Nonclinical Studies for Plasma Derived and Analogous Products

SLIDE 1

This presentation will cover the Division of Hematology or DH, in CBER's Office of Blood Research and Review, and how the Division reviews the nonclinical and preclinical studies performed to support the applications for the DH products.

SLIDE 2

As you are probably aware, CBER has three offices: the Office of Vaccines, the Office of Cellular Tissue and Gene Therapies, and the Office of Blood. The Office of Blood has three divisions: the Division of Blood Applications or DBA, the Division of Hematology or DH, and the Division of Emerging and Transfusion-Transmitted Diseases or DETTD.

SLIDE 3

This talk will review the regulation of products in the Division of Hematology. This division has four laboratories. The Laboratory of Plasma Derivatives regulates immunoglobulins, antitoxins, antivenoms and some other products, such as alpha-1 protease inhibitor, used in a congenital deficient disease.

The Laboratory of Hemostasis, or LH, has a wide breadth of products that are involved in coagulation, such as clotting factors which could be plasma-derived or analogous proteins produced from recombinant technology. Also, this laboratory regulates wound sealants and combination device-biologics. These devices are different from the devices mentioned in the presentation entitled, "Medical Device Review at CBER". For the device-biologics reviewed in the Laboratory of Hemostasis, only the biologics part is reviewed, not the device part. The device part is under the purview of CDRH, the Center for Devices and Radiological Health. The Laboratory of Biochemistry and Vascular Biology regulates vascular proteins, volume expanders, artificial oxygen carriers, and enzyme inhibitors, such as Hemin.

And, the Laboratory of Cellular Hematology, or LCH, regulates cell products, such as red blood cells, white blood cells, plasma storage devices, anticoagulants and collection devices.

So, as you can see, there is a wide array of products that are regulated in the DH division, starting from products derived from blood and plasma, which have been used for a long time in the clinic with a good safety record, and analogous

recombinant proteins. Also, there are some very novel products, such as artificial oxygen carriers.

SLIDE 4

What is the legal basis for FDA asking for animal studies? First, the term "animal studies" is commonly used interchangeably with the terms "nonclinical laboratory" or "preclinical" studies. So, you will hear all these terms used throughout this presentation.

For biologics, there is the Public Health Service Act. It establishes the requirements for biologics to be licensed, a requirement for introduction into interstate commerce. The Code of Federal Regulations, or CFR, specifies that for a company to obtain a biologics license, they need to file a biologics license application, or BLA. In this BLA, they are required to submit data derived from nonclinical laboratory and clinical studies that will demonstrate safety, purity, and potency -- in other words, safety and efficacy of these products when used in humans.

In order for these clinical studies to actually be performed, the sponsor needs to file an IND, which stands for "investigational new drug application". In this application, the nonclinical studies have to show adequate safety of the biologic product under consideration. This means that the animal studies show that it is reasonably safe to start the proposed study in humans. Only then can the clinical study begin.

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What is the objective of performing nonclinical studies? The nonclinical studies in animals -- also called "pharmacology and toxicology", or PT studies -- are performed to assess possible toxicity in clinical subjects.

SLIDE 6

You cannot talk about toxicology without talking about the general toxicity test, which is the basic unit in toxicology testing. The general toxicity test is an animal study which can be done in rats, but also in mice, and sometimes in dogs and monkeys. You can think about the general toxicity test in terms of its design as a clinical study. It contains treatment and control groups. It contains a method of bias control, such as randomization. It has dose-ranging from low doses to high doses. This is done to establish a dose response of the toxicity observed. For example, you may see this type of response: low dose, safe. Middle dose shows some toxicity. For high dose, the toxicity is confirmed and even exacerbated.

Also, the general toxicity test is used to establish a "no observable adverse effect level" or a "no observable effect level." These are levels that are used in toxicology to decide at what dose a compound is safe to use, thus making sure that the compound is safe to use at the pharmacologic dose.

