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Open Session

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P R O C E E D I N G S (8:05 a.m.)

DR. MULE: Welcome to the second day of the Cell, Tissue and Gene Therapies Advisory Committee. Again we have a rather full agenda on a couple of timely topics.

I'd like to begin by having Gail read the conflict of interest statement.

Agenda Item: Conflict of Interest Statement

MS. DAPOLITO: Thank you, Dr. Mule. Good morning. This brief announcement is in addition to the conflict of interest statement right at the beginning of the meeting yesterday, February 9, and will be part of the Cellular, Tissue and Gene Therapies Advisory Committee on February 10, 2006. This announcement addressed conflicts of interest for the discussions of Topic II on the National Toxicology Program on retroviral vector-mediated mutagenesis, and Topic III, an update of the review of the research program of the Office of Cellular Tissues and Gene Therapies.

In accordance with 18 USC, Section 208b.3, waivers have been granted for Topic II to Dr. Jeffrey Chamberlain and Dr. Anastasios Tsiatis. Dr. Richard Mulligan was granted a limited waiver; he may participate in the committee discussions, but will not vote on Topic II.

This announcement also addressee conflicts of

interest for Topic III, the update on the recent review of the research program of the Office of Cellular, Tissue and Gene Therapies. In accordance with 18 UCS, Section 208b.3, waivers were granted for Topic III to Dr. Jeffrey Chamberlain, Dr. James Mule, Dr. Anastasios Tsiatis and Dr. Kenneth Cornetta. A copy of the written waiver may be obtained by submitting a written request to the agency's Freedom of Information Office, Room 12A-30 of the Parklawn Building, Rockville, Maryland.

Dr. Kurt Gunter serves as the industry representative, acting on behalf of all related industry, and is employed by ZymeQuest, Incorporated. Dr. Gunter is also a part time employee of the University of Massachusetts Memorial Medical Center, and a scientific advisor for ViaCell, Incorporated. Industry representatives are not special government employees and do not vote.

With regard to FDA's guest speakers, the agency has determined that the information provided by these speakers is essential. The following information is being made public to allow the audience to objectively evaluate any presentation and/or comments made by the speakers.

For Topic II, Dr. Christopher Baum is employed by the Cincinnati Children's Hospital Medical Center and Hanover Medical Center in Germany. He conducts clinical

phase one trials to explore retroviral vector-mediated gene therapy in Fanconi anemia and in children with brain tumors.

Dr. Rich Irwin is employed by the National Institute for Environmental Health Sciences, NIH, in Research Triangle Park, North Carolina.

This conflict of interest statement will be available for review at the registration table. We would like to remind members and consultants that if the discussions involve any other products or firms not already on the agenda for which an FDA participant has a personal or imputed financial interest, the participants need to exclude themselves from such an involvement, and their exclusion will be noted for the record. FDA encourages all other participants who advise the committee of any financial relationships you may have with the sponsor, its product and if known, its direct competitors.

Thank you, Dr. Mule.

DR. MULE: Thank you, Gail. I would like to introduce Dr. Goodman, who will recognize committee service of the retiring members.

DR. GOODMAN: Thanks very much. I assume they are not all getting fired or something, because there are four at once, which is a bit unusual.

Anyhow, I have the pleasure of being here, both

that I can stay for a little while and hear some of the discussion this morning on this NTP program, and I appreciate that. I have heard some about the discussion that you all had yesterday concerning product characterization and potency.

We, and myself particularly, I have said this before, but tremendously value the advisory committees. I would like to be able to be here at all of them for the whole time, and not just for the cell and gene therapy one, but for our others. But we view this as an important opportunity to get input.

Your advisory committee is particularly unusual, in part because of the nature of the products, where you are participating in something that I hope is exciting. At times it may seem a little ill-defined perhaps, but I think it is exciting, because it is not just about evaluation of a product or evaluation of our analysis of a particular situation, but you are contributing and helping define new fields go forward.

It is interesting that this is the centennial of the entire FDA this year. I think the products this committee has expertise in are truly products for this next century of medicine. So I think your role is both unusual and particularly important. You are also mostly very busy people involved in that exciting science on various ends,

whether it is clinical or developmental or statistical, et cetera. So we recognize your time is important, and we need you because we can't live on an island with all this activity going on around us and do our job well.

There are four members retiring, and we will miss them. I would like to recognize them and thank them for a number of things. They are Jonathan Allen, David Harlan, Butch Tsiatis and Mahendra Rao. I think I had some notes here, but I pretty much said what I wanted to say.

In addition to serving on the advisory committee, I know Dr. Allan was on the Secretary's Committee for Xenotransplantation. That is certainly a challenge. I like to say any time I go downtown it is a challenge, but that is a very interesting cross-disciplinary area. Then of course, Dr. Rao for exceptional service as the chair, and also being located close to us until recently as a nearby colleague and often collaborator.

So I think together, these different individuals have done different things, but I know you have provided us with input on things like safety, which is very important, models, clinical trials proposals, analysis. As I said, you have helped us try to find pathways forward that allow these products to be developed and evaluated in a safe and scientific way.

The other thing I know is that you do work and

interact well with our staff at various levels, and that you support them, that you are part of the process that helps us evaluate our programs, our science, our regulations, so we appreciate that. So you have really helped what I hope will be the beginning of a series of real successes in this area, and I thank you for that.

That is about all I have to say. What I would do is maybe ask you to come up one by one, but maybe we can do it all together and make it not a prolonged affair. If you wanted, you could even have a picture together, which might be nice. So Dr. Allan, Dr. Harlan, Dr. Tsiatis and Dr. Rao, I will give you each your plaques.

Agenda Item: Topic II: National Toxicology Program, Proposed Study on Retroviral Vector-Mediated Insertional Mutagenesis

DR. MULE: Thanks, Dr. Goodman. Before we start Topic II, I just wanted to recognize Dr. Richard Mulligan, who has joined the committee today. Welcome, Richard.

Topic II is related to the National Toxicology Program. There is a proposed study on retroviral vector-mediated insertional mutagenesis. The committee had obtained a copy of some documents related to this topic for review. There will be a series of three speakers, and then we will tackle a couple of questions that were provided to the committee at the end of this topic.

The first speaker is the FDA introduction by Carolyn Wilson.

DR. WILSON: Thank you, and welcome to the second topic. As was already mentioned, we will be discussing a program of research through the National Toxicology Program designed to look at an animal model to assess retroviral vector-mediated insertional mutagenesis and more specifically tumorigenesis.

I want to be brief in my remarks today, because a lot of the background and rationale for why this study is important has been laid out in a series of previous committee meetings discussing the results of XSCID clinical trials, but I will briefly go through the rationale for why we think this is an important study that the FDA needs for regulatory purposes, briefly discuss the goals of the study, and then finally remind the committee of what our goals are for today's meeting.

I will be talking today only about gamma retroviruses, but I want to acknowledge that of course lentiviruses are also in clinical trials and are being developed preclinically and will be coming to us more in the future. There is one question for the committee to consider that is a little bit more forward looking, but this initial study is focused only on gamma retroviruses.

Just to quickly remind the committee about how

gamma retroviruses are involved in altering gene expression, in retroviral vectors at either end in the DNA form is an element called LTR, long terminal repeat. This LTR carries an element called the U3. The U3 carries very strong enhancer sequences, and these sequences have been shown through a variety of studies in animal models that you can cause activation of gene expression at very distal sites, up to 300 kilobases away. At the 3' end, because this element is repeated in the 3' LTR, you can get read-through transcription, causing activated gene expression of downstream sequences, and then also just by virtue of where the particular vector integrates, disruption of a gene may also lead to disregulated gene expression.

Now, these events may or may not translate into an altered phenotype, either for the cell or for the organism. But in some cases, and this is associated with presumably other events that occur within a particular cell, this may lead to tumorigenesis.

To just remind you, retroviruses are widely acknowledged to cause tumors. The first tumor causing virus was identified in 1908 by Payton Rouss as a filterable agent causing tumors in chickens. So it is not that people didn't know that these were tumorigenic. They have also been used as models to induce genetic aberrations in a variety of species such as zebrafish, for example, to

study pathways important in developmental biology.

As retroviral vectors were first entering clinical trials in the early 1990s, everybody acknowledged that this was a risk. Most people assumed that in the absence of replication, the finite number of sites for genomic integration would translate into a reduced risk. As we now know -- and again, this has been presented in great detail in previous discussions to this committee -- the X-linked severe combined immunodeficiency clinical trial in France has demonstrated that this is no longer just a theoretical risk.

In that clinical trial, modification of transduction conditions were resulting in increased transduction rates, combined with a clinical disease that provided an in vivo selective advantage for cells that were carrying that transgene, initially resulted and translated into therapeutic benefit for most of the children who were treated in this clinical trial. Ten out of 11 showed both clinical and laboratory evidence of immune function, but unfortunately as we also now know, this translated into an increased risk, where three out of ten of the children who were successfully treated later developed leukemias, and one of those children has subsequently died from relapse leukemia.

So with this in mind, the FDA talks about

development of products to successful clinical implementation as being on a critical path. We feel that this successful implementation of retroviral vectors in the clinic is currently limited by this potential for tumorigenesis, and that it is a challenge to the field to try to identify a way to minimize the actual risk and maximize the therapeutic benefit.

We think that on this path to developing a successful clinical product is to develop a valid preclinical model that can be used to assess the risk of retroviral vectors that may be modified in the future with the aim to reduce the likelihood of tumorigenesis. This is consistent with previous advice that we have received from this committee, as well as other advisory bodies that have met internationally and nationally. You have encouraged investigators in the field to explore alternative retroviral vector structures to reduce the risk of tumorigenesis, things like dilution of this U3 element which we know is important for gene activation, introduction of elements such as insulators that may reduce that activation activity of the U3, or introduction of suicide genes that could be used in combination with a pro drug to eliminate vector positive cells.

Then in conjunction with that recommendation, it was stressed that such alternative vectors should be

adequately tested in animal models. That is where the challenge is, is trying to identify what animal model will be most appropriate to perform these investigations.

So as we move forward in our own internal thinking about what preclinical model would be important, there were several considerations. As you know, there have been a number of studies that have done in vitro mapping of the genome to look at insertion sites. While this is certainly important for providing some critical functional data for the pattern of integration, these don't give you any information on the biological effect of those integration events. So we felt that any model should be able to show a biological effect, in which case you are talking most likely about an in vivo model.

Large animal studies of course are limited by the feasibility of doing large sample sizes, long term observations, so that brings us to doing rodent studies which allows both for the assessment of biological effects and use of a larger study size in order to get statistically significant results.

Finally, we also thought that the model should mimic human clinical studies. We thought that CD-34 or hematopoietic stem cells would be a good target cell to focus initially on. Approximately one third of current clinical trials using retroviral vectors use this cell, and

furthermore, the XSCID clinical trial used this as a target cell, so we think it is the most relevant place to start in terms of looking in an animal model. Then finally, such a model should also have a measurable rate of tumorigenesis in animals.

So with these considerations in mind, we found that a study that was originally published in Science a few years ago by Christopher Baum and his colleagues fits many of these requirements, actually, all of the requirements. This model has also been subsequently tested and shown to be reproducible in additional publications.

Dr. Baum will speak after me to provide some additional preclinical studies that are relevant to this discussion. Importantly, what he showed is, by doing long term observations combined with serial transplantation of the hematopoietic stem cells that have been transduced, he found that in six out of ten animals in secondary transplants, that an acute myeloid leukemia-like disease was observed. This correlated with retroviral vector integration into a gene called EVI-1. This also correlated with the activated expression of this in the leukemic cells.

So this brings us then to the goals of the study that we are proposing to do through the National Toxicology Program. First and foremost is to develop and assess the

sensitivity of this particular preclinical model to look at the risk of retroviral vector mediated insertional tumorigenesis. We plan to do this in a study size of a sufficient number of animals to give us at least a 90 percent confidence interval and a negative result. Again, following the protocol developed by Chris Baum, we will include secondary transplants in order to enhance the sensitivity of detecting a tumorigenic event.

In addition, we want to look at two variables, which is the effect of after dose on tumor frequency, which is something that Dr. Baum has investigated in small sample sizes and seen an obvious effect on reduction in tumor incidence, but this needs to be explored in a large study and a larger sample size. Then finally, the effect of deleting U3 from the LTR is an example of one particular approach that might reduce the likelihood of tumorigenesis.

The details of this study will be presented by Dr. Rick Irwin from the National Toxicology Program.

Let me finish then with reminding the committee what we hope to do today, which is to discuss three questions. The first and really most important is that we would really value your advice and comments on the general scientific approach that we are proposing here to evaluate this particular model for its feasibility. In addition, more detailed feedback is of course also welcome.

The second question is more forward looking. As I mentioned, lentiviral vectors are being explored, both in clinical trials and preclinically.

A recent paper by Thamus and his colleagues has shown that in utero gene transfer has caused a high incidence of liver tumors in the resulting animals that were born from those experiments. We would like the committee's consideration of whether or not this is an animal model that might be useful to explore through the National Toxicology Program to assess lentivirus tumorigenicity.

Then the final question, again more forward looking, would be to comment on other toxicology models for more broad cell and gene therapies that it would be useful to study through NTP, and generally the use of NTP as a resource for development of toxicological testing models for novel therapies.

I thank you for your attention. I am happy to answer any questions, or we can go ahead to the second speaker.

DR. MULE: Thanks, Dr. Wilson. Any questions? We will move ahead to Dr. Chris Baum.-

DR. BAUM: Thank you very much for the invitation to this conference. I would like to introduce you to our pre-NTP data that we have accumulated in our labs in

Hanover and in Cincinnati Children's Hospital to develop a mouse model that is able to detect side effects caused by random transgene expression.

It is known, as Carolyn Wilson pointed out, over the recent years increasingly known that retroviral transgene delivery causes a variety of side effects. So far we have discovered two major manifestations. One is that insertion mutagenesis could enhance the fitness of stem cells after infusion into patients, resulting in still-benign clonal dominance. This we have observed in the mouse model, and it is also being seen in a recent clinical study. And Copeland's lab has shown that similar phenotypes can be produced in vitro based on retroviral gene transfer into primary bone marrow cells of mice.

The second, much more concerning side effect is of course the induction of leukemia, which as Carolyn Wilson pointed out we have first shown in the mouse model. We are always talking here about non-replicating retroviral gene transfer, and subsequently the same problem has been observed in these XSCID-1 clinical trial in Paris, but interestingly, so far not in London. And very importantly, Cynthia Dunbar's team has also described a monkey, a single case, where after transfusion of retroviral modified hematopoietic cells, a sarcoma developed six years after delivery of the cells.

Moreover, we do have some concern that some of the genes we use in human gene therapy could cause side effects simply by ectopic expression, so not related to the integration side just because they are over expressed, which we called phenotoxicity to distinguish it from insertional genotoxicity. That then leads to concerns that there is a scenario of combinatorial tumorigenesis, where either multiple insertions in the same cell could accelerate leukaemia development, or there is a collaboration of side effects of the expressed transgene with insertional genotoxicity.

Now, over the recent years, very important data have accumulated that viral factors do determine the integration pattern in the genome. The most famous observation is that HIV-based vectors as opposed to mouse leukemia virus based vectors, have a higher propensity to integrate into transcribed areas of the gene, transcribed genes, while they have a twofold lower propensity to integrate close to the promoter. And of course, integrations close to the promoter, within five KB for instance of the transcription start sites, are considered to be more likely to deregulate the effector gene.

So there was some hope that maybe using HIV-based vectors, you have a lower risk of activating genes, but you could also conclude that maybe NIV-based vectors enhance

the risk of disrupting genes, because they more often integrate into transcribed genes.

As Carolyn Wilson pointed out, it is completely unknown at this point whether these vectors really differ in their transforming potential. There is some preliminary evidence by Luigi Nardini's group to suggest that. We also do not really know what the impact of the transgene cassette is. I will come to that in a minute.

So that is why we proposed for the National Toxicology Program to look into a mouse model based on C57 Black 6 mice, in which we can distinguish retrovirally modified donor cells from host cells based on chimerism in the CD45 antigen.

This is one major advantage, because all mouse models that you can think of do have a residual risk of endogenous tumor formation, and here in this case you can distinguish modified donor cells from endogenous cells. The other major advantage of this mouse model is that this particular strain, Black 6, is not known to be very likely to develop endogenous leukemias even after irradiation. There is a residual risk, but it is low.

How did we develop this model? What are the data that we accumulated so far to validate that this model will be informative? The idea is to retrovirally modify primary cells that are taken from steady state mice, put into

cytokine containing cultures to activate them to allow MLV medicated gene transfer to occur, and then have the vector introduced at low dose or high dose and look and see where there are transplantations, what type of manifestations related to insertional side effects we will get.

