the agency before exclusivity expires.

There has been some interest, by the way, of some people on the legal side to try to see whether or not these provisions can now be extended to biological products, but thus far biologics do not have to deal with the exclusivity issue.

The very last slide I had is on a pediatric advisory committee. Our regulations calls for the development of a pediatric advisory committee to tackle important questions about pediatric studies. And that has been done now. There is a pediatric advisory committee that is a subcommittee of the Anti-Infective Advisory Committee. That subcommittee is made up of expertise from a number of different advisory committees.

I tried very, very hard to get somebody from the BRM Advisory Committee added to this committee, but it was very difficult because of the timing. There were not many options in terms of when the first meeting of the pediatric subcommittee could be held, and there are people who are SGEs who can be used for the pediatric advisory committee like Malcom Brenner, Joanne Kurtzberg, people with relevant pediatric expertise in some of the types of therapies that we have.

This committee is supposed draw on expertise, like I said, from existing advisory committees. It's going

to include people with ethics backgrounds, clinical pharmacology backgrounds, an industry representative. And they are supposed to tackle a range of important pressing issues in the area of pediatric development. The idea, though, is that a disease specific application for pediatrics would go to the right committee. If it's for an asthma drug, it would go to the Pulmonary Advisory Committee, for instance. If it's an oncology drug, it would go to ODAC. But this committee would tackle issues on a much broader basis.

That's my final slide. I also just wanted to mention that in addition to all the things that I mentioned, there are a number of other activities that are going on with respect to pediatrics. There's a pediatric pharmacokinetics guidance document that was issued as a proposal in the end of this year, and comments are being incorporated and a final document should issue.

There is a big effort going on in international harmonization. I'm a member of that delegation. We're going to be meeting again in Washington in October of this year to hopefully finalize an international guidance on pediatric studies. It's very interesting because we're trying to harmonize with Japan and Europe, and there's a big difference, particularly among the Japanese, with respect to putting pediatric patients on clinical studies

and their views. So, it has been a very good learning process and hopefully it will be a very useful document as well.

So, with that, I'll be happy to take any questions the committee might have. Thank you for your attention.

DR. SALOMON: Thank you, Karen.

I just wanted to make a point for those of you who are following time there was a policy to allow the time to expand just a little bit having to do with just efficiencies a little later so that people shouldn't be over-concerned that we're a few minutes late. So, there is time for some discussion here.

MS. MEYERS: If biologics are exempt from the pediatric rule, does that mean that FDA doesn't really have the right to say you want all the new biologics --

DR. WEISS: Excuse me. Abbey, biologics are not exempt from the pediatric rule. They're exempt from the exclusivity provisions. Biologicals have to comply with the provisions of the 1998 rule, meaning that they have to conduct studies in pediatric patients. If it's likely to be used or offers a therapeutic benefit, they have to have those data either at the time of the approval or it could be deferred to some time later on. But they have to comply.

MS. MEYERS: I'd just like to comment that it's a very interesting phenomenon because it's about 20 years now that we've been advocating to do something about the pediatric uses of drugs. Our organization has worked for it. We're just so happy to see that something was finally being done about it.

All of the excuses that the industry used all of those years about it not being ethical and being too expensive and all of these things that could be well documented, every one of them didn't even complain on the day that FDAMA passed because they knew that they were going to get six more months and essentially a patent extension. And for some of these drugs, actually a half a billion dollars in six months, a billion dollars, or $2 billion for six months.

Now, when the regulations were issued -- and it just confirms the same principle as the Orphan Drug Act which is: build it and they will come. Tell them they're going to make money and they're going to do it. Suddenly it's very ethical to do these types of studies.

Now, this has just caused a big problem in the consumer community in two ways, first, the healthy consumers and then of course the sick consumers. But the healthy consumers who are drowning under the cost of drugs, particularly the elderly, are just waiting for these

patents to expire so they can buy a generic. And this has put off generics for six more months buying some drugs that are huge, big sellers, I mean, ulcer drugs, et cetera.

Then FDA said if you get this pediatric exclusivity, you're going to get it on a form of your drugs. So, somebody came along and did some exclusivity trial. I forget if it was Advil or Tylenol, some unbelievable drug. I think they did it on the liquid formulation of pediatrics, and they got six more months of exclusivity on a pill that all these other people need, and they have caused a tremendous uproar because that means they have to pay an inflated price for six more months. That's major in their minds.

So, I wonder, if you're going to do a pediatric study and you get exclusivity on the liquid form on something like aspirin, you shouldn't get it on all aspirin. You should get it just on that liquid form. So, I don't know what led FDA to come to the decision that you were going to do this which is going to cost United States consumers multi-billions of dollars every year.

The second issue is exempting the orphan drugs, and I don't understand why that was done, especially without consultation with the orphan disease community. We are American citizens. FDA is a consumer protection agency. When we get orphan drugs on the market, we want

them to be as safe and effective as any other drug. We don't want you to say let's make an exception for orphans and put out dangerous drugs or drugs that don't work.

But you've done this here with exempting the pediatric requirement for orphan drugs even though a huge number of orphan drugs are cancer drugs that get approved for cancer, and once they're out there, they used on every type of cancer and we have no pediatric data.

So, I'm a little bit upset about that. Can you respond?

(Laughter.)

DR. WEISS: I think you have raised good points. I don't know if there are any avenues at this point with this regulation being finalized to address them. Exclusivity -- I can't really talk a lot on it. I don't know what the thinking was and what the negotiations were that were going on. Given the fact that biological products were not eligible for the exclusivity, I haven't paid as much attention to all of the legislative history with it.

I suspect, though -- and I don't know if anybody from industry who is in the audience knows or can comment on that -- that I'm sure that as part of the negotiations under FDAMA, you're right, people probably looked at the incentives and said, we need a whopping big

incentive and this is what we need.

So, most people here in this room were probably not involved in those discussions. So, I really can't specifically comment on that.

With respect to the orphans -- and it's too bad that somebody from Orphan Drugs is not here -- we're actually going to be meeting and have some more discussions about this issue about exempting orphans for pediatrics because I think there is some concern, just what you raised. I don't know what's going to happen with that, but I think there needs to be some more discussion.

I can tell you from personal experience with even some of our particular products that you have orphan indications that have been studied for adults. We haven't had problems thus far with talking to our sponsors and saying, you know, yes, you actually could be exempt and are exempt, but you really need to get these data and it's the right thing to do. And we have not met with obstacles in terms of people not doing the studies.

