DR. PILARO: The therapeutic dose, I actually only touched on in the hemophilia study, and the therapeutic dose was actually tenfold lower than the no observable effect level dose. You had therapeutic levels of the gene being expressed at lower doses. That's what gives you your margin of safety.

CHAIRMAN SALOMON: So, that is a good--

DR. PILARO: Yeah, that is a good thing. That's a good thing.

CHAIRMAN SALOMON: So, if your effective dose is lower than your no adverse effect--

DR. PILARO: If you're tenfold lower than your toxic dose, then yes, you're in great shape.

CHAIRMAN SALOMON: The last question is when you compared all those species, it was in the cystic fibrosis trial where you had the NOAEL's for mouse, hamster, cotton rat, rhesus monkey, baboon and human, how significant are these comparisons then? I mean, the rhesus monkey, for example, gave you 8.2-times-ten-to-the-seventh and the baboon, 1.8-times-ten-to-the-ninth, and the human, 1.2-times-ten-to-the-seventh. Does that mean that the baboon is not a good model for the human?

DR. PILARO: Actually, the data out of that study are somewhat old now. The rhesus monkey no observable effect level dose is probably at least two logs higher now.

Those studies were conducted with early generation of vector, and it is probably not fair to have that one in there because that was actually done very early on.

The baboon, the monkey and the mouse actually showed the same pathology in the lung. They all had perivascular infiltrates. They all had peribronchial or cuffing of leukocytes. There was inflammation present. There was edema present in the lungs. When you actually looked at the one patient that developed the toxicity, she had a white-out of her lungs on X-ray. She had basically infiltrates all over the place, too. It was very predictive when you looked at it, but the mouse was just as predictive as the baboon in that particular group of studies.

CHAIRMAN SALOMON: Not to belabor it, but the data for the human, though, is 1.2-times-ten-to-the-seventh, which is two logs lower.

DR. PILARO: Well, and that was the only dose that was studied lower, so at this point in time we actually have additional data that shows that the NOAEL, the later study that I showed you, the NOAEL is three-times-ten-to-the-ninth PFU after repeated administration. It actually is higher. It is up in the same range as what was seen in the animal studies now.

CHAIRMAN SALOMON: Thank you. That clarifies things.

DR. JONES: Hi. My name is Estella Jones. I am a primate veterinarian and I'm the chief of the primatology lab for CBER, Center for Biologics Evaluation and Research. Today, I am going to discuss the advantages and disadvantages of using primates in biomedical research. I will frequently try to stay away from acronyms, but the animal care world has its own life of acronyms, as you will see. You will see me using NHPs, and that means nonhuman primates.

I try to stay away from the use of the word monkey, because it means a lot of things and a lot of times every nonhuman primate is not a monkey to us. Now, we know--the things that come to our head when we say advantages. We know that nonhuman primates closely resemble us, genetically and biologically, so we think that they are good animal models for a lot of diseases and we have obtained a lot of relevant information, and they're valuable research subjects for a lot of diseases, certainly.

But, to date, we have really only used about 30 of these species out of 200 that are available. There are some disadvantages that come to mind, especially when you talk to primate veterinarians. There are increasing animal welfare regulations and legislations, cost and supply limitations. They are comparatively lower with reproduction rates. They have very long gestation periods, if you compare that to a

mouse, every 20 days. They have a lot of special regulations, as you're about to see.

We will start with federal agencies. You can decide for yourself whether that is a or a con. Department of Interior, some of these will be surprises. Fish and Wildlife Service, actually, Fish and Wildlife regulate the Endangered Species Act. They also enforce the Lacy Act. Some of these things don't seem like they need enforcement, but, for instance, the Lacy Act came up with the idea that animals do need ventilation when they travel overseas. That one should be a no-brainer, but actually that one needed an act.

There's something called CITES, which you will see if you have ever worked with chimpanzees. By the way, chimpanzees are not monkeys. Most veterinarians get very offended if you call chimpanzees monkeys. They are great apes. CITES stands for Convention on International Trade in Endangered Species of Wild Fauna and Flora. For that one, I will use an acronym. Department of Health and Human Services, under that one falls the NIH.

NIH is responsible for developing PHS-funded organizations that are ALAC-accredited. That one, I will also use an acronym. ALAC stands for--it used to stand for American Association for the Accreditation of Laboratory Animal Care, but now they're calling themselves ALAC

International, and that one is too long to say. There was OPRR, which is now OLAW. We like to make up acronyms and then change the name, too. That keeps everybody confused.

There is the National Chimpanzee Management Plan that also falls under NIH. The Interagency Animal Model Committee for Review of Research Protocols Utilizing Chimpanzees--there is the NIH Intramural Nonhuman Primate Management Plan, and then there is the SPF Rhesus and Cinemologous Breeding Program that NIH is responsible for, and that started in 1988.

Then, of course, there is the Centers for Disease Control, and they are responsible for overseeing the importation of nonhuman primates into the United States and preventing the introduction of new diseases; one of those is--some of you may remember the Ebola-like scare that we had back in 1989. I was around for that one--yellow fever, monkey pox, there are quite a few regulations with CDC, and they were kind enough to share some statistics with us, and I will get to those later.

FDA, we have GLP regulations and criteria for viral vaccines that a lot of you are familiar with. Department of Transportation--we're facing some problems now with importation because airlines are deregulated and they can pretty much choose what they're going to ship now. A lot of them are refusing to ship nonhuman primates because

of the disease risk.

The Justice Department is involved with the controlled substances that we have to use to transport a lot of these species. And a lot of states have developed laws to try to protect the cities that the primates come into with some of the communicable diseases. If you can get past the federal agency involvement, then we go to national laws, regulations and policies.

Really, I am only touching on these subjects, because we have a limited amount of time today. The document that you see on your right is a guidebook for everyone who uses laboratory animals. It is pretty extensive and it has been revised many times. It started off being called the Guide for Laboratory Animal Facilities and Care in 1963, and it was revised in 1972, and now it's called the Guide for the Care and Use of Laboratory Animals, and this is pretty much our Bible.

This is a new revision and it is revised about every five years. In 1966, we had the Animal Welfare Act, and there are so many revisions on that that I cannot list them all on this document, but you can find out a lot about the Animal Welfare Act, if you're interested, on the Internet. Every revision is listed. There is some current legislation on pain management that is in a comment period, and if you like to comment, now is the time. There is IPSC,

and that was renamed to IRAC, and that is currently a group that meets quarterly, I believe.

Then there is the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals. That is a published document that is amended currently, as well. So, you should be familiar with all of these documents if you plan on using laboratory animals, including nonhuman primates.

The Animal Welfare Act is a very important document in using especially nonhuman primates, because as you know they are a very visible animal with the animal rights activists. I chose to pull out Policy 12, because when you write an animal study proposal, or some people call it an ASP, you have to fill out a lot of sections and justify why you're using--why you have chosen a nonhuman primate, particularly.

If you look at number four, the written assurance that the activities do not unnecessarily duplicate previous experiments, this particular wording means that you have to categorize your pain classification into column C, D or E. C means minimal pain or distress, to the USDA. D means pain or distress that you will alleviate. In other words, if you're going to classify your animals under column D, that means that you're going to use an analgesic or have some means of relieving pain. E means that you do not plan on

intervening at all, and if the animal dies while that animal is on your experiment, then it has to be justified and you have to provide a narrative with your protocol.

The trick is if you have any nonhuman primates or any animals at all that go under column E, these are the protocols that are targeted by groups like PETA, and there are lots of them. It is not just PETA, but animal rights groups frequently pull all large animal protocols that are classified as column E under the Freedom of Information Act, and you may be a targeted scientist, i.e., they may follow you home and protest on your lawn and threaten your children and all of that stuff.

That is an important thing to know, should you choose a column E protocol--that is a very sensitive category. Importer requirements, this is a CDC requirement and you have to be registered with the CDC to important nonhuman primates into this country. Their use has to be certified. You have to implement disease control measures that the CDC approves of. They have to be isolated for a minimum of 31 days. That is if they are healthy, if they are not showing signs of clinical disease. You have to report suspected zoonotic illness and maintain records regarding distribution.

Also, it is important to know that if you have animals that you have arranged to come into this country,

and there is an outbreak, a lot of times these animals are caught in nature and there's an outbreak of a stress diarrhea, and that might look like some type of zoonotic illness, it is your responsibility and your expense to have these animals in containment until you can prove that they are healthy. The CDC does not take on that expense. That is up to the shipper--I mean, up to the person that is importing.

This was the total number of primate imports over the last six years. CDC was kind enough to provide these statistics for me. As you can see, we're going into a decline, because there is a fear, a concern, that we are eventually going to get some kind of zoonotic disease outbreak from a nonhuman primate. Like I said, we had our Marberg scare about 11 years ago.

Right now, we only have 26 registered importers. The slide on the right was from a colony in Africa. This species is now endangered, so you would not see this species used in research in the United States. That is the black and white collibus monkey. Out of all the imports last year, fiscal year 2000, ending in September, there was only a .4 percent mortality, but 2.9 percent morbidity.

So, carriers are decreasing the numbers that they're willing to carry and this is creating a problem of availability, unless we breed our own animals at breeding

institutions. Also, this slide is just designed to show how many shipments these were spread across and also how many species came into the country.

There were 10 carriers in the last 12 months across the continent that were willing to carry nonhuman primates. It is not just across the continent. There was one airline that carried nonhuman primates within the U.S. 24 times within the last 12 months. That statistic was provided by the CDC. As a matter of fact, my facility had chimpanzees come in last month and the researcher had to go and pick up the chimpanzees himself. He drove to New Mexico to pick up five chimps. From what I understand, it was not a pleasant trip. That is dedication.

By the way, chimpanzees are now costing $120,000 each. Macaques, these are rhesus macaques on the right. These are imports by country, and this is just giving you an idea of the majority of the cynos and macaques that are coming in and what countries are importing these. This, of course, will change based on any trade restrictions that go into place in the coming years.

If you have been reading the Post, then these numbers may definitely change. This is also by country for African Greens and baboons. These numbers are not nearly as high as the cynos and rhesus numbers. I was fortunate enough to spend some time with World Health at the Institute

for Primate Research in Kenya, and there they have a vast availability of baboons. They are considered pests, kind of like roaches are here. They bargain with the pineapple farmers not to kill them and they use them for research there.

Another import-by-country slide, and you see the owl monkey is almost not in importation at all. So, now we're on to quarantine, and there is a need for quarantine. If you're lucky enough to get them here, okay, now we're into quarantine, and this is pretty self-explanatory. You have to have a staff that is separated from your healthy staff, because if you go through all of the requirements to get them here, and you bring a sick colony in and your whole healthy colony dies, it does you no good. So, you want a healthy colony to come in, but there is a requirement to keep them quarantined.

Standard at NIH is 90 days and our facility is on NIH's campus, so we do abide by their standard quarantine rules. If you get a positive TB test and they do have to pass three negative TB tests every 30 days at NIH, you have to start your quarantine all over again, this is at the investigator's expense. This can get pretty expensive if this were to happen.

If you get an illness in quarantine and it's nondescript, that starts your quarantine period all over

again. Also, you cannot have cross-contamination of personnel, equipment and clothing, because that just defeats the whole purpose of having a quarantine, obviously.

Cost factors, and this really is what is driving the cost of primate research up so high, and that is really what the animal rights activists would like to see, is to make it so expensive that we just cannot do it at all, and then they have reached their goal. Animal procurement is expensive. The average rhesus monkey, you can expect to pay anywhere from $4,000-to-$6,000 per animal these days.

You also have to expect to pay--these are actually NIH numbers--46 percent of your cost in caretakers' salary and benefits. Now there's discussion about the hazards in this job, because just normal macaque work is a very hazardous job. I'm sure most of you know that macaques carry diseases that are not threatening to them, but are very deadly for us. So, a very innocent exposure can cost a human its life. I lost a classmate to a monkey disease.

Caging is very expensive and we will discuss that in a minute. Of course, you have to provide veterinary care and all of these expenses have to be taken into account. Housing is something else that a lot of people do not realize can be very, very expensive. There are lots of different types of housing. We're trying to go back to the more natural habitat.

Again, a lot of the language in the Animal Welfare Act and the guide is to provide a more natural environment. We're finding that you get more natural behaviors if you can provide a natural environment. Some of these are outdoor group caging that is designed to create a more natural environment for the animal. They seem to thrive, to be able to breed and do better, perform on the trials better, if you can do that.

Some of these cages are designed to provide enrichment where they can be pair-housed. Actually, pair-housing is now a requirement of the guide. If you do not pair-house your animals, you have to provide a scientific justification of why that is not occurring. This cage on the right is averaging about $6,000 to $7,000 right now.