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The general toxicity test is comprehensive and detailed. What does that mean in practice? Here is a list of components that are part of this test: It incorporates clinical, cage-side observations of animals several times a day.

These observations are based on a pre-established protocol, with the lab personnel handling the animals and performing detailed observations for signs of toxicity at predetermined times every day, let's say before and after dosing, on day five, and before final sacrifice.

The general toxicity test includes looking at measurable parameters. These parameters include clinical chemistry, such as liver enzymes; blood chemistry, such as coagulation; hermatocrit; gross pathology, such as organ appearance and weight; and histopathology of main physiological organs, in other words, making slides for each organ or tissue of interest and microscopically examining them for signs of toxicity.

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General toxicity testing can be exploratory and confirmatory. For example, it is not informative to use doses that are so toxic that all the animals perish during the experiment. Often, in a first study, called the exploratory study, several doses are used, and the doses that could be meaningfully used in a confirmatory study are determined. Then, in the main study, three groups may be used - one with a dose where no effect in animal well-being is seen, then one with a dose where animals show some toxicity or signs that precede full blown toxicity, and then one with a dose that confirms toxicity. Such a design would be very informative in not only determining the toxicity profile of the compound, but also in designing a clinical study that ensures the safety of the patients.

The general toxicity test could be acute, but is usually a repeated dose test, because a repeated dose study is the most informative study. Also, it could be customized. You could add safety pharmacology end points, for example, measure the pulmonary gases. This presentation will cover the safety pharmacology a little later. Also, you can add immunogenicity end points and measure the antibody response.

If concerned, you can add more detailed histopathology. For example, if the toxic effect of the biologic or drug includes central nervous system toxicity, then you can collect and analyze more slides of the brain, and examine them for signs of toxicity.

After listing all these attributes of the general toxicity test, the question one asks should be: Is there any one-size-fits-all design? Can one actually design a general toxicity test that could be used to test the toxicity of all of those products? The short answer is no.

The reason for this answer is the breadth and scope of the products that CBER regulates. There are very old products for which the toxicity in the clinic is very well known. So, you don't need to perform a very extensive study. Instead, take a more focused and directed approach. But, there are products that are so novel that you actually do need to probe more exhaustively, because you do not know the possible constellation of toxicities that could occur.

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There is another factor that makes regulation of biologics more complex than that of small molecules. That is the structural complexity of the biologics. Here is an example:

This is the structural formula of Warfarin, which is a small molecule blood thinner. The structural formula is a graphical representation of the chemical structure of a compound. Once you know the chemical structure of warfarin and have its formula written down, you know what the pharmaceutical is. Synthesizing this chemical structure means you synthesize warfarin.

This is a cartoon rendition of antithrombin III structure, a protein and a biologic that is licensed to be used in some of the conditions where Warfarin may also be used. However, the similarity ends here.

When you talk about antithrombin III chemical structure, you could mean its sequence or "the primary structure". However, note that its structural formula is not displayed the same way as warfarin. There is not enough space in this slide to display the full structural formula for antithrombin III. The reason is because the antithrombin III primary structure is big, much bigger than warfarin's. It contains 430 amino acids, it contains sugars, and it contains ions. Furthermore, even explicitly writing down these 430 amino acids, sugars and ions would not give you antithrombin III. More importantly, synthesizing these 430 aminoacids, sugars and ions would not give you antithrombin III.

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That is because, in addition to the primary structure, antithrombin III also has a secondary structure. This means it has helixes, loops, and beta sheets in different parts of its sequence. Furthermore, in addition to that, antithrombin III has a tertiary structure, also called a global structure, which in this case, is kept together by the three disulfide bonds that it contains. A lot of proteins may also have a quaternary structure due to homodimerization, or heterodimerization.

Thus, the structure of antithrombin III and other biologics is very complex. Because of this complexity, when one assesses their potency and safety of biologics, one has to take into account their structure. One has to make sure that the primary structure is correct, the secondary structure is correct, and the tertiary structure is correct, in order to be sure that the biologic is safe and effective.