In pediatrics, I think for oncology from my personal experience in the field -- I don't know, Ed, maybe you can comment more. People who have spoken a lot in this area have actually felt that many of the studies for cancer have actually come through first in pediatrics. I mean, there are differences of opinions about that, but I think

most of us on the cancer side are really happy with the fact that there are these big, cooperative groups ongoing and a lot of effort in studies of pediatric cancer drugs.

DR. VOSE: I just want to say I haven't had such good luck in trying to get sponsors to use that clause of FDAMA because the last three lymphoma drugs, like diffuse sparse cell lymphoma drugs which does affect the pediatric population, I've tried to just get them to do studies down to 13, and they won't even do adolescents. So, I think I can't say I've had very good luck at all with that.

DR. SAUSVILLE: If I could continue with the discussion. I was actually very concerned that if you look at the Federal Register list, forgetting orphans, but just the diseases that waivers are automatically granted to, 12 out of the 20 categories are cancer. So, one could imagine a scenario where, to elaborate on the point made, that you go for an indication in, say, pancreatic cancer and then clearly the pediatric patients might then not be addressed because the indication gives you an automatic waiver. So, I think this issue needs to be thought through, and I agree that sponsors, sometimes where the perceived market is in an adult population, might not actually be very motivated, as it were, to pursue the pediatric --

DR. VOSE: But even in lymphoma where it does

affect pediatrics, they weren't willing to.

DR. SAUSVILLE: So, that would only augur more unfavorably for drugs that might have a perceived larger market share --

DR. WEISS: I can tell you the exclusivity has a sunset clause of 2002, and in 2001, there's supposed to be a report to Congress about this to see how good it's working.

Part of the controversy, when we finalized 1998 regulations, on particularly, I think, part of the industry's side was, hey, we have these incentives now. We don't need a mandatory, forced-down-our-throat type of regulation. We're going to do it because we have the incentives. So, I think a big part of this report to Congress is going to see how well the incentives have worked and whether or not that will continue.

DR. SALOMON: Dr. Miller?

DR. MILLER: I have just a clarification. If you have the rule and it applies under the rule, can they still get the incentives? Why do you give incentives if it's something that's already a rule?

DR. WEISS: It's felt that the rule was still going to be necessary because, for instance, biologics are not eligible, certain antibiotics and things that are off patent would not be eligible. There may be things that

there would be the exclusivity, but maybe studies would have to be done in neonates. Neonates in particular have been real therapeutic orphans, the feeling is, in terms of actually having clinical trials conducted in those populations. So, the feeling, as outlined in the preamble of the regulation, was that despite the fact that we have the exclusivity, there was still going to be a large number of gaps and that the regulations, if you will, might help fill in those gaps where the information was not being collected.

DR. MILLER: That wasn't my question. My question was, once you have the rule, are they still eligible for the exclusivity? Once you make a rule, you don't have to give carrots and sticks anymore.

DR. WEISS: Because you do it.

Well, the idea, though, is that if the sponsors meet the provisions of exclusivity, they will get the exclusivity. It's a little bit confusing and every time I think I understand it and I reread it again, I realize that it's still quite confusing. But the idea is to actually allow everybody the opportunity first to have exclusivity and then to apply in those situations the rule when they have not gone forth with the exclusivity.

The exclusivity is probably more for existing products, for the marketed products, because the rule

really isn't really go back and do much for the marketed products.

DR. MILLER: Right, but why do new products --

DR. SALOMON: One way would be, if you're thinking about the negotiation process that led up to the rule, whether we agree or not, it's going to cost the sponsors money to do these trials and submit them to the FDA and go through all the different changes to comply with the rule. It doesn't surprise me very much that in negotiating the final wording of the rule, that this got incorporated for a large number of drugs already out there.

DR. SIEGEL: Well, now, it should be noted -- please correct me if I'm wrong. Dr. Weiss understands this a lot better than I and that may be why I'm more able to understand the confusion.

(Laughter.)

DR. SIEGEL: Exclusivity is a law. So, you could question whether or not Congress did the right thing and they're going to revisit it in a couple of years. But to the extent that the company does something that meets the criteria in the law, they will get exclusivity.

Now, the agency in promulgating the rule recognizes that that law does not cover all the things that are needed and that financial incentives may not get all the things that are needed, so additionally provides for

some requirements, not all of which necessarily have all the teeth that it might take to enforce it or all the carrots that it might take to enforce it, but that's how we come to this combination of approaches.

DR. SALOMON: The concern I'd like to express is when you start applying this rule that you go overboard with it. I was just on a site visit for a series of trials sponsored by the NIH, and I know it's not the FDA, but it's pertinent in that they put in the description of the RFA that it should be done in children too, which was to me the NIH's interpretation of the pediatric rule, for a complicated trial involving stem cell transplantation in patients with autoimmune disease. And it was completely ridiculous to be doing this in kids, and it was entirely experimental. But the people applying for the grants were being compliant with what had been put out in a federal document describing the RFA.

So, my only point is that I think there's got to be an expert committee, and the FDA really needs to pay attention when the pediatric rule is necessary to be applied. Otherwise, the other side of the coin here is you're going to force a lot of children to undergo experimental therapies. It's inappropriate.

DR. WEISS: I think there has to be a balance. It's not an easy call to make. But we're finding the same

thing that you just outlined, Dr. Salomon, that we're in the position sometimes of saying to our companies, back off. I think I'd like to have more safety data or I'd like to see how this performs in some of the adult settings first before I want you to go into pediatric patients. So, it's a little bit of the shoe on the other foot from what we've had experience in in the past.

Because there is the rule out there and everybody knows they're required to have these studies, but for something that's a me-too type of therapy, you might want to wait because you know that post-marketing you might see adverse events that haven't been determined or elicited in a smaller database pre-marketing.

DR. SCHWIETERMAN: Dr. Salomon, I'd just like to add I think you raise a very good point. It's another useful role of the many useful roles that I think these committees have. In fact, this very issue has come up and I think we're very likely to bring it to a committee, probably the Arthritis Advisory Committee, supplemented with members of this committee, to discuss that because I think you're absolutely right.

MS. MEYERS: It's an example of why politicians shouldn't do things involving medicine when they don't understand medicine.

(Laughter.)

DR. SALOMON: They thought they were doing the right thing I'm sure and that's usual.

I was in a debate in Switzerland. The guy was a politician; he was a senator. And he said to me, when politicians have questions, they have to act. And my only response was that I hoped that reasoning never got across the ocean.

(Laughter.)

DR. SALOMON: Anyway, with that, unless there's anyone else with a really pressing comment, I'd like to thank everyone for the first part of the morning and take a 10-minute break and then we'll get started again.

(Recess.)

DR. SALOMON: So, as we restart this session this morning, I'd like to introduce Dr. Hugh Auchincloss who's going to present the report of the Xenotransplantation Subcommittee which he chairs. Dr. Auchincloss.