Environmental enrichment is a requirement now under the law. It has to be natural. You have to be able to assess their well-being. That includes keeping records of what you're doing to enrich these animals, also what you're doing to assess their psychological well-being. You have to give consideration to species differences and what is natural for them and be able to record their cognitive and motor enrichment, because now we're finding that psychological distress can give you an adverse research results. That is actually not surprising. That is something that has been shown in humans, as well.

These are just some of your options for what we consider to be enriching. That is a puzzle feeder. Most nonhuman primates can figure that out in under two minutes flat. Humans, maybe a couple of days. These are toys. You do have to watch the size of your spaces, because you do not want them to swallow this thing whole and then have to take them to surgery to remove it.

We're going to touch a little bit on taxonomy. Like I said, monkeys are not really all monkeys. There are prosimians. These species, I just wanted to show you, they vary so much in size, some of them are really just not much bigger than a rat. If you're looking for vascular access, that might be hard. All of these are considered endangered now, so you're not going to find these guys in research; same thing for the Tarsiers. You're not going to find these in research.

There are few places that are running breeding programs, but they don't breed very readily in captivity.

CHAIRMAN SALOMON: Dr. Jones, as long as there is a natural break here, how much long do you have? I just am trying to stay on time here.

DR. JONES: I think I'm about four slides from the end here.

CHAIRMAN SALOMON: Okay. Thank you.

DR. JONES: You can read these for yourself. I

won't read to you, but we split monkeys into two groups, platyrrhines and catarrhines. These are new world versus old world. It's just a fancy name for a new world monkey from the left, versus old world, and they are characteristically different. If you're going to select a group, you have to know these differences, because they may impact your research results.

As you can see, some require vitamin D3 in their diet, the others don't. You see the ischial callosities, just pads for them to sit on, and the others don't have them. Some have them, some don't. The opposable thumbs just help them peel bananas and things like that.

This is a marmoset. Also, you have to know you that you cannot house squirrel monkeys with marmosets because squirrel monkeys carry very generic diseases that are fatal to marmosets. Owl monkeys are used in research, especially for antimalarial drug development.

That is a squirrel monkey. Also, I wanted to mention that there are so many species and subspecies within each group, if they have a lot of subspecies like the African Green, we recommend that you do karyotyping because you can get different results based on just having different subspecies within the same research group.

This into the old world monkey species here, African Green, or Cercopithecus, rhesus monkey, which is

very commonly used in the U.S.; 80 percent of these macaques carry the herpes B virus, which is deadly for humans. If you're scratched or bitten or even exposed with urine or feces through a mucous membrane, you are at risk for herpes B, which can be deadly in humans. It causes a fatal encephalopathy. Apes and humans are not monkeys, and the chimpanzee is used for hepatitis research.

This is a first-response posting that we're required to have in all of our facilities at the NIH. You should enroll your employees in what we call an animal exposure surveillance program, because this is a big risk if you have employees working with nonhuman primates, and these are the zoonotic diseases that come to mind when you have nonhuman primates in your facility.

I also listed the various uses of nonhuman primates in biomedical research, and that list is there for you to read on your own. And I believe we are at the end here.

CHAIRMAN SALOMON: Abbey, point of clarification?

MS. MEYERS: Before you sit down, I really want to know, the Animal Welfare Act and all of the regulations, do they apply to privately-funded research, as well as publicly-funded?

DR. JONES: Yes.

MS. MEYERS: If you have monkeys, you have a

pharmaceutical company and you have these things at your headquarters, you still have to follow them through?

DR. JONES: Yes, you do. That applies to industry, as well as government agencies.

MS. MEYERS: I would also just like to comment that there's no equivalent to the Animal Research Act for human beings.

DR. JONES: No, there are actually more regulations for animal study protocols than human protocols. I used to work at Baylor College of Medicine, and we frequently got that complaint from the people who reviewed the human--the animal protocols versus the human studies.

MS. MEYERS: Yes, we do not have any enrichment.

DR. JONES: Right, no human enrichment.

CHAIRMAN SALOMON: Thank you very much. I think everyone has been sitting for quite awhile. There are two more talks before we get to the discussion, so I would like to call a 10-minute break and ask people to really be back in 10 minutes this time. Thank you.

[Recess.]

CHAIRMAN SALOMON: I would like to introduce one addition to the panel at the table. It is Dr. Joe Tomaczewski, who is the chief of Toxicology and Pharmacology Branch, the Developmental Therapeutics Program of the National Cancer Institute, National Institutes of Health.

Ed Sausville had an internal site visit going on and asked to--that he had to leave for a few hours, but gave us his super expert, and so I think we're well-served and we appreciate your joining us. You have to answer one brilliant question sometime in the next hour-and-a-half.

Before we start with the next speaker, Dr. Anderson, I pointed out, had stolen my chance to make a big dramatic introduction to his concept that we could get the Human Genome Project and Dr. Collins to begin the sequencing of these larger vectors. He says he has a follow-up, so Dr. Anderson?

DR. ANDERSON: Yes, Phil Noguchi and I had additional phone calls over the break, and we have now gone from a very possible to a very probable for getting these herpes simplex, pox and so on that are directly involved, not only in disease, but also vaccine development and gene therapy.

Phil now has the ball firmly in his own hands to make direct contact tomorrow, to go about the process of determining which viruses will be done. That's it.

CHAIRMAN SALOMON: I think this is the government at its best. I appreciate that very much. Okay, Dr. High, to talk to us about the use of the canine model of hemophilia, and then that will be followed by Dr. Whitley, and then we will go on to the discussion.

DR. HIGH: I'm a hematologist on the staff at the Children's Hospital of Philadelphia and a member of the faculty at Penn and our laboratory has had a long-standing interest in the molecular basis of blood disorders and on the use of gene transfer techniques to treat hemophilia. As Dr. Pilaro mentioned at the outset, we have use the hemophilia B dog model to do safety, efficacy and toxicity studies of AAV vectors used to treat Factor IX deficiency, and the short version of what I am going to talk to you about over the next several minutes is that the major complication of our current protein-based method of treating hemophilia is the formation of inhibitory antibodies, that it is likely that this complication will not be avoided by gene-based approaches to treating the disease, and that it is, in my opinion, an obligation of investigators to assess the likelihood of inhibitor formation using their gene therapy technique in an animal model of the disease.

As I mentioned, the major complication of the current protein-based method of treating hemophilia is the formation of antibodies that inhibit the activity of the clotting factors. Those occur 20 percent of the time in individuals with Factor VIII deficiency and in three percent of individuals with Factor IX deficiency.

The reason that there are serious complications, this is a picture of a boy with a compartment syndrome as he

has bled into the soft tissue in the thigh, and you can see also he is unable to straighten his knees because of repeated bleeds into these joints. The concern over inhibitory antibodies is that normal therapy fails. It is much more difficult to control hemostasis.

There are products that can do it, but they are much more expensive than they already very expensive clotting factor concentrates. Individuals who develop inhibitors suffer increased morbidity and mortality. They suffer from much reduced ability for us to maintain hemostasis during either necessary or elective surgery, and individuals who develop antibodies to Factor IX can develop anaphylactoid reactions to the infusion of concentrates. It is a serious problem, if it develops.

Clinically, inhibitors are measured in a unit called the Bethesda unit, which measures--one Bethesda unit is defined as the quantity of inhibitory antibody that can result in the loss of 50 percent of factor activity when the test sample is incubated with a sample of normal plasma. Clinically, these are divided into high-responding, which is over 10 Bethesda units, or low-responding, and that is important because they are managed differently.

They can also occur either as long-lasting persistent inhibitors or more transient inhibitors, which tend to be lower titre. It is known from 30 years of

experience with protein concentrates for the treatment of hemophilia that there are certain risk factors that predispose toward them, and first and foremost among those is the underlying mutation causing hemophilia in the individual, and so, for example, in Factor IX deficiency, many individuals who develop inhibitory antibodies have large gene deletions or early stop codons in the Factor IX gene, and if one views the underlying mutations on a gradient moving from large gene deletions through stop codons, missense mutations associated with no circulating protein or missense mutations associated with a circulating protein, there is a gradient with many inhibitors occurring here and much less frequent as you move to the right.

The induction of inhibitors can be understood in terms of moderate concepts of tolerance. Inhibitors are promoted by T-helper cells. In a normal individual, during fetal development, self-reactive T-cells are deleted or energized, but depending on the underlying mutation, a hemophilic individual may not express the epitopes recognized by those T-cells and thus they persist, and on encounter with the antigen during initial treatment with Factor VIII or Factor IX concentrate, these T-cells can promote induction of a neutralizing antibody response.

Clearly, the nature of the underlying mutation and the amount of coding sequence that is lost is a risk factor.

In protein based therapy, certain inherited characteristics of the immune response are also risk factors. We know this because there are many individuals who have this same underlying mutation, but not all of them will form inhibitory antibodies when exposed to factor concentrate.

The clotting factor itself can be a risk factor for antibody formation. There was a so-called outbreak of inhibitory antibodies in the Dutch population some years ago, following the introduction of a new method for viral inactivation of the plasma used to produce the product. Finally, there is a great deal of information in the clinical literature for hemophilia that suggests that individuals exposed to clotting factor for the first time, under situations where there is extensive inflammation or tissue injury, may be more likely to form inhibitory antibodies.

I believe that it is likely inhibitory antibodies will also be a problem for gene-based approaches, as well as those that we currently use that rely on the intravenous infusion of Factor VIII or Factor IX protein, and one can list many ways in which antigen presentation may differ between a donated gene approach versus intravenous infusion of a protein, and this slide illustrates one of those.

For protein-based treatment of hemophilia, the protein is exogenously synthesized and infused intravenously

and antigen presentation occurs almost exclusively in the context of MHC Class II, which has as its job the display of peptides derived from proteins that are taken up from the environment and a gene-based approach where now, for the first time, the individual will begin to synthesize the protein endogenously.

Antigen presentation will surely occur in the context of Class II determinants, just as it does for intravenous infusion of the protein, but may now also occur in the context of Class I determinants which have as their function the display of the peptide fragments that are derived from proteins synthesized inside the cell that displays them. That is just one example of the differences between antigen presentation and these two different approaches.

Work by a number of investigators over the past 10 years has demonstrated that a number of these factors listed here may be risk vectors for inhibitory antibody formation to clotting factors in a gene-transfer approach for the treatment of hemophilia. I should stop and say that I am talking about hemophilia, but these remarks in general apply to genetic deficiency states characterized by the absence of some circulating secreted protein.

The vector itself can be a risk vector for inhibitor formation. The target tissue that is chosen, the

dose of vector, the presence or absence of tissue-specific promoter elements and immunomodulatory maneuvers at the time of antigen presentation. I will not have time to go through all of these factors, but I will show you one example of experiments that we published earlier this year, looking at the role of the vector itself and immune response to the transgene product Factor IX when a vector is introduced into skeletal muscle of mice.

For these experiments, we made either an AAV Factor IX vector or an adenoviral Factor IX vector and injected it into the hind limbs of mice and at time points later sacrificed the mice and examined them for the presence of CD8-positive cells, and you can see these were present in the context of adenoviral vector, but not in the context of the AAV vector. In additional experiments, we harvested lymphocytes from the draining lymph nodes and the splinocytes of these animals and carried out experiments looking for cytotoxic T-lymphocyte response specifically to Factor IX, so use target cells expressing Factor IX and used the harvested lymphocytes as the effector cells and were able to show that there was Factor IX-specific CTLs in the lymphocytes derived from the Ad-injected animals, but not from the AAV-injected animals, and in additional experiments we looked at CD4 profiles, again from lymphocytes isolated from animals that had been injected either with Ad Factor IX

intramuscular or with AAV Factor IX intramuscular, and you can see that when these lymphocytes were stimulated with Factor IX, lymphocytes from the Ad Factor IX injected animals produced interleuken-2, interferon gamma and IL-10, whereas the animals that had been injected with the AAV Factor IX had at most a limited IL-10 response to Factor IX antigen.

Clearly, the vector itself can be a determinant of the immune response to the transgene product. As Dr. Pilaro mentioned, we have done a number of studies in hemophilic dogs looking at other determinants of inhibitor formation in the setting of gene transfer in this approach to treating hemophilia. I'm going to talk a little bit now on some of our studies that analyzed dose as a determinant.

For these studies, we were using an AAV Factor IX vector, and you talked about AAV earlier in the day, so I'm not going to spend a lot of time, but the strategy that we are currently using involves the introduction of an AAV Factor IX vector into skeletal muscle. In earlier experiments, we had demonstrated in immunodeficient mice that the introduction of the vector into skeletal muscle could result in long-term expression, and actually this went out for more than a year, long-term expression at levels that would be therapeutic in humans. These correspond to levels of five-to-seven percent of normal circulating Factor

IX and would convert an individual with severe hemophilia into one with mild hemophilia.