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FDA has promulgated guidance to help design a program to assess the toxicity of biologics that acknowledges the complexity of the biologics. The battery of nonclinical studies to evaluate safety of pharmaceuticals is set forth in two guidances. The one used the most is the ICH S6 and is titled Preclinical Safety Evaluation of Biotechnology- Derived Pharmaceuticals. This guidance applies to most of the products regulated in the Division of Hematology, because most of those products are in fact derived by a biotechnology manufacturing program.

Another guidance addressing the preclinical evaluation of safety is the ICH M3, titled Nonclinical Safety Studies for Conduct of Human Clinical Trials for Pharmaceuticals.

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This is a side-by-side comparison of the ICH-S6 and ICH-M3 guidances (it needs to be noted that at the time this presentation was written, revisions to both guidances were underway within ICH). It becomes clear that there is a lot of overlap between the two. Both guidances recommend safety pharmacology studies be performed, exposure assessment by means of pharmacokinetic and toxicokinetic studies, single-dose toxicity studies, repeated dose toxicity studies, productive performance, general toxicity, and carcinogenicity.

However, there are two specific areas in the S6 document that are of particular importance to biologics: the specification of the test material and immunotoxicity studies. Why?

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First, why is the specification of test material important? As mentioned with respect to the complexity of the structure of a biologic, the specification of test material needs to account for the structural integrity of the biologic. That means the primary, secondary, tertiary, and quaternary structure, which are all very important in determining the potency of activity of your biologic.

Specifying the test material also means taking into account the glycosylation and other post-translation modifications, which play a very important role in the clearance of the biologic. The clearance becomes an essential parameter when determining the dose of the biologic.

Closely related to the test material specification is the issue of impurities. Because the biologics are produced from such complex starting materials, such as blood or cell culture, there are a lot of proteins in that mixture. From this mixture, you need to purify your protein of interest, the biologic. Thus, impurities in the final product could derive from substances that co-purify with the biologic during purification.

Another concern arising from blood and cell culture starting materials is the issue of the advantitious agents, such as viruses or bacteria, that may be present. Thus, the manufacturing process needs to ensure that all potentially harmful agents are removed. As a result, the purification process is very often very complex, and impurities arising from the purification process could actually be present in the final product.

The S6 guidance specifies that the emphasis should be placed on purification. It is not efficient to design a preclinical program that aims to assess and evaluate the impurities. The manufacturing should ensure a robust purification of any and all impurities. But, if the purification process does not take care of everything, then you need to perform an analysis -- a risk assessment of what is the risk of exposure to humans from this impurity. In such an analysis, all the data from such things as animal studies, literature, and clinical studies needs to be taken into account.

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Safety of excipients is another issue of special interest in biotech products. Excipients are very often added to the final formulation of a biologic because of the very nature of the biologic. They are often unstable. Biologics need to be stable to ensure the potency, so there are excipients added in the final formulation.

There is a guidance issued by FDA called the Guidance for Industry Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients. This actually clarifies and sets forth the program that should be followed to make sure that excipients are safe. Having said that, let's mention that new excipients are uncommon. The manufacturers very often use excipients for which a safety database has already been accumulated.

So, when FDA receives a new biologic application, the excipient is checked, both its amount and the expected exposure. Then a comparison of the exposure to other licensed products with a known clinical safety profile is done.

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A very useful resource is the TOXNET Database of the National Library of Medicine, which is part of the National Institutes of Health. This is a web page that is fully searchable. And, it's free.

On the left side of this slide, there is a list of all the databases that can be searched, including a hazardous substances database, a carcinogenicity database, and a development, reproductive toxicity, genotoxicity database. You click on the database, enter the name of a chemical excipient of interest, and you will then have access to a wealth of information about the known studies for that chemical.