DR. AUCHINCLOSS: So, what I'm doing is I'm bringing to you a report of a subcommittee of the Biological Response Modifiers Advisory Committee. That subcommittee is the Xeno Subcommittee, or Xenotransplantation Subcommittee.

I should point out to you that Abbey Meyers was present as a member of that committee and your chairman,

Dan Salomon, was a member of that committee.

And just to tell you about the report, following the meeting, I prepared a summary statement of what I thought had transpired. That was circulated to all members of the committee and, in fact, many of the visitors as well for some clarifications and extra points which were added. Then that report was approved by the subcommittee members, at least by a majority of them, and everybody who was a member of the committee had an opportunity to send in some additional comments. We have one additional comment from Leroy Walters which is included with the document that you received, and I think Dan Salomon himself may want to make some additional comments as well.

Because you have had the document and had a chance to read it, I'm not going to read it in full. What I want to do is give you the three-minute version and then answer questions, as much as you'd like, again for clarification.

There were essentially three areas for discussion. The first was the FDA came to us and said, we have a lot of data that has been presented to us by various potential sponsors relative to the question of PERV infection of potential human recipients or in fact human recipients of animal tissues. Can you help us to evaluate the data?

We looked at a large amount of data that, in sum, indicated that there was no evidence at this time that any human or non-human primate recipient of the xenograft had demonstrated evidence of an infection with porcine endogenous retrovirus. So, that was the conclusion that the data suggested.

The question then became in the face of a large amount of negative data, how valuable, how reliable is that data, particularly in a situation where you don't have a positive control? And so, a substantial amount of discussion took place in which experts who were members of the subcommittee suggested kinds of assays and improvements in assays that should be sought over time that would give us increased confidence that negative data was as reassuring as it seems on the surface to be and that those kinds of recommendations are included in my summary document. So, that was question number one.

Question number two had to do with what I've termed a concept of relative risk. In effect what the FDA said to us is we've set up a definition of xenotransplantation which is reasonably comprehensive, and it means that we've got a lot of regulating to do. Can you point out some areas in xenotransplantation that are so unrisky that we might be able to be a little bit less stringent? That's probably not the right word, but where

the review process could perhaps be streamlined in some fashion.

I would say that the subcommittee probably had trouble with that concept. If the potential benefits of xenotransplantation were like this, our judgment about the real level of risk was something very small like this. To try and make gradations of risk within this narrow band of very small risks didn't seem to us a particularly useful concept at this time. That isn't to say that there aren't some forms of xenotransplantation that are more risky than others, but that the difference between them in the grand scale was too small.

I'm not sure that we have fully grappled with this larger issue. There may, in fact, be forms of, quote, xenotransplantation that are encompassed by the definition in which the same types of review process and the application of the guidelines suggested by the FDA might not be fully required. One example was that contact between human cells and those from invertebrate animal sources probably would not require the same degree of scrutiny, but even that point I think the subcommittee members ended up saying we're not terribly expert in that particular issue.

The third question that came to us was the FDA basically said we have a number of potential sponsors of

solid organ xenotransplantation trials who are beginning to indicate that they are thinking about initiating clinical trials. What they're asking us and, therefore, what we're asking you is, what do you expect to see in the way of preclinical data that would be thought of as reasonable as a basis for initiating clinical trials?

There there was again a great deal of discussion, and I don't mean to suggest that there was any unanimity.

First of all, let me make a point that I think is very important for clarification. There are trials of clinical xenotransplantation being conducted at this time under supervision by the FDA that involve cellular transplants. So, this conversation that we were having about preclinical data and how good should it be applied only to solid organ transplantation, effectively pig hearts, kidneys, or livers, and it really came down to kidneys or hearts that we were talking about.

In general, what the committee ended up saying was that data from non-human primate experiments is probably the best data that you're going to get. It's an important set of data that you need. It has some limitations, but it probably is the right model to be working with, and that frankly the results that we would expect to see in a non-human primate model should be pretty

darned good and should be in the realm of what you would expect to see in human recipients before initiating clinical trials.

Again, I will not try to go through the wide range of opinions that were expressed by committee members -- and there were, indeed, a range of opinions -- because that's not so important to air here. It's in the document. I think the important thing was to air for the FDA and for the potential sponsors here's the kind of range of feeling that you will encounter when you come back to us with real proposals for clinical trials when you have a subcommittee or a committee of experts, somewhat like us, listening to your proposals.

So, Mr. Chairman, I think I'll stop there and basically respond to questions, and I believe you may want to make some additional comments as well.

DR. SALOMON: Thank you, Hugh.

Essentially, as presented, I would think that was a very fair representation of what happened at the committee. I think some of the interesting things that are challenges in these things came up during the committee discussions that I just wanted to add here.

One issue has been an assumption that cellular transplantation of xeno-derived tissue, specifically pig extracorporeal circulation, pig liver, extracorporeal

circulation on whole livers and cells, use of fetal pig cells in mesencephalon transplants, that these which are now approved and ongoing clinical trials somehow represent less risk of transmission of porcine endogenous retroviral infections than the next major step that xenotransplantation will take, and that is of actual whole organ transplantation or eyelid transplantation.

Based on a general approach, taking that as a starting assumption, there was then a lot of discussion about what was the quality of data in an animal model that would be required to justify clinical trials. I would say that there are many of us in the field that, number one, don't necessarily accept this starting assumption, that we think that the risk of transmission of this endogenous retrovirus is as great with cellular transplants as it is with organ transplants or, I think as Dr. Auchincloss also did a very nice job, we can get into arguments that then come down to very small, incremental risks very difficult to quantify.

Should we move forward to clinical trials then became a major focus of the latter half of the subcommittee's discussion. And it is true that in that subcommittee, the majority felt that we were not ready to move forward into clinical trials of organ transplants. I just would like to point out in closing that there is a

minority of that subcommittee, which obviously I represent, that does not agree with that position, and it would be a long discussion about the details of why. I think Dr. Auchincloss has not gone into the details, and so I won't either. But I think it's just important for the record that majority/minority -- does it really represent majority or the minority of the transplant community? I'm certainly not in a position to speak to that today, but I think that overall I've already noted to Dr. Auchincloss privately that I think he did a very fair job in the report itself, showing that there was discussion. I just wanted to highlight that there are some differences in opinion that will have to get sorted out over the next few years.

DR. SIEGEL: I'd just like to rephrase the summary of the second point in a somewhat different language.

DR. AUCHINCLOSS: Thank you. I think it needs it.

DR. SIEGEL: I didn't want to offend you. I'm glad you welcome me to do that.