The evidence that this model will work is published in the -- besides that 2002 first case report, in the recent paper published in Blood by Wudje Moedlich and colleagues. What we have done here is to compare two different vectors, one expressing the multi-drug resistance gene, the other one a DS red fluorescent protein. We introduced the vectors at low and high MY in exactly the model that I pointed out in the previous slide, and then showed that there is dose dependent risk of leukemia development in this mouse model, and that this is associated with insertional mutagenesis of non-replicating vectors.

One theoretical consideration is important here, what is the rationale for dose escalation using gene transfer vectors. Of course, dose escalation is a classical approach in toxicology, but should we do that when thinking about manipulation of cell products? The rationale is mathematical. If you consider that every cell that receives a retrovirus has no entry limitation, so all cells can easily take up the virus and integrate it

into the genome, then even when you have a very low average copy number of only two, where you transduce the majority of the population, you have a considerable risk based on quassant statistics that many of the treated cells will have more than five integrations, so more than one percent.

Now, if you consider that in clinical trials you treat ten to the eighth to ten to the ninth cells, one percent is quite a big amount of cells. So we are talking about one million mutants potentially. But this only applies to vectors that have no entry limitation. Fortunately, so far the stem cells that have been modified do have an entry limitation, and we rarely see multiple integrations with conventionally used vectors.

That more than five integrations matter was seen in the mouse model. Upon dose escalation, the tumors go up. They typically had more than five integrations, shown here for each single mice, where we investigated tumors that developed in the mouse model. Then when you look into the type of integrations that you get, you have a clear-cut over representation of proto oncogenes, and often more than one proto oncogene being hit, and also signalling genes, some of which it would make sense that they could also contribute to looking at development if they are deregulated. When you further increase the number of integrations in a particular tumor, you also get maybe

innocent bystander mutations, where some other genes are also hit but that do not necessarily contribute to leukemias.

However, I have to point out that we still have to formally show in these mouse models how the genes that are hit cooperate in leukemia initiation. So far, the evidence is only based on genetic findings and not on functional studies, that these proto oncogenes caused the leukemia.

Another interesting point of this mouse model is that as you have seen before, it can hit many different proto oncogenes. It not only leads to side effects when the integrations occur in the promoter proximal window. We do see these hits in signalling genes shown in black or proto oncogenes shown in red close to the promoter, as would be expected, to be more frequently seen with mouse leukemia virus, but we also see them far upstream or far downstream of the promoter. So I am confident that the model would be able to pick up side effects also of other vectors that don't have a propensity to go into the promoter proximal window. You also should keep in mind that for many of the proto oncogenes, this is not the only window that can lead to a critical deregulation.

We also have this element of serial bone marrow transplantation here in the model. Why that? Often after

six months of observation, you will have preleukemic stages, where the pathologists will tell you that there are nests of potentially leukemic cells, for instance in liver, or the spleen will have an altered architecture, but this is difficult to distinguish from reactive conditions sometimes.

Then at that stage, the peripheral blood may look normal or even anemic, but it wouldn't be informative in terms of leukemia. However, when you serially transplant bone marrow cells from such animals, in the second cohort you have an increased likelihood due to the additional proliferative stress and longer observation time, where you end up with a manifestation of an acute leukemia with all the diagnostic parameters that you want to see -- severe alterations of spleen architecture, infiltration into other organs such as liver or kidney, and of course also release of plus into the peripheral blood.

Another point to validate the model was to look whether we were just dealing with spontaneous genetic instability, rather than retroviral vector induced problems, but this does not seem to be the case.

As shown here by the rather sensitive spectrokaryotyping performed by Professor Schlaegeberger's lab in Hanover, the leukemias that we have investigated so far, three out of those did not show genetic instability.

Here you see the two cases that did show additional genetic instability, but this is rather limited. So maybe one or two translocations have been seen, but not severe alterations of the karyotype. Remember, three out of the five leukemias that had these multiple insertions had a normal karyotype within the limitations of this assay.

So what is the incidence of adverse events in our mouse model so far that are all in an academic lab and not in GLP labs? The low dose gene transfer using this particular vector that causes leukemias at high dose with the DS red trans-2 gene, more than 20 mice remained free of disease when investigated greater than 12 months in a primary transplantation. More than 50 mice that received bone marrow cells transfused with other marking vectors were also okay over prolonged observation periods.

Occasionally as I have mentioned initially we see host derived lymphoblastic leukemia cells, so far always lymphoblastic, and we think they are irradiation induced, which is comparable with the literature.

There is however a reproducible induction of leukemia even in the low dose gene transfer situation, if the cDNA encodes a potential oncogenic approaching. That is of course expected.

In the high dose situation which is relevant for the discussion today, we have seen in the published paper

one out of seven mice that had a transplanted leukemia at week 26, and you have seen the many insertions into oncogenes.

I should also point out that we had another mouse of this cohort that was found dead in the cage, and somehow escaped this way the molecular analysis. We think that this was also leukemia, but we are not exactly sure. Four out of the other remaining five animals had been serially transplanted into secondary recipients, and there we had another manifestation of insertion mutagenesis with is clonal dominance, so-called pre-leukemic expansion of cells related to insertional upregulation of proto oncogenes such as HOX-A7 and BCL-11A.

The incidence of fatal outcome was reproduced in a second yet unpublished experiment, where two out of six mice died, and yet another vector with encoding the MVR gene that gave exactly the same incidence; two out of six mice had come down with leukemia. We therefore expect leukemias with an incidence of ten to 50 percent. The great advantage of the GLP conditions is that we won't miss a single mouse that dies unobserved. Of course, in an academic setting that occurs, specifically when the mouse tends to die on the weekend. But in the GLP lab, that should not happen.

The other manifestation that is important is this

B9 clonal dominance, as published in a recent paper, which challenges early interpretations of mouse gene marking studies. When looking into serially transplanted mice with normal hematopoiesis after retroviral vector-mediated gene transfer at rather low dose, we find that some mice have oligoclonal to monoclonal hematopoiesis, as many others before have seen that. So we asked what are the integration sites in these dominant clones.

Using the LM PCR technology, we looked into the integration sites and made the surprising observation that the clonal drift to clonal dominance seems to be dependent on the integration site. When you look in the primary bone marrow transplantation animals, you already see an over representation of hits in proto oncogenes; two percent. This gray area here is roughly the contribution of proto oncogenes to the genome, but there were more than ten percent recovered in the primary bone marrow transplantation cohort as integration sites.

We do see a number of signalling gene hits, but also an equal number of hits in genes with other or unknown function. When you then serially transplant the same cells and look which clones tend to dominate in the secondary bone marrow transplantation, then you see that the number of the clones in proto oncogenes increases and the clones with hits in neutral genes are counter selected, they are

simply gone in these animals.

You also see that sometimes clones are only visible in the secondary bone marrow transplantation as dominant clones. They haven't been observed as dominant clones in the primary cohort. In these cases, the contribution of proto oncogene hits is even bigger.

Here we have a genomic and statistically sound approach to look also in the absence of leukemia manifestation into side effects caused by insertion of mutagenesis. The genes hit by these retroviruses in these cases were deregulated -- by real time PCR, and these dominant clones, and you see deregulations to various levels, up to one thousand fold, but often it is mild, just two to threefold, but still, in none of these clones it was as found in the controls. So the genes that are hit tend to be upregulated, sometimes down regulated.

So what are the advantages of this model? To repeat, it is clinically relevant. It uses a normal genetic background, and it is a bone marrow transplantation model. It gives you reproducible induction of leukemias, and it is relatively unbiased in terms of the phenotype of leukemias that you get, which I find very important. You get myeloid leukemias of various maturation stages, monocytic or random cytic. You get erythroid leukemias and lymphoid leukemias. So far we haven't seen B-cell derived

leukemias; the lymphoid leukemias have all been T-cell derived, but maybe that will change when we look into more animals.

You have the second readout of clonal dominance, which is very interesting not only from a toxicology point of view, because it may lead you to the discovery of genes that enhance stem cell fitness, which is of course scientifically and also practically for regenerated medicine a very interesting finding. So we do have here a very nice approach, specifically when performed in the large scale, to look into the insertional genomics of leukemia induction and stem cell fitness.

What is not necessarily a disadvantage is that the mouse model is known to be more susceptible to leukemia development than humans, so we wouldn't have to wait for ages. We will see manifestations within a year of observation time. Again, keep in mind that humans receive far more cells than the mice, so it is maybe a good idea to have a model that over reports side effects.

What type of vectors could be tested? There is a big hope in the field that we can make the scenario of gene therapy much safer when changing vector design or vector backgrounds. So we can test gamma retroviruses. A mouse leukemia virus belongs to this group. Lentivirus such as HIV or SIV or spumavirus such as human formivirus based

vectors, and then the first idea is to take out this strong enhancer promoter from the U3 region and to put it into an internal position, thereby deleting the repetition of the enhancer promoter and the long time repeat. It is called a self inactivating SIN vector.

And of course, you can also ask what happens when you introduce a morphysiological promoter instead of the viral promoter, or when you introduce these famous insulator sequences. What is important for a large scale study is that we are able to design these vectors and produce them even in large scale conditions at sufficient titers for those escalation studies.

Is there any evidence that SIN or LTR vectors have different infectivity? This is very important when comparing vectors. We have to make sure that we have the same number of hits in the cells when starting the experiments.

This is an experiment to look into that question. This is an LTR product and a SIN vector product, used at increasing multiplicity of infection in 32D myeloid progenitor cells, using ecotropically pseudotyped supernatants. You see that there is no detectible difference in the infectivity of LTR and SIN vectors based on the flow cytometry here for the fluorescent protein encoded by the vector, and also based on southern blot this

can be validated. The infectivity of vector stocks can be made identical whether you encode a SIN vector or an LTR vector.

This is relevant for lineage minus cells, can be introduced many copies into lineage minus cells of LTR and SIN vectors. This is shown here in vitro, so these are the primary bone marrow cells of the mouse. Again, you see after dose escalation you have really high transduction rates with the self activating vector and the LTR vector.

This model turned out to be not only important for validating the supernatants, but also it gave us a second readout for insertion mutagenesis, inspired by a report of Neil Copeland's lab. We continue to culture these cells and look whether there might be clones that survive serial replating. Usually bone marrow cells tend to die upon serial replating.

Now we have managed to increase the sensitivity of this assay. We were able to discover individual clones transduced with high copy numbers of LTR or SIN vectors that survive these conditions, and are by definition mutants, because the mock cells would die under these conditions. This may lead us to another very important model to evaluate side by side with the in vivo model.

This model is quick and will allow you to screen vectors before you go into the large scale in vivo

analysis. What we do do here is again, in minus cells harvested from steady state mice, it is a viral gene transfer under exactly the same conditions as for the in vivo studies. Then an expansion culture to somehow rescue and expand the mutants in the first step, followed by serial replating under different growth conditions. Then if you do recover clones you can further expand them for genetic and phenotypic analysis. Again, this is inspired by a report from Neil Copeland's lab.

What we do see then is that there is a significant difference in the transforming capacity between an LTR and a self inactivating vector, even if the self inactivating vector as the same strong enhancer promoter internally. It requires a higher MOI to mutants than the LTR vector, and also, the number of mutants that you get is significantly lower than with the LTR vector.

I should say that the assay does have some variability, but please keep in mind that we are dealing with primary cells and with stochastic integration events, so this is not unexpected. Based on statistical analysis, the difference between LTR and SIN vector is significant.

To further evaluate what is going on, we have to develop then the LM PCR, not only for the LTR vector as previous performed, but now we can also run the LM PCR, which was easily done for the self inactivating vector, so

that we can pick up the integration cells of the SIN vectors.

I would like to point out that our method is a little bit different from what others are using. We focus in our analysis on the reproducible dominant bands, which we directly cut out of its LM sequence as PCR products or sub-clone them individual to sequence them, but we neglect smaller hardly visible bands. Using this approach, we reproducibly identify hits and important things.

For the SIN vector transfused clones that came up in the in vitro analysis, of course we had to show what type of integrations do take place. Here you see southern blot analysis of transformed clones recovered in vitro after gene transfer with LTR vectors or with SIN vectors. In both cases you have more than five integrations in most of the clones. Interestingly, this model as Copeland has shown before does select for integrations into EVI-1, which is also relevant for in vivo studies. When you get a clone with a SIN vector, it will also show you an integration into EVI-1 in exactly the same positions that the LTR vectors sit in EVI-1. So that tells you that the SIN vectors can hit the same proto oncogenes, but still their transforming potential is reduced, even when they have a strong internal promoter, but there is residual transforming potential also.

To summarize these in vitro studies, their replating ability reflects competitive fitness, which in our study design is the function of the incidence of transformed clones and the fitness of these clones. The SIN vectors are significantly less transforming than LTR vectors, but have a residual capacity to introduce insertional side effects. We think that we can now test further vector modifications that hopefully lead to enhanced safety and also other types of vectors.

Still, we need the in vivo readout because it is less biased for integrations into EVI-1, and should give you a more neutral observation of what types of scenarios can occur with integration of retroviral vectors.

So we would therefore propose to use these vectors. First up would be the LTR vector compared to the SIN vector that has residual transforming capacity in the mouse model, and then test it in this mouse model, which will be explained in detail by Rick Irwin.

This is a repetition of the slide that you have seen before, so we wouldn't change anything from the academic setting because the model works in the academic setting. The only thing that we are doing now is to upscale all the procedures that are required here in the cell processing prior to transplantation, which is a little challenge, but so far the development of the upscaling

procedures looks good. This occurs in Cincinnati Children's Hospital.

There will be a pilot study which will be explained in greater detail by Rick, in which we simply ask the questions, are we able to transplant mice in these experimental conditions with retroviral vector modified cells, and do we have high engraftment rates and high gene marking rates.

We will terminate the cell early after 12 weeks, and there will be a second study arm, where the mice are observed in Cincinnati Children's Hospital where the cells are processed. A major study arm is the Batelle GOP lab, so we have to ship the cells from Cincinnati to Columbus, Ohio, this is a two-hour drive by car, where they will then be injected. We just have to validate that the cells have maintained their transplantation ability by having a second cohort transplanted in Cincinnati Children's Hospital.

In the definitive study, all the mice will be kept in the Batelle GLP lab. In addition to the study design that will be explained in a minute by Rick, we will propose to do some investigation side by side in this in vitro immortalization model, which will hopefully then help us to validate both models against each other.

In Batelle, most of the leukemia related diagnosis goes on, which is very important, so this is

unbiased GLP. We have developed a very precise plan for the molecular and phenotypic followup, which according to the input can be modified still. But I think the plan is quite good and advanced at this stage. It went through many different peoples' heads before it came down on paper, as you have seen it.

The service offered by Cincinnati Children's Hospital is the third processing, because Batelle toxicology lab cannot do this. It is not experienced with this part. So vector production, cell processing and cell shipments conditions are all validated currently by Cincinnati Children's Hospital, and these services will be offered based on SOPs with batch records done by GLP trained technicians, but this is not GLP, to be kept in mind.

Southern blot analysis and molecular analysis will be performed in Cincinnati Children's Hospital. For the molecular studies this occurs SOP driven in a clear-cut certified lab. This will address quantitative PCR, RCR diagnostics, and ligation mediated PCR. We have here a German mafia looking into the database management, but I would be happy to have more scientists involved in this, and we need input from many experts in the field to develop the database and to look into the genomics of this study.

So to summarize, what is the value of the NTP

study? Who do think it is relevant? Gene therapy is promising, but the long term consequences are insufficiently known. We have to address that prospectively. It is also somewhat pioneering, this study design. It is the first GOP study to my knowledge that addresses side effects of a complex stem cell product, maybe of any complex gene modified product. It is informative, because it will give us a rationale to increase vector safety. Also, it is a unique resource for genetic insights into mechanisms of clonal dominance and mechanisms that induce leukemia or maybe sarcoma.

It is fully consistent with the mission of the NTP. That is a sentence from the briefing document. The NTP wants to move toxicology from a predominantly observational science to a predominantly predictive science focused upon the inclusion of target specific mechanism based biological observations, and that is exactly what this study suggests.

I would like to conclude with acknowledging all the people contributing to the development of the model, and of course to all the experiments associated with it. This is a collaboration with Boris Feiser in Hamburg University, Christov von Karler, Manfred Schmidt and the National Genome Center in Heidelberg, now in Germany. My small team but significant contribution here from

Cincinnati Children's Hospital, Elka Vill, Joanna Clark, Brendon, and coordinated by Lilah Thries and David Williams in Cincinnati Children's Hospital. This is my team in Hanover. I would like to specifically point out Olga Kristikarva, Ute Morisch and Setra Lee, who had a major contribution to the development of the mouse model and to genetics behind it.

We are supported by the European Union, the German Research Council, the German Ministry for Research and more recently also by the National Cancer Institute.

I would like to thank you for your attention. And of course, I would be happy to take some questions.

DR. MULE: Thank you, Dr. Baum. Questions?

DR. MULLIGAN: The frequency with a neutral transgene is very key for this. The DS red vector is probably the best beta that you do have. But it seems limited. There are one out of seven in the first published work.