Based on those studies, we wanted to extend our studies into the hemophilic dog model, and as I said before, one of our major goals in using the hemophilic large animal model was to determine the likelihood of an inhibitory antibody response using this particular strategy of introducing AAV Factor IX into intramuscular sites.

For these experiments, we collaborated with the canine hemophilia B colony, with a group that runs the colony at UNC Chapel Hill. The defect in these dogs is known and was determined some 10 years ago. They have a missense mutation at a highly conservative glycine residue in the catalytic domain of the protein. They have normal Factor IX transcript levels, but no circulating Factor IX antigen, and they have severe hemophilia B, less than one percent activity.

The experiments that we did made use of the vector that is diagrammed here. It directs the expression of canine Factor IX--this is a very important point--that without the use of a species-specific transgene, these animals will rapidly develop antibodies, and it is not possible to continue to follow the work. Using a CMV enhancer promoter and a synthetic intron to derive canine Factor IX CD in an expression, we carried out the

experiments outlined here.

Our initial experiments in dogs were actually done as a dose-escalation approach, starting with about ten-to-the-eleventh vector genomes per kilo and moving up to nearly ten-to-the-thirteenth vector genomes per kilo. The dogs ranged in size from six-to-20 kilos, so the larger ones were as big as a nine-year-old child, and then they underwent intramuscular injection of vector at day one of the protocol and have been followed since using a number of coagulation, hematologic and chemistry clinical pathology measurements, as well as serial biopsies of the injected muscle tissue.

One point that I would make, which is an advantage of the dogs over mouse models, is that, of course, dogs are very long-lived compared to mice, and we've been able to follow these out now for a period of over three years. What we've seen in the dose escalation study is that the more vector injected into intramuscular sites, the higher the circulating levels of Factor IX. The blue line across the middle here denotes 50 nanograms per ml, which would be a level of one percent in humans, and levels above this, we know from experience with clotting factor concentrates, should improve the clinical phenotype in an individual with hemophilia.

So, we have been able to follow these animals for long periods, over three years, with the study continuing as

long as we continue to be funded. We have also done serial muscle biopsies in the animals and these are muscle biopsies done six weeks after injection and again two years later, and you can see there is no evidence of inflammation or degeneration in the muscle, and the dog vector was co-injected with carbon particles, which shows up here as a sort of reddish material and allows us to go in and biopsy exactly the right location, and you can see again the carbon particles are evident here two years later, and although there is freeze artifact in this muscle, again, there is no evidence of any inflammatory infiltrate or evidence of deterioration of the tissue.

Then, we also did immunofluorescent staining for Factor IX and injected muscle tissue, and you can see cells that demonstrate the fluorescent stain here. We did serial chemistry studies in these animals, again over a period of years. Except for transient elevation of the creatinine kinase immediately after injection, which is also seen when saline is injected in the animals, there was no evidence of any changes following vector injection, no changes in hematologic parameters.

We did viral shedding studies in the animals, including collection of semen from the male dogs that were injected and saw no evidence of transmission of vector in the semen and other serial studies have all been negative

for any toxicity. In addition to the toxicity studies in dogs, we obviously did toxicity studies in other species, as well, including mice, rats and rabbits. Dogs were important for these studies, and also another important issue we were able to address in the muscle biopsies was to look at gene transfer on southern blot from the biopsied muscle, and without going through it in detail, I will just mention that using undigested DNA, vector sequences are detected as a high molecular weight smear, and the genomic DNA is cut with either a single-cutter or a double-cutter within the mini-gene cassette, it releases fragments of the appropriate size and allows us to estimate gene copy number, which in a dog is generally about one-to-six copies of vector per diploid genome in the biopsied muscle.

Finally, this other issue, the issue of the formation of inhibitory antibodies, in this first sequence of dogs, we measured both on western blot and using that Bethesda assay that I referred to earlier, the presence of an inhibitory antibody in an animal that received the highest dose. As you can see on the western blot, two weeks after injection, we first detected an antibody on western blot, and it peaked at about five-to-six weeks and then slowly receded.

The data on the Bethesda assay tracked very well in terms of the temporal cores with what is seen on the

western blot, and it peaks at about seven Bethesda units and then slowly recedes. During the time the inhibitory antibody is present, it's not possible to measure Factor IX, but as the antibody recedes, now you can see Factor IX appearing in the circulation.

We viewed this as an important cautionary note and, as you will see, we did additional studies on it later on, but this looked to us as something that would be important to follow up on and probably a dose to stay below in human studies. So, a human study has been initiated based on this, and this is a study done in collaboration with Avigen and with investigators at Stanford University and is an open labeled dose escalation study with three subjects in each of three dose cohorts.

The study is essentially an outline of what you had seen in the dogs. The individuals undergo intramuscular injection of vector into the vastus lateralis and then we do a battery of hematologic, chemistry and collaboration studies, and periodically these individuals undergo muscle biopsies to look for evidence of gene transfer and expression.

One thing you will see here is that in the initial studies, we did make the decision to use individuals with missense mutations, and the reason for that was because the dog studies we had done had been in animals with these

relatively less severe mutations, missense mutations, and we felt it was important not to get out too far in front of the animal data that we had on this important safety issue.

Fortunately, missense mutations account for a large percentage of individuals with hemophilia B, so we were able to do that. First of all, in terms of inhibitory antibodies, these have been measured weekly, initially, and then monthly since the first vector injection. Fortunately, none of the individuals on the trial have developed any evidence of inhibitory antibodies at the doses tested so far, which are still about fourfold lower than the dose where we first saw a transient inhibitory antibody in the dog study. and then southern blot on muscle DNA harvested at the muscle biopsies has demonstrate findings similar to what was seen in the dogs; that is, if you look at uncut DNA harvested from these, one sees again a high molecular weight smear, and if you cut with a single-cutter, echo R1, within the mini-gene cassette, you release the unit length 4.5 KB fragment and again copy number can be computed from this and has run about one-to-four copies per diploid genome.

Note that these were actually subjects that were in two different dose cohorts, but because the dose per site is held constant, there's not really a substantial difference in the copy number per diploid genome. Again, I will say little bit more about the dose per site in just a

minute.

If you look at immunofluorescent studies for Factor IX in the biopsied muscle, this may be a little difficult to appreciate, but I want to make the point here that immunofluorescent stains for Factor IX, and by the way, these look quite similar to what we've seen in mice and dogs, the expression appears to be primarily--these are two adjacent sections, one stained for Factor IX and one for slow-twice miocene, and you can see that it appears to be the slow-twitch fibers that take up and express the AAV vector.

I won't say more about that at the minute. I will say that, for the muscle biopsies, both immunofluorescent stains and immunoperoxidase stains are done and the immunoperoxidase stains show similar information; that is there is a typical checkerboard pattern where there are positive fibers directly adjacent to negative fibers, probably reflecting the mix of slow and fast fibers in the injected muscle.

The reason immunoperoxidase stains are done, of course, is that they are very long-lasting, compared to immunofluorescent stains. The importance of the studies that have been done so far is simply that they do suggest that the dogs accurately predicted what we saw in terms of gene transfer and expression in the human data that are

available so far. We did want to follow up on the evidence of dose as a possible risk factor for inhibitory antibody formation, and in additional studies done in the Chapel Hill dogs, which are demonstrated here, and this is a busy slide and it is difficult to sort through, but this is gradually increasing doses per kilo in the series of dogs, and we assessed both anti-canine Factor IX antibodies on western blot, which may or may not be inhibitory, and inhibitory antibodies, and you can see that in this series of dogs, the three animals that got the highest dose per kilogram, diagrammed here, one had no evidence of inhibitory antibody formation, one had a transient inhibitory antibody--that is the one I had shown you earlier--and one had a longer-lasting inhibitory antibody.

Without showing you all of the data, I will indicate to you that it appears the best predictor of inhibitory antibody formation may indeed be the dose per site. These two animals got two-times-ten-to-the-twelfth vector genomes per site, and it had either no inhibitor or a transient one. This animal got a five-times-higher dose per site and had an antibody that lasted for a period of nearly a year and had a much higher titre, and these are diagrammed together here.

This is the animal that I had shown you earlier in red, and then this other animal had a much higher titre and

longer-lasting antibody, following a higher dose per site, and based on these data, we do recommend to the clinical investigators that they confine the dose per site to well below the dose given to this animal here.

Hypotheses as to why the dose per site may matter are listed here, and we're trying to, in the laboratory, sort through these. Is there a contaminant in the prep that can act as an adjuvant? Does a higher dose per site lead to better transduction of antigen-presenting cells? Could a higher number of viral particles at a local site change the cytokine milieu and lead to increased danger signals as the Factor IX is being produced or, finally, could the higher levels of local Factor IX expression, combined with the high number of viral particles, trigger an immune response against Factor IX; that is, could the antigen be essentially now presented in the context of a viral infection?

We're working to sort through this, but it was actually through the dogs that we saw this as a potential problem. Let's see. I would just maybe mention one more set of experiments. With intramuscular delivery of AAV vector, the likelihood of inhibitor formation appears to be dose-dependent and dose-per-site-dependent.

To follow up on our observation that we're concerned about using individuals or admitting to the trial individuals who had mutations more severe than missense

mutations, we did a series of studies with Clint Lottrop and Auburn University in Alabama, and they have dogs that have a very severe mutation, essentially an early-stop codon, and they have essentially no Factor IX transcript. These are normal Factor IX transcript levels. These are from the Auburn dogs.

What we saw in those studies is that even at this dose per site, but at a much lower total dose, inhibitory antibodies developed in these dogs with a more severe underlying mutation, and even if the dose per site was lowered, this still occurred. This is again the Bethesda titre in these animals and you can see following injection the inhibitory antibody appears, and it does seem that this can be altered by dosing the animals with cytoxan at the onset of the injection, so they got four doses of cytoxan, one dose every two weeks immediately after vector injection, and following that, they had sustained correction of the whole blood clotting time and no evidence of inhibitory antibodies.

We have additional mouse data that suggest--that actually was the basis of performing this experiment--but the important aspect is that when we went into dogs with a more severe mutation, we did indeed see this problem of inhibitor formation, I think giving support to the idea that it was probably not wise to admit individuals with severe

mutations to this trial.

I'm going to conclude summarizing that inhibitory antibody formation is the most common complication of the current method of treating hemophilia, that is, intravenous infusion of protein. Neither theoretical considerations nor experimental data suggest that gene transfer approaches would avoid this complication.

One can attempt to assess these things in murine models of hemophilia, but these models are essentially very limited, because all of them are murine models, hemophilic models due to gene deletions, which are found in only a small percentage of the human population and which do not mimic the gene defect in most individuals with hemophilia, and moreover, strain differences in these mice may confound interpretation of data, and there is some very nice published work by Sheila Connolly and her colleagues at GTI Novartis, that demonstrates using a third-generation adenoviral vector, no inhibitory antibody formation in hemophilic mice, but the presence of inhibitory antibodies in hemophilia dogs using an adenoviral vector with a tissue-specific promoter.

The dog model of hemophilia does allow assessment of inhibitor risk, which I think is really the most likely complication. It is the most likely complication of our current method of treatment. It is likely the most common

complication of a gene-based treatment. As I said before, I think that investigators have an obligation to design and carry out experiments that will let them assess the risk of inhibitory antibody formation, using their gene transfer technique, that requires the use of a species-specific transgene, and those are cloned and available.

The vector, the promoter used in the mini-gene cassette, the dose, the route of administration and the underlying mutation in the recipient all will influence the likelihood of inhibitor formation, and it is probably that each of those will need to be evaluated independently. With that, I'm going to stop.

CHAIRMAN SALOMON: I had one question before you step down. I don't understand, how did you explain getting a transient antibody response in the first set of dogs that effectively cleared circulating antigen, and then it spontaneously resolved and antigen was detectable. That's not an immune response I'm familiar with.

DR. HIGH: I can't tell you how many immunologists we have talked to about this, but I will tell you that using protein-based therapy for the intravenous infusion of protein, this is a very commonly observed phenomenon, and some substantial proportion of individuals who develop inhibitors have these transient inhibitory antibodies, and despite 30 years of using these protein concentrates, the

immunologic mechanism of that phenomenon is not worked out and people fight about it.

One explanation I have heard is that it is a B-cell response with no T-cell help. If you have any other ideas, I'm interested to hear them.