The issue that we raised is really less one of graduation of risk and are there some things that are so low risk, but just that what we're seeing, particularly in the area of cellular therapies, is a broad spectrum of products with a broad spectrum of types of risks. What we

sought and, I think as Dr. Auchincloss acknowledges, began to receive in a process that undoubtedly will continue is guidance as to which of those factors are likely to make a difference and what sorts of differences they're likely to make, not to say that there are necessarily high or low risk factors, but that, for example, exposure of cells or use of an animal cell line may require different types of controls from use of fresh animal tissue.

Certainly the many controls in our guidance document regarding veterinary care and feed records of source animals may be less critical regarding cell lines, and the committee indicated that, versus other controls that can be done in terms of studying cell lines for presence of adventitious issues.

And similarly issues such as vertebrate versus nonvertebrate species raising different issues that may for certain controls that we've recommended because of concerns about certain pathogens, be they, say, trions or retroviruses or herpesviruses, may well apply differently to different species.

We also discussed other issues such as duration of exposure, dose of exposure, use of barriers ex vivo and in vivo exposure around which I think the committee largely said, as summarized, that, well, in general there's not enough about any one of those to make a general comment

that one could significantly lower guards or significant concerns on the basis of those issues.

DR. AUCHINCLOSS: Thanks, Jay. As soon as I used the word "stringency," I knew that I was in trouble because that clearly is not the point of this. It was not a matter of lower degrees of stringency, it was a matter of are there different kinds of controls that should be in place.

Abbey, you were there also. Would you like to any other --

MS. MEYERS: Would you believe I have nothing to say?

(Laughter.)

DR. SALOMON: No. No, I don't actually.

(Laughter.)

DR. SALOMON: That's why I'm looking at you incredulously here.

(Laughter.)

MS. MEYERS: Actually I thought it was a terrific report. It really was. It was very accurate and very unbiased, and I thought it was terrific.

DR. SALOMON: As I already said, I endorse that. I also think it was fair. Dr. Auchincloss and I routinely disagree. We expect that from each other, and there has been a long record of that, collegially I hope.

With that, I'd like to call this committee to a vote on accepting the subcommittee report by Dr. Auchincloss.

DR. MILLER: Second.

DR. SALOMON: Second?

We have a vote. Do we go around? Okay.

Dr. Auchincloss, do you accept your own report?

(Laughter.)

DR. AUCHINCLOSS: Yes.

DR. O'FALLON: Yes.

DR. CHAMPLIN: Yes.

DR. SAUSVILLE: Yes.

DR. VOSE: Yes.

DR. SALOMON: Yes.

DR. BROUDY: Yes.

MS. MEYERS: Yes.

DR. MILLER: Yes.

DR. SALOMON: Dr. Goldsby?

DR. GOLDSBY: Yes.

DR. SALOMON: Yes. Was that yes, Dr. Goldsby, or yes, you accept the report?

(No response.)

DR. SALOMON: Well, as a triumph of technology, we'll accept that as a yes from Dr. Goldsby.

(Laughter.)

DR. SALOMON: Then with that, I would say unanimously we've accepted the report. Thank you very much, Dr. Auchincloss, for all the work.

DR. SIEGEL: Not to interrupt that concept, which is what I wanted to add to, that I know for myself and many others in the agency that the written summary -- and I think for everyone at the meeting -- that we saw of that discussion that came from Dr. Auchincloss was one of the best summaries of an advisory committee meeting or, in fact, of any meeting that I've ever seen. And what's more it issued within a day or two of the meeting. I was totally flabbergasted, and I think that we really owe a great debt because those written summaries, as well as transcripts, are important reference documents for us to make sure that we can do our best to follow the advice of the committee.

DR. SALOMON: I don't believe it's in my purview to vote a special commendation to Dr. Auchincloss --

(Laughter.)

DR. SALOMON: -- at this point, but perhaps at the break you can inform me if I have that power. But if I did, I would confirm it now from the chair.

Well, then the next thing that we're going to do is invite Dr. Zoon and Dr. Siegel to submit the

presentation of certificates for retiring members.

DR. ZOON: Well, this is one of the few pleasurable events we have at our advisory committee.

(Laughter.)

DR. ZOON: And it's one that we look forward to which is special recognition and special thanks for those serving on our committee and the time and dedication that they have spent working on important public health issues.

I would just like to say that while this is a new tradition of giving certificates from the Center to our advisory committee, I think that it's one that has long reflected our appreciation for the hard work and the devotion that many of you do and we recognize for very little.

We thank all the ongoing committee members and hope they keep staying with us, but also special thanks to those who have served.

I'm going to let Jay say a few words before we present the certificates to the departing members, but one, this has been a committee that has a very special place certainly for me. I was there when this committee was first formed, and it's very impressive to see the evolution of this committee and the importance of this committee and the subcommittees that were created especially as we just finished discussing on the xenotransplantation issues.

So, I want to thank all of you very sincerely and special recognition for those who are leaving the committee today.

I'll ask Dr. Siegel to say a few words.

DR. SIEGEL: I suspect that the politically correct thing to do would be to allow my boss to do this job, but she graciously consented to let me participate as well because I do feel extremely strongly about the value that your services and advice and contributions over the year have added to the process. I wanted an opportunity to formally acknowledge that in a manner as I have hopefully done, although I'm sure inadequately to the way I feel, in many informal discussions and conversations.

I would also like to say that in fact while often contentious and stressful, I almost always enjoy advisory committee meetings. I think there is a lot of pleasurable experiences. They're certainly, at the very least, intellectually challenging and stimulating. I look forward to them. There's always anxiety as to whether we'll get ready in time, but they are always interesting experiences. As I've commented on many occasions, it beats working.

(Laughter.)

DR. SIEGEL: So, we haven't exactly planned out the way we'll do this, but let me just say a couple of

quick comments then about the individuals who are leaving the committee.

Abbey Meyers who has been our consumer representative, I think maybe the first one on this committee, and a tireless advocate always on behalf of good issues and good causes, somebody who, like most of our best committee members, has not been reluctant to speak her mind, not been reluctant to ask for explanations or information when needed, and to give opinions when needed, and whose input has been tremendously valuable.

Virginia Broudy, who has always been a sound voice of good scientific judgment and advice, of reason, a builder of consensus, somebody who has I think never made a comment that I wished hadn't been made --

(Laughter.)

DR. SIEGEL: -- which occasionally happens, not that I'm the final arbiter of what are good comments or bad ones.

DR. ZOON: You better stop there.

(Laughter.)

DR. SIEGEL: What I mean to say has always made comments that have been extremely helpful and useful to the committee and to the agency.