You made a comment about a non-published study, where there was incidence of fatal outcome. What does that mean, exactly?-

DR. BAUM: We have the same as before. We have one validated leukemia with insertional event into a well-known proto oncogene, and one mouse that preferred to die on the weekend and escape molecular analysis.

DR. MULLIGAN: So it is two or three out of 14 then so far?-

DR. BAUM: Yes, it is exactly the same incidence as in the first. If we count the unobserved death as a leukemia induced death, it would be two out of six roughly, so 30 percent . If we don't neglect that, we have ten to 15 percent at least. That is why I said we will have tumors developing with an incidence greater than ten percent, but likely lower than 50 percent.

DR. MULLIGAN: Was the DS red retrovirus use for the second work produced in the same way as the first? I don't remember, did you use 293T cells for the first?-

DR. BAUM: Yes, we are using transient transvection inter-293T cells. I should mention that we make sure that there is no replication of retrovirus around when these leukemias come up.

DR. CORNETTA: Christopher, the SF promoter enhancer, can you explain a little bit more what modifications went there, and do you think there is any connection between the ability to detect erythroid, myeloid and lymphoid leukemias and potential modifications that were made in the promoter enhancer?-

DR. BAUM: We have previously done some studies where we wanted to dissect the enhancer in the spleen focus forming virus. The background is that the spleen focus

forming virus seems to be the most potent mouse virus promoter enhancer that expresses high level of transgene in primitive hematopoietic cells as well as in many mature lineages.

So far, we haven't made studies to address what role specific enhancer motives have in transforming cells. We would like to do that in the in vitro model first.

But I should mention that the HARV MDR vector that has been detected in the same setting and gave the same incidence of leukemias uses a Maloney mouse leukemia virus enhancer promoter, which is about four times lower activity in stem cells maybe than is being focused on the virus, but still gives you the same leukemia incidence. But this was a different transgene, of course.

DR. ALLAN: I may have misunderstood this, but you had talked earlier about your clonal dominance, where you are looking at secondary bone marrow transplants. I think you mentioned the fact that in the animals that got the secondary bone marrow transplants from animals that didn't have any clonal dominance, they had a higher number of hits in the proto oncogenes. Do you know why that would be? Do you have any idea why? -

DR. BAUM: Why we have that selection?

DR. ALLAN: Yes. You have a higher number of hits in the proto oncogenes in the secondary bone marrow

transplant.-

DR. BAUM: Yes, because we think that the hit in the proto oncogene led to clonal dominance, so was the cause of enhanced fitness of that cell. So if it if you wish a pre-leukemic expansion of a stem of a progenitor cell clone without leading to overt alterations of hematopoiesis. All these mice have been shown by the pathologist to be perfectly normal in their hematopoiesis. We even did tertiary transplants from some of these mice, and they remained healthy.

DR. ALLAN: Maybe I got it wrong, but it looked as though you had a higher number of hits in the animals -- when you transplanted from animals that didn't have any clonal dominance, you had a higher number of hits in the proto oncogenes.-

DR. BAUM: No, that is maybe also too shortly described here. We look here in mice where the secondary transplant recipients have just a couple of clones contributing to hematopoiesis, one to form maybe. So these are monoclonal to oligoclonal in their hematopoiesis, and there you find the striking over representation of hits in proto oncogenes or other interesting signalling genes.

DR. CALOS: The secondary transplantation gives you increased sensitivity. Could you quantify that? I guess it is the additional proliferation of those cells.

Do you have some feel for how much additional proliferation you get out of that secondary transplantation?-

DR. BAUM: No, that is difficult to say. Of course, if you look into the situation of the normal cells here, a single stem cell clone would have to be plenished, maybe hematopoiesis, but then it depends on the number of repopulating units that you transplant, how strong the proliferative stress really is. But we can't give you any number here.

It may lead to the question, can we further modify the model with the serial transplantation conditions, like shortening the observation period and have more serial transplants or infusing less or more cells to modify replicative stress or at-growth factors. There are many ways to do that, but we haven't done this so far.

DR. MULLIGAN: In the case of the SIN construct that you talked about, if you look at the mean fluorescent intensity, is it equal to that that you get with the LTR based vector, or is it reduced?-

DR. BAUM: That was important in the study design. It is equal. The difference is just maybe ten percent. That can be explained by the fact that the SIN vectors we have published, they have better RNA processing. Although they have one enhancer less, they process their RNA better, nuclear export of the transcript is better.

DR. MULLIGAN: Does the LTR based vector have the WPRE sequence?-

DR. BAUM: Yes, we made sure --

DR. MULLIGAN: So both of them have it?-

DR. BAUM: Yes.

DR. MULLIGAN: The last question is, when you do your infection, what is the decrease in the stem cell content after you do your culture and then transplant, relative to absolutely untouched? If you do a competition, how -- the question is, is this a system where you reduce the number of stem cell clones to a point where there may be stress on the stem cells to amplify? So is the reason why perhaps this gives you a better re-value for leukemia, especially in the secondary, it is that you are wiping out the activity of the few clones that you reconstitute with.-

DR. BAUM: Of course, like all cytokine conditions for hematopoietic cells, the in vitro growth period for about five days reduces the number of repopulating units by a factor of about 90 percent. Eighty to 90 percent of the input cells are lost in this culture period, but still we have repopulating units to transfuse into the animals.

I have references of the paper from your lab, where you have shown that when you use lentiviral transduction protocols with highly purified stem cell

populations, you can maintain the number of repopulating units, based on improved in vitro conditions. It will be very interesting for instance to see, as you suggested in the discussion of the paper, whether when you go into a more purified cell population, whether you then reduce the risk.

We do have some circumstantial evidence that the risk exactly comes from the otherwise short-lived progenitor cells, which are the majority of the treated population. This would be clinically relevant, so we would have to define better ways of stem cell purification for clinical studies.

DR. MULLIGAN: One other question about the enhancer activity. One difference between the SIN and the LTR is, you have twice the number of enhancers. Have you ever put two enhancers in the SIN position to see if there is any difference? We will get to this when we talk about the study design, but I would have thought that is an odd thing to compare, the LRT vector to a SIN vector that still has the enhancer. I would have simply thought you would have looked at something that doesn't have an enhancer, versus something that has some enhancers.

So I wouldn't expect it to be different, is my point, other than that you have two in one case and you have one in the other case. So I wouldn't think just

because it is sitting in the center of the protocol sequences that necessarily there is going to be any different effect.

DR. BAUM: The major difference of course is that you get rid of this active three -- that can lead to an initiation of a downstream gene by its own activity, promote activity, not only enhancer mediated.

But of course, you are right that these SIN vectors with these very strong internal enhancer promoters are not expected to be tenfold safer than an LTR vector, but even a factor of two or three would be clinically meaningful if you translate that into the SCID X1 study. That might lead to a situation where no patient has been observed so far, because of the low incidence of the events.

We do have many cases where in gene therapy we still need very strong promoters. We have been looking for strong promoters over the last decade or so, in order to get certain applications to the therapeutic level of gene expression. Of course, we can test weaker promoters in the same vectors, but the question would be whether they will have high levels of expression as required for a therapeutic setting.

DR. MULLIGAN: That is another topic that we could talk about, people who know something about

enhancers. My sense is that whether you have -- if you are going to have a vector that makes a protein, you are going to need transcriptional activity. If you have transcriptional activity then you will have something close to enhancer activity.

So the details of how one transcriptional element interacts to enhance something is very, very key here. The paradox of this is that I don't see really how the SIN vectors particularly are going to help, because by definition most people are going to want to have good gene expression. So whether they make a SIN vector or an LTR based vector, they are probably going to put in something they think works very well. There are some obstinate points about using PGK or something.

So ultimately, I think the issue is that you are putting in by definition for gene therapy something that has transcriptional activity. Until we know much more in detail about the real fine issues related to how a transcriptional sequence enhances adjacent sequences, I'm not sure we are going to do much.

Therefore, I'm not sure that these kinds of things will address the SIN question. I think the very basic work, some of which was done probably ten years ago, on the details of what it means to be an enhancer and what the elements are that makes something an enhancer, those

may be most important. If that information could be put into a retrovirus vector, it may be way less important whether it is a SIN vector or an LTR.

The thing you mentioned previously is a pretty unknown issue, that is, what is the relative risk of the 3' driven transcription. That is true that it would be a clear difference between a SIN vector and a LTR driven vector, but I'm not sure that anyone has looked at that carefully enough to know whether that specific aspect of things weighs comparable to say the general enhancement of vectors.-

DR. BAUM: Well, of course that is now referring to the philosophy of the study design. Our idea was to go step by step, first of LTR versus SIN, and then look into the different promoters or addition to insulators. You could also take the more radical approach and say you don't expect too big a difference between LTR and SIN when they have the same promoter. Why not first test a vector that has a considerably weaker internal promoter such as BTK, and then show that this is clearly different from the LTR. But it would still leave you with the question, can you discern a vector with a strong internal promoter as you might need for HIV gene therapy, or expression of chemo resistance genes, or even the gamma chain, interleukin-2 receptor needs by profound levels of expression to work.

Your uncertainty will remain. You still will have to check vectors with strong promoters.

DR. MULLIGAN: We can talk more about this, but I think no enhancer, no transcriptional activity versus a lot is the simplest thing first to test the hypothesis. That would presumably directly implicate the transcriptional activity. I think although it is a simple thing, it may be a nicer thing to get out of the way quickly.

DR. MULE: Dr. Wilson, do you wish to respond?

DR. WILSON: Just one point of clarification, which is to say that the choice of vectors here is not necessarily to say that this is the definitive test or the definitive vector, but just choosing one place to start, with the idea that there would be followup studies presumably with other vector designs.

This particular choice was thought to be in some ways a good control for just taking it out of the LTR context, but still having that strong enhancer activity, and as Chris pointed out, still having similar transgene expression, so you can rule out any transgene specific effects. As you pointed out, if you are having very different levels of transgene expression, then that is another variable you have introduced. So you can just keep that in mind and we can discuss it again later.

DR. MULE: A number of these issues will come up

after Dr. Irwin's presentation.

DR. CALOS: I think Richard makes a good point, that having the -- we now have one copy rather than two copies of the promoter, and that might account for the relative difference. But as you said, we have to have probably a strong promoter enhancer in it.

So for the sake of argument, you might say that looking at a lot of different promoters, you are still going to get tumors. It is just a matter of degree. Maybe the more definitive thing would be to bring in the insulators, because we are always going to have to have a promoter within the retrovirus.

Should we bring in the insulators and see whether that blocks it? I guess in some ways you could say that might be the most promising strategy for getting around the problem, rather than messing around with the matter of degree on the promoters and enhancers, because we can't really get rid of the promoters and enhancers. As you say, we need it for the expression.

The data with insulators over the years has been -- I don't know, maybe it is fair to say fairly consistent, that they work, that they do tend to isolate the -- I don't know how other people feel, but certainly I am familiar with some situations where it has worked to relatively isolate the transcription unit.

DR. WILSON: If I may comment on that, in our early considerations of study design, we consulted with David Emory who is at University of Washington, who has done a lot of work on insulator elements. We brought into that discussion several consultants from NIH to work on models of hematopoietic stem cell transduction.

Their consideration was that while the ability of insulators to protect from position dependent effects has been very well documented and supported, the ability of insulators to prevent enhancer activation is still at a somewhat premature stage.

So that was why, partly because of feasibility of study design, we decided that that would be presumably a second step, once we felt we had sufficient data to move forward with this particular model, that we would bring in other vector designs to test, one of those being on the table.

DR. CALOS: So you are saying that there isn't any guarantee that those insulators are going to protect from the enhancer effects within the vector?

DR. WILSON: At this time there wasn't sufficient data to definitively demonstrate that.

DR. MULE: We have a comment from the floor. Can you introduce yourself and your affiliation?

DR. LU: Sure. My name is Xiaobin Lu. I am

currently not associated with any company or institutions. I do have a possibility to exercise VirxSys stock options within a number of days, so that is my only connection here.

I have a question for Chris. Have you ever seen in your mouse model tumor incidence derived from the disruption of a tumor suppressor gene, where you knocked out two genes at the same locus?-

DR. BAUM: We do have cases where it seems that the gene is disrupted, but that is here the difficulty in the model. When you have multiple insertions, you can't really say what the individual contribution of such events is. But maybe in the low dose situation, you may find these tumors. So far we haven't seen that.

But of course, there is concern in the literature, if you down regulate transcription factors such as GATA-1, even haplo insufficiently, can cause trouble in hematopoiesis. It is a valid concern that needs to be addressed.

DR. MULE: I think we will go ahead and move on to the actual study design, Dr. Irwin.

DR. IRWIN: Good morning. Carolyn asked me to give a little brief introduction to the National Toxicology Program to begin with, thinking that perhaps there were several of you who may not be too familiar with it. So I

thought for the first part of my talk, I'll bore you a little bit with some of our standard PR here.

The NTP, National Toxicology Program, was established in 1978. A major motivating factor for the establishment of this program was much of the legislation that was passed in the late '60 and early '70s requiring that new chemicals that were brought into commerce would be given in-depth toxicological evaluations.

At that time, there were several hundred compounds that were already widely used that were grandfathered in under the legislation. One of the reasons for establishing the NTP was to provide a mechanism where the government could conduct the in-depth toxicological evaluations of these particular compounds.

It consists of three major components from the DHHS. The NIEHS, it is headquartered at NIEHS, so it involves components from NIH, CDC and the FDA. In fact, the National Center for Toxicological Research, NCTR, is a separate physical facility in Jefferson, Arkansas, and which we have extensive collaborations with .

We are not a regulatory agency, but much of the data that we generate is used by regulatory agencies. We have a process in which initially this was begun. The large group of chemicals that were grandfathered in were evaluated by each of the regulatory agencies, and the ones

that were considered most important were essentially nominated to the NTP and arranged in a priority order so that we would have some idea of which ones were the ones that needed to be evaluated first, and what types of evaluations needed to be done.

This process has now been expanded, so that if a particular citizen has a compound that they feel is really important, they can call us. There is a formal process you can go through to nominate various compounds. One of the more interesting ones we had about 12 years ago, the Michigan Department of State Police nominated Luminol. Anyone who has ever watched CSI knows what Luminol is; it is used to detect various biological products. Fortunately it was quite safe.

Our general mandate was initially -- although the need to test this backlog of chemicals was a major motivating factor, we were given a fairly broad mandate, essentially to coordinate toxicology testing within the federal government to try to strengthen the science base in toxicology, to develop and validate improved testing methods which I think is very apropos to the project we will be talking about here today, and to serve as an information source on toxic compounds to other government agencies and the public.

One of the things that we have tried to do

rigorously is to control every variable that can conceivably be involved in conducting one of these tests, so that tests that were conducted in the early 1980s are of equal validity to those that we are doing today. Certainly there has been an advance in testing technology and the number of types of assays and so forth that have been used, but the basic elements that are involved in standardizing these tests have basically been the same.

This is just a brief list here, but all aspects of the physical facilities in which these are done are specified in our statement of work, the personnel requirements with regard to education and training of the personnel, the use of SOPs, the establishment of SOPs for protocols and procedures, animal husbandry, every aspect of animal husbandry is specified, even down to the point where we use the same strains of rats and mice for almost all of our testing. We do complete chemical analysis. We confirm the structure of everything we get, and the purity and so forth.

We have really systematized the microscopic collection and analysis of tissues histopathologically. I will speak a little bit more about in a minute. We have established minimum standards of study design, again in an attempt to make sure that everything is highly standardized. All of our studies are done GLP.

An in-depth toxicology evaluation may involve pre-chronic studies, which for us are three months or less. These can be regular toxicology studies, the developmental and reproductive immunotoxicology, and the behavior and neural toxicology studies we do are generally pre-chronic; they can be longer if necessary. These are tailored to the specific chemical or substance that we are evaluating, and the types of questions that have been posed on that.

Then probably what we are most famous or infamous for, if you will, are our two-year toxicology and carcinogenesis studies. Typically a standard pre-chronic toxicology study will involve somewhere between 250 to 300 animals. We use both sexes of rats and mice.

In a two-year toxicology and carcinogenesis study, a bare bones study in rats and mice, it would involve 800 animals. Typically the studies we do contain extra groups, and generally they will run somewhere between a thousand and 1200 animals, depending on exactly what is being done.

We have very extensive documentation of all of our studies. You can go to the NTP website. You can go to any one of our two-year studies. You can look up any animal you want and get a complete dossier on that animal. There will be all the in-life data that was recorded, feed consumption, body weight, water consumption, any clinical

signs that were observed in that animal, the necropsy record for that animal, and a complete histopathologic evaluation of all 45 tissues that were collected from that animal at necropsy.

This is a searchable database. Anyone that is interested in that can certainly try that.

Also, we have a complete record for animal room environment, feed and water analysis, chemical vehicle analysis. If a compound is administered in feed, then the stability and concentration of that compound is monitored regularly in the feed or water, depending on what the route of administration is. We also have extensive interim and final reports. We are in constant contact with the contract laboratory where the work is done, and we are aware of any irregularities. Any time an animal dies in this study, we are aware of it right away.

