them out and put them in co-cultures with cells that would be, you know, H9 or--

DR. KORDOWER: No, that is something we have to do.

DR. SALOMON: Do you have any studies at all that would address the issue of replication-competent lentivirus?

DR. KORDOWER: None that have been currently finished.

DR. SAUSVILLE: You introduced in a prominent way the possibility that having

regulatable expression would be important, and you used the tet system as an example. Although tet promoters are used very avidly in preclinical models, they tend to be at either the On or Off.

Is there evidence that you can actually grade the level of expression using that particular promoter system, or do you think this would be relevant, for example, to use in humans?

DR. KORDOWER: My answer is totally speculative. This is such a potent trophic factor. My guess would be that you would not be able to dose it with the tet-On system.

DR. SAUSVILLE: Although it's comforting that you can turn it on and turn it off and regulate it, whether that would be practically have value in terms of grading doses is unclear at this point.

DR. KORDOWER: Right. For me, the necessity to have it is totally a safety issue.

DR. RAO: I was also curious about the fact that the lentivirus seems to be relatively more specific towards the neurons. I mean would you care to say? I mean the relative ratio at least published would be 10 to 1 for astrocytes and oligodendrocytes.

DR. KORDOWER: In culture.

DR. RAO: Even in the brain?

DR. KORDOWER: No, I think Dr. Naldini is here, I don't know whether he has data, but I know the original rat studies, I believe were also predominantly neuronal, and I think that is quite consistent. That is my understanding.

DR. VERMA: That may have to do with the promoter off.

DR. KORDOWER: The PGK.

DR. VERMA: But some PGK, CMV, many of them have very often, but some of them, like EFN-alpha, does not do as well in neurons as it does in other cells. It is a matter of the promoter, too.

DR. RAO: But it seemed better in neurons.

DR. VERMA: Depending upon the nature of the promoter you use.

DR. RAO: Is there any culture data from this lentivirus suggesting that there is a cell bias?

DR. KORDOWER: I am not aware of any.

DR. VERES: If anything, I think this is related to the envelope, the VSV envelope. In this regard, I think there is some data published, at

least from meeting reports, they are using either rabies or the other retroviral envelope which claim to have tropism to the glial cells.

DR. MULLIGAN: You mentioned on several occasions that you didn't think neurogenesis was responsible for the effects. I thought one of the effects of GDNF purported in the past was indeed neurogenesis. Why isn't that happening or why wouldn't that happen?

DR. KORDOWER: We have actually pulsed a couple of animals with BODU and didn't see anything, and also, the cells are always in the exact cytoarchitectonic location that they should be, and you never see any streaming.

You saw, I guess the best example was the nigral injection where basically, half the midbrain was filled with GDNF, and so you would figure if it is going to cause neurogenesis, it should do it throughout. You don't see that. It is only in the nigra.

DR. MULLIGAN: What were the original data suggesting that that was a GDNF effect, was there injection made in the past by other people suggesting that this occurred?

DR. KORDOWER: Yes, I think in neonates.

DR. ZAIA: Can you repeat one more time, in terms of your rationale, is the GDNF inducing dopasynthesis I presume?

DR. KORDOWER: The GDNF is preventing neurodegeneration, and GDNF is increasing tyrosine hydroxylase expression, which is the rate-limiting step of dopamine synthesis, causing regeneration of fiber, so it is doing three things.

DR. ZAIA: But then are you suggesting that if you had gone in with the enzyme that you needed to increase dopa, that may have failed? Had you done the control of using whatever the dopasynthetase is, I don't remember the enzyme--if you had gone in with TH after the challenge, would you have protected?

DR. KORDOWER: You wouldn't have protected.

DR. ZAIA: Why not?

DR. KORDOWER: Because TH isn't a protective enzyme, it's a synthesizing enzyme.

DR. ZAIA: But it would raise dopa levels, wouldn't it?

DR. KORDOWER: It would raise dopa levels.

DR. ZAIA: And so you are saying that that is not sufficient to protect?

DR. KORDOWER: I am saying because the cells are going to die anyway, so if you are not preventing their death--you may get a bump in symptomatic benefit, but you are not going to--

DR. ZAIA: I see. So for the rationale, then, it requires the trophic factor.

DR. KORDOWER: Correct.

DR. ZAIA: Okay.

DR. SALOMON: But if we follow that, then, again deferring to my neurology colleague, the way this model was set up is he creates an acute injury, so during that period of time, there is cell injury death and, you know, this quasi-state that maybe some cells can be rescued, and that would be your target, right?

You give your GDNF gene therapy then, right, it is not--you didn't show us any data where you caused the injury, waited for two months, at which point the animals have the 30 second or greater fruit-sorting test, and then gave the GDNF therapy.