CHAIRMAN SALOMON: We will fight about it later. It's very interesting. It's very interesting, because it suggests that you have some sort of tolerance induction or an antibody getting turned off, but that's not the purpose of today's meeting. I had to ask in the context of trying to use these models to model what might happen in human patients. It is atypical.

DR. MILLER: Very nice, but I have a question about whether or not it is clear that the dog model is valid until you reach your first immunogenicity in humans, because right now you have it in dogs, but you don't know it is modeled in humans because you never see it in humans, and not that I want the humans to get antibody responses, but building on that, and it's a beautiful model, but without having some positives, it's hard to say that it models.

DR. HIGH: That brings up a very important question, because I didn't have time to show you, but again Sheila Connolly's data from GTI Novartis would suggest that using an adenoviral vector, there's also a dose-dependent increase in likelihood of inhibitor formation, and it does

appear from many studies that I did not have time to show you, that as you escalate the dose in animals, first you see, if you're going to get inhibitors, first you see transient antibodies and then you see more persistent higher-titre antibodies.

There are two ways to look at that. One is that you will just keep dose escalating until you begin to see that in humans, and I have real concerns about that because of the risk of inhibitory antibody formation and the lack of certainty that the first inhibitor you encounter would, in fact, be transient. The other way you can use the data is to try to define situations that either enhance or reduce the risk of inhibitors and use that information to construct the clinical trial, and we have opted for the second strategy, not the first.

CHAIRMAN SALOMON: Excellent. Thank you. Very good presentation. Then it is my pleasure to introduce Dr. Richard Whitley from the University of Alabama, who is going to talk about the use of aotus monkeys to assess neurovirulence of a replication-selective herpes vector.

DR. WHITLEY: [Off microphone.] --vaccines for the whole family of herpesviruses, I think it is essential to weight the risks and benefits of what has been learned over the last 20 years using a variety of animal model systems to take engineered viruses from animals into humans,

and this has been done both in the vaccine arena, as well as in the gene therapy arena at the present time.

For those of you who are not aficionados of the herpesvirus family, please remember that there are eight viruses that are herpesviruses. Three are in the alpha herpesvirus family, three are in the beta herpesvirus family, two are in the gamma herpesvirus family. These viruses have been sub-classified according to their predisposition infect and establish latency in target issues.

For example, for the alpha herpesviruses, we know they have a neuronal predilection and therefore establish latency in neuronal tissue and can be reactivated in that site. That is very different from the beta herpesviruses who tend to establish latency in endothelial cells, lymphocytes and macrophages from whence they are reactivated. Gamma herpesvirus is EBV and HHVA--excuse me--establish latency, for the most part, in lymphocytes.

I want to make four points about this slide. The first and the most important one is please remember that animal models for all of these viruses have been developed for the purpose of studying pathogenesis and antiviral therapy, historically. It has only been recently that animal models of herpesviruses have been used to study gene therapy. That is very important to remember, because if you

stop and ask the fundamental question, which is really what you're being asked to address this afternoon, and that is do animal models correlate or predict benefit or harm in human disease? The answer is only in the context of antiviral therapy.

Whatever I say about the aotus is relevant only to the studies that are ongoing now, that are being done at three or four hospitals in the United States. The second point that I want to make is even though there are members of a subfamily of viruses, namely the alpha herpesviruses, they behave very differently in animal model systems.

Herpes simplex Type I is less likely to establish latency than herpes simplex Type II, although we can drive it to latency and we can reactivate it in that animal model system. The last point about this slide is just to pursue the discussion of this morning and the ongoing phone calls that have been taking place. There was a program project grant that has just been funded to go to Lyn Enquest, Tom Shank, Bernard Roizman and Elliott Keith, relating to the sequencing of several of these herpesviruses, and I just point out that that grant was funded for five years, not six months.

Okay, with that in mind, I think you really need to began in looking at the relevance of animal models, whether it is in the mouse, the guinea pig, the rabbit or in

the aotus, with what is the biology of human disease? What are we trying to prove here? Why are we doing this in the first place? What are the risks and benefits when we think about the predisposition of herpes simplex, which is the common vector used in these studies to establish disease?

Remember, herpes simplex viruses live on mucosal surfaces. They cause oropharyngeal disease and they cause genital disease, for the most part. However, they can cause herpes simplex encephalitis in adults. That will occur in approximately one-in-150 or one-in-200,000 individuals annually, and that is what we have to prevent when we are talking about using herpes simplex as a vector for gene therapy.

We also know that if it causes disease in the genital tract, newborns can become infected, and if they do, disease can be life-threatening. With that in mind, let's go one step further and let's ask ourselves about the natural history of this disease, and when we think about the natural history of disease, infection enters the body from a mucosal surface. Virus replicates as a function of intimate contact, either kissing or sexual contact, in the oropharynx or at a genital site, with initial penetration of nerves and then accession of virus to a sensory dorsal root ganglia.

It is at this site that virus will replicate and either be transported back down to skin sites to cause

lesions or, ultimately, become episomal and resident in the ganglia until it is subsequently reactivated. Why do I worry about this? I worry about because I have spent the last 30 years of my life trying to treat this disease, which is herpes simplex encephalitis, and it is the one disease that if it occurs in an individual who is on one of our gene therapy studies, we all have to be held accountable for understanding why this disease occurred.

With that background in mind, I want to look at how attenuated herpes simplex viruses can be used for direct gene therapy, for vectors for foreign gene expression or as attenuated vaccines. I will just begin by saying that for 20 years Bernard Roizman, a colleague of mine at the University of Chicago, and I have been working on the latter area, namely attenuated vaccines utilizing principles from his laboratory on the engineering of herpes simplex, and then taking them into animal models that we have tried to establish at the University of Alabama at Birmingham.

Here is a listing of some of the animal models we used. I don't intend to bore you with it, but I do want to point out that you can adequately and, in fact, in detail study these animals to get a better understanding of the safety, efficacy and ability of these viruses to establish latency and be reactivated. For example, in the mouse, we clearly know that we define safety following intracerebral

inoculation, either in an immune-competent or in an immunocompromised mouse, particularly a skid mouse.

We can study efficacy and challenge experiments. We can study latency by harvesting ganglia and reactivating them in vitro, and the systems we can use then are those of immune-competent, immune-compromised mice. We can study genetic stability, and I'll illustrate that for you in a minute, and we certainly can study neurovirulence to get an assessment of that before progressing into subhuman primates.

The guinea pig is a good model for latency and recurrences. The rabbit is a great model for using the eye and the issue of establishment of latency at the trigeminal ganglia, and in my opinion, when we use the aotus trivirgatus or a nancymae, we're really looking at safety more than efficacy or the establishment of latency. These are difficult systems to use. This is not a system that I would recommend for the casual investigator, but we can use immune-competent, immune-compromised animals in both systems and assess neurovirulence as well as pathogenesis.

I want to illustrate this for you with a couple of examples. Here at two viruses that have been taken from bench into humans. The top is the example of a virus that has deletions in the inverted repeats of a gene identified his gamma 134.5, and I'll show you how that virus behaves

upon inoculation into the central nervous system in a minute.

This virus was developed in the laboratory of Bernard Roizman about a decade ago. Remember, the problems that we were talking about sequencing other, this is 150 KB of DNA. There are inverted repeats that bound the unique long segment, inverted repeats that bound the unique short region, and it is going to be very confusing to sort this out, although I'm sure sequencers can do that with facility.

The second construct is a virus that was initially known as R7020, that was a candidate vaccine that consisted of both HSVI and HSV Type II. It currently has been reconstituted, reformulated as a virus known as NV1020, and it's entering into a clinical trial for metastases of colorectal carcinoma. I would point out that both of these viruses have herpes simplex virus thymidine kinase, and as such they are susceptible to acylovere, and that is a fundamental principle when considering herpes simplex viruses.

TK-negative viruses, in my opinion, should not be used in human experimentation because you cannot treat them with the drugs that we currently have available, unless you want to go to potentially toxic medications. Why use these viruses and what is the safety data that allows us to advance them forward into human investigations? The purpose

of this study was to define the platforming LD 50 ratio, in other words, the number of viral particles required to kill 50 percent of the animals.

This is an assessment of neurovirulence and neuroattenuation. Those data are shown in the column on the far right. These are viruses that are deleted in gamma 134.5, and that is shown here, compared to the parent virus, which is known as HSV1F, it is wild-type virus. For wild-type virus, 200 viral particles inoculated intracerebrally will kill 50 percent of the mice.

For a virus deleted in both copies, because remember this gene maps in the inverted repeats of the unique long segment, identified R3616, you cannot kill the mice with over one million particles of virus inoculated into the brain. If we put a stop codon into gamma 134.5, it remains its avirulent phenotype, as we have to do with these viruses, restoring 34.5 restores neurovirulence to these viruses.

With that as background then, we have a candidate virus then that potentially could be used either as a backbone for gene therapy or as a vector for foreign gene expression. Here are the experiments that we do to establish genetic stability, and these are very straightforward experiments that utilize the mouse to determine whether or not we can address one of the two

fundamental issues that herpes virologists always worry about; one is a revertant to a wild-type phenotype and the second is a second site mutation which would lead to virulence in the animal system.

We will take a mouse, we will inoculate herpes simplex intracerebrally, on day three of our avirulent, our aneurovirulent virus will harvest brain tissue, isolate virus in cell culture, re-raise it to a stock titre, inoculate it intracerebrally and repeat this process eight times. We will then, as we do this, continue to calculate PFLUD50 ratios. If we see no change in PFULD50 ratios, it indicates the virus has not regained neurovirulence.

Our ability to detect genetic variance of reversion or second-site mutations is one-out-of-ten-to-the tenth viral particles. This is a sensitive way to screen genetic stability in the candidate herpes simplex virus that have been used. So, where are we then with the viruses that have been developed for human administration? R7020 was the first virus, and I'll illustrate data for you from this candidate vaccine strain from aotus. It was administered to 33 volunteers in Lyon, France, in studies that were done with Institute Merieux. G207 is a study that was just finished and reported in Gene Therapy; 21 volunteers who had glioblastoma multiformi were inoculated directly into the tumor stereotactically with this construct.

This study was done by Bob Martuz of Mass General and Jim Markert of my own institution. NV1020 is a virus that I showed you at the bottom of the viral construct slide, entering Phase I studies in volunteers with liver cancer. The first volunteer went on that study this week. Then the last candidate strain is a virus known as AB9395, which is a herpes simplex Type II deleted in gamma 134.5, which is undergoing evaluation for potential vaccination.

What do these animals look like that we use? Here is the aotus nancymae. This is an HSV hypersensitive animal. I want to emphasize that for you, because this represents both the pro and the con of using an animal like the aotus in evaluating potential herpes simplex vectors; 10 platforming units of wild-type virus given intracerebrally will cause fatal encephalitis. These animals will die in a period of 10 days.

At the present time, whether it should be or not, it is the standard for preclinical evaluation of genetically engineered herpes simplex viruses. We can inoculate virus intracerebrally, intraocularly, intravaginally or intramuscularly and then we can perform a variety of studies that are relatively routine for other animal models in assessing outcome.

For survival, we can look at dose dependence, site of administration versus long-term survival, clinical signs

of disease, radiographic evidence of encephalitis that would occur in these animals. For those animals that are sacrificed, we can look at histopathology, in situ hybridization, immunohistochemistry and certainly we can look at cellular pathogenesis and differential jean expression within the central nervous system.

For virology, it's not just viral quantitation and viral isolation, but it is also PCR evaluation of where viral DNA and what viral DNA is distributed in the brain itself, and certainly we can look at foreign gene expression within the model, and I will illustrate each of these principles for human studies that we have done. Over a period of 14 years, there have probably been four different types of studies that have evolved. The first, Bernard Miniet, Bernard Roizman and myself evaluated R7020 in the aotus, and I think it was probably the first time the aotus, both the immune-competent and the immune-compromised, was used to evaluated herpes simplex and get some understanding of the pathogenesis of the viruses under those circumstances, and I'll illustrate those data for you in a minute.

Four years ago, we began evaluating 9395, which is a deletion in gamma 134.5 and HSV2. Sam Rabkin and Bob Martuz evaluated G207 and published their data in Journal of Virology last year, and then currently we're working with a

herpes simplex virus that expresses IL12, and I will show you how that particular virus behaves in animal model systems.

The first is deleted in the joint region. The second is an HSV2 deletion with a deletion in genes responsible for recognition of the virus by the host cell. The third construct is a 34.5 deletion, as well as a second site segmentation, and that second site mutation is a ribonucleotide reductase and it was done intentionally to avoid second site mutations that would occur naturally when administered to the host.