One of the things that was most challenging when this was first started was to decide how to review the pathology. If you consider a study with 800 animals and we collect 45 tissues from each of the 800 animals, the number of slides and diagnoses involved in one of these studies is a little bit overwhelming.

The process we use is to start out with -- at the contract laboratory there will be an ACVP -- American College of Veterinary Pathology -- certified veterinary

pathologist. Generally there is one for rats and one for mice. They will go through and they will read all the tissues and make preliminary diagnoses on all these.

These diagnoses are then reviewed by a second pathologist, again one for rat and one for mice, which we call a QA pathologist. This is generally someone who works under contract for a company that specializes in pathology under contract to us, and they are not at all connected with the person at the laboratory.

Finally, we conduct what is called a pathology working group, which will be approximately ten pathologists from government, academia and industry. They will sit down and they will review the diagnoses, and they will resolve any differences that were observed between the laboratory pathologists and the QA pathologists.

There is a standard set of descriptors that has been developed by consensus over the years that are used to describe these lesions, so that you don't run into any conflicting diagnoses as far as that is concerned. What is a pedacellular carcinoma in a study that was conducted in 1982 is the same as a pedacellular carcinoma that you would observe in a study conducted in 2005.

This gives you some idea of the types of studies we are involved in. We have a fairly substantial program on herbal medicines and dietary supplements, because these

compounds are not -- the testing is not necessarily required for these. We have a study that will start very soon on cell phones. We have had to construct a facility in which to do this study, so in about five years you will get the definitive answer on cell phone radiation.

We have a state of the art photo toxicology laboratory at the National Center for Toxicological Research. We have programs in endocrine disrupters. We have a program in which we are evaluating nanoparticles and other nano scale materials. Finally, the program which is relevant to our discussion today, it is on DNA based therapies and it is being conducted in collaboration with the FDA.

This is our website. We have conducted 540 two-year studies plus probably a thousand pre-chronic studies. They are in databases that are all searchable if you need any type of normative data, clinical pathology data, body weight data, food consumption data, anything that you might want for rats or mice, you can probably find it there. There are also instructions for nominating anything if you are so moved to do that.

This is the topic for today. Our role in this is to conduct and validate this assay in a toxicology testing laboratory under GOP conditions.

To do that properly, we will need to do a study

in which we use a large number of animals. So Chris and Carolyn and myself and a number of other people have put together a study protocol in which ultimately we will evaluate at least 700 mice.

One of the things that we learned early on in the NTP is that before you start a 700-mouse study, it is better to look at maybe a 50- or 60-mouse study. So the first thing we will do is conduct a pilot study. Chris has mentioned this before.

A critical part of this whole thing and success depends on our ability to engraft a high percentage of our primary recipients. So our initial pilot study will involve evaluating 60 animals in which the primary recipients will be injected, and they will be then observed for 12 weeks. So the goal of the 12-week pilot study and the reason for doing it is to allow the contract laboratory -- and actually, to allow us to evaluate the proficiency of the contract laboratory, in conducting the various procedures, methodologies and assays that are going to be required in this study.

The results will tell us what types of changes we need to make, and will give us a much better idea on what will happen and how successful we expect the definitive study to be. It also provides us an opportunity to make any modifications to the definitive study design, since we

will have actual data that we will collect under the GLP conditions. Success will be determined by engraftment of a high percentage of primary recipients.

These are the two vectors that we will examine first. Chris has talked about these, and they have been discussed in detail, so I won't bore you anymore with this.

This is the schematic of what the design will be. We will test the LTR and the SIN vector. We use empty particle controls. There will be 15 animals that will be processed at Batelle for each vector, and ten controls. As a processing control, five animals will be treated at Cincinnati with the same cells and under the same conditions that are used at Batelle, and this will allow us to determine if there are any problems here, what might be the cause. It is a built-in safety valve for us. So there will be a total of 60 animals. We will give a single high infectious titer. The donors will be male C57 Black 6's with a CD45 .2 marker. The radiated recipients will be females with different makers.

There will be a six-week interim evaluation, in which blood will be withdrawn. We will evaluate hematology. We will do transgene expression. We will look for donor chimerism and myeloid lineage differentiation. This will all be done by flow cytometry. This will give us a preliminary idea after six weeks of what we might expect,

and what type of success we might see.

The 12-week necropsy will be very similar to the necropsy we actually perform in the definitive study. One of the reasons for this is to allow the lab to determine whether they have their teams assembled properly and whether they have enough people.

If you take a look at what is going to happen, each mouse is going to be handled extensively. To begin with, we will draw blood for hematology. We will do immunophenotyping, transgene expression, and cells will be frozen for DNA isolation. Bone marrow will have to be isolated. It will be examined for pathology. There will be immunophenotyping and transgene expression. Cells will be taken for PCR and for DNA isolation, and any remaining cells will be archived. We maintain a frozen tissue archive, so we will be able to keep this tissue and have it available.

The spleen and thymus; we will have to take organ weights. We will have to take a section for histopathology. We will have to take some and make a single cell suspension to look at immunophenotyping and transgene expression. Cells will also be saved for DNA isolation. For lymph nodes we will take a section for histopathology, and if the lymph nodes are enlarged, they will be processed also for immunophenotyping and DNA

analysis.

The liver, kidney, brain, ovary, heart and small intestine, sections will be taken for histopathology. They will be weighed, and tissue will be saved and frozen for DNA isolation.

In the pilot study, all the remaining organs will be collected and preserved in formalin for possible future microscopic evaluation. In the definitive study, we will actually collect and process all of these organs and a complete histopathologic read will be done on all of these. But for the pilot study, we didn't think that would be particularly necessary at first, unless the results will dictate later that we go back and take a look.

In the definitive study, we will use 50 primary recipients per group. These animals will be injected with transduced cells. They will be held for seven months, and we will assume we will get approximately 50 percent incidence of leukemia. One of the things that we will find out from this study is what we do get.

Any of the animals, the primary recipients, which are asymptomatic after seven months, bone marrow will be isolated from these animals, and that bone marrow will be injected into two secondary recipients. These secondary recipients will then be held an additional seven months to look for additional leukemias that may develop.

In the definitive study, we will have 50 animals per group. We will be examining two vectors. We will be examining a low and a high infectious titer, so we will have approximately 200 animals in phase one of that. In phase two, we will have -- it will involve 100 animals that -- we are assuming now that in phase one none of the animals develop leukemia, so at the end of phase one we are saying if we see no leukemias, we will need 100 animals in phase two, since each one of these 50 will have bone marrow isolated and transplanted into two recipients. We don't expect we will need this many, but for planning purposes we have included 100 animals here.

Our controls will have a phase one control. At the end of the seven month, we will randomly pick 50 controls. Bone marrow will be isolated from these animals and they will be transplanted into two animals per each, so half of that will be 25, and again that will be 50. So if the study goes as we have outlined it here, we will lose about 700 animals. We are anticipating that we will see leukemias at the end of phase one, so we will have less than 100 animals here for the phase two.

In order to monitor for leukemias, we will take peripheral blood samples every six weeks, and we will do differential counts. We will evaluate hematology and we will do transgene analysis by either flow cytometry or

QPCR, so at the first six weeks it will be flow cytometry, at 12 weeks it will be QPCR, at 18 weeks it will be flow cytometry, and so forth.

The necropsy again will be similar to or pretty much the same as what you saw for the 12 week study. You have to appreciate that you cannot necropsy 50 animals in one day, so the lab will probably be able to necropsy somewhere around 15 animals per day. It won't be so difficult to irradiate and inject 50 animals in a day, but at the end of the study we will probably be able to necropsy about 15 animals per day, so it will probably take somewhere around a week to necropsy each group. That is one of the limiting factors in this study and one of the critical components that we will evaluate, certainly in the pilot study.

Again, histopathology will be performed on all the tissues, and any remaining tissues from this study will be maintained in the NTP frozen archives, and will certainly be available to other members of the scientific community who might be interested in conducting various types of analyses with these tissues.

That is all I have to present. I'll be glad to answer any questions you might have for me at this time.

DR. MULE: Thanks, Dr. Irwin. Perhaps we can go ahead and put the questions up for the committee, because

they are relevant at this point to the discussion. So we can open up the floor for the committee if there are specific questions.

DR. MULLIGAN: Is there a contingency for not being able to reproduce the frequencies of tumor formation? I am a little lost on the pilot study. The pilot study I know was to make sure that the infections and the bone marrow can get safely to wherever, and the injections go okay, but I am curious about, will there be a point where there is a stopping of the activity if you don't detect what is anticipated?

Let's say very few if any of the animals get tumors by the end of the phase one. You made a comment kind of suggesting you are aware that could be the case, since you said you would go to the phase two. But did anyone make any decision about that, like would you try a variation, a repilot plan, or that sort of thing?

DR. IRWIN: Yes, I have thought a lot about this in particular. I guess my answer is, what we are trying to do is validate this particular assay.

I guess if we don't see anything at the end of phase one, particularly in the definitive study, we will have to scratch our head a little bit, but I think we can still move forward with the phase two. I think it would be -- I can't really think of a cutoff point here. I think we

need to try to give this every opportunity to succeed.

One of the things that I like to point out sometimes is, of the 540 chemicals that we have tested for carcinogenicity, fewer than half are actually carcinogens; most of them are negative. In many ways, you can look at that is a -- it gives you an answer, but it doesn't tell you much other than that. In a sense, I view this in the same way. This will tell us whether this is going to work or not, and if it doesn't work, then we have to go back to the drawing board on it.

DR. MULLIGAN: In the pilot though, I think you said it only goes for 12 weeks?

DR. IRWIN: Twelve weeks. What we are really just looking for is engraftment at this point. If we get a high percentage -- if we go through the pilot study and don't get a good percentage of engraftment, then we really have to rethink what we are doing. If we get a high percentage of engraftment, that is what we are looking for.

DR. MULLIGAN: Shouldn't that also look at tumor formation? Does that occur in a 12-week time period? No.-

DR. BAUM: We have so far not seen tumors within 12 weeks observation when the transgene was a neutral transgene.

DR. MULLIGAN: is there a stopping in the pilot? During the pilot, if you get reconstitution that is all

okay, but the gene marking, which is something you can test, isn't whatever you are looking for, some low dose, but you are setting the upper dose, then what will you do? Is there a game plan for what happens if that is the case?-

DR. BAUM: With the protocol that we are using here, we have extensive data from the academic labs that the in vitro marking rate largely reflects what you get in vivo.

The major concern that we have is whether the cell survive the shipment, and whether they transplant conditions are reproducible in Batelle, as we have seen that in the academic settings. So the major concern would be an increasing proportion of host-derived hematopoiesis in the animals, because you always have survival of some host cells after irradiation. We would expect that we see within the donor population the marking levels that we can monitor already in vitro after a few days. The chimerism analysis, that is really important.

DR. MULLIGAN: Another question is, will the definitive study look at this issue of the stem cell content upon transplantation? That is, will you know whether or not there is variations in the actual numbers that are put in?-

DR. BAUM: We thought about establishing recompetitive population assays, but of course they are

very demanding, and we gave up on that idea. But if the panel advises to do so, it should be done, of course.

DR. IRWIN: At the end of the pilot study we will look at our results very carefully. That is one of the reasons for doing it, because we need to make modifications and so forth. But our intention is to move forward.

DR. GUNTER: I may be at risk of showing my lack of knowledge here, but one thing to think about, and maybe we are doing it, I just need to understand better, is, in the definitive study I understand the positive controls can have an intact LTR. What I am suggesting is that the positive control in that study be the same or as close as we can get to the vector that was used in the French SCID study.

The reason I am suggesting that is, that is the most human experience we have, and we know what the incidence of tumorigenicity was in that clinical study. If this model proves to be predictive, it might be useful to translate that human risk into an animal risk. Then when we evaluate new vectors, we can make maybe some semi-intelligent comparisons between the tumorigenicity risk of new vectors compared to one that was known to be tumorigenetic in humans.

DR. BAUM: Maybe I can comment here. In the specific case of the gamma C knockout mice studies, the

mouse models have not been informative to predict the carcinogenic risk that occurred in the humans. The model simply is not valid as it stands so far, so that is why we would suggest to go into this wild type situation, with the neutral marking that they are not under disease specific conditions until we have clear evidence that a mouse model is available that reports the problems encountered in the gamma C clinical trial.

DR. ALLAN: Just a basic question on animal losses. What do you figure is an acceptable number of animal losses during this type of study, because you are doing irradiation in bone marrow transplants, and you are manipulating these animals a lot. So I am wondering what you expect to get in terms of the losses.

DR. IRWIN: Even though we are specifying a group size of 50, we will probably irradiate and inject 65 or so animals to make sure we at least start out with a reasonable group size.

Once that is completed, once the initial injections are made and the animals are back and survive a week or so, we feel fairly good that anything we would see would hopefully involve just the leukemias. But obviously, if we have a lot of mortality, you can only stand so much, and we will have to stop the study at that point. It wouldn't make much sense to continue.

DR. CORNETTA: I think in terms of the question about the SCID trial, the transgene in that situation is under question in contribution. So I think looking at this model, the selection of vector for the positive control vector I think is a good one.

But maybe to step back, what is the data that we are looking at get out of this, we have heard the study, but what is the question that is being answered that is going to help the field here? Is this just a study that is designed to see if this study design can be used in the future for other questions? Or are we trying to answer some particular question with this study?

DR. MULLIGAN: To amplify on the question, I think it is a great one, because I am having difficulties with it, too. If for instance it was the case that the target for oncogenesis in your system is an expanded parent cell that is cultured during a couple of days, let's just say that if you were to do the same thing, everything was the same, same vector but you used a purified stem cell population, and you used the same vectors and you did the transplants and nothing happened. Then the question is, what is the model? Then what?

So there is a presumption I think that you have that it is probably enhancer activation. So I think one of the questions to hear is, is that what you think? Is that

why you are setting up this, and is that what the test is? If someone else has a different point of view, if you thought it was the population that you affect or the cytokines that you use, then would this still be a good model?-

DR. BAUM: If we consider the current state of the art and clinical gene transfer of hematopoietic cells, this occurs with cultured cells that are stimulated and that are not pure stem cells. We are using CD34 plus cells which contain more than 90 percent non-stem cells.

It is very unlikely that we will have clinical situations where we can infuse purified populations of human stem cells into the patients very soon. In that respect, the choice of the mouse model and the particular population of LIN minus cells largely reflect what is used in the human. The CD34 antigen in the mouse is not equivalent to the human, and the LIN minus population is rather equivalent to the currently used clinical conditions.

The other question is cytokine stimulation. We do know that we will soon see more trials using lentiviral vectors. It is an important question, but it is also completely unknown whether some aspects of lentiviral transduction protocols increase or decrease the risk. You could also argue, if you increase the engraftment index

because you stimulate the cells less in vitro, you could increase the risk even when using a lentivirus.

So we don't have the perfect situation here in the mouse model. It is hard to predict what will be used in the future in five years from now. We have to start from somewhere. We think the mouse model so far in the literature is the only one that showed reproducible incidence of insertion leukemias, and that is why we chose it. It can be further improved, I'm sure, but if you want to start soon, we should take this model. That is my opinion.

DR. TSIATIS: In none of the documents that I saw were the statistical analyses laid out, or the rationale for the sample sizes. As a statistician, that is usually how I get an idea of what the primary goals are of the study. I didn't see that. It seemed all very vague.

DR. GUNTER: I feel obliged to ask a general question. It is stuff that I would like the FDA to think about. That is, if this model and system prove to be informative, and let's say the retroviral vector construct that is tested is much less tumorigenetic than the positive control, will that vector be accessible to the community as a whole?

Specifically, as you know, I am the industry representative, I am thinking of companies. So how is the

choice of the safe vector going to be made, and will that be generally accessible to others?

Then it leads to another issue. If success is shown in that way, is that going to be the de facto approved FDA vector? What are the steps going to be in the future to test other vectors? So probably there are no clear answer to this, but it is something I would just like our FDA colleagues to think about.

DR. WILSON: If I could comment on that, and also maybe some of the earlier questions from a few minutes ago, I just want to be clear that the choices of vectors here are not meant to say that we are saying that these are the definitive safest vectors. They are meant to just be model vectors, where we expect a positive rate of leukemogenesis in one case, and a reduced rate of leukemogenesis in the second.

The primary goal of this study is not as much to actually test a hypothesis of change in enhancer or insulator element and so on. I know this is kind of a hard thing to put our heads around, because we are scientists and we want a real hypothesis, but the real thing we are testing here is the robustness of this particular model, how exportable it is to other labs, what is the sensitivity when we go with a vector that would have a reduced incidence, what is the reproducibility, and so on.