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Another very good resource for evaluating the safety of excipients is the National Toxicology Program, or NTP, Database. The NTP is part of the Health and Human Services Department. It conducts research with regards to carcinogenicity and genotoxicity of chemicals. The database is fully searchable, very well annotated, and also is free of charge for the public. There are other free and proprietary databases that FDA may use; the two mentioned here are just a few examples.

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This slide shows an example of an issue FDA faces many times. A new application comes to the FDA, with all the animal studies done but using material that, due to changes in the manufacturing process, is different from the final product material for which licensure is being sought.

For example, during the product's development, the manufacturer may have added a final heat treatment or nanofiltration step during the manufacturing process to make the product safer. But, this extra step in the manufacturing process may have changed the final product. Because biologics are so complex, that may change the chemical structure of the biologic or may change the impurity profile of the biologic. So, FDA needs to find out how the final product compares with the product with which the animal studies were performed. Is it as safe and effective? This comparison is called a comparability study.
The comparability studies could be done in vitro and in vivo.

Often FDA receives in vitro studies comparing primary, secondary, tertiary, and quaternary structure of the two products. The glycosylation pattern of the final drug product is checked to see how it compares to the predecessor. FDA asks sponsors to measure the potency, such as enzymatic activity, to see how it compares to the predecessor.

If FDA is not satisfied with the biochemical comparability, that is, if the two products do not show comparability in the in vitro studies, then comparability studies can be performed in vivo, in animals. Often these are pharmacokinetic and biodistribution studies, meaning that you compare the disposition of the predecessor with the final drug product in an animal study by looking at how it will be distributed, what is the total exposure, and so on. These parameters are more related to the efficacy of the biologic. But also, a comparability study could be a comparison of the safety profile. In such a study, one can compare the safety and potential toxicity of the predecessor and the final drug product in an animal. FDA may ask for such a study to be performed if deemed necessary.

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On to some specific considerations in immunogenicity. Why is immunogenicity important for biologics? Human biologics are recognized as foreign in an animal body. Thus, they will elicit an immune response in animals. Simply put, the

animals will form antibodies to the human biologic, and the presence of antibodies could confound the results and interpretation of the study, especially the repeated dose toxicity study.

Dose studies are repeated because the immunogenicity does not appear immediately, most of it is delayed. Because antibody formation could actually neutralize the effect of the biologic, the data you are getting from the study may be confounded. You may think there is no toxicity of the product when in fact the product is not even active in the animal due to being neutralized by the antibodies. That is why in biologics, very often there are repeated dose toxicity studies that last only one or two weeks, with two weeks observation time after the repeated study, because of the onset of antibody response.

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Even though you know you will get an immune response at one or two weeks, this immune response could provide important information, especially when comparing two different formulations of the same biologic. Thus, the immunogenicity response often should be characterized. The type of antibody response, the number of responding animals, as well as the neutralizing activity all are important pieces of information in actually interpreting data from the study.

SLIDE 20

Another area of importance when analyzing the immunogenicity of a biologic is the comparative immunogenicity study in animals. For example, if there is already a plasma-derived protein or biologic on the market which shows a good track record in its immunogenicity in patients, it is essential to compare the immunogenicity of the new product, such as a recombinant product, in animals, side-by-side to the product with the good safety record. This can provide important information about the immunogenicity for the new recombinant product.

SLIDE 21

Another area of specific interest for the products regulated in the Division of Hematology relates to the new route of administration of an existing biologic. Let's say that there are compounds already on the market which have been approved, such as, for intravenous application, and a company wants to use the same product but now administer subcutaneously, for example.

There is a very good guidance, now still in draft form, that has been posted on the FDA web page called the Guidance for Industry and Review Staff, Nonclinical Safety Evaluation for Formulated Drugs and Products Intended for Administration by an Alternate Route. The guidance sets forth the ways of evaluating the safety of a new route of administration.

One set of studies recommended is animal pharmacokinetic bridging studies comparing the old and the new route of administration. What do you learn from such a study? You learn several things regarding dose and efficacy of the new

dose, but also information that could be important for safety analysis. If you, for example, see a larger exposure with the new route of administration, let's say the area under the curve for the new route is larger for the same dose, then you may need to ask the sponsor to look at the toxicity of the new route of administration, because now you have larger amounts of the biologic available in the body.