The last viruses is one that I just mentioned and that is a virus that expresses IL12. What do you learn from these experiments? This is the aotus trivirgatus. It was R7020, so it's HSV1 in the long segment, HSV2 in the short segment. Virus was given by one of a variety of different routes, intradermal, intravaginal, HSV1, HSV2, the period of viral shedding was relatively brief. These animals all died in a very short period of time with very low exposure to replicating virus, ten-to-the-one, ten-to-the-two.

With R7020, we could administer up to ten-to-the-sixth to ten-to-the-seventh viral particles by one of several different routes, with all mice under these circumstances surviving. This should be S, not five. All those animals survived following administration of virus.

If we look at multi-inoculation with a TK-negative or a TK-positive virus, we can actually quantitate duration of shedding by site.

We know the longest shedding will occur about 22 days in all animals, but we also have the opportunity to determine whether or not virus is picked up from ganglia when animals are sacrificed and dorsal root ganglia harvested, and you can see here that approximately one percent of ganglia expressed latent virus. That latent virus is R7020 or the TK-negative virus, R7017.

There are other things that we can do. We have standard procedures for intracerebral inoculation of virus, and I won't go into the details of what we've done, but I just illustrate some of the work that was done first with G207 and then with virus constructs that we've made in Birmingham. Here's the virus and the does that was employed. You can see up to ten-to-the-ninth viral particles were put in.

The animals were followed until they either died naturally or were sacrificed. One animal died, for example, from an aneurysm; the other was sacrificed at 20 months to determine whether or not virus could be detected at latent sites, whether it could be reactivated from the central nervous system and whether there was PCR evidence of virus. This is in contrast to wild-type virus, HSV1F, ten-to-the-

three viral particles inoculated intracerebrally led to death at five days from encephalitis.

You can also see that with mock infection, these patients lived their natural life course. For those animals sacrificed here and in other experiments to detect evidence of latent virus, one can find latent virus, but it only in approximately one-to-five percent of all ganglia which are assessed.

We can also do repeat intracerebral inoculation. Here, ten-to-the-seventh viral particles was put into the central nervous system to determine whether or not there was an additive effect if virus was given a second time, and the animals surviving the first series of experiments were reinoculated, followed for ten months, to determine whether or not there was any additive effect and these animals are perfectly fine and well.

What we've done is taken this one step further, and that is pose the question what happens when you use herpes simplex as a vector for foreign gene expression? Here, we have used IL12 to determine whether or not it had adverse effects when expressed in herpes simplex upon inoculation into the central nervous system. No changes in behavioral or feeding patterns. The monkeys remained--excuse me, the aotus remained normothermic throughout the period of time they were evaluated.

There was a period of temperature to 104, which was associated with increased activity. If we look at MRI scans on the monkeys that we evaluated in our studies, this was 10 days post-inoculation of ten-to-the-seventh, both sagittal sections, as well as sections that were coronal sections, looking for evidence of encephalitis. There's no evidence of hemorrhage. There's no evidence of edema. There's no evidence in shift in midline structures, all characteristics of herpes simplex encephalitis.

The conclusion that we reached from the encephalitis component of this study was there was no evidence of central nervous system disease. We did follow up this monkey. We found that the monkey developed an infection that was treated with antibiotics, and this makes an extremely important point. It was treated with presumptive antibiotics, developed a diarrheal illness, ultimately went into renal failure with nephritis, secondary to the antibiotics that were administered.

The reason I bring this up is the aotus is a difficult monkey to deal with. They are fragile little creatures and one has to manage them very carefully. If we looked at the brain upon evaluation of this monkey, there was no evidence of encephalitis or necrosis. There was an inflammatory response in the choroid plexus, but no ventriculitis. We did not find any evidence that led us to

believe that herpes simplex expressing IL12 led to disease in the central nervous system of this animal.

We looked for additional evidence of encephalitis at multiple other sites and we found absolutely nothing that was indicative of disease. Studies that we will do in the future will expand the histopathology in specimens from this one monkey, to try and get a better understanding of what the nature of the initial site was, particularly as it relates to PCR and in situ hybridization on brain tissue, and we will try and push this does probably in one or two other monkeys before going on.

I just want to make one point, and that is as a clinician who has to take care of patients with herpes encephalitis, primum no nocere. We have to remember that herpes simplex can cause disease in the brain and we have to be exceedingly careful. I just want to end with a couple thoughts, and that is what is okay with herpes simplex using a mouse, guinea pig, rabbit and aotus certainly is not going to be okay if you consider cytomegalovirus, human herpesvirus-6, human herpesvirus-7, EBV or HHVA.

Each one of the members of the herpesvirus family has a very, very different spectrum of tissue trophism and susceptibility in animal model systems, and unless that is well-understood by all parties involved, you should not embark upon these studies casually. The last slide is there

are both pros and cons of using the aotus. The pros are that this is an exquisitely sensitive animal model for preclinical toxicology, but the con is the biggest one, and that is it is too sensitive, and therefore ultimately we're going to have to find a happy medium that we can use.

Thank you.

CHAIRMAN SALOMON: Rich, before you sit down, I had two quick questions. One is, with retroviruses, frequently many of the viral particles are not infectious. In comparing your titres between wild-type and your vector, the question is obvious, so I have a follow-up to that.

DR. WHITLEY: That is the standard problem that we have with all the herpesvirus, and that was one of the rate-limiting steps, I think, in my opinion, and the folks CBER don't have to comment on it, in the licensure of the Occa vaccine strain, was because the vector particles account for a significant volume of that virus, and it is a problem with herpes simplex, but to a lesser extent, and that is an issue that we deal with all the time, very difficult to quantitate. The only thing you can do is look at infectious particles that are put into the tissue.

CHAIRMAN SALOMON: When you did your titres, those were infectious titres, so when you say you put in ten-to-the-sixth or up to ten-to-the-ninth, got no infection, that was of actual infectious titres.

DR. WHITLEY: That is known virus. It titred before it went in. It was titred from the syringe after the inoculation was done.

CHAIRMAN SALOMON: That is perfect. The other question I have is you have a mouse model and you have this nonhuman primate model. One of the things you and I talked about before is, is this now an example in which we have added to the preclinical development by having a nonhuman primate model, or are we find this is a nonhuman primate model that is excellent, but we could have actually found out everything in the mouse?

DR. WHITLEY: I think, at this point in time, the aotus adds very little to what we learned in a mouse system, particularly a skid mouse system, which is also exquisitely sensitive to HSV. It was developed because of the concern of both Bernard Roizman and myself, that we didn't want to take herpes simplex into people and have something bad happen, but we're having to re-question that at this point in time, just to be honest.

DR. GORDON: Have you ever tried interrupting either wild-type virus or looking at latency rates for the recombinants--for the vector--with acyclovere in the monkeys?

DR. WHITLEY: I haven't done it in the monkey, but I've done it in the mouse and I could tell you what we've

learned in the mouse. We did it in our tumor model in the mouse and we've done it with both acyclovere and gancyclovere, and just to tell everyone what the experiment is, we take a skid mouse, we put in human tumor cells, establish the tumor for five days, actually MRI the mouse brain to make sure we have a tumor, then put virus directly into the tumor itself, and then we can follow survival as a function of dose and as a function of other manipulations that we have tried.

If you administer gancyclovere within a day or two days of putting virus into the brain, you lose any potential anti-tumor effect of the virus itself, and, in vitro, all of the viruses I have described to you are as sensitive as wild-type herpes simplex to acyclovere and gancyclovere.

DR. BREAKEFIELD: What is the frequency of mutation of the TK locus?

DR. WHITLEY: It is very, very, very low in the normal host. In fact, there's a nice study that was just submitted to NEJM, looking at the development of resistance in the normal individual after exposure to acylclovere, both episodically and chronically, and these are people who were on drug for five or six years, and it is less than .03 percent, and that is--don't forget, these are acyclovere-exposed patients. It is not the normal population. It is even lower in the normal population.

FLOOR QUESTION: If the difference between the mouse and the aotus is not that great, what is the advantage of using the aotus at this point over the mouse?

DR. WHITLEY: To make those of us who are taking this into people feel more comfortable.

CHAIRMAN SALOMON: Excellent. Thank you. I think this was really a series of superb talks that focus on the issue in front of us. To finish the day, our job is to get through two questions, and the key one is to discuss the appropriate basis for determining whether safety studies of a gene therapy product should be in small animals, for example, rodents and/or nonhuman primates, which was clearly what I was getting at with my question to Rich and to the others along the way.

I think it is important, one of the agreements that I had with the FDA in discussing sort of these kind of questions was that we deal specifically with the idea that we don't get into defaulting to nonhuman primate models, for reasons that I think are obvious to everyone. At the same time, I think we're all grappling with the same issue that Rich responded to, and that was, yes, he now knows that the nonhuman primate model told him the same things that the mouse model did, but how would he have made that kind of conclusion confidently prior to doing these nonhuman primate studies?

Even when you have a good mouse model, one could make an argument, and obviously I'm putting this out here for discussion, could make an argument for going forward to a nonhuman primate study, even with the unknowns, and then the question to the group is what do you think of that kind of a statement and how could we do that intelligently and reduce the use of nonhuman primates, if possible?

That is kind of the issue. Does someone want to pick up on that? Let's start with how do you tell when to request a nonhuman primate model, versus a mouse model, at all? Let's just start with the basic decision of I have got a great product and I've got proof of concept.

DR. GORDON: I just want to make some comments on this. I think selection of an animal model--I think it was very interesting, Dr. Whitley's last comment, why did you select a primate, and the answer was because it made us feel more comfortable before going into humans. I think that this is a very historically common reason for choosing primates, but if I were to make a recommendation to the FDA, it would be to discourage doing it for that reason and to look for other reasons.

These reasons may be what about the organ system you're actually studying, and we don't need to think only about primates, but about other larger animals, does the organ system physiologically resemble the human more in a

dog or in a pig than it does in a mouse? What about, in the case of gene therapy vectors, the distribution of receptor for the vector? You don't really want to test gene therapy vectors in a dog for liver gene therapy if the receptor is not on the liver of a dog, and it is on a mouse and it is on a human.

I think the physiologic, biologic criteria are the ways to select a model, not because the animal is closer in size to a human, not because the animal's eyes are both in the front of its head, as they are in a human, and not, because it just makes you feel better in some sort of nondescript way, which I have a lot of sympathy for that feeling, may I say, but I think it is costly and potentially more controversial and also much less efficient.

Let's remember that if we're looking for a rare effect, something that would occur one-in-every-50-animals, it is very difficult to do that in a primate and ever see the result. We saw a bunch of studies this afternoon where there were fewer than 10 primates used, and that is only natural. I think one should discourage going to animals like that unless there is a demonstrable reason for doing it, and that would relate to the biology of the disease, the biology of the organ system and the biology of the vector.

DR. JONES: If I can speak on Dr. Whitley's behalf, too, what he mentioned at the beginning of his

presentation that we have not considered is when he chose the aotus, it was not just to make them feel better. It was also because they're very susceptible to herpes diseases, as well, and I think you have to take that into account when you are selecting an animal model. If the dog were a model that were susceptible to that, then maybe the dog would be your first choice in a large animal model, so I don't think that that was the entirety. Would you agree?

DR. CHAMPLIN: There may be some unique features about--you know, herpes, obviously, is a dangerous virus that can cause human disease and to mimic that in an animal species makes sense, whereas some other viruses that are not toxic on their own, the AAV, for example, would not make sense in the same rationale.

DR. WHITLEY: No, I think that's exactly the point. I think you have to understand the pathophysiology of disease and that was the point that I made with the third slide. If you don't understand the pathophysiology of the disease, you should not be doing these experiments in the first place. I think the key issue is that we didn't know when we began these studies is what we learned in the mouse and what we learned the rabbit, the same is what we learned in aotus. It wasn't until we got the experience of three different groups now that have used this animal and have basically reached the same conclusion, that we feel like

we're on firmer ground.

DR. ANDERSON: Just historically, this same thought process as you went through is what happened with the original gene therapy trials with retrovirus. We did a lot of studies in monkeys, took a lot of criticism from our colleagues, because I was at NIH, we could afford it. People on the outside couldn't afford it, but the issue was, was something going to happen when we put retroviruses into nonhuman primates that didn't happen when we put them into mice and rats and so one, and the answer was no, there wasn't.

There is not a need now to continue doing nonhuman primates, unless, as the example here, unless there is something unique that could only be answered in a nonhuman primate. Short of that, we have answered the question, so we don't have to keep reinventing the wheel.

CHAIRMAN SALOMON: One thing that has come out in these conversations is that there are different study outcome objectives. One would be safety of a vector, which I think loud and clear was what Rich was telling us. He was concerned about safety of the herpesvirus. Then, there are efficacy issues, which, for example, the dog model was one way of very clearly addressing the efficacy issue without using a primate.