And Julie Vose, who has worked several years as our Chair and before that as a member who was selected as

Chair based in significant part on her performance as a member and who has as Chair I think -- Dan Salomon's comments are correct. She's a wonderful role model for a Chair and has done an outstanding job of keeping the committee focused, of ensuring that we get the advice we need, and providing sound answers to questions, both her own answers and summaries of the consensus expressed by other committee members.

So, we have wonderful new people joining the committee. I look forward to their contributions, but I must say that this also represents a significant loss. We will, in fact, be asking each and every one of you from time to time to come back as consultants to continue to share your expertise with us. And thank you.

DR. ZOON: I just want to say once you've been on an FDA committee, you're never off.

(Laughter.)

DR. ZOON: It's tradition.

Abbey, could you please come up? Abbey, thank you very much. We have a plaque and a letter of appreciation.

(Applause.)

DR. ZOON: Virginia? Thank you very much for all your service.

(Applause.)

DR. ZOON: And certainly but not least, Julie, thank you. It has been a pleasure.

(Applause.)

DR. SALOMON: Miraculously we're basically five minutes off time, which is I guess good.

Well, then what I'd like to do is begin the third topic of today's meeting entitled Immune Reactions Against Therapeutic and Diagnostic Biological Products. This will be introduced by Dr. Zoon in a talk entitled Immunogenicity of Therapeutic Proteins.

DR. ZOON: This is an area that has a great deal of history and one which we would like to bring to this committee, one, to acquaint you with the issues and, second, to get your advice on a number of particular matters. This deals with the immunogenicity of therapeutic proteins.

To start, why is immunogenicity an important property? And it's important because it distinguishes many biological products for most small drug molecules. As macro-molecules, often even those that have great similarity to naturally present proteins in the body, they present themselves in such a way to be immunogenic, and the consequences of this are something that we would like to share with you today as a result of our experience of at least a decade and a half on recombinant and DNA-derived

proteins and monoclonals.

But I think it's clear, as we look at this data, even from these new molecules that are currently used for therapy and diagnosis, that this is not an issue that was just a result of the biotechnology era. Many of the issues that we have seen actually occurred in the pre-biotech era, going back to the earliest days of the 1900s looking at equine antitoxins and the fact that antibodies were raised against these causing serum sickness.

Initially other products, plasma-derived products, such as Factor VIII for hemophilia, was also an issue where antibodies were raised against these macromolecules, and in fact with natural Factor VIII derived from plasma, up to 30 percent of individuals treated had antibodies raised against this particular product. Of those, about 18 percent had clinical significance and patients were actually changed to porcine immunoglobulin, to porcine Factor VIII.

So, I think there is a history even with naturally derived products that we have in the experience from the past and ones again such as we've seen not only with Factor VIII but also with insulin where the presence of these antibodies could actually abrogate the biological response or clinical effectiveness of those particular molecules. In addition, they could have adverse event

consequences, such as immune complexes being formed that might have some clinical adverse events.

So, in the age of biotechnology and particularly early days of not only products such as insulin and growth hormone that we had experience with, but also products that you'll hear about in greater depth this morning, such as interferon and some of the monoclonal antibodies, have raised important clinical issues both from the efficacy issues surrounding the presence of the antibodies and their ability to neutralize the biological activity as well as some of the adverse events.

We actually have for you today a summary of all the major products that CBER regulates with respect to these. Some of these products will be looked at in greater depth, and hopefully this will set the stage for the issues that we would like you to address.

While we're looking at a number of these particular areas, I would just like to say that this particular topic of the relevance of immunogenicity and immunotoxicity has been a question that has been raised and has waxed and waned in importance I would say over the past 15 to 20 years. I think our experience of late, although the issues come and go, has raised a new particular attention to this, and I would like to particularly alert this committee that it's something that we have always been

concerned about. Although some of those concerns might have been theoretical in the past, our experience to date now has enough in our database to suggest that this is something we need to pay greater attention to.

Really the purpose of the presentations today will be to provide you an overview of the data that we have available, to serve as a basis for the discussion of issues in product development related to immunogenicity, and to provide background material for what I believe are important future discussions related to these products.

Clearly the issues to be discussed will include factors which contribute to immunogenicity, how these antibodies are measured and some of the important factors surrounding both the timing of measurement of the antibodies, as well as the methods themselves, the immune response data on a number of therapeutic products, and also their clinical implications.

The agenda for this morning will cover presentations related to antibodies by Dr. Kathryn Stein, a presentation on interferons by Joseph Bekisz, and a presentation on all the rest of the products by Amy Rosenberg. I think it's very important to put this in the context of the whole, and while we have a great deal of experience with antibodies and interferon, some of the issues Amy is going to raise in her presentation actually

raise a number of serious concerns. And then it will finish up with a short presentation by Dr. Bill Schwieterman regarding the clinical issues.

We'll then go on and have the committee discuss the implications of these data looking specifically at assays, some of the preclinical models and development issues we'd like to see, some of the clinical development issues that we might want to look at, as well as product labeling and claims.

I think this will be a very fruitful discussion. I myself am very interested in following this issue and working with the committee on this topic. So, thank you very much.

Now, I'll ask Dr. Stein, if Dr. Salomon agrees.

DR. SALOMON: Thank you, Dr. Zoon.

DR. STEIN: Thank you very much and good morning to members of the committee and the audience.

This morning I'm going to discuss the immunogenicity of monoclonal antibody products.

Over the years, we've seen an evolution of monoclonal antibody products from entirely murine to entirely human antibodies. In the evolution of these products, we've seen various combinations, including chimeric antibodies in which the variable region of the antibody is from the original murine source and the

constant regions are from a human source, and more recently humanized antibodies in which only the complementarity determining regions, shown here as the purple stripes, responsible for the antibody specificity are derived from the original murine antibody and the rest of the molecule is human. And then there are a variety of sources of human antibody, although to date we have less experience with fully human antibodies in the clinic than we do with chimeric or humanized antibodies.

Among the other products we regulate are bi-specific antibodies where half molecules of two different specificities are combined to form an entire antibody that sees two antigens, various fragments, recombinant fragments, or naturally derived Fab or Fab prime fragments.

This is a three-dimensional structure of an antibody molecule showing the two heavy chains in blue and red and the two light chains. It shows that the antibody combining site is formed by the union of the hypervariable regions or complementarity-determining regions derived from both the heavy chain and the light chain. This region confers the specificity of each antibody clone and thus each antibody has a unique site related to its specificity that has the potential to form an antigenic determinant.

Thus, antibodies are inherently immunogenic and each monoclonal antibody has the potential to be

immunogenic. These antibodies are unique. They must be evaluated individually, and comparisons across antibody products for immunogenicity can be quite problematic.