Also, another thing that is compared in such a bridging study is the clearance. For example, if the biologic used in the new route of administration is cleared faster than the old route of administration, then you may need to adjust the dosing in the clinic, because now you have less amounts of this drug in the body. Another area often looked into is the possibility for neoantigen formation, which means that by applying the biologic via a different route of administration, you can make it more immunogenic, and thus make more antibodies to it. That is a possibility, and FDA does look for such a response using different methods, including local site histopathology. So, you check the local site immunogenicity using histopathology, in addition to looking at systemic immunogenicity.

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Now we'll discuss a little bit about safety pharmacology studies, which is an area of studies where a lot of information is provided for the safety of biologics. These are studies that focus on organ-specific toxicities such as cardiovascular safety, hyper and hypotension. Thrombogenicity potential of the new biologic is another area looked into very closely.

The standard battery of these studies is included in the ICH guideline known as "7A" which, as with all ICH guidelines, FDA has implemented. Another thing that the sponsors could very well do, and often do, is incorporate these safety pharmacology end points in the general toxicity study. This way, you could use fewer animals by actually combining the studies together, and thus, refining the studies.

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Here are a couple of real-life examples that FDA has encountered in day-to-day practice, when reviewing applications. Let's start with some findings in animals that were not seen in the clinic. The example is that of a human immunoglobulin preparation that caused a dose-dependent red blood cell hemolysis, followed by severe anemia in rabbits, as in hemolytic anemia.

The sponsor used an approved immunoglobulin formulation, completely safe in the clinic and with a proven safety record, as a control in a rabbit study. However, in this study, some rabbits suffered hemolytic anemia where the red blood cells were liced. Upon investigation, it was found that this toxicity was dose-dependent and, at high doses, it resulted in the actual demise of all of the rabbits.

Rabbit red blood cells contain 1-3 galactose in their cell walls. However, human cells do not contain this modification, and 1 percent of immunoglobulins

circulating in our blood are anti-gal immunoglobulins. So, it was determined that the human derived product contained antibodies that bound into the rabbit red blood cells, thereby causing their hemolysis. This explains the dose-response effect observed - higher dose, higher amount of antibodies, and more severe hemolytic anemia.

What was learned from this study? That the dose needs to be limited in rabbit studies. A smaller dose in this model also allows for a more realistic picture of the toxicity that is not masked by the model dependent artifact, that is the hemolytic anemia. As an aside, these rabbit studies were very important because they were a model for a specific disease. So, using a different animal model was not an option.

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The third example is one of a successful approval. The application was for a biologic with an active ingredient that is a recombinant human protein. It was indicated for the prevention of thrombolic events in patients that were hereditary deficient to this protein. The purification process included a nanofiltration and a terminal dry heat treatment for viral removal and inactivation.

SLIDE 25

Several safety toxicology studies were performed to support the application. There were single-dose studies both in rats and dogs. There were repeated dose studies. There were two 28-day studies in rats; one included toxicokinetics to measure the exposure. There was a fourteen-day study in monkeys. Also, there were reproductive studies in rats, as well as genotoxicity studies in vitro and in vivo.

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The design and the conclusion of the safety studies are presented in this slide. These studies were performed according to good laboratory practices with dose-ranging and control. The highest doses used in acute studies and repeated studies were approximately 9 times maximum daily dose, and approximately 5 times maximum daily dose in humans, including reproductive studies. More importantly, the toxicities observed at the highest dose were due to an exaggerated pharmacological effect at the multiple human dose level. These were all transient, and there was no neutralizing effect due to antibody formation, which means that the results from the repeated studies could be well interpreted.