I think the early retroviral gene therapy data,

French, you would agree, was safety again. You were concerned about replication-competent retrovirus, for example. Can we start to maybe become up with some things that we would say then would be principles upon which one would suggest the appropriateness of a nonhuman primate model?

You started with saying that you certainly would have to document reasonable expectation of a similar distribution of receptors or permissivity for the vector that is being chosen in the primate.

DR. GORDON: That is right, I would say that. That would relate both to efficacy and safety. Efficacy would also relate to whether or not the organ system models physiologically that which you are treating in the human. I just want to say parenthetically that I completely sympathize with the choice of the aotus monkeys here, and I would have been the first person to do the same thing after I heard they were that sensitive, so it's not like I'm criticizing. But, yes, again it is the physiology of the system.

Perhaps cardiovascular disease is better study in a pig than in a monkey, because the system is more similar to a human.

DR. BREAKEFIELD: I had a few things to bring up for discussion. First of all, I think we don't know a lot

about the receptors that are present for different viruses in different species, and if we had all that information, it would be very much easier to decide which model--just concerning safety, would be the best model. I think sometimes it's hard, though, and it especially gets even harder to know now when people start changing the surface properties of the virus to try to target them to different tissues and start putting essential viral genes under different types of tissue-specific promoters, because some animals that weren't infected before, I mean, that is a trophism issue that has to do with entry of the virus and also whether the virus replicates or not.

If you change those properties, you really don't know what to anticipate. I would say I felt, when they showed the studies with a replication-defective adenovirus and they compared it in--I guess we don't want to use the word monkey, but you know, but monkeys and mice, and it was the same, I thought that was great. It gives us a baseline. We feel confident now that this type of vector is the same. Somehow, you know, we talk about it, it is, in some ways, a little unrealistic or something to think we don't feel comfortable.

After all, that is part of the public domain. If we don't feel comfortable, we have to ask ourselves does the public domain feel comfortable? Why do we feel comfortable

testing monkeys? Since we think they're closest to us? Especially, in my opinion, if there is a disease where there is medical treatment available that is pretty good, hemophilia would be one of those or cystic fibrosis, that people are not in a life-threatening situation, those would not be the first people I would try a new route of administration or a new vector type or something very different without having a little more confidence in maybe multiple animal models.

I'm not saying you have to use nonhuman primates, but using different models as kind of a first line of is this or isn't this safe, so I have other comments, too, but I'll--

CHAIRMAN SALOMON: Again, I'm trying to pick up on just trying to come up with principles then that the committee would agree on. That principle would suggest that as a new vector is developed or a known vector is modified in a way that would significantly affect its range of trophism for different cell types, for example, or its expression in different organs, those would be points at which a trial might consider preclinical work in a nonhuman primate, providing that the first principle was correct, that there was a reasonable expectation that the vector was still permissive in the nonhuman primates.

DR. JONES: And making sure that we choose the

correct species of the nonhuman primate, making sure that that nonhuman primate species is appropriate for what we have chosen.

CHAIRMAN SALOMON: So, another principle then would be, with 200-plus nonhuman primates, though, maybe only about half-a-dozen really available to any kind of research regularly, do you have a suggestion as to how we should do that? How would you choose a species?

DR. JONES: I would say lots of consultation with your colleagues and people who are in the field, lots of information sharing and literature searches. There is a lot of available information out there on what is being used for different areas of research.

CHAIRMAN SALOMON: Joe, did you have a comment?

FLOOR QUESTION: I would tend to agree with all that is being said in terms of trying to use some type of scientific rationale for picking the species. In drugs, it is a somewhat different scenario, but we have actually gotten away from simply doing rats and dogs with drugs, as well, and we look for a rational scientific basis for using an animal model, rather than just arbitrarily saying we are going to do rats and dogs and that's it.

The same thing with the biologicals, we had looked at a protein last year in which we knew the sequence of the human protein, and so we looked at the sequence of the mouse

and of the cyno monkey, as well, and decided that the cyno much more closely approximated the human situation. We were able to utilize the monkey then, we felt, as a reasonably good prediction of what would happen in man.

You are talking about trophism. We are actually working with another group on a trophism-modified virus at this point in time. The majority of the studies we're probably going to be doing are going to be directed in mice, but there is also an imaging component with this, as well, so we're going to wind up doing some additional primate work, to a much more limited extent, in which we're going to be doing some imaging in addition to that to see whether or not the distribution of the trophism-modified virus is different than of the normal virus.

CHAIRMAN SALOMON: So there, the rationale or the principle you're going to use is you know that the virus is tropic in the mouse and in the nonhuman primates, but you now want to use the nonhuman primate to demonstrate if there is a distinct difference in the trophism, in terms of tissue specificity.

FLOOR QUESTION: Yes, to decide whether or not we need to target particular tissues in our evaluation of toxicity that we may have overlooked previously.

CHAIRMAN SALOMON: As a principle, that would get back to your talking about if you know the receptor or have

some sort of measure of the receptor, even if you have not cloned and sequenced it, that one should argue that there is a reasonable expectation of a similar distribution of the receptor, and if there are second receptors, such as we know for certain, like, lentaviruses (ph.), that that would be important, as well. Right? Okay.

DR. GORDON: I would say I think primates should be chosen the same way other animal models are chosen. There is a scientific basis for choosing them. They are much more cumbersome to work with in a variety of ways that we have not yet discussed, however. For example, at my institution everybody is screaming that every mouse has to be behind a barrier so they can be uniform with regard to what pathogens they have been exposed to in the past. You start importing monkeys from Samoa and let me tell you, you don't know what they've been exposed to. You don't know how old they are. You don't know anything about your genetic background. You're in a much less well-controlled system.

CHAIRMAN SALOMON: To what extent, if we think about the universe of vectors that are currently in front of us--to what extent do we know, based on what we know about these vectors, are they different between, let's say, mouse and dog and let's say nonhuman primates, in terms of the questions we have been asking? Is there some obvious background knowledge? Are certain classes of vectors more

or less likely to require nonhuman primate work because of a species-specific distribution of receptors for the vector?

DR. PILARO: I wasn't going to hit on that issue specifically, I was just going to speak to the two different classes--well, two of the different classes we heard about today, the adeno and the gutless adenos--are lumped together because basically the outside of those viruses is what causes the toxicity, and it is the same for the two of those and the AAV, and they have been studied across a various variety of species, for lack of a better word, and really, with the adenovirus, what we have learned from the data we have looked at in the past seven or eight years is they are inflammatory no matter what route of administration you give them, no matter what species you give them in, and the doses are always very close, the dose at which you see no toxicity and the dose at which you see frank inflammation and pathology.

There is a very sharp threshold. They are very close when you scale between the species. We have learned that adenotoxicity is basically comparable, no matter what species you're looking in. There, you see the justification for doing the studies in the smaller animal models. You can actually get higher numbers of animals treated. You can do more things with them. You can sacrifice them at interim time points and look at histopathology, whereas within a

nonhuman primates you would really be limited if you wanted to do an interim time point to just doing either a biopsy or blood work.

With AAV, we have learned across mouse, dog, nonhuman primates, I believe it is both rhesus and cinemologous monkeys now, and even human, that there is no known pathology with this vector. It can be given into the lung. It can be given into the muscle. There is no inflammation seen. That is another one we feel comfortable with. You don't necessarily need a nonhuman primate study for that. I believe rabbits have also been tested for that one, too. That's in the literature, as well, there's no pathology with it.

We are comfortable with those classes of vectors, saying that we understand what is happening based on the biology of the response to the virus. We know what's going on here. We do not feel that nonhuman primates would be added value to these two particular classes of vector, unless there is a specific question you're trying to ask.

DR. WHITLEY: I think there just needs to be a sidebar that is added just for the herpesviruses, because herpesviruses behave differently according to the strain of mice selected and the rodent species utilized. I guess what that does is lead me to the conclusion that, when you're developing both safety as well as efficacy systems, they

have to be individualized according to the virus and optimized for the information that can be retrieved, and to pursue what Xandra was saying, is we try and target viruses for different tissues, that is not to say you won't use an aotus at one point or another in time, but you better have a reason to use it and understand what the added value is going to be in that system.

CHAIRMAN SALOMON: Again, that is what I'm trying to do, is come up with a series of principles that would generally be--that everyone would agree with.

DR. BREAKEFIELD: Just, I mean, in general, I agree with what Anne Pilaro says. I think sometimes, though, you come up with something that is not quite the same, like in mice, they always tell you well, we can't really do those hepatic arterial injections, you know, we do the portal vein, so your route of administration isn't quite the same and you wonder can that be a factor.

I think the other thing, with replication-defective vectors, like adenovirus, once you know where it goes, it's going to go to the same place. But I think by the time you start changing promoter elements that control critical genes and targeting elements on the surface, I think those are open questions again, and the whole distribution in different species may vary. You just don't know.

CHAIRMAN SALOMON: Are you saying, again, species and tissue-specific differences in vector interactions--I mean, in enhancer and promoter interaction.

DR. BREAKEFIELD: If new issues arise in a protocol, then it has to be reevaluated, if the data we have speaks to it or doesn't speak to it.

CHAIRMAN SALOMON: It seems like we've come up with another principle, and that would be if a clinical trial that you have now specifically designed requires a type of administration that it would reasonably, on the part of physicians or the public or any regulatory agency, constitute something that was a specific risk for the administration route, and you could not do that in a mouse, that that would be another potential rationale for a large animal, not necessarily for a nonhuman primate, but at least for--

DR. MULLIGAN: I think another way to classify vectors is simply replication competence, that if you have a replication-incompetent adeno AAV retro, the issues are very different than if you're looking for something that you want to have a, you know, Pac Man function to chew up tumor or something. Those are issues that are tough, because we know so little about the normal determinance of tropism, in the herpes case, in particular. I think I would look to any replication-competent vector as very different in terms of

the animal model system, and really look not very hard at the replication-incompetent systems.

I think we've talked about the necessity to have receptors, the route of administration, but in the replication competence, I think that even for efficacy sake, which, you know, we're take a back burner to in this discussion, but for tumor approaches, where there's an effort to have actual replication just in tumor tissue, I think those systems that are now being looked at are very unsophisticated, and while from a safety point of view, things may be okay, I think looking from the point of view of efficacy, it may be very important to really look more carefully at the different systems.

CHAIRMAN SALOMON: Picking that up, what Anne told us was that there is such a body of information suggesting that the data between the nonhuman primates and the mouse, let say, for the adeno and the gutless adenovectors, is similar, that she is basically taking the position that she would be comfortable with that sort of data, yet in the context of the gutless adenoviral vectors, where you're going to be putting in helper virus at some percentage, I mean, do you want to comment on that, Dr. Chamberlain?

I mean, then you've got replication-competent virus and the principle, I think, Rich was saying was that that might be necessary to do some limited amount of

nonhuman primate data.

DR. MULLIGAN: Just on that point, I think the amount of replication-competent virus he is talking about is not important. I'm talking about a gene therapy approach where you're attempting to have replication competence.

DR. CHAMBERLAIN: Yeah, it's a different issue, because the helper virus we're using is essentially a conventional adenovirus, so that would not really change anything.

DR. GORDON: Can I just say one little more thing about that, that even in regard to Richard's comments, I think when you look at a nonhuman primate, the key question is, is the response of the animal, on the basis of its toxicity response or its efficacy response, going to tell you something about what the human will do better than or for the first time, as opposed to another species of animal model. If it tells you that, you should use it. If it doesn't tell you that, you should not necessarily use it.

DR. MULLIGAN: I did not mean to suggest that that was a rationale for going to a nonhuman primate. In fact, I think the discussion, if there is a consensus, as Ed said before, it all depends. I was just saying that for replication-competent viruses or vectors, you definitely want to look at the system, any system, and make sure that you're as close to looking at the characteristics that are

important as you can be.

CHAIRMAN SALOMON: Rich and Xandra, what do we know about the other herpesvirus vectors, CMV, EBV? I guess you knew I was going to ask that.

DR. WHITLEY: Let me take a shot at it first and then you can jump in. CMV is being developed as a vector, but we have a really, really fundamental problem, and that is we don't have a model to begin with to look at that virus, and we had a meeting at CDC about three weeks ago that was really quite a productive meeting, but at that meeting, it was very clear that we do not have an adequate model to begin to probe develop CMV as a vector.

For EBV, we have tumor genicity models in nonhuman primates. The question is, will they ultimately be used for the evaluation of gene therapy approaches, and it's just not that far along yet. It is the next step, because you do not have a rodent system to evaluate those viruses, and that is true for HHV-6, HHV-7 and KSHV. I think there you are going to be stuck using some form of nonhuman primate.