The types of antibodies and the jargon associated with that are human anti-mouse antibodies, human anti-chimeric antibodies, and human anti-humanized, or anti-human antibody.

Such antibodies can inhibit function, for example, anti-idiotypes that see the antigen combining site. They can alter the bioavailability, either antibodies to the idiotype or to the Fc region. They can also cause adverse events, again, both types of antibodies.

What contributes to the immunogenicity of antibody molecules? Impurities can contribute, those derived from the cell substrate or media components that co-purify with the antibody. They can be directly immunogenic. They can act as an adjuvant. There could also be product-related impurities such as fragments and aggregates that are more immunogenic than the intact molecule.

Modified antibodies such as conjugates of drugs, toxins, chelators, or antibody fusion products can have added immunogenicity due to the added substance, can create new antigenic determinants at the conjugation or fusion site, and antibody fragments, for example, Fab or

Fab prime fragments, can expose new antigenic determinants.

Antibodies used as ancillary products, which may not be readily obvious in the final product, can also contribute immunogenicity. For example, antibodies used to deplete cells either in solution or immobilized on some device can either leach off the device or remain bound to cells and be immunogenic, and antibodies used in affinity chromatography can leach off in the purification process of the final product. We try to control for these as best we can, but in the case, for example, of cell depletion antibodies, there may be undetectable amounts of antibodies bound to a cell that later turn out to have been immunogenic.

The measurement of immunogenicity of antibodies is very critical, and it has a number of parameters and influences. First, the detection assay. The sensitivity and specificity of the assay is important and this will differ from product to product. Again as I mentioned, comparison of immunogenicity across products can be very problematic because the sensitivity and specificity of the assay is not always known and it may differ widely from product to product.

For antibodies, there are HAMA, HACA, and HAHA antibodies that can be formed against, in the case of HAMA, species-specific antibodies for murine antibodies,

antibodies to the class and subclass of the product, antibodies to polymorphic determinants in the constant region, the allotypic determinants, and as I mentioned, antibodies to the idiotype. I would stress here again that all antibodies have idiotypic determinants and even fully human antibodies can be expected to have some immunogenicity related to the idiotypic determinant.

The measurement of antibodies to products are influenced by the timing of sample collection. I think as you heard this morning from Centocor, the presence of circulating product itself may inhibit the assay that's used, and therefore measurements taken with high levels of circulating product may not be meaningful. Many of the sponsors who are measuring HAMA, HACA, or HAHA antibodies use a sandwich type of assay where the product is immobilized on a plate, the product is labeled as a detection system, and they use the patient's serum to cross-link if antibodies to the product are present. These type of assays can be very highly inhibited by the presence of circulating product and complexes.

There are also many clinical factors which influence the immunogenicity of antibodies. For example, the patient population, the genetic background. As I mentioned, constant regions of antibodies have allotypic determinants, and depending on the patient's genetic

makeup, they may or may not make antibodies to the allotype. This is a particular issue in the case of patients with rheumatoid arthritis who have preexisting rheumatoid factors which are IgM antibodies directed against IgG constant region determinants. Autoimmune diseases where patients may be prone to make antibodies to a greater extent than patients without autoimmune diseases.

Preexisting antibodies not only of the rheumatoid factor but, for example, for antibody conjugates where a bacterial toxin may be coupled to an antibody, for example, diphtheria toxin. Patients may have and usually will have preexisting antibodies to diphtheria toxin, and those will be on board prior to treatment.

Intercurrent illnesses that disrupt the distribution of proteins, for example, kidney and liver disease, may contribute to the immunogenicity of a product and disruption of the blood/brain barrier may affect immunogenicity as well and the consequences of antibody formation.

Concomitant medications are an important consideration. Chemotherapy will suppress the ability of a patient to make antibodies, and immunosuppressant drugs even when given with products, as you'll see with OKT3, can have effects or not have effects. A highly immunogenic antibody such as OKT3 is still immunogenic in the face of

immunosuppressive therapy; whereas, you heard this morning from Centocor, their antibody product given with methotrexate had lower immunogenicity than without methotrexate.

Generally, immunogenicity increases with dose and frequency of administration, but there may be exceptions. And we've seen some indications of high dose tolerance; that is, very large amounts of antibodies and given for a long period of time may be less immunogenic than lower doses. We heard some of that from Centocor as well about different doses of their product. Their talk this morning really set the stage for this discussion.

The route of administration is also a potential contributor toward immunogenicity. In general, subcutaneous routes are more immunogenic than intramuscular or intravenous, but we don't have a lot of solid data on that.

What are the consequences of antibody formation? First of all, they can limit the usefulness of preclinical animal studies. Even in non-human primates and even including chimps, human or humanized antibodies can be immunogenic. So, starting with the most closely related species to humans, you can still have problems related to the immunogenicity, and that will limit the interpretation of PK bioavailability and also will confound the safety

data.

Antibodies can affect the bioavailability of a product both by increasing and decreasing. For example, an Fab product, for which an antibody is present, will effectively be increased in size. A whole antibody directed against the Fab will increase the size of the Fab and therefore increase its bioavailability. Antibodies can also decrease the availability of an antibody by increasing the clearance.

There can also be changes in the initial volume of distribution at the first time point due to removal of complexes from the circulation, and so the timing of sampling is very important.

Antibodies can cause loss of effectiveness neutralizing antibodies, in particular anti-idiotypic antibodies. However, antibodies to the constant region where an effector function is important for the function, for example, ADCC, could also have effects on the effectiveness of the antibody.

We are, of course, concerned about adverse events that are related to antibody formation: injection site reactions, systemic reactions which range from mild to life-threatening anaphylaxis.

If you have antibodies on board, it can limit the utility of another monoclonal antibody of the same

species.

It also has the potential to interfere with monoclonal based diagnostic tests. This is particularly true for murine antibodies where many diagnostic tests, particularly for cancer antigens, are murine monoclonal antibody based.

I'd now like to turn to the reported data that we have on immune responses to murine chimeric and humanized monoclonal antibodies and again would like to caution that comparisons across products are fraught with problems. So, what we're going to look at is the data reported for a given product with the assay used by the company during their clinical trials. I've separated them by species or by type of antibody.

So, the first is murine monoclonal antibodies. You may not be able to read all this, but these are the licensed products going from OKT3, which is the first licensed murine monoclonal, which is an anti-CD3. It's an IgG2a kappa and it's a whole molecule.

Another whole molecule, the other whole murine antibody, is Oncoscint, which is an antibody to TAG 72. This is an IgG1 kappa.