That is why I think there is some concern -- and I hear you and I understand, and we have thought about a lot of these questions in terms of what vectors to test. But I think at the end of the day, it is almost not as important, because we really want to be able to say, is this going to be a useful model, so that when sponsors come to us with their own version of a vector that they want to test to say it is safer, we can say this is a valid model for you to do that analysis in, as opposed to saying, this is the vector that is safest and we want everybody to use it. That is not the goal of this study.

Does that help clarify some of the questions, I hope?

DR. MULLIGAN: Well, for instance, if you set up this and someone comes to you with a different vector and a system, I am harping on the purified cells for no good reason, I'm not totally an advocate of this, but it is a good way to make the point, and comes to you and says, we think this is safe, because we think it is the stem cells and it is not the vector. We have done this little clinical study that we could do in a mom and pop laboratory, what do you think?

If you were to tell them, you should use the model, it doesn't have any meaning to them. You would say, we think your model is not the safe model, it is the way

you do it to elicit the tumor formation that we think is the innovation that we have.

So I'm not sure how then we would test it, meaning, how would this model serve to help on anything other than say insulator function if that is what you thought was -- I guess I am trying to focus on exactly what this model can tell you. I think it can tel you probably something about insulators and about enhancers. I think it can also tell something about pro-viral insertion per se. But if you think that it may be something else about the protocol, the cell source, the cytokines, then by definition it can't.

DR. MULE: I guess, Richard, if you lock this in and it is statistically powered, to get to your point, then any modification in the actual transplant protocol I guess can be compared -- if you use similar vectors, can be compared with this particular study to determine head to head whether or not it is the transplant that is the source of cells, or whether it is the vector.

DR. MULLIGAN: I think the positive thing is, if you want to craft this as a community activity, you could vary all these things, and you could have purified stem cell populations versus this.

I guess I am just thinking that by the time this study gets done, everyone is going to have more gray hair.

It is going to be years from now before you get this all done, and things are going to be moving very quickly before this is all finished. Before this is all finished, there is going to be much more sophistication in everything, I think.

DR. WILSON: That is one of the reasons why I am sounding like a broken record and saying that we don't want to put this forward as saying this is the definitive vector construct, for the very reason that we recognize that this is a multi-year study, that new vectors will be coming along by the time this is complete, and so on.

I certainly accept your point that the primary role of this model would be to test alternate vector backbone structures, but as Dr. Mule pointed out, you could if you use the same positive control vector also subsequently vary other transduction conditions or stem cells and so on.

So I think down the road we have opportunities to test more variables, but for this initial study, given that it is already a large number of animals -- in fact, we did actually start out with a much grander scheme in terms of testing a lot more variables, and realized that the feasibility of doing that as an initial study was probably not a very good idea. That is one of the reasons why we have scaled it back a lot, to do one vector construct

comparitor, and assessing the feasibility of this model, as opposed to trying to address every possible variable that could be addressed, because certainly there are many.

DR. MULE: Carolyn, how would you tackle Dr. Tsiatis' point about, was there a statistician involved?

DR. WILSON: I'll let Rick comment on that, because I know they did.

DR. IRWIN: The number of animals we chose is a compromise between what is feasible to actually conduct in a contract laboratory and statistical power. This is the best combination, it gives us the best statistical power for a reasonable number of animals.

Obviously if we included more and more animals, we would have greater statistical power. But you introduce a lot of other variability in that, in terms of processing the animals and handling the animals.

DR. TSIATIS: Statistical power for what?

DR. IRWIN: To detect negatives, basically. In other words, if we see no result, what is the significance of that, is it really a negative. We don't have any trouble -- a positive is a positive.

DR. TSIATIS: So if there were no leukemics --

DR. IRWIN: If there were no leukemics, that's right, that would give us -- this is the best compromise between statistical power and the number of animals it is

feasible to work with.

DR. TSIATIS: So you are not interested in comparing the groups to each other, the four or five groups that you have constructed?

DR. IRWIN: That can be done at some point.

DR. CORNETTA: I think the FDA needs to be commended for bringing this study forward. I think something needs to get started, and this is a really good starting point. I think you have got two folks involved that can bring this forward.

There are two things I struggle with. One is the choice of the SIN vector having the enhancer in it. I think it is a lost opportunity to try to understand whether read-through from the 3' LTR contributes. I think that is something that you might be able to at least start to address here. There are technical issues with that, but I think by putting in the enhancer even in the middle, where it is half of the strength, I think you may well get a lower incidence of leukemia, and people will just argue that you have a lower dose of the enhancer, and I'm not sure that is going to add much information.

The second thing is leaving out lentiviral vectors in this context. I think many folks in the field are moving there, and that is going to be the challenge, that folks will -- by the time this study is done, how this

is going to add to where the field is going may be a lost opportunity here.

DR. MULLIGAN: I agree completely, precisely with what he just said.

On the first point, I don't think it is at all clear that it will turn out to be that proviral insertion per se won't turn out to be very important. I think if you don't address that right up front, then it shifts the way the field looks at things.

So for so many reasons, I think you ought to have a completely transcriptionless vector right up front versus the LTR. I think that is very, very important. I think it is under appreciated that proviral insertion per se can be very important.

I know the retort is that you have to put in something, but you have to know the mechanism, you have to know the frequency of a mechanism. I think that would be very helpful.

On the second one, the lenti, he is exactly right; people are running past this, and there are a lot of people for whatever reason that will think it is safer or whatever. I don't think there is any reason to think it is or will be, but I think people are immediately on level with the retrovirus in terms of the paucity of activity.

So there, there is going to be a whole gap that

this is not going to help with probably half or three-quarters of the effort that moves ahead.

DR. BAUM: If I can comment here, we do have a vector available as the gamma retroviral backbone that has absolutely no enhancer promoter, but is still the same transgene internally. This is currently in the end of the first cohort of analysis in the academic scale, so mice have observed for six months, and we do not have the integration pattern so far, but that will be available soon.

The vector can be produced at sufficient titers to have the same number of hits in the bone marrow cells as the LTR vector. This is what we know. You sometimes see -- in about a third of the transduced cells, you do detect transgene expression, probably by a mechanism involving a cryptic endogenous promoter in the retroviral backbone.

So that would be available, if the panel decides to ask the question up front, what insertion of an enhancer-free vector does to the cells. It could be tested in this very model.

Lentiviral vectors could also be tested, but here we have an issue to define the same infectivity between gamma retrovirus and lentivirus. So if the question is which of the two vectors is safer, that would require at least a couple of months preclinical studies to define

transduction conditions under which both types of vectors have the same level of gene transfer into the in vitro treated population.

But you could also have the same study started with lentiviral vectors, where we have an LTR or an internal promoter or no promoter. All that is possible. All the vectors have been cloned, and it really comes to the point now what you think is scientifically more sound.

I do think that the original design has proposed here still makes sense. The idea was to reproduce what we found in the academic lab. An academic lab can always make mistakes and can be biased somehow. This study will give you a reference point against which you can compare all the potential variables like cell type, cell dose, novel vectors that you think of for your future clinical studies.

DR. MULE: For the sake of time, let's move to the second question and continue the discussion, which has to do with the point Dr. Mulligan made with respect to type of cells, the cytokine mix, other parameters. With respect to this question, what other models in in utero gene transfer model was used as one example.

DR. MULLIGAN: One thing I thought the FDA might be interested in getting much more information on is the gamma C preclinical activities which, although they are limited, I would really like to know why no one ever

detected this.

One thing I was thinking would be perfect for a large scale study is to craft something to see if you can determine the basis for what was seen in the patients. So there you have the transgene being potentially very key, the LTRs perhaps being important, and the background, the actual immunodeficient recipient.

So it just seems like that is lost in the literature. There was a disappointing preclinical understanding, and it will be interesting to put more resources on that activity, I think.

DR. HARLAN: I had two questions that sort of pertained. One is, Dr. Mulligan is saying I can have more gray hair. I wonder how much more gray hair. How long will it take for this study to take place, one?

Two, I was extremely impressed with the thoroughness of it, but that also means this must be a very expensive study. If this becomes the model, how do we test other things down the road? Is this going to be a reiterative process, that NTP does this?

DR. IRWIN: We expect this study to get started this spring. We expect to actually have animals exposed. So by the end of the year of 2006, we will have preliminary data, at least to begin with. There is still much more that will have to be done, but we will certainly have all

the in-line preliminary data. We will have an idea of whether if we got leukemias, how many we got and so forth, those types of things.

I think the NTP is very much interested in doing these types of studies. I can't speak -- unfortunately I can't speak for the entire organization, but certainly this is a major area of interest of FDA. We feel that these types of activities help support their regulatory function.

So from my point of view, and I think other people within the NTP, we consider these to be important. We certainly cannot test everyone's vector, unfortunately, but we can do these types of general studies and validation types of studies. I think if there are studies that the people in gene therapy collectively feel are really important and will advance the field, certainly we would be very interested in looking at these and trying to conduct some of these.

DR. MULE: As a followup, if someone like Dr. Mulligan were to come to you with a variation on the protocol as it is up and running, what is the availability of resources and maybe prioritization if you will that might allow additional models to come in as this study is going forward?

DR. IRWIN: Once we get an activity like this underway, it is generally much -- I won't say much easier,

but it is easier to supplement it, shall we say. There is a lot of momentum that is required to move these things forward.

This is a cross-agency study. It will cost a few million dollars. NTP typically pays big money for these studies. It is not unusual for a chronic inhalation study to cost four million dollars, let's say. So I know the numbers sound astronomical, but it is not out of the realm.

I would encourage anyone who -- Dr. Mulligan if he wanted to do that -- to let us take a look at it. We can see what would be involved in trying to implement it. I think we are as sensitive as we can be to peer review, and that is why we are interested in having things that generally are more or less a consensus within the field. We talked to a number of people before we even delved into this work at all.

So with that proviso, I would say certainly we are open to looking at anything people think is important and will advance the field.

DR. MULLIGAN: To follow up on the gray hair question, isn't it the fact that the data will not really be collected for two years, because there is a three-month activity, and then there will be another couple of months. By the end of the year there will be a seven-month first

transplant, and then there will be a second seven-month transplant.

DR. IRWIN: That's right. The data from the pilot study will certainly be --

DR. MULLIGAN: By the end of the year the pilot study, but then the definitive study will initiate for 14 months or something.

DR. IRWIN: Right. It could be two years, because the pathology will take quite a bit of time on that. But there will be preliminary data, in the sense that we will know what the leukemia incidence is. We will know a number of other things.

DR. CORNETTA: The in vitro studies that are combined there, looking at the potential of other assays besides doing this, will be key to that. I think the cost of these, adding those on to the development of new products, is going to be very significant. So it may not be that the in vitro assays that are envisioned will give us the information, but if they do, I think that will be something that potentially will be very helpful for the field, and to have that corroborated with a GLP study will be important.

DR. IRWIN: And remember, there will be tissue left from the study, and this tissue will be in a frozen archive and can be used. We have a process where

investigators can apply for the use of that tissue for research projects.

DR. MULE: This is more a question for the committee. is there a danger that two years from now, the way the field is moving forward, that data generated from this -- I'm not going to say rather expensive effort, but a significant effort, may yield information that may not be valuable?

DR. MULLIGAN: Yes, I think so, but I also am very supportive of this. I think it should definitely happen. It is just the way technology moves ahead. Ken can attest to this; they put in a request to make a vector manufacture, then they begin to lose interest in it after a year or so because there is something they think is a better vector.

I'm sure that there is going to be better vectors here, and models that are different, and insights into what is all happening. But I don't know how else you could -- you could talk yourself out of it, I suppose, but I think it is likely to tell you something.

DR. CORNETTA: Looking at the requests that we have been getting in the national vector lab, no one is talking to us about retroviruses. There is one center that is still very involved with them, but most folks are talking about lentiviral vectors.

NHLBI has been looking to develop some contract facilities for production, and they have outlined AAV and lenti, and they are not asking for retroviruses in that. So I think that is one of the concerns we expressed before, is that something that we can wait two to three years before -- or where is the plan there, because the field is moving toward that direction. I'm not sure it is based on hard evidence, but that is where a lot of folks are going.

DR. MULE: I guess at the least -- and we have raised this before -- that this study could serve as a critical standard whereby other vectors can be very carefully compared. So at the minimum, I think it is a very important study from that point.

DR. GOODMAN: I was going to focus a little bit on what somebody brought up before, which is the biomarker approach to the toxicology study.

I think that obviously, leukemia in a way is an ultimate biomarker. I had to step out for some of the presentation, but I assume you are seeing that many months, or you can see molecular evidence of that many months before you have the end point of the two-year traditional toxicology study, or is that not correct?

DR. BAUM: We don't have a good surrogate for leukemia development before it actually is present.

DR. GOODMAN: What is the time curve for the

leukemia development?-

DR. BAUM: Roughly five to six months in the first cohort, and three months --

DR. GOODMAN: So that is not two years, is what I am saying. It is not two years. That is a major end point of interest. I'm just saying, where does that fit into the analytic plan and the understanding of the ability of this as a tool to look at interventions about vectors or new vectors.

So there may be an opportunity, for example, if you are able to develop a lentiviral model -- and again, I don't know much -- but that is relevant and useful in this construct, to do that and study it if it is available at a time before the two years. So if you know that the model is up and running, you may not have the final histology in tumorigenesis, but you may have a good leukemia model in which you can have studies that have some overlap. So I think if those aren't ready now, that would be something to consider.

The other thing I was going to ask, maybe I just missed it, is, is it impractical, or is there a plan or the possibility of a plan to say for example RNA as well as DNA. You do want to get at some of these issues about what is going on at an expression level. You are creating an incredible amount of scientific data here, and it would be

nice to exploit as much of it as possible. Also, that may allow you to determine what are some biomarkers for what is going on.

I do agree, it would be nice to have a model -- with the pace of vector development, a model that could give you these answers in less than a year, rather than two years, et cetera.

DR. ALLAN: I want to pin you guys down. It is obvious that the studies need to be done, but I keep hearing lentivirus. So if you had your druthers, would you say let's just skip the retrovirus and go right to the lentivirus? No?

DR. CORNETTA: No, but I think especially trying to do them potentially in the same study -- one of the arms in this study using the SIN retro with at least having the enhancer there, i'm not sure is adding more. Do you substitute a lenti in there, or do you have another arm that potentially has a lenti in there now, or do you just get ready to do a second set of experiments? Obviously if you are doing everything together, that helps to know whether this was some variability with the transduction protocol of the cells.

But I am concerned about the field moving ahead and having lentiviral trials on, and then we are debating about the tumorigenicity of them when some patient develops

a tumor down the road.

DR. CHAMBERLAIN: Yes, I agree with that. I think the thing that has to guide us is the model where we have the most clinical experience, which is with the retroviral vectors, and we did see leukemia, so logically that seems to be the best place to start.

But I am worried, as you both have brought up, about the limited nature of what we are going to learn. The fact that there is still an enhancer in there, I agree that my prediction would be that you are going to get a lower incidence of leukemia, but it is not going to go away completely. Yet, you can't do a million variables all at once because of the expense and the logistics. But along with Dr. Goodman's comments, is there a wag to start introducing a few more variables a little sooner in this, rather than two years from now looking at the data and deciding, let's try a third vector. Once you get this underway and the mice are incubating, and you are waiting to see what happens, can we bring in a third vector that maybe has some insulators on there to really try to knock down the enhancer activity, or a vector that has no enhancers whatsoever, or a lentiviral type vector, just start bringing in new variables at six-month intervals, rather than at two-year intervals.

DR. CALOS: I support the study and its design.

On the other hand, I have to say that it is certainly my expectation out of this study and the future studies that people discussed that we are going to see leukemias, and we may be able to reduce the frequency of it, but I think it is inherent in a randomly integrating system, where we require gene expression for the transgene, that you are going to see it.

So I just want to say that I don't think this is really the path of the future, longer term, that we have to get away from these vectors. They aren't really appropriate for gene therapy if we could design our ideal vector. I think the situation at the moment is, as far as I know there isn't any other integrating system that has been shown to work in long term repopulating HSC right now. I think that will probably change over the next few years.

At the risk of sounding self serving, I just want to bring up the system that my own lab is developing, which is a totally different integration from a phase that requires a lot of sequence recognition to integrate, so it has far fewer integration sites. Although this is relatively new and it certainly hasn't been tested rigorously in terms of safety, at this point there have been quite a few animal studies done, and so far no tumors. But we haven't got it working in the hematopoietic system, though looking in the liver and some other tissues we don't

see anything.

I think this system and this idea, and we are modifying it even further to increase the site specificity, is really the wave of the future. So I just think, do wait. We are spending millions of dollars investing in these retrovirals, looking at the safety of these systems, that I think are inherently unsafe. So we can improve them, but I don't think that is really where we want to be in ten years. Probably we will be somewhere else, I hope, in ten years.

So I think it is part of the process to understand what the risks are with these systems, but I think the work that Chris has done has been very careful and convincing that the risk is there, and it is really tough to get rid of that risk just from the way the system works.