CHAIRMAN SALOMON: There, I guess, the principal would be one the one that we already discussed, and that is if it is not--if the tissue itself in the mouse, let's say, is not permissive for the virus, then you can't use it.

DR. BREAKEFIELD: But isn't that the only way an adenovirus--that it can infect mouse cells, but it can't

propagate in them, so if you try to look with the replication conditional, you know, replication-competent vector, are you really evaluating toxicity in the mouse? There may be, I think we've gotten to this before, the cotton rat is the only one where it does replicate and that is very hard to get, but, you know, these are the issues we get into, they are difficult to--

CHAIRMAN SALOMON: I didn't know that. I didn't realize that, after everything we said about the mouse, that you were not getting replication in it. Maybe Rich was thinking that when he made his comments.

DR. PILARO: Can I make a clarification on that? With a cotton rat, we know we get a limited amount of replication of wild-type adenovirus in the lung. However, no one has systematically looked to whether or not we get replication of the other tissues in any of the species, including nonhuman primates. So, we're really at ground zero with that. We know if you put a replicating adenovirus into a mouse liver or into a monkey liver, that you would get the same results. We don't have that data.

CHAIRMAN SALOMON: But if I understand right then, really what you have said then, and I think Dr. Lyons, GTI Novartis, said something very much along the same lines with adenoviral work that they did, that from an adverse event point of you, it looks like the mouse and the nonhuman

primates and even the human is fairly predictable, and that is a very good thing in terms of not having to do nonhuman primates for those studies, but it doesn't seem anything like that is true if the question one wanted to ask is what would be the risk of injecting a replication-competent adenovirus? Can we agree on that?

DR. PILARO: You've got it. That is the point.

CHAIRMAN SALOMON: Well, that was, I think, what Rich was trying to say and I missed it, so that was just me not understanding it.

DR. PILARO: We just don't know what an appropriate model to look at replication-competent adenovirus would be right now.

CHAIRMAN SALOMON: Do we know that a nonhuman primates is a model for it?

DR. PILARO: No, we don't, we don't have that information. No one has really systematically looked across the different species, going into the different tissues, to see if you put a replication-competent virus in here, does it replicate in a mouse liver, does it replicate in a human liver, does it replicate in a monkey liver? We just don't know.

DR. CHAMPLIN: Now you have human trials with adenoviruses.

DR. PILARO: What we know from those trials is the

data that have been discussed before the RAC, is it appears that replication-competent viruses replicate in the tumor, but not in normal tissue, and that is the best data we have got out of those studies.

DR. ANDERSON: I'm not telling you, you know, but that is a correct statement, but that is a non-data statement, because those replicating adenovirus were designed not to replicate in normal cells.

CHAIRMAN SALOMON: Right. These are the P53 mutant. French, would you agree, with respect to retroviral vectors, I think you kind of said that, but just as long as we were going through each of the vector classes, to try and come to some sort of consensus, would you agree with the retroviral vectors that, at the moment, as long as one didn't do something again along the principal that Xandra came up with, that you didn't profoundly change the vector in some way that would raise concern, that the retroviral vectors do not need to be tested in nonhuman primates at this point?

DR. ANDERSON: They had considerable testing, including testing with RCR, with replication-competent virus, to the point they inadvertently did the definitive experiment of seeing what level of replication-competent virus would produce lymphoma in the animal. All of that data was a report that I wrote, along with Gary McGarrity,

to the RAC and the FDA, analyzing all of the data of retroviruses into monkeys. We went into over 30 animals and many of those animals are still alive and still being followed.

The total data now that represents well over 100 monkeys, or well over 100 monkey years, suggest that we understand sufficiently about what the risk factor is that probably no additional monkey studies need to be done, or nonhuman primate studies need to be done.

CHAIRMAN SALOMON: I have quoted some of those studies in grants and gotten mixed results. Some people do not think that it is absolutely established that injection of replication-competent retrovirus produces lymphoma in these animals. There are a relatively small number of animals in the end and there isn't spontaneous incidents of lymphoma in the animals, as well, particularly in captivity.

DR. ANDERSON: Let me refer you to the report that I am first author of that went to the RAC and FDA that analyzed all that data, as well as additional data out of the lab notebooks that was never published anywhere else, and the conclusions are sufficiency solid that it would be hard for me to believe that anybody who has analyzed the data would still question what the situation is.

You could always say you could do 100 more animals and what if you did this and what if you did that, but there

is no question that it was the replication-competent virus that caused the lymphomas. There was no question that the retroviral vector did not cause them, and all the conditions required, including severe immunocompromised, the lack of antibody response, the necessary (sic.) for a long-term retroviremia lasting over 100 days, it was a clonal event in each animal. The virus was actually isolated out of sequence.

I mean, thorough studies were done.

DR. BREAKEFIELD: But if you just subtract out the lymphoma component and say that is established, let's say you had a retrovirus vector that had like a different targeting moiety on its surface and now you are going to inject very large amounts, IV, do you think it needs to be retested in primates or nonhuman primates or do you think the data is there that supports that would not be toxic?

DR. ANDERSON: Well, that is actually a question that we are directly facing, because we have now developed a targeted vector that can be injected directly into the bloodstream, and that will be discussed at the RAC in December, and is at the FDA, and our feeling is that we would not obtain additional information. This is replication incompetence, to go along with Richard, that it is not necessary to go into nonhuman primates.

If, however, we or someone else were to develop--

well, I shouldn't say we or someone else--Dori Kasahara, in our program, has developed a replication-competent retrovirus specifically for treatment of cancer, and that is an interesting question. I don't want to bias either us or the FDA by speculating at this point, but we're going to have to face that issue over the next several months.

DR. NOGUCHI: Not to prolong the discussion too much, but regardless about what people think about the lymphoma, what is clear and has been demonstrated is if you have replication-competent murine retrovirus that you give to a nonhuman primate, you will get chronic infection at the very least. Some precipitating events like severe immunosuppression may then lead to further replication and activation out of that persistent infection.

I think in at least a general sense we know what will happen with any contamination of replication-incompetent retroviruses. I think French has also identified, though, just as adeno went from replication-deficient to replication-selective, they may an approach--approaching that with a retrovirus, then I think the question is open again. But just talking about adenovirus, as an example, again it may or may not be the nonhuman primate that is the appropriate model. That is still open for much discussion.

DR. TORBETT: I was just going to as Dr. Anderson

if he felt comfortable with the many different approaches in his discussion, that many different types of targeting vectors now coming online, whether it's VSVG, whatever, which can probably hit a cell with much higher frequency than others, that simply using a mouse model is adequate? That is to get back to your point that mouse models would be adequate.

DR. ANDERSON: Yes, yes, right. Well, now that I'm sitting on this side of the table instead of out there, that is really sort of tough, because that is the other issue we have dealt with, because our targeted vector is much more efficient in our mouse models by having a mixture of VSVG with our chimeric targeted 4078. I would tell you my gut feeling. I would be a lot more comfortable going into primates for just the reason that Dr. Whitley said, is before we going into a patient that we get into a monkey, but I don't know if I could justify that on scientific grounds.

DR. TORBETT: We're back where we started. I was just curious.

CHAIRMAN SALOMON: I'm glad to say this day will not end with consensus, any more than the morning did. At this point, we have been talking about safety, and I think that we have come up with at least a series of principles that might at least be used to guide an FDA decision along

the lines of whether a nonhuman primate or a large animal model should be used.

Principles would include that you have permissivity, that you have a reasonable expression level of the receptors for the vector in different tissues, that if there is extensive experience with the vector choice in nonhuman primates to the point that there seems to be no evidence upon which to argue that the nonhuman primate adds information for safety, that you could not get in a mouse or a dog or another model, then there would not be a reason for doing it.

But that changes--significant changes in a known vector class that could be argued scientifically would generate additional safety concerns, replication-competent virus whose behavior could be very different in a nonhuman primate than in a mouse, or where a clinical protocol would specifically require an administration route that would reasonably generate concern on the part of anybody looking at the study. All those would be reasons--yeah, please, add one.

DR. BREAKEFIELD: The only thing I would add to that is if you had a transgene product that was active in humans and not in mice and was active in nonhuman primates, that might be another.

CHAIRMAN SALOMON: Right. I was doing something

specific here. I was kind of ending with the safety and then I wanted to finish maybe a couple minutes talking about efficacy issues that I think have a whole other set of principles. Does anybody have anything to add to what I sort of reviewed quickly? Basically, it wasn't my idea. I was trying to review what came out of the discussions here that would be then, as I said, some set of principles upon which to consider this, and I think we did answer most of the questions then about class of vector and when do it.

At the end, like I said, I would like to talk about efficacy for a minute.

DR. NOGUCHI: I think the reasoning and the discussion has really been excellent here. I'm reminded a little bit about sometimes we discuss questions about surrogate endpoints, and it seems to me the principle is the same, for our conclusion that you have come to today about where we can safely use a model other than a nonhuman primate in this particular case or any other model, someone had to do the studies in the first place or else you can't really make that evaluation.

I think that overall it is a very good lesson for a lot of different things, not just for this, but things like surrogate endpoints and everything else.

CHAIRMAN SALOMON: Yes, I agree, and I think again, really I think an important principle here is

whenever you would introduce a new viral class of vectors or non-viral vectors or really a significant change in the vector, then those would be another place where one would have to consider again the model. Let's then segue at the end to are there any additional principles that should be added to the discussion for efficacy parameters?

It is one thing, then, to have a model in a mouse in which we're comfortable that the adverse event profile is similar, but now how do we think about other issues with respect to expression and gene delivery, antibody responses, for example, really came up and I challenged Dr. High about the idea that, well, you know, this is a novel antibody response and then you're trying to reassure us this is a model for humans. My response is, well, it might be, but then you have to see that happen in a human, which I think was Carol's point. Can we discuss for just a minute what do you guys think about what does efficacy issues add to model choices?

DR. TORBETT: I think it depends on the system, if it is going into a human and you were using a cytokine, for example, or whatever that doesn't cross-react with a mouse, that mouse is going to see as foreign, you're already biasing the study and getting information that might not be relevant. However, I think in all these situations, you have to judiciously pick the system that you're using, again

if it something that is very toxic to mouse again, you're going to bias your study, so in these kind of situations, if it's a cytokine, IL-12, whatever, going into a nonhuman primate system, it might be the only thing that will work.

DR. CHAMPLIN: Even there, it may not work, I mean, immunogenicity isn't really, at least in my experience, it's not very predictive from animal models to humans. Sometimes you have problems on one end or another and you just have to do the study to see. The other issue is the model system for--you know, there are some nice diseases, hemophilia would be a classic example, where the model system could be very helpful, whereas there are many other diseases, cancer, where the animal tumors don't predict necessarily for human responses, particularly for immunotherapies or cytotoxics.

I think, depending on the disease system, animal models may or may not be predictive and you really want to look for safety and for feasibility of expression of the transgene.

DR. WHITLEY: I was going to try and deal with one principle, and I think that when animal models exist for disease, they should exploited and they should be studied in the context of the natural immune response. Having said that, if I just take a different hat and put on a vaccine hat, rather than a gene therapy hat, I can take a mouse and

immunize a mouse with water and that will protect the mouse from challenge with wild-type herpes simplex infection.

What we've learned from the mouse to humans with sub-unit vaccines has not been applicable. Sub-unit vaccines don't work in people, they work in mice, so we have to be little bit careful about the analogies in terms of the meaning of the immune response in rodent systems compared to man.

CHAIRMAN SALOMON: That would underline the principle I started with, and that is that when we start talking about efficacy, the game is again different. There may be very clear situations in which we would need to do some nonhuman primate studies to demonstrate efficacy or reassure ourselves that an immune response was similar. Dr. High, I thought you might want to make a point.

DR. HIGH: I just wanted to make one point, and that is that in data that I didn't have time to present, using those two different dog models of hemophilia, we actually purified dog Factor IX protein that showed that, if you infused purified canine Factor IX protein, similar to the product currently used to treat humans, except that it is species-specific, if you infuse that into the Chapel Hill dogs, the ones with the missense mutation, they don't make inhibitory antibodies, but if you put it into the dogs with the early stop codon, they do make inhibitory antibodies.

This correlates with what we have observed treating humans, that is a dog with a more severe mutation has a greater likelihood--so that speaks to the point raised by Dr. Miller, because actually the immune response to the transgene product in hemophilia is really a safety issue, so it is a safety issue related to the transgene product instead of the vector.

CHAIRMAN SALOMON: It is an efficacy issue, as well, right, there's no point in doing gene therapy if you're going to get an antibody and inhibit it. Right?