Both of these antibodies have shown antibody formation in a high proportion of patients, over 80 percent in the case of OKT3 which is given over multiple days in

the presence of immunosuppressive drugs and Oncoscint which is given once and has been reported to be immunogenic in 55 percent of patients.

The other murine antibodies are Fab prime fragments or Fab fragments, and the reported immunogenicity ranges from less than 1 percent for CEA-scan to 6 percent for Verluma.

Now, in general and as expected, murine antibodies are much more highly immunogenic than either chimeric or humanized, and I think you'll see on the next two slides that there's really very little difference in immunogenicity between the chimeric and the humanized antibodies in contrast to the whole antibodies that you see of the murine species.

Now, what you'll see on the next slides is that most of those antibodies are in fact whole antibodies and given multiple times for therapeutic as opposed to diagnostic reasons. They show immunogenicity in the low range comparable to the one-time use of murine Fab fragments.

These are the chimeric antibodies: ReoPro, which you heard about earlier this morning; Rituxan, Simulect, and Remicade, which you also heard about. They're IgG1 kappa antibodies. ReoPro is an Fab fragment. The others are whole antibodies. The reported

immunogenicity ranges from less 1 percent to 13 percent.

Humanized antibodies. We have three licensed products. Zenapax, which is an anti-IL-2 receptor. This is an IgG1 kappa. Synagis, an anti-RSV, an IgG1 kappa, and Herceptin, an antibody to the HER-2/neu receptor. This is also an IgG1 kappa. The immunogenicity reported for these antibodies ranges from less than .1 percent to 8 percent. These antibodies -- the 8 percent directed at the Zenapax antibody -- are anti-idiotypic antibodies, as might be expected for a humanized antibody.

We've also seen some unusual reactions. You heard some reference made to Remicade earlier. In a small group of patients who were treated 2 to 4 years after their initial treatment with Remicade, 10 of 40 patients had delayed reactions 3 to 12 days following readministration. These were serious reactions consisting of myalgia, rash, fever, polyarthralgia and some other symptoms in a smaller number of patients. This may be an unusual situation because of the long interval, but it's something that we must consider in evaluation of other products and what happens with a patient who has been untreated for a long period of time.

DR. CHAMPLIN: (Inaudible.)

DR. STEIN: Yes.

MS. MEYERS: What did you say?

DR. CHAMPLIN: I just asked if it was a serum sickness-like syndrome with antibodies to the antibody.

DR. STEIN: Yes. The answer is yes.

We also saw some reactions with Enbrel which is not a monoclonal antibody intact entirely, but it's a soluble TNF receptor fused to the constant region, the Fc region of an antibody. For this product, some recall injection site reactions were seen consisting primarily of redness at the site of a previous injection in 15 of 213 patients studied in a controlled trial. This was most prevalent during the first or second months of treatment, but not subsequent months.

So, what are the issues that we have to deal with? I have these listed as issues for the future, but they're really ongoing issues with regard to the potential for immunogenicity of antibodies.

We're seeing more and more therapeutic antibodies. In fact, most of the antibodies we see now in clinical trials are for treatment, and many of those are for treatment of chronic diseases. As I mentioned, even fully humanized antibodies may be expected to be immunogenic in the idiotypic region, and so this is an issue that we need to be concerned about and to gather more data.

We have made an effort in the Division of

Monoclonal Antibodies and other divisions in the Office of Therapeutics to encourage sponsors -- and I guess throughout the Center -- to remove all animal and human-derived, blood-derived products from their process of manufacturing. Although we don't have any specific data to date that any immunogenicity is directly attributable to approaching contaminant from media or cell substrate proteins, we think this is an issue that must be kept in mind not only for immunogenicity considerations, but also transmission of infectious agents that may be associated with these products.

Finally, we do have new methods of production of antibodies including phage display libraries. With this technology, we can expect to see new combinations of heavy and light chains or new idiotypes that are not found in the repertoire due to the way this technology is employed. There are many rounds of antigen selection from these phage display antibodies, and any combination of heavy and light chains that happens to bind antigen might be selected even though it might not normally be expressed in the repertoire.

Finally on this list, we are seeing production of antibodies in plants which is a relatively new technology. Plants, as I'm sure you're aware, have unusual sugars. We don't know whether this is going to have an

effect on the immunogenicity of antibodies, but it may very well and this is something that we need to pay attention to.

I think I'm going to stop there.

DR. SALOMON: When we discussed how we were going to present this, the idea was that if anyone had a specific question of the speaker, so specifically on what Dr. Stein has just presented, that we'll go ahead and take a couple questions with the idea of not generating discussion, but mainly making sure that everyone, when it was fresh in our minds, was clear on what had been presented and save discussion issues. So, let me open it for that.

Dr. Auchincloss.

DR. AUCHINCLOSS: Well, this issue of comparing products -- and let me just give you one example from your two slides, and you can tell me whether they're real differences or not. Under the chimeric monoclonal antibodies, you have Simulect anti-IL-2 receptor with evidence of less than 1 percent frequency of antibody responses. And then under humanized monoclonal antibodies, you had Zenapax with an 8 percent incidence.

Do you believe that those differences are real? Do you have an explanation for why in that case the humanized antibody to the same receptor should have such a

high rate of antibody, or do you think it's just assay differences? Or how do we explain that?

DR. STEIN: Well, let me answer the last part first which is because it's an anti-idiotypic antibody, theoretically a human idiotype or a humanized idiotype could be more or less immunogenic. It really depends on the primary sequence of the CDRs. We know that there are many species conserved residues in those regions, and so I don't think it's possible to predict that humanized antibodies or even fully human antibodies will be necessarily less immunogenic than chimeric ones.

I think if you look at the numbers across those two slides, I don't think we can say that any of those are significantly different from others. We don't have the data comparing these products side by side in a single laboratory, and as I mentioned, because antibodies are unique and you have to have the specific product to measure anti-idiotypic antibodies, it's really impossible to compare.

So, I would say that comparing all the products -- the only thing that we can say is that whole antibody of a murine origin is far more immunogenic than chimeric or humanized antibodies. I think beyond that, it would be a real stretch to say that there were any significant differences.

Also to go further and just allude a little bit to the discussion coming up later, we would not allow comparisons in advertising of those types of differences.

DR. AUCHINCLOSS: Well, I know that's where you're going, and so that's what I'm trying to figure out here. You do not believe that there's a difference between less than 1 percent and 8 percent frequency.

DR. STEIN: I think we don't have the data to know that.

DR. SALOMON: Dr. O'Fallon?