DR. HARLAN: I take that point, and the points from Drs. Mulligan and from Ken. I have heard lots of variables, vectors, cell types, cytokines, and I also just heard that this is a two to four million dollar study.

I am going to assume that this validates what Dr. Baum does. Then all of these other things, people are going to want to test them, and I'm sure companies are going to have strategies they want tested. I think we should be talking about what do we do when this study

validates what Dr. Baum has already done, and we want to test all these 50 other variables. How are we going to do that?

DR. GUNTER: Just a brief comment. First of all, I agree that the field needs to move forward. We need to look for better vectors. We need to look for vectors that maybe can go to targeted integration sites. But there is always sgoing to be risk in any therapy, so we really need to remember the concept of relative risk here.

That is why I think the FDA and NTP should be commended for thinking about this study. It is an attempt as I see it to try to quantitate risk. That is what the patients need and that is what the physicians need that are going to be giving therapy. So let's not forget that every day, patients with cancer get therapies that are very risky and could cause tumors. So I just wanted to mention that.

DR. MULLIGAN: Two points. Quickly on the lenti thing, I think that probably there may even be a consensus that if you could -- this first thing can be complicated, but a lenti vector, squeezing that into the study actually might be a good idea. Also, removing the enhancer from the SIN vector.

I think David has said on the most important point, though. The way things do work, if there isn't an upfront message from this group here that, don't tell us in

a year and a half or two that there is no money now to make use of the great system, because that is what I imagine will inevitably happen. Inevitably, you will do this thing and it will be painful, and let's say that at the end of it, it is, so what. Then the opportunity will be lost completely in the future. People will say, you just spent two, four million bucks, and look at what happened from this. I know you have ten different things that are way more exciting than you had two years ago, but what are you going to do now?

So I think a message ought to be that we strongly support this, but because we assume that eventually we will be able to make use of it.

DR. WILSON: If I could just comment on that, I think the ultimate goal is not necessarily to say now each sponsor can feed into NTP, and NTP sponsors each and every vector modification, but it is really more also for our own internal use, so that when sponsors come to us and they have developed a new vector, we can refer them now to a study that is in the public domain, that has been done with incredible rigor and with statistical analysis
and so on to say this might be useful. Not to say they necessarily need to do it on that grand a scale, but from that data, we may be able to say you can scale back to this or that.

But I think that is really the primary goal from our perspective in terms of regulating these types of products.

DR. IRWIN: I really don't know right now what the cost of this study will be. I just used two to four million dollars, it may be half of that.

Originally when we talked about this, these were two vectors that Carolyn and Chris thought would be appropriate to use. The original idea was to look at a number of vectors. This initial study is as much to establish that this can be done in a GLP lab and to get people trained and in to evaluate the SOPs and so forth as it is to actually generate data.

So as I said before, once we have something that is workable like this, certainly we would look forward to extending it, and hopefully be able to make modifications that will keep up with developments in the field at the same time.

DR. MULLIGAN: Is there a definable budget, in fact? That is, if you are going to tell people --

DR. IRWIN: I wish I could tell you there is. I hope at some point there will be. At this particular point, no. This is the first study like this that we will have ever conducted. We have done some small pre-chronic studies with AAV vectors and ADD vectors, but we have never

done anything quite like this.

But there is an interest and a commitment to work and to try to do these types of studies.

DR. MULLIGAN: Can you tell us who makes the decision, or what is the group that makes the decision about something like this?

DR. IRWIN: Well, we recently got a new director of the institute and a new director of our program. I haven't been told that anything much has changed at this point. There was a substantial commitment prior to this, that is all I can say. I would be surprised -- this is one of the most clinically oriented studies that NTP has ever been involved with, and it is in direct support of the FDA and their regulatory role.

So I feel fairly good about this, but this is the beginning, and hopefully will continue along.

DR. GOODMAN: I think it is important not to raise totally unrealistic expectations. I think part of the point here is to try to provide a useful tool and some approaches, and to get like here today, your best input about, is this a useful tool and what are the best ways to test it.

Ultimately it is controlled by Congress and their appropriations, what is available to the government and what are the competing needs. It could be that even in

gene therapy in two years, people would say we have this tool, but right now there is this other more important problem or priority.

So I think we have to be realistic about the limited resources, but the question really is, can this provide a useful tool, could it provide for example for a sponsor a better way to answer questions that they are going to have to answer anyhow to develop a product in human beings.

So I think ways to try to keep down the costs, make the model simple and relevant, look at biomarkers in preclinical development, those are directions that are good to go in.

DR. HARLAN: Well, because I don't have a dog in this fight, I'm not a gene therapy person, and because there are minutes of these things, I just can't imagine any academic lab or even a large company being able to do the rigorous study that we just heard described. If it pans out that it is a useful paradigm to build protocols around, I would like to move that a steering committee be developed to test other strategies to run through a system like this, rather than try to ask a company or an academic center to do studies like this.

So down the road, different vectors, different cell types, different cytokines could be tested. It would

be most cost effective to do it at one center doing it right.

DR. MULE: Before we move away from this topic, I would just like to commend the FDA and the National Toxicology Program for this partnership. We certainly look forward to hearing many more good things about it.

Are there any questions or issues that you may have of the committee before we move ahead?

DR. MULLIGAN: Are we going to get to the other questions?

DR. WILSON: We didn't really do question two because it is a slightly different question, not related to the hematopoietic model at all. So we would appreciate if the committee has any comments on that.

DR. CHAMBERLAIN: This is a simple answer from my point of view. It would be that I think it is a little premature to take on this type of a study with the in utero gene transfer. The reason for that is that the observations that Mike Themis' lab have made have been with the FIV vector, rather than the HIV vector. It isn't clear yet whether it is going to hold true for the HIV vectors.

How widely used the Themis vector is going to be is a little unclear, because it is a proprietary vector of a private company. So I think until we have a little more data on that, we might need to wait.

The other thing is that the mechanism of what is going on is completely unresolved at this point. We don't know why he is seeing those tumors. It might make sense to get a little more basic research in there to try to get at the mechanism of action before embarking on a large scale study of that vector system.

DR. CORNETTA: I agree. I think there are some things about that that aren't explained. That is the kind of study that is good to do in a GLP, but I think we need to know more. Whereas, the study we talked about today is really not based on observations of one lab; it is really observation from multiple labs, from now clinical trials suggesting clonal dominance. There is a lot to back up this moving forward in such an expensive and intense study.

Plus, I think for lenti vectors, people are looking at bone marrow as a target. It is not clear what exactly this will be used for, for clinical therapy in the next five years.

DR. MULE: Other comments?

DR. MULLIGAN: For other potential systems, my point of view is that being able to kill the transduced cells is the best safety feature. At the end of the day, that can get a lot of attention.

So I think the NTP activity could be very helpful. There are a lot of fancy suicide systems, but

they have never been tested in the rigorous way you would have to test them to think -- they compare one to the next one, so that would be another place where you could do some of the things, quick studies, but where lots of models would be important, and looking at the escape of tumor cells, that sort of thing, would be very key.

DR. CALOS: I just wanted to discuss another model that I am aware of in liver that was from Mike Bishop's lab, and Dean Felsher works on it at Stanford. That is this MIC inducible model, where you can turn MIC on at various times during development just with a dot cycling type of thing.

What they have observed is, if you turn MIC on early, for example, you get a higher incidence of tumors and all this. We are actually planning to use that system to look at the 5C-31 system, since we are looking at liver as a target, and whether that might be able to add -- or whether that would be considered useful data to have if we do the 5C-31 system with the MIC induction, which basically creates a lot of sensitivity for tumorigenesis.

That is just another system that I wanted to raise, that seems to achieve a similar aim for that organ of just creating a lot of sensitivity to pick up tumors.

The other is just, Luigi Aldini has a tumor prone mouse. I don't know how you feel about that in relation to

Chris' system.-

DR. BAUM: May I comment on the tumor prone models? They are certainly valuable. They may give you a readout quicker than when you mutagenize wild type cells, but they are also more biased, because you only look for a subset of cooperation partners for this particular leading oncogene in the model. They might not at all be clinically relevant in that respect.

DR. MULE: Other comments? Carolyn, do you have what you need?

DR. WILSON: Thank you, this discussion was very helpful to us for both questions. I don't know if time permits, if the committee wanted to go on to question three to have a broad-ranging discussion about other possible investigations for cell gene therapies generally. I know that is a big topic, but that would be our third question.

DR. MULE: Any comments from the committee there? It seems like we touched on some of those aspects during the discussion. The point always is, what are the resources available. We can certainly come up with a number of exciting and interesting areas that could be plugged into the system, but unless there are specific examples of what we can do, then we can move on.

DR. WILSON: I think the idea about using NTP as a resource, wow, that is fantastic. If someone has got the

skills and the money to do these kind of studies, great, let's try to take advantage of it. I think it would be just to watch the field as other systems come along that look promising, that look like they could address some of these issues, if consensus arises that we could make a case for testing them, that would be the general way to go.

DR. CORNETTA: It is probably even in the larger view, because this can be a partner for the FDA. This partnership and the use of this program has generally been for agents that are not biologic agents. I think the agency is now challenged with how to bring and test these biologic agents. It seems like it is a good mix, because there is certainly interest in NTP to look at novel agents, and it meets a need for the agency.

DR. GUNTER: As someone said earlier, small companies and small academic labs could never afford the study we have proposed here today, so I think it is a great idea to have NTP involved.

I would just add that I think there has to be a good mechanism for insuring a competitive and equitable process to compete for that resource.

Agenda Item: Open Public Hearing

DR. MULE: Thanks. So we will move ahead. There were no prior requests for comment at the open public hearing, so if there are any individuals in the audience

who wish to comment specifically on this topic, we have a few minutes. Otherwise, Dr. Rao.

DR. RAO: My name is Mahendra Rao.

DR. MULE: Dr. Rao, before you comment, I will have to go ahead and read the announcement.

This is the open public hearing announcement for general matters meetings. Both the Food and Drug Administration and the public believe in a transparent process for information gathering and decision making.

To insure such transparency at the open public hearing session of the advisory committee meeting, FDA believes that it is important to understand the context of an individual's presentation. For this reason, FDA encourages you, the public, the open public hearing speaker, at the beginning of your oral statement to advise the committee of any financial relationship that you may have with any company or any group that is likely to be impacted by the topic of this meeting. For example, financial information may include the company's or a group's payment of your travel, lodging or other expenses in connection with your attendance at the meeting.

Likewise, FDA encourages you at the beginning of your statement to advise the committee if you do not have any such financial relationships. If you choose not to address the issue of financial relationships at the

beginning of your statement, it will not preclude you from speaking.

DR. RAO: My name is Mahendra Rao, and I am currently an employee of Invitrogen Corporation. I have no direct financial involvement with any of the companies under discussion here.

The only point I wanted to make was related to question three that was raised by the FDA. That is looking at alternative models for screening. One point that was made throughout the committee's deliberations was that you can use in vitro models to stress the system and look at this. The assumption was made that we could look at hematopoietic models.

What is forgotten now is that in the last couple of years, there is a general availability of human cell lines specifically related to stem cell populations or specific derivatives of all of the tissues or organs that one is looking at. It may be very useful to use that as an additional or complementary set of experiments that are done with the toxicology program, because one could set that up and be able to do it.

There is some evidence from the European Commission on Evaluation of Alternative Methods, which has used for example embryo body formation to look at embryo toxicity at early developmental stages using such a model,

which can be expanded to using human cell lines. So certainly for viral toxicity, that may be a useful way to go.

DR. MULE: Thank you, Dr. Rao. So we will move ahead and take a break, and reconvene at about 11:05.

DR. WILSON: If I could just thank the committee one more time. We really appreciate the input and comments from everybody.

(Brief recess.)

Agenda Item: Topic III: Overview - Research Program, Office of Cellular, Tissue and Gene Therapies, CBER

DR. MULE: Let me start by acknowledging Dr. Rao, Dr. Carbone and Dr. Epstein, who have joined us at the committee table.

We will go ahead with the next topic, which is Topic III. It is the overview of the research program of the Office of Tissue and Gene Therapies. Dr. Kathryn Carbone will present the research program for CBER.

DR. CARBONE: Let me just give a little foundation for why I am talking about this.

When Dr. Goodman became Center director and I became Assistant Director for Research, one of the things that we discussed is that we had spent many years evaluating our research program at the

investigator/laboratory level, but that it was a very important task from a management and research leadership point of view to evaluate the research programs at the larger level of the entire office. So we are going through each office that does active laboratory research in the Center, blood, vaccines, and gene therapy, in going through this process.

As many of you know from having dealt with laboratory

level and investigator level site visits, we always bring the discussion to the full advisory committee and the site visit group brings their report for final approval. So today, the goal is to present a high level discussion of research at CBER. I will be presenting the CBER perspective, and Dr. Epstein will be presenting from the office. I think some of this is very relevant to what we were discussing with the NTP project.

I will just start by sharing the vision that Dr. Goodman created when he came to the Center. I think this shows quite clearly that the novel and innovative products, particularly in areas where we don't have good regulatory pathways or historical regulatory pathways, are very important to actively help notice that there is an active component, the facilitation of development, which is also reflected in the FDA's mission statement, which was

formerly not included. So that is part of our goal, to advance public health.

This is just a very quick structure. This is the major office, and then there are several different offices which either have management functions, support functions, or actual product review functions and research functions, as I mentioned. We also have computational research, statistical research, clinical trial research, clinical trial evaluation research. So we have clearly run the gamut in the Center.

One of the things that we feel is important in advocating for an active role in the FDA for public health is that there is an overall disturbing trend over the entire FDA. Despite the terrible diseases that we deal with, many of which still have no cure, and despite a very great boon in basic medical research and wonderful candidate products, and innovative technologies leading to very innovative products that are being developed, there appears to be some disconnect or some inability to translate the products in the same booning way into medical use for patients. Some of that is the natural dropout, et cetera, but the question is, are there ways we can actively step up the process to improve these odds.

What I am going to start with is citing a very interesting article by Dr. Murvis in Science, where he

interviewed many of the individuals from industry -- and I use the statement sponsor, because we recognize fully that our sponsors include academia and other units other than the pharmaceutical industry, but in this case they were interviewing mostly the larger pharmaceutical industries. So he developed this discussion of drug discover and development.

Now, when the critical path initiative was published by the FDA, and I'll talk about that in a bit, the number was quoted as $800 million to develop a new drug. In some ways we were criticized in some circles as being way, way overinflated. So I was happy to see that somebody actually had a number than we did, not that I am happy to see that it is higher, but I think the point is, it costs a whole lot to make a drug.

In fact, I was talking to a group that was very interested in helping support drug development. They were very excited to have a billion dollars to apply to this effort. I said, okay, half a drug. But anyway, lowering that number is the holy grail of industry, and it is the holy grail of everybody, because making the process more efficient and more economical suits everybody to make good products.

So how can CBER do this? From the sponsor perspective, it is not the number of targets validated or

the number of chemicals selected; it is the proof of concept in patients. So having a lot of great potential drugs is good, but in reality, needing the information to cross the doorstep and actually use them safely in patients.

Unfortunately, there are those, I think many, who believe that selecting that right one to pursue is -- and I'm quoting -- a crapshoot, and that picking a winner is something that is an art, not a science. I think all of us find that believable, but distressing.

In our case, keep in mind that although we are very encouraged and are very thrilled to see candidates crossing the threshold, we are also very cognizant of the fact that that is just the beginning. A huge proportion of those drugs where there is a lot of investment fail. I use drugs generically, mind you; I am meaning biologics as well.

The really sad news is that when they fail, they should fail early when there isn't a great deal of investment. How to tell which ones are going to fail in a prescribed scientific and predictable way is a big problem. So that is part of our focus, is to make the process more efficient and more predictable to better serve the American public.

There is a general feeling in science that it is

a creative process, and management of such a creative process destroys it. But clearly, not managing it leads to inefficiency, so how to balance the creativity and the efficiency.

This is a very scary thing to read, but like I said, there is probably general agreement that in many cases, especially in complex biological products like the ones you deal with, that intuition plays a great role, and they are coming fast and furious in how to predict. In some cases, this is the genesis of the interest in us applying to the NTP for a collaboration to study a test that might help us with predictability, or at least be a model of a type of test to help in predicting.

So consider the value of actively and intelligently managing science and research specifically targeting at ways to develop products. Keep in mind, when sponsors come to us and make value statements that may be quite accurate of safer and better, we need ways to measure safer and better that we can rely on. So having an attention to measurement and the tools to measure is very critical for us, and why we call it critical path science.

There is an initiative at the FDA, and there has been recently funded that has been allocated to CDER, Center for Drugs, in the early stages of discussion, for this critical path. It currently is unfunded, and we are

doing our best to support it with the funds that we do have, and by leveraging with phenomenal partners like NTP.

Basically, the goal is to identify, focus and manage the results of specific regulatory and scientific challenges, which will actively improve the development process and product availability across categories of products. We need this science to lead policy, if you will. Policy sometimes precedes or lags behind science, but it always should be informed by science, because uninformed policy is generally not a good idea.