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This is a tabulation of the pharmacokinetics and biodistribution studies performed during the pre-clinical stage of development of this biologic. These include toxicokinetics in dogs and rats, a comparison of different development process batches, and several studies comparing recombinant and human plasma biologic, for example, in monkeys, but also in rats.

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Some of these pharmacokinetic and biodistribution studies were performed following a change during the manufacturing process, and after the animal toxicity studies were completed. This manufacturing change included an addition of a nanofiltration step and terminal dry heat. This caused changes in the aggregation and the deamidation profiles of this biologic. Because of this, the company performed pharmacokinetic and biodistribution studies in animals. Pharmacokinetic parameters from two different studies using heat treated and non-heat treated product respectively, show similar values for clearance, systemic exposure, and half life in rats. Thus, it was found there was no difference in exposure and the distribution of this biologic after the changes took place.

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As mentioned, comparability studies include a comparison between the old product and the new product, which is a recombinant product. This often means comparing a product for which there is a substantial safety database with a new product which is more novel. This was the case in this application as well. There is a plasma-derived product analogous to the product in this application. This existing product had been on the market for many years, and FDA knew of its long history of safe and efficacious use. Thus, a comparison was done with the pharmacokinetics of the existing plasma derived product and the new prospective biologic.

From this comparison, it turned out that the clearance mechanisms for the plasma-derived and the recombinant products were different. The recombinant product was cleared faster because it was recognized by receptors in the liver that did not recognize the plasma-derived product. Thus, its clearance was six times faster than the plasma-derived product. This clearance information was very useful in determining the starting human dose in the clinical study, because it was now known that you had to dose differently, and dose more often, due to the faster clearance that occurred.

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Here's a general picture of what FDA learns from the pharm/tox studies. In nonclinical animal studies, FDA learns information about possible toxicity of the product. In other words, FDA looks at the specific toxic responses that could accompany the use of the biologic.

FDA learns about the dose where these toxicities become apparent. Even more importantly, FDA learns about the shape of the toxicity/dose response curve. This is important because if you find a steep curve, meaning the initial toxicity is low but increases quite fast, then you should proceed very carefully in escalating the doses in the clinic. That's important information to know before you conduct the clinical study.

FDA also learns about the signs and symptoms that could precede full-blown toxicity. For example, you know that if you see thrombocytopenia in a patient, you have to look carefully, because it could precede disseminated coagulation, a much more severe toxic response. Also, you learn about organ-specific toxicity, for example, pulmonary toxicity or liver toxicity.

You also learn about reversibility of the toxic effect, for example, whether an increase in liver enzymes is related to an increased enzymatic activity due to clearance, thus reversible, or whether it is due to permanent liver damage because of an organ specific toxicity.

These are all very important data points to make the decision regarding such things as clinical use, dose, and clinical study design.

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Now, how do you use all this information? Of course, FDA uses it to make decisions about the biologic. The key word throughout the presentation has been safety. So, decisions are made about the safety of the biologic. FDA safeguards the safety of the patients or clinical trial participants by using a safe starting dose, by adding specific tests to monitor for specific signs of toxicity and, ultimately, by not allowing the clinical study to proceed if it is unsafe. In some cases, that's a decision that is made if the toxicity is too high and FDA feels it's not safe to proceed into humans. The term used is for the investigational new drug to be 'placed on hold'. In this case the biologic is not used in the clinical trial until it is proven safe and the clinical study design is safe. Information from animal studies can help FDA make the study design safe. For example, as mentioned, incorporating exclusion criteria to exclude high risk populations could improve the clinical study design and make it safe to proceed from a clinical hold.

In conclusion, this information would help the patient and medical investigators make an informed decision. That is why this information is included in the package insert and the patient package insert, so that both the investigator and the patient can make informed decisions regarding medical treatment.

SLIDE 32
So, we end where we began: "Toxicology points the way to safety."

SLIDE 33
These are useful web sites.

Slide 34:
This concludes the presentation, "Nonclinical Studies for Plasma Derived and Analogous Products".

We would like to acknowledge those who contributed to its development. Thank you.