So, when we now make the jump between how one would use that animal model to what is going on in a human patient with Parkinson's disease, a lot of it has to do with where in the state of the

disease progression we are at, which you acknowledged right at the beginning, but it also goes to what extent is neural cell loss and destruction occurring.

DR. KORDOWER: Versus phenotype.

DR. SALOMON: Versus, you know, just changes as I think Dr. Zaia was getting at, where it would be metabolic or enzymatic pathways that are being altered, so what's new, you know, animal models are tough to do. I didn't mean to go too far beyond it.

One thing that I find sort of interesting is you do this injection and then it's a little tricky with the slides, because what you are doing a lot of times is you are showing GDNF staining.

DR. KORDOWER: Right.

DR. SALOMON: And what you don't show a lot of is how many cells actually got hit by the vector and how that relates to where you find GDNF. I mean it's too wonderful, but you do this injection and you get only the putamen or only the substantia nigra.

So, how much spread of the original lentiviral vector occurs outside the needle site and how much spread afterwards occurs of virally

infected cells, and how much is the rest due to just spread of the GDNF?

DR. KORDOWER: We are getting a handle on that. Part of the vector system has the woodchuck-enhancing element, and we have an in-situ probe against that. So, you put the injection in and you probably have 3 to 4 millimeters on either side of the injection filled with cells, labeled cells, but the secretion is much farther than that, and we can fill out the entire striatum.

In fact, there is even more--what I showed you immunocytochemically is an underestimation of what is there, because when we do our punches, and we don't know where the injection is, I am just doing it on a piece of fresh tissue, sometimes you get a punch that is outside the area of immunocytic chemistry, and although the level of protein there is greater than background, significantly greater than background, although it is not as much as what is in the number of the staining, it is still much greater than background.

So, it is even greater than what I showed you, and we can basically fill the entire striatum with GDNF.

DR. VERMA: Didn't you have a construct

with GDNF, area of GFP?

DR. KORDOWER: No.

DR. VERMA: Oh, you haven't. I thought you had that construct that would tell you.

DR. KORDOWER: No.

DR. SALOMON: He threw us--I am kind of disappointed in you guys actually, because Jeff set you up with the statement you cannot go forward with lentiviral gene therapy unless you have a regulatable promoter, and the resounding silence here--

DR. VERMA: Or trophic factors--

DR. SALOMON: I don't know. Okay. I mean do you guys want to take it or--there is consensus here from the Committee that you have to have a regulatable promoter.

DR. CHAMPLIN: Here, there is the functional possibilities, as well as dangers from systemic effects. In the brain, obviously, you can make things worse symptomatically, as well as better, and if they get worse, you could turn it off by using the tet-Off system, so it is not so much worrying about the killer virus emerging as much as the functional effect on the patient.

DR. KORDOWER: I must say that the reason

I feel that way now, I didn't feel that strongly about it six months ago, before the Fried, et al. report, but you put in a fetal transplant, you have got five patients, and I don't know if anyone has seen the videotapes, they are horrific, they are horrific, and you don't want to be doing, you know, you don't want to have put a dopaminergic trophic factor in, have something similar happen, and you can't turn it off.

DR. SALOMON: You ought to do a suicide gene.

DR. KORDOWER: Now you are getting complicated.

DR. SALOMON: In a fetal cell transplant you can. I mean I think the principle here is really important, and there is two principles. One is okay, I mean I was partially being facetious. I realized that Dr. Kordower was making the point specifically for intraneural applications, but still that is really a bold point from a regular point of view to say that.

The second issue is to what extent do we have confidence in tet-On/tet-Off systems. I mean I thought this was, man, this is a lob for you guys. I mean everybody goes nuts every time you

mention a tet-On/tet-Off system is leaky, it turns off, it gets silenced, and no one said a word.

DR. SAUSVILLE: I did protest a little bit about the tet, if you remember, and we established that pharmacologically, it is probably not going to allow regulation, which leads to what I think you stated it was the worst case scenario regulator rather than something that you are going to--but, also, isn't that rather context-dependent?

I mean one could imagine replacement therapy is where the consequences of having more or less are not quite the same, but that I guess needs to be judged on a case-by-case scenario.

DR. VERMA: Also, I think in the case of the tet, it is not really a question of people have been talking about 100 percent off and on, that is not what they are asking for. If you have a small leaking, it is very different than absolutely zero. So, these systems don't have absolutely zero, but small leaking is tolerable in many cases.

DR. KORDOWER: As long as your biological effect can be reversed, you are fine.

DR. VERMA: I tend to agree with you that it's a good idea to have regulation in general, but it is not necessary for every disease candidate,