Many people assume that the critical path occurs very late in the process of the official FDA filing, but in reality, it should occur very early in the process, because having better preclinical tests to help identify the products that will succeed and ultimately those that won't will be a huge savings. We of course at CBER get involved very early in the process, and have as many of you know pre- pre-IND meetings with sponsors to try and resolve or prevent issues from arising later down. So it is quite an extended process.

It is not meant to supplant or say it is better than any type of basic discovery or, quote, NIH-type research which is obviously phenomenally well done. In fact, it is used to support the fact that this gets translated all the way to success. So in effect, it is a

very symbiotic relationship.

So scientific research and science -- and you know it better than anybody -- and the kinds of products that this committee deals with is sometimes seen as a little arrow off to the side. In fact, probably the most common question I get is, the FDA does research? Then the second question always is, why does the FDA do research?

What we try to make clear in this critical path initiative is that an application will come in. We see problems across the sponsors. We see opportunities to solve problems across the sponsors. We identify those challenges, and then we turn to scientific solutions, or at least the beginning of scientific solutions. It doesn't mean we solve every problem, but we are often in a good place to help identify problems across different categories of products, but academia, other government, science, industry, we are all working on this. So by working on this, we can recommend a scientific solution. We can go and get public input.

This is one great example. We identified a problem, as Dr. Carolyn Wilson and her group have identified a problem. We have brought it to the committee to discuss best ways of resolving the problem. When we get some information -- and as Dr. Goodman said, there will be early phase of information and late -- that can apply back

to the guidance and standards, and then can be used within the review process.

Typically, we don't say to sponsors, here, do this test and show us the data. We say, we need to know this information, here is an example of some test which seemed to have been valuable, show us what you have, please provide us with the information. They are welcome to go and do anything they want, but I have generally found in my years with the FDA, it is very much appreciated to have some specific guidance about ways to approach things which have been shown to be successful.

So why CBER? Why the FDA? Like I said, why does the FDA do research questions? The innovators create these phenomenal scientific tools based on their products, and then expend their resources to evaluate their products, and this all remains in the proprietary trade secret boundaries, appropriately so, since this is the way the system works, but in general, we can't necessarily require -- in fact, we can't require general important questions to be answered in the context of specific product questions. That information that comes in on specific products remains proprietary.

What we have in CBER is not just expertise in standard scientific disciplines, which we do, but what we also have are those expert people who also understand

product development and how to regulate a product. That is not standardly seen in most basic biomedical settings in discovery and research arenas.

CBER research regulators see successes, failures and opportunities across whole classes of exciting, innovative products, and want to help. As you noted, the lag time between the time we can start the NTP study and get the final answer could be years. Therefore, you can't wait to start these studies until it becomes apparent in the public domain that there is a problem brewing. These problems need to be identified quickly and addressed quickly, so that the problem doesn't arise down the road; it is already resolved.

The guidance documents that we can base on science will provide a clearer and more predictable regulatory path. Sponsors in industry and other people who are investing in these products appreciate having a clear path to walk down. When the path becomes murky, it becomes inefficient and often becomes expensive, and that slows the development of valuable new products for the public health.

So CBER can play -- if you will a disinterested party, since we are the lack of conflict party here, we can play a convenient coordinating role for scientific interests across sponsors. Many people have expressed a doubt to me as to whether sponsors in an economic setting

would cooperate in this manner. There are many examples where the FDA has already led groups of cooperation. There were several sponsors applying for digital mammography readings. Not one sponsor had enough data to use with their method in order to validate it. So the Center for Devices got together, got a consortium together, everybody pooled the data, and every sponsor was able to use the pooled data to validate their method. So that was an example of where the FDA coordinated, and so the process moved ahead.

What we do at CBER in order to have people who truly have this expertise in the regulatory realm as well, and product development realm as well as science, we have a pretty unique -- I won't say completely unique, but fairly unique among the FDA centers, this research regulator model, which means our people working in research and creating new science and resolving these critical path problems are also the people actively reviewing the INDs and PLAs, doing the inspections, helping write the guidance documents, participating in the evaluation of adverse drug reactions and risk assessments and license products. Along with their regulatory scientists, partners, clinical review partners, are part of the review team that actively sees the product. This gives I believe our researchers a specific insight into the issues.

How do we actually get the rubber meeting the road? We have a small and very hard-working intramural research program. I will leave the details for Dr. Epstein to present, but we do what we can in the high priority areas that we can answer.

But I think importantly, and an NTP study is a clear example of this, we find something important that needs to be done. We propose to the NTP that this would be a good study. They agree to work collaboratively, and we take off and we get something done that we could never do on our own.

But in addition, simply talking about these sorts of issues, raising the awareness, if at any point the program was funded to do so, we could actually actively engage in an extramural program to support this kind of activity. So the outcomes are numerous, and I won't elucidate those.

I have undergone an effort to do some cultural change in thinking about this kind of science, because it is quite novel, and people often say, NIH is just basic science, you guys do applied science, it is clear. Well, it is not that clear, because a lot of what we do will sometimes be a basic activity, but it is directly applicable to a product.

There are many examples of this. There was one

example, there was a problem with developing a particular product. It was manufactured in a random way. The product was very variable, very hard to measure, very low yield and resulted in shortages. So our biochemist said, it strikes me that if it was done in this way, you would improve those problems. Sure enough, they tried it, a uniform product, two to three times the yield, and that now is being used in developing countries to manufacture products, or at least test products manufactured that way. So it is basic biochemistry directly stemming from a manufacturing issue in a product and now going out to improve public health. So regardless of the type of science, the key is the question that it resolves and the problem that it resolves.

We track now specific applications that are directly supported by the research programs, and I will let Dr. Epstein talk about those specifically. We have in our center, because of our service as a public health agency, a lot of work on biodefense and pandemic influenza.

The major goals in this case for OCTGT particularly, to create efficient high quality regulatory pathways where there are none, applying 21st century science to improve the efficiency and accuracy of established regulatory pathways -- some products we deal with were licensed over 30 years ago and we want to continue to work with manufacturers to improve those --

focus on the outcomes, is the key.

I went through and analyzed the program that we have. A program is basically divided amongst the problems that we see and the issues we need to resolve -- safety, quality, efficacy and other, other being often products that are not yet licensed, that we have more mechanistic work.

We have a formal review process which we are working to improve and continually modify to make it as on target as possible. So we are developing and have in place a current, but we are also improving a formal process for internal expert evaluation of proposed research plans, based on priority and scientific quality, the internal and external evaluation of past research achievements coming out of the research programs, internal management reviews which are done on a yearly cycle using annual research program reporting in a web-based system we have, external site visits, in which many of you have participated at the research regulated level, but now the office, and then we look very clearly at outcomes that directly impact the regulatory process, publications, policy guidances and research QA/QA, meaning the quality assessing that we do.

In managing CBER research goals -- I'll just finish with a few slides on this -- we developed through management goal ways and our research leadership guiding

research principles for the offices and then the whole Center. We formed a CBER leadership research council that coordinates the development and implementation of these principles in the management practices. We have met a couple of times now, and have made some good headway, and we have an agenda set up for the next 12 months.

The guiding principles are one of those things where everybody goes, well, naturally, but I think it is very important for our staff to be very explicit. So the research program should be of necessity, because we have a large variety of stakeholders that we work with, collaborative and include all sorts of sciences. Its scope encompasses both the scientific basis of the preclinical and clinical studies, but areas which are somewhat under appreciated in basic biomedical research of manufacturing, regulatory submissions and how to evaluate them, the science of clinical review, for example, inspections, part of manufacturing science, and then postmarketing surveillance and guidance development.

The research that we do must be of necessity high quality, efficient and very directed, and managed to provide outcomes that address our scientific and regulatory challenges in product development, including safety, efficacy and quality.

The research leadership council is composed of

the Center's scientific leadership and an accompanying regulatory science leader for every office. So Dr. Epstein serves, along with a member of her regulatory scientist leadership.

Tasks for innovative processes for insuring stakeholder input into the research program, of which this discussion is one, prioritization, goal setting and developing tools for assessing the work load both in the regulatory and research arenas. We have because of the necessity of our small program developed -- which is appropriately in product silos in terms of administrative lines, but because of scientific expertise goes across the Center in many cases, we have developed a matrix virtual team program where all the scientific expertise experts are identified in particular areas. The goal is to bring them together to enhance the critical mass in that particular scientific discipline.

I will just end with some opportunities to give you an idea of some of the things that have reached our interest in a critical path meeting that we held, a workshop, with stakeholders in 2004. But we always encourage continued advice and input into these sorts of agendas.

For OCTGT, I pulled out some of the better characterizations of cell therapies and links to

standardized clinical lab outcomes. In other words, can you test the cell with some kind of test and provide a biomarker of a cell which would then tell us how cells are going to act once it is given to a patient. It is interesting; in many settings biomarkers are clinical measurements of patient responses, but I finally managed to raise the thinking in some areas, that this is different, in that we seek out many times biomarkers of the products themselves.

Appropriate toxicology approaches for complex biological products. Again, you have heard an example of that today. New assays, standards, biomarkers, surrogates, always a good idea if you have surrogate markers in terms of efficiency and quantification and prediction of development. For tissues particularly and cells, multi-pathogen detection methodologies, and even inactivation which is out there, but something that could be very valuable, improved longevity and storage, this is a problem with cell therapies, and enhanced clinical trial design and analysis.

So I just want to thank the committee for listening to our presentations today. I want to thank the site visit group for coming and giving us their opinions. I am happy to answer any questions if you have any.

DR. MULE: Thank you, Dr. Carbone. I think what

we will do is hold the questions until we finish the presentations, and then ask you to come back to the podium. Our next speaker is Dr. Witten, and she is going to discuss with Dr. Epstein, who will follow up, on the Office of Cellular, Tissue and Gene Therapy research program.

DR. WITTEN: Thank you. I am going to give a brief overview of the Office of Cellular, Tissue and Gene Therapies, and then introduce Dr. Suzanne Epstein, who is going to talk about the research program in the office.

As you all know, the mission in OCTGT is to facilitate the development and access to safe and effective medical products. As you have also heard over the last couple of days, our products are so novel that we are in the unique position of seeing the products develop in tandem with the development of the assessment tools, which don't currently necessarily exist.

I am going to briefly cover these three topics: How we use science in research and review, what our products are, and an overview of the office.

I think Dr. Carbone has already covered this, why do we have research in our office. Since our goal is to bring scientific advances to the medical product development process, we are in the position of seeing what some of the gaps are for what I would call in particular some of the assessment tools that might be used for these

products. They are the ones you have heard about the last couple of days, assessment tools for potency of some of these unique products, as well as models for assessing safety before you might even go too far with your study of your gene therapy product.

The goal is to use this science in the development of regulatory guidance and practice, so that we can give sponsors the best advice and help as these products develop for medical use.

I just would like to mention that under the concept of science, we would consider not only the science that is done directly in our own labs, but collaboration of the sort that was being described this morning with the NTP.

This is just to say we recognize we don't do it alone. Some of our research is in collaboration. Certainly we have many stakeholders, both in terms of their interest in the products, in the science, and in the developmental research from the public as well.

These are the products that we regulate in our office. We mostly discussed cellular therapies, tumor vaccines and gene therapies today, but we do have many other products in the office. Many of these aren't really mutually exclusive categories. That is, some of the cell therapies could be xeno products, for example.

The office is organized into three units. The largest division, the Division of Cellular and Gene Therapies, is the part of the organization where there are basic laboratory science as well as product review. The Division of Human Tissue Products is the division that developed and administers the rules for the regulation of human tissue, and the Division of Clinical Evaluation and Pharmacology/Toxicology has both medical officers and pharmacology/toxicology experts who review products in their development stage.

There was since the last meeting of this committee in which the site visit took place a reorganization of the laboratories in DCGT, the largest division that I just mentioned on the page before that. There were basically three goals which were accomplished: To consolidate the research reviewer, investigators who had similar backgrounds into similar laboratories, a change in the name of the branches to reflect the regulatory as well as the scientific goals of each laboratory respectively, and also to achieve a critical mass for each lab for efficient operations of the laboratory grouping in the division.

As my slide, I will provide you with the new structure. You have this in your handouts, too. There are now three laboratory

branches and two branches that focus on product review. However, the reviews are done across the entire division and the review policies and the science, there is a lot of cross fertilization between the laboratory/review branches and the purely review branches.

So that concludes my brief remarks. I want to introduce now Dr. Suzanne Epstein, the Associate Director for Research in our office, who will briefly mention the site visit and give an overview of research in our office.

DR. EPSTEIN: Thanks very much. Those of you who participated know that we had our office-wide research site visit in September. That group was convened to review the entire research program. There were some members from this advisory committee, and then there were a variety of invited experts in a variety of fields.

At that time, we presented our critical path priorities and projects. We discussed research practices within this office, and then gave some examples of some accomplishments. I am going to do that in much briefer form today.

You have already heard why there is research within CBER. In this particular office, cellular tissue and gene therapy products call for -- especially because they use new regulatory paradigms, which are still evolving, that has been mentioned, and then some scientific

issues important for progress in this field are not being adequately addressed by other segments of the community, so CBER participation can be very helpful.

Our research can address entire product classes, rather than individual sponsors' products, that can avoid the need to revisit it, but it also means we will put the data out in the public domain. So CBER research can help fill some gaps.

In terms of research management, Dr. Carbone brought this up. The idea is conscious management of the research programs rather than just letting it run free. So we have some communication tools to try to foster a coordinated program. Within the office we give work in progress presentations. There are also abstracts of each research program that are available to the entire staff, and they are on the website. Then the annual reports are available to the entire staff.

Our communication tools beyond the office are of several types. Within the FDA, there are briefings of CBER leadership, for example, Drs. Goodman and Minton, and then of agency leadership when it is called for, and we have also participated in a series called CBER grand rounds, that is attended Center wide.

We also participate in information exchanges with stakeholders. This includes publications in the scientific

literature as well as regulatory publications, talks at scientific conferences, workshops which we either sponsor or may just attend and participate, and then advisory committee committees like this one. So we have a variety of ways to get our information out into the public domain.

Another area of research management is the use of tracking and measuring tools. This was described in somewhat more detail in September. I am going to make it very short right now, but if there are questions, you can ask them.

Within OCTGT, there are several items that are now being tracked. I track publications of each investigator once per year, also the external funds that are brought in via grants, CRADAS or interagency agreements or patent royalties, and then staffing levels. That includes staff that are brought in using grant funds. That way, we have a picture of a particular investigator's enterprise and their productivity in publications.

Also, we just recently started tracking patents. There is workload tracking being developed.

In terms of research strategies within OCTGT, our goal is to anticipate needs within the field, as well as addressing current problems. As you heard this morning in the NTP discussion, we will be playing catchup even if we do our best. We certainly don't want to be behind the

times, addressing only the problems of today when the problems of tomorrow will be upon us before we know it. So we are trying to stay ahead of the curve as the products and technologies change, and the change is very rapid in this field. We want to perform studies relevant to entire product classes as I mentioned before, make the results public and thus accessible to all.

Some of our research uses current product systems, and it may be obvious, the connection of a study to a specific product, but some other projects address underlying issues that we must understand if we are going to move these products forward. It is not always going to be one for one, but if we can't understand fundamental issues about the biology of these systems, our regulatory decisions and policies will not be well informed.

Our process for identifying priorities is indicated here. We receive input about new products on the horizon from a variety of sources. This can include pre-submission inquiries, and you heard earlier that we have a lot of contact with sponsors very early, through pre- pre-IND informal inquiries as well as pre-IND meetings. We also learn about such new initiatives at scientific conferences and through the literature.

Given those new products on the horizon, we identify anticipated areas of major product activity and

the related critical path issues that we can see coming. We then monitor our existing staff and programs for gaps and weaknesses in expertise or redundancies, and then we address them.

Just to give one example, a few years ago we identified the need for adenovirus expertise, and considered that a gap in our existing programs. So we recruited an adenovirus research expert to fill the gap and address those issues.

So using that type of horizon scanning, what priorities have we identified recently? Over the past year, here are some of the areas that we had identified as important for future product review needs. One is tissue engineering. There, we are exploring partnering and leveraging. There is also some adapting or as Dr. Carbone sometimes calls it, flexing, going on by some existing staff who are reorienting some of their projects.

Another important area is cancer biology. The clinical staff identified cancer biology models for surrogate end points as a critical need. We have a very large number of products in the cancer area. In this area, we are building on our existing research programs. We already had some existing in-house expertise and the capabilities have recently been expanded by acquisition of an imaging system by CBER in a coordinated center-wide

effort. So that can be used in those studies.

In the area of bioinformatics, another area of need, we are enhancing and leveraging some existing FDA capabilities, but we will also need to collaborate outside the FDA.