DR. HIGH: Right, so it is both, but the point is this is a safety issue related to expression of the protein, and we do have information, from using purified protein, that what happens in the dogs is similar to what we have already observed in humans. I think there is a rational basis for believing that the data generated by gene-donated approach may predict what happens in humans.

CHAIRMAN SALOMON: That would raise then a principle that I would put out for comment, that if in the roll up to a clinical trial you could convincingly argue, with data, of course, that the model you had chosen, that was a nonhuman primate, where it is a mouse model or a dog model or a guinea pig model, I don't care, reflects a clinical experience at a molecular, cellular, protein level in such a way that it would really give a convincing

argument to people that this was a model, that you would not necessarily need then to go on to a nonhuman primate model to make your point.

DR. HIGH: I might turn that around a little bit, just to say that that fact alone would not exclude the necessity for nonhuman primates, but I would argue that it would support the use of an animal that was deficient in the protein.

CHAIRMAN SALOMON: That is the principle.

DR. BREAKEFIELD: I mean, I want to stress that point, too. I think several people have said it, but if there is a non-primate model, a mouse model, of a disease available, I think it is very important to do the safety studies in those animals because sometimes, due to their illness or whatever else, they become especially susceptible to the virus. If the nervous system kind of degenerates and the virus infects those cells, they may be much more affected, and it is a very important model to include if it is available.

On the other hand, I don't think you can hold the investigators accountable if the model doesn't exist because it takes a long time to developed these models and sometimes you develop them and they don't even look like the human disease model.

CHAIRMAN SALOMON: I think that the issue I was

trying to come up with was not if you don't have a model what do you do. I can't help that. I mean, that is not for this group to discuss. But what I was trying to argue was if we agree that if you had a model and if you could demonstrate that that model paralleled what one saw in clinical experience, which is what Dr. High was saying, could we agree that you could then use that reasonably to argue for the use of that model as a surrogate for nonhuman primate data prior to going on, let's say, to a Phase I or clinical trial.

I feel comfortable with that, if it would, of course, be an individual thing. I would have to be convinced by the results and I would be skeptical, but I'd have to be convinced.

DR. MILLER: I mean, my point was, from the animal studies, is that you can use that data to suggest you don't need to go to a maximal tolerated dose. I think you can say as long as you are getting efficacy, you can look for the minimal effective biological or pharmacological dosing, but using that model system, if you didn't get efficacy at the level which you felt was, based on your model system, not immunogenic, you would be forced to go up, so I think you can use the model system to try and help you as long as you're getting a biologic efficacy effect.

While you are saying it is safety, it is also

giving you--as long as you have efficacy, it can model only safety, but it has to be evaluated through both safety and efficacy.

CHAIRMAN SALOMON: I think it's a good point, too. I think part of what I didn't say, but I agree with, is that if you are going to make this argument not to go to nonhuman primates for a new vector system, it shouldn't be just based on safety alone. Picking up on what you said, there should be an animal model of the disease and you should be able to demonstrate efficacy, because I think at this point, in general, I think the public is getting shy of the idea to going on to risky trials when efficacy has not been demonstrated, even reasonably, in a model of the disease.

DR. NOGUCHI: I would just like to clarify what I think I hear Dr. High saying, and it is actually just slightly different than what we have been discussing. What we were really discussing is in the absence--how best to put this? There is ample evidence that inhibitors is one of the worst things that could happen with treatment of hemophiliac patients, because that can literally destroy any potential or real benefit that current technology can provide.

What Dr. High is actually addressing is do we need to have the experience with gene therapy of developing inhibitors in a human first to say that then the animal model is valid, or can we had instead use the wisdom of

previous experiments, and what Kathy High is saying is that we know that under certain conditions, with a big deletion that gives you no protein, puts a person at more risk, and she now has evidence that in the animal model with a gene therapy vector, that you can, under those conditions, induce inhibitors.

The question of whether it is valid or not is a genuine one, but I think it's going a little bit beyond the issue here, is the question of do we need to have the human experience before we say let's not go there?

CHAIRMAN SALOMON: Actually, I don't agree with this at all. What I'm saying is specifically that if you want to argue that you do not have to go on, that you have got a model that predicts what is going to happen in the human, you have got to tell me on what basis you made that prediction. Look, we can cure all kinds of diseases in mice. I've had enough of that in 20 years of curing mice. There should not be a sick mouse in the United States, and yet my patients are not doing so well.

I think that the point here is I would buy the principle that if you had a model in the humans--I'm not saying do it in the humans until you get a side effect and then congratulate yourself as part of a workup to a clinical trial, but I'm saying if there is a clinical experience in humans through the administration of exogenous Factor VIII

in hemophilics, that now your model parallels and now you want to tell me now I've got a valid model and I don't have to do a nonhuman primate, that is what I'm saying I accept.

If there's no human data, then all the arcane, beautiful, molecular baloney that you come up with for your animal model is just that.

DR. CHAMPLIN: Why would you need to do a nonhuman primate for the Factor IX approach?

CHAIRMAN SALOMON: I actually was saying I don't think you do. I think that was a perfect example. That would be a model in which I'd say you've got human correlation, you don't have to do nonhuman primates. That was the whole point. I agree with that.

FLOOR QUESTION: What I do not understand is, in the event that there was no canine model of Factor IX, what would you gain by a nonhuman primate study of a gene for Factor IX with regard to inhibitors? I mean, I don't understand if there is no model, then a nonhuman primate does not help.

DR. CHAMPLIN: That's what we said. We said we would not recommend it.

FLOOR QUESTION: And if there is a model, how does it help?

CHAIRMAN SALOMON: I could answer, but Xandra, do you--

DR. BREAKEFIELD: Well, from probably most of the primitive viewpoints, but just that if you been working with a vector and different species have different kinds of immune responses, I would imagine the nonhuman primate has an immune response more similar to humans with some subset. Maybe that is wrong.

FLOOR QUESTION: But that is a safety issue.

DR. BREAKEFIELD: Well, it is efficacy, too. If you make inhibitor, then your vector isn't going to work in those people that have the deficiency. Right? I see. You're trying to just argue for efficacy.

CHAIRMAN SALOMON: Can you spell out what you're saying? If you don't have a model--we were talking about if you had a model and how you ratify that model without having to do nonhuman primates. That is not what you are getting into now. You're suggesting now that what is the situation where you don't have an animal model for the disease, and I'm not going to demand--

FLOOR QUESTION: Dr. High has made the case that we have this experience with the canine Factor IX, et cetera, and you have said there is a syllogism you can follow with regard to the canine Factor IX that might persuade you that maybe in that case you do not need a nonhuman primate model for Factor IX. What I do not understand is in the absence of having a primate hemophilic

model, what a nonhuman primate model for Factor IX would do.

CHAIRMAN SALOMON: Nothing.

FLOOR QUESTION: Okay.

DR. GORDON: I just wanted to make a little comment on efficacy and that is the one that was brought up briefly with the cystic fibrosis mouse, and here is an animal that does not have CFTR, but when it comes to efficacy at the level of alleviating disease, it is not a useful model. I think if a primate presents a form of disease which can be examined for efficacy at the level of alleviating symptomatology, then it clearly is a model that should be sought. I want to make one other political point, if I could.

I think it is great to look for non-nonhuman primate animal models, but I don't think that it should be implied that one would hesitate for a moment to use a nonhuman primate if it was the best model. I don't want to get into that dangerous ground of suggesting that we would not do adequate animal testing just because somebody does not want us to use a primate or something like that, not that anyone in this room agrees with that, but I just want you to know there are people who do think like that.

CHAIRMAN SALOMON: I agree. I think that the principle that everybody is trying to grapple with is what would be the circumstances in which just sort of a knee-jerk

response would be, we have to do a nonhuman primate because we're uncomfortable, versus we're going to do a subset of nonhuman primates to answer a specific question necessary for the safe introduction of this agent, to a question, as this gentleman posed, from Avigen, is that if you don't have a disease in it and in this particular case, the issue is you can't look for an inhibitor without an absence of the native protein, then it doesn't make any sense and I agree with that, as well.

DR. GORDON: Well, let me just make a brief amendment to the way you responded, although I do agree with it. I think if you're in a situation where you are looking at vectors and looking for a suitable animal model and you don't have one yet, they shouldn't be left off the list, that is if you don't have a suitable animal model for looking at response, looking at toxicity, looking at supportive gene expression, then they must be included in the list and it would be a rationale for at least examining those species.

CHAIRMAN SALOMON: Right, and then I think again you would turn to the principles that we've kind of articulated this afternoon to ask critically whether if you fulfilled a number of those principles, whether a nonhuman primate model was reasonable, a new vector, a change in the vector, something specific that is species-specific. One

thing I wanted to comment on, and I think we're getting pretty close to the end here, but one thing that always bothers me is when we look at these animals models and when you look at these nonhuman primates, when you do these data, am I supposed to go into the nonhuman primate model with human Factor VIII or Factor IX or we have kind of beaten these factors to death, let's say IL-12 or any of these different things, if you're going in with the human protein into the nonhuman primate, I'm not getting that quite straight.

At the same time, however, is then do I have to stop and remake my vector using the primate equivalent of the protein, which I can see a couple of the sponsors in the back fainted already, but anyway this is all Rich Whitley's idea, by the way. I didn't have anything to do with it.

DR. PILARO: Can I address at least part of that? It's been our long-standing policy that if you have a homologous gene available and you have done some preliminary efficacy work with that, that it is permissible and actually encouraged to do toxicity studies with that gene, so that you actually understand in the system you're looking at what the toxicity of that protein would be.

There are differences between human and nonhuman primate IL-12 or interferon or any of these biologic proteins. The monkeys usually all develop antibody against

the protein within several weeks of administration. So, it really--

CHAIRMAN SALOMON: Are you saying when you use human IL-12?

DR. PILARO: I'm saying when you use human IL-12.

CHAIRMAN SALOMON: But not if you use monkey IL-12?

DR. PILARO: If I had monkey IL-12 available, I'd give you an answer there. I don't. That is something that we have not got data available on yet.

DR. CHAMPLIN: Antibodies to cytokines in humans--and, you know, that may or may not be biologically relevant to their therapeutic use, and clearly things can be very different in primates and non-primates, in terms of their antibody response. So I personally don't see that--any animal system really predicts for the human immunogenicity-related problems.

DR. PILARO: Okay, I did want to give the other half of that, though--that if you did not have a homologous gene available and the only gene you had was the human gene and the transgene product is species-restricted and only active in human and nonhuman primates--that would be a call where you would have to use a nonhuman primate. We certainly wouldn't make you go clone the monkey gene and insert it into a vector and then do studies. It is sort of

six of one, half dozen of another.

DR. HIGH: Actually, since she brought up clotting factors, maybe I'll just mention it because it harkens back to another point that I had tried to make. Monkey Factor IX, I know I said the wrong word, but Monkey Factor IX is about 97 percent identical to Human Factor IX at the sequence level, and it turns out that if you give an AAV vector expressing Human Factor IX into the liver of monkeys, they don't make antibodies to Human Factor IX. If you use some other vectors, they do.

CHAIRMAN SALOMON: Well, that may be a reason we could talk about it, that we can get back to later as a resin-immune site, but that is another story. I guess, just to pursue this just for another second and then we're done--if I, as a sponsor, did a study, then, with monkey IL-12--because these days, it is not that hard to insist on cloning a monkey homolog, to be honest. But let's just say I did that study with homologous protein and I got this and this result. And now, of course, I want to go to a human study, but I'm not going to use monkey IL-12. I have got a fresh construct of human IL-12 in the same vector. Is that okay? I mean, is the FDA going to roll with that one?

DR. PILARO: Are you asking me to design your talks program for you? If you are, what I would tell you is, if you had available that vector or that protein, the

recommendation that I would make to you is, if you're going to go for the nonhuman primate studies, you build your talk study and you do your dose response with the monkey study, but you add in one dose group with the human protein, preferably close to the maximal dose that your giving, so that you can see what the differences are, what the similarities are.

The human protein is what you're going to go into the clinic with, so it's always useful to have that data. You want that information. When you're dealing with a species restriction or when you're dealing with significant differences between a nonhuman primate and a human, you would want the information with the homologous molecule, if it's available--big caveat.

CHAIRMAN SALOMON: Good. Okay. Did we answer the questions that the FDA wanted? Is there anything hanging out there that you want us to deal with?

DR. PILARO: You have basically given us some, what I call, red flags for when you think nonhuman primate studies are appropriate. And, I want to actually commend the group because you go along pretty much with what the guidance in the ICHS-6 document for biotechnology-derived products is. That document does not address gene therapy products, but it is basically what we use in biologic as our Bible. So, I'm happy to see that we've been kind of going