Then finally, the area of protein chemistry and proteomics was identified. This advisory committee I believe participated in a site visit of an individual who has now left the agency, and we were advised to replace that type of expertise. We concurred with that. A recruitment was undertaken, and even in these difficult times, that has now been successful, and a new investigator will be arriving in April.

I will just mention that that investigator like the previous four most recent tenure track investigators brought in, is form outside the government, from a university.

I am now just going to give some very, very brief examples of the types of projects and accomplishments that we have.

In the area of gene therapy, some of the major issues include vector safety and characteristics, as you heard earlier, and then patient immune responses, which can have an impact on safety and efficacy. Our strategies and projects include adenoviral studies, retrovirus studies of

safety and detection, herpes virus studies of vector safety and characterization, and then studies of host immune responses that are induced by viral and plasmic vectors.

Here is just one example. In the adenovirus field, the public health issue includes unexpected toxicity which was observed in a clinical trial with a death, and there was not a good animal model for understanding that. So the CBER investigator performed studies which have had some very valuable outcomes, insight into how adenoviral vectors can cause toxicity. They provided an animal model for gene therapy in the specific context of pre-existing liver disease. That is important, because that is one of the contexts in which vectors were being used. Sponsors are now utilizing that information in clinical trial design. Reviewers can use it in advising sponsors. And the model can be used for safety testing of new vectors by comparison.

You heard a lot about the NTP program this morning, so I'm not going to go through this in detail. But I will just mention that it is another form of leveraging. The retroviral model was discussed this morning. The last bullet on this slide refers to a different study, a quantitative assessment of plasmids for bio distribution, persistence and expression of transgene. So that will also provide a platform for comparing new

vectors bridging to new modified vectors and formulations. if we have a validated model for that, each sponsor won't have to start over.

Moving on to cellular therapies, in this field, some of the major issues include controlling the growth and differentiation of the cells, product characterization and immune rejection; how will you know where these cells will go when they are put into the patient, how they will change, and what they will do.

The strategies of our scientists to address these include studying key signalling pathways that determine cell fate, what do the cells differentiate into, cell death, you don't want your product to go in and die immediately without having done its job, and then development of anatomic structures in the cases where the cells are supposed to form part of a structure. In addition, we have studies of immune cell activation and the immune responses to cellular therapy products, because that can be a barrier to therapy.

The outcomes of these various research programs have included approaches to identifying markers for product characterization and process control. We need markers better than viability or a vague phenotype. We are in an era where we can have molecular markers for the cellular phenotypes.

Going on how to the area of tissue engineering, in this case, some of the major issues include the interactions among components that will yield proper tissue structure and function if a product is put into the body.

Some of the strategies here include studies of tissue anatomy and factors that control joint development. You heard yesterday about some products involved in joint repair, so this is a current field. In addition, molecular signals determining liver development. The outcomes here for example include identification of some novel factors that contribute to successful joint formation at anatomic boundaries.

Going on to xenotransplantation, the major public health problem is of course organ supply, the deficiency of sufficient organs. Some of the scientific barriers in the field include transmission of infectious agents between species from the donor to the human host, and then immune rejection impeding xenotransplantation.

Strategies we have used to address them include studies by an investigator of porcine endogenous retrovirus or PERV, using assays for detection and studying species tropism in transmission. Another approach has been studies of transplantation immunology, how can we modulate and reduce rejection reactions. So the outcomes here include for example a useful and practical assay for PERV and some

approaches to minimizing rejection.

Going on now to another product area, tumor vaccines, in this complex field with a wide variety of product modalities, the problems include product characterization and the need for accurate tests for identity, purity and potency. You heard some about this yesterday with the potency assays.

Some of the in-house strategies include studies of animal models of targeted interventions in tumors, markers of tumor growth that can be used for marketing both in preclinical studies and potentially in clinical studies, and then immune response assays that could potentially be used for potency tests, such as T-cell activation.

The outcomes have included in one case a marker of identity for tumor vaccines and serum biomarkers for monitoring.

Now I'll just mention briefly the application of new technologies. This is not to a specific product area, but to all product areas. A variety of new technologies offer an opportunity for better product characterization. For example, gene expression microarray and flow cytometry which were both discussed yesterday. Flow is in a more advanced stage of use in actual product characterization, microarray is in an earlier stage, but both need to be developed.

So high throughput screening can give you much more detailed information than traditional assays. This can be used for characterization of cellular products, also of cell substrates being used to produce vectors or other materials, and it can also be used for patient sample analysis in the trials.

In the flow cytometry area, which is further advanced, as we have mentioned, there has already been significant standardization. The assays need to be standardized to permit comparisons. CBER has been a leader in participation with NIST and CDC in a federal standardization initiative. There is now a fluorocine solution available as a standard reagent, and there are standard microbeads available for calibration of the instruments. These standard materials make it possible to compare data across different times, different assay dates and between different laboratories, making both product and clinical comparisons more reliable.

We have participated in additional reference material development. There is a retroviral reference material shown on the left. This is now available from ATTC, and it can be used to show that RCR, or replication competent retrovirus, assays are of a given sensitivity. That reduces the volume of vector supernatant that has to be tested without compromising product safety, so that has

saved sponsors money and made the results more reliable.

In the adenovirus area, there is now an adenovirus reference material also available from ATCC. This allows different sponsors to normalize their detection and sensitivity of viral particle counts and infectious titers. Currently, external RNA spiking controls are being developed for use in microarray and RT PCR.

The outcome of all this work is providing sensitive consistent testing, which in turn can facilitate progress toward successful products, and can save sponsors money.

So to summarize, we see the management and prioritization of our research programs as an ongoing process. These new product classes present novel scientific opportunities, but also novel regulatory challenges. We try to identify scientific questions that are of regulatory importance and address them. The solutions to certain key problems can facilitate product development, and also of course inform our regulatory decisions and policies as we develop them.

We welcome suggestions and advice from the committee.

DR. MULE: Thanks, Dr. Epstein. Comments from the committee? Dr. Calos.

DR. CALOS: Kathryn mentioned this phenomenon

about products failing late in the cycle. What is the main reason for that? Is it just the numbers game of more patients?

DR. CARBONE: I think that is a very good question. In fact, I might direct you to an article that was published by our staff. I think it was specific to vaccines, but it looked at clinical holds, which is a surrogate for failure because there is a reason why a product is put on clinical hold, and they evaluate the reasons.

In many cases, I would say the problem is often an untoward and unexpected safety concern which arises. Safety becomes a big issue. It depends, it is all a risk-benefit analysis, but for example in vaccines it has become terribly problematic. A recent study was done of 60,000 children, which based on a safety paradigm, that is the number that was needed. You certainly don't want a 60,000-person trial to fail in that stage. You would like to have a better predictability early on. So often it is an untoward adverse event that wasn't particularly expected.

Sometimes it is a very late failure, where the product is safe, the product is effective, and it can't be manufactured in the scale that is needed. So often there are very experienced manufacturers who know about manufacturing, and there are sometimes sponsors who come in

whose expertise does not lie in that area, and the scaleup has not been considered and the product fails on that level.

But I think the larger question is evaluations of specific reasons of biological products for failures would be a very valuable pursuit. I know that has been done on the vaccine end, but we certainly could go further.

DR. MULE: Other comments, question?

DR. URBA: I just had a question which I think you sort of answered at the end. Looking at it from the outside, how hard is it to recruit people and place people, keep people? You did mention you were able to track four people in a row from outside. That sounds good, but when I look through this, there do seem to be a number of open positions and acting positions, so how can we help with that?

DR. EPSTEIN: Historically, CBER is a fairly attractive research community, and is viewed that way by people at NIH and outside. So I don't think there is a severe problem of recruiting. It is of course a very different environment, and people have to come in with expectations of smaller groups, fewer resources and different responsibilities.

But I think the research staff has pretty good retention. When you refer to actings, that is in terms of

people being selected into additional roles, I presume. It is not that there are openings, vacancies, but we face a constant challenge of resourcing the programs we have, keeping them viable, keeping them competitive.

DR. PURI: May I add to the point, that many times the acting positions are told to get the appropriate person to come into that position. So for example, in the Division of Cell and Gene Therapy which I direct, I am an acting branch chief of tumor vaccines and biotechnology branch, because there are a number of junior people in that group, and perhaps I identify someone from outside in the future.

DR. GOODMAN: I think one thing the scientific community can help us with is when we are interested, when we have openings. Right now, the federal budget is obviously very tight, and that is not going to change very quickly. But I think when we have openings or through your collaborations with our scientists, are ways of attracting people who can like and thrive in this unique niche.

I was an academic scientist. It is not a place typically to build that kind of program, but it is also a wonderful place where you can be involved in some really interesting problems, help advance things. I think there are some people who really take to that well.

Now, that said, your work in supporting this,

helping us identify the important issues can also help us build support. I think it is an area where there is incredible opportunities to do a lot more, if we could.

In the agency's critical path initiative, I think it is very important that different scientific communities help us keep things on that agenda, such as what we are doing with the National Toxicology Program, that can benefit these other areas of science.

So I think you can play a helpful role there. But we are very conscious of the limited resources. We often have resources that are more than limited, where we have to deal with some of the highest priority issues that come along, and we have to shift resources. So it is tough. We also need people who can function under those circumstances.

DR. GUNTER: I have a question about clinical reviews. This is more of a regulatory question. First of all, you have a wide variety of different kinds of products that you have to deal with, requiring a wide variety of clinical expertise.

Also, we heard yesterday about some tumor vaccines that are coming at us pretty quick, and we are going to have to make some efficacy and safety decisions on clinical data pretty soon, probably.

So my question is, can you speak to the clinical

review that you could apply to those kind of products, and is there opportunity for cross-talk between CDER and CBER or other parts of CBER? I'm just curious as to how that works.

DR. WITTEN: I guess I should answer that. We have a number of clinicians, both oncologists and people with expertise in internal medicine and other subspecialties in internal medicine in the office who review as you say a wide range of products. They work extremely hard. We are actually recruiting for an additional oncologist, so I'll just mention that just in case you know anybody, but I know that is not the purpose of this meeting.

But we do have active dialogue, both with the clinicians in CDER and also in CDRH, because some of these products do share some of the issues of implants that you might want expertise from the device area as well, for example, in the cardiology arena. We also have available for us GEs for consultation that we have also called, and on occasion, people who are GEs like advisory consultants to the advisory committee for advice on specific matters, too, during the review process.

So I think we are quite skilled at jumping into a new area and in getting the expertise that we need for that. We do have some regular formats for discussion with

CDER, in oncology in particular. There is a cross-FDA group that includes both our group as well as the CDRH people with an interest in oncology, and the CDER oncology people, to look specifically at coordination for some of the oncology clinical programmatic issues.

DR. CALOS: We see a lot of press about the FDA commissioner and stability in that office, or instability. I am just wondering how affected are you by all of that.

DR. GOODMAN: We have got a lot of work to do. We are fully engaged in that. Dr. von Eschenbach is supportive of that. I think we are forward looking and driven by the work. I think that suffices. I think things are going well. I think that you can hear some of the kinds of things that we are doing to build cell and gene therapy and support this agenda.

Dr. von Eschenbach is very interested in what he always talks about as the pathway from discovery through development to delivery. My view is that is a perfect match for what our Center does. But certainly you like to have stability. All of these things can present specific challenges, but I think the centers and the scientists and our staff and office directors and division directors, we have our time lines and deadlines and important public health issues, and that is what we focus on.

DR. HARLAN: It has sort of been said already,

but I will just emphasize it. I buy hook, line and sinker, 200 percent the need for CBER research, and all of the reasons you gave, Dr. Carbone in particular. But I didn't hear, and just wish to emphasize that if I have a product that is being reviewed, I want it being reviewed by someone that is in the business and doing it day to day so that they know the problems and the issues and the science. The only way you can do that is by being in the business. I would just emphasize how important I think that is.

DR. URBA: I just had a question on the previous topic, not the FDA leadership topic. Pazdur, oncology -- I don't know exactly how to describe that, but the idea that there is a separate way of doing, is that the connection between CDER and CBER you were mentioning, or is that something separate, and will that involve us if a vaccine cellular product in oncology comes to this committee?

DR. WITTEN: I'm not sure of your exact question, but maybe I should clarify what I said previously, which is, Dr. Pazdur is both the office director for the Office of Oncology Drug Products, as well as the director of the oncology program. In that role as the director of the oncology program, he has convened a group across the centers to look at issues that we have in common, make sure that there is good communication on review issues as needed, and policy issues as well. So that is part of that

role that I think you are asking about.

Does that answer the question?

DR. GOODMAN: I can add, since some of the organizational changes that occurred a couple of years ago, we have really put a lot of effort into -- just in general, because it is the right thing to do and it is the way to leverage resources.

We do have very close interactions. For example, there are products where we may get official consults. This isn't just in cell and gene therapy, but in other areas. There are regular meetings of the oncology groups, where our reviewers and reviewers from CDER sit together and discuss specific clinical issues. So there is a lot of cross fertilization.

On the advisory committees, I think we would again approach that like we frequently approach it, which is how do we get the best input in the group of people at the table for considering a product, if an oncology product was to come. That could include additional outside experts, like you have seen in specific areas. It could include members of other advisory committees or some amalgam of those things.

Again, we would also welcome your input as some of these things and how best to address them, but the point at the end of the day is to get the best science, the best

advice, the best review. It doesn't matter to us where that comes from.

DR. PURI: I just want to add, we have an interagency oncology task force between the FDA and NCI. I am a subcommittee chair of a joint training and fellowship program, and Dr. Pazdur is part of the committee with me as well.

DR. TOMFORD: How is the research funded, by contract or what? Does CBER have laboratories, or how does that work?

DR. CARBONE: CBER has an intramural research program that comes out of our operating funds. The staff, who are also regulators and reviewers, are select members of the staff, depending on qualifications, why they were hired, what are the interest areas they represent, are offered the opportunity to be fairly minimally supported in terms of laboratory staff and space.

We have three buildings on the NIH campus and a research building off campus. We have BSL-3 laboratories, animal facilities, a core facility, NMR; we are pretty well staffed from a laboratory setting. The operating funds that support the research are fairly limited. Since we are not intramural NIH, we have the opportunity, and are selected and reviewed and evaluated in circumstances to obtain outside funding. So we have a process within the

FDA to review at the center and at the FDA level any sorts of granting opportunities for lack of conflicts of interest, appropriateness of the research being proposed. The National Vaccine Program Office provides funding to inter agencies for research purposes, and we tap into that, as does NIH and CDC.

We have some IAG work that stems out of some research in cell substrates, for example, with NIAID. But I would say approximately 70 percent of the day to day support of the laboratories in terms of supplies comes from external sources, but there is an intramural program.

We publish about 200 to 300 publications a year in about 100 different journals. This is all fairly well tracked.

DR. CHAMBERLAIN: I just have a general question about the scope of the Office of Cellular, Tissue and Gene Therapies. You referred specifically to the regulation of tumor vaccines, but there is a growing interest in using what might be considered gene therapy vectors for developing vaccines against infectious diseases. Who regulates that?

DR. GOODMAN: The way we have it structured now -- again, one of the nice things about being a pretty small center is, people work together and talk together, and we try not to have a lot of silos. But in general, because

the paradigms in preventive studies for infectious diseases and vaccines for those line up with the typical vaccines. I think our current construct is, those would be done through the Office of Vaccines.

But for example, using a plasmid DNA vector or whatever, if we had other expertise in the house, like Dr. Epstein, in the area in which that product was, they could be part of the review team, they could be specifically consulted for advice about the product, et cetera.

What I always tell people is, let's not make a round peg fit into a square hole. Let's get the right people together. As Celia said, we will go outside the center for that, too. We just don't have the resources to have expertise in absolutely everything in every place in the center.

So for example, in tissue engineering right now, just as an example of an innovative approach, we have a joint team from our center with expertise in biologics and cellular products, working with people from the Center for Devices, who have expertise on the device part or the matrix part. I think we take this approach internally.

It is a long convoluted answer, but basically because the clinical trials for prevention of infectious disease, the infectious disease expertise, there is a tremendous amount of that in our Office of Vaccines, so

that would probably be the primary site for those reviews. But it would involve experts in the science in other areas. We have experts in the Office of Vaccines as well on DNA vaccines.

DR. CARBONE: Just to mention, in our research management teams, because we are a relatively small program, it is important to leverage expertise even between the offices. So coordinating the associates for research for each office is my job in communicating what expertise is across the center. We may have a malaria expert in blood, but they may serve as the review expert in other offices when malaria issues come up.

So we solve some of this stovepiping problem by developing these virtual teams of expertise across the offices. So we will utilize people broadly when we have a limited number of people in that area.

DR. MULE: Thanks. On behalf of the committee, I'd like to thank Dr. Carbone, Dr. Witten and Dr. Epstein for providing us with the overview of the research programs.

This concludes the open session. At this juncture, we will take a five-minute stretch, and then continue with Dr. Rao's presentation at the closed session.

(Whereupon, the open session was adjourned at 12:10 p.m.)