DR. O'FALLON: My question pertains to your statement that specificity and sensitivity are important. Specificity and sensitivity could relate directly to the question that Hugh just asked. If the specificity of two different assays is different, you're going to have quite different results and the difference between 8 percent and 1 percent could be due completely to specificity.

So, you must have hundreds of different assays here. Do you have data about the sensitivity and specificity of these assays? I don't need an answer, but I think in our discussion that's going to be a very important concept that has to be discussed.

DR. STEIN: The assay for HAMA, HACA, or HAHA is something that is reviewed by the product reviewer for each product. We encourage as sensitive an assay as

possible with appropriate specificities; that is, not only should the assay be able to detect class and subclass specific antibodies, but the assay should be able to detect anti-idiotypic antibodies. Beyond that, we just don't have comparative data, and again because idiotypic assays would have to be unique to the product, it may be impossible.

We could develop standards of some sort that would translate back to micrograms per ml of antibodies, and that may be something that should be done. We just don't have that kind of comparative data. But all of these differences in specificity and sensitivity could contribute to differences in the results.

DR. SALOMON: Dr. Broudy?

DR. BROUDY: I think one very important point is not just what percent have HACA or HAMA, but what is the effect of the HACA or HAMA on the biological effect of the therapeutic antibody product. That comes up, for example, very well in Factor VIII where 30 percent have antibodies and a small percent have antibodies that are neutralizing and then the patient has tremendous bleeding problems due to their anti-Factor VIII antibodies. So, in addition to just the percent that have the HACA or HAMA, we'd like to know how does that impact the therapeutic efficacy of the infused antibody product. And that must be known, for example, for OKT3.

DR. STEIN: For OKT3, there was a report in 1991 which surveyed all of the studies where titers in excess of 1 to 1,000 were associated with loss of effectiveness. But I think in many of the other cases, we don't know that a particular adverse event or loss of effectiveness is associated with these antibodies, and I think that's a problem that we have to face. And even for some of these antibodies, I think it's fair to say we don't know the exact mechanism of action, so we don't have very good assays to measure that.

DR. SALOMON: Dr. Vose?

DR. VOSE: I just want to say I think that's a very important point, that we shouldn't automatically assume that these antibodies dictate and therefore describe that the patient won't have a benefit, and that for each individual specificity, we need to really try to take that into consideration, so that we shouldn't just try and put that in every antibody.

DR. STEIN: Right. I think particularly for new products, but for those existing products, it's very important to know what the antibody status of the patient is. I think in many instances for imaging agents, we've seen loss of the ability to image a patient a second time even though the product may be indicated for single use. If a patient is re-imaged and has a HAMA titer, you may

lose the ability to image with that antibody. So, there are some instances where we may have a better idea of the effects than others, but in most cases we don't really know to what to attribute this.

DR. SAUSVILLE: And with that specific example, also to follow on on Dr. Vose's comment, the dose of administered material becomes variable because a given titer might have more import if you're giving a very small amount of the labeled product as opposed to milligrams and milligrams of a therapeutic product. So, I think that's a factor also.

DR. STEIN: It certainly is.

DR. SALOMON: Again, I think these are all great discussion points. We will return to these really and try and focus on them to answer the FDA's questions.

I'd like to also put on the table for later discussion the whole idea of showing that there's an antibody against a biologic is one thing, but we haven't talked about showing what that antibody is against since these preparations are often contaminated by fragments of the proteins, aggregates of the proteins. These may, indeed, be more immunogenic than the intact properly natured form of the protein itself. I think the implications of that and where we think about where we should look at specificity and how that applies on what the

sponsor does in terms of production control I think is another unique element of the biologics. I hope we'll return to that one as well.

DR. STEIN: One thing that I didn't mention today and primarily because it's a large issue in and of itself, but proteins undergo post-translational modifications. You lose amino groups, so there's deamidation. There are oxidations of methionines. There's decarboxylation and there are a variety of other modifications that could contribute to immunogenicity. And that's a three-day symposium I think, so I didn't get into that. But one should be aware that these are other possible causes of immunogenicity.

DR. SALOMON: Well, we've got a rich number of topics to discuss later. Thank you very much.

I'd like to introduce Joseph Bekisz to discuss the immunogenicity of interferons.

DR. BEKISZ: Good afternoon. I'm going to be discussing the immunogenicity of interferons. I am going to discuss some important issues associated with the formation and detection of anti-interferon antibodies, give an overview of the eight CBER licensed interferons, and discuss a few ongoing issues. But I will begin with some background information on the three types of interferons.

To begin with, interferon alpha. Its actions

are antiviral, antiproliferative, and immunomodulatory. There can either be 165 or 166 amino acids. This is because in some interferon alpha subtypes there is an extra amino acid at position 44. There are two disulfide bonds, one between positions 1 and 98 or 99, depending upon the size of the subtype, and between positions 29 and 138 which has been found to be essential for biological activity.

Interferon alpha is unique in that it consists of a family of at least 13 subtypes encoded for by 13 functional interferon alpha genes which show 70 percent homology. It contains one potential N-linked glycosylation site and there are 30 percent homology in amino acid structure to interferon beta.

Interferon beta. Again its actions are antiviral, antiproliferative, and immunomodulatory. There's only one interferon beta gene. The molecule has 166 amino acids with one glycosylation site. We see one disulfide bond between amino acid positions 31 and 141, and this has been found to be necessary for biological activity. Its sources are fibroblasts and some epithelial cells.

Interferon gamma again has the same actions as interferons alpha and beta, but its antiviral and antiproliferative activities are less than that in interferon alpha and beta. Interferon gamma is involved in

virtually all immune and inflammatory responses, for example, activation growth and differentiation of T- and B-cells. It has 143 amino acids with no disulfide bonds and two glycosylation sites. There is no or very little homology with interferon alpha and beta.

It should be mentioned in discussing interferon alpha, beta, and gamma that interferon alpha and beta show the same receptor, whereas interferon gamma has its own receptor.

Types of interferon antibodies. There are binding antibodies which have been found to be IgM and IgG, and for detection of these particular antibodies, we see at least two different in vitro assays, an ELISA, which is an enzyme linked assay, and an IRMA, which is an immunoradiometric assay.

In neutralizing antibodies, they are usually IgG and they are directed against the biologically active or antiviral site of the interferon molecule. They are detected by a cytopathic effect assay. Interferon has the ability to protect cells against viral challenge. If there is a neutralizing antibody in patient's sera and it's mixed with the interferon, that activity is neutralized.

With respect to preexisting antibodies, these are not usually seen or seen at very low titers in healthy individuals, but they are seen in some cancer patients and

in some patients with viral diseases, specifically in HIV patients.

The important issues which should be considered in discussing antibody formation and detection are route of administration, patient population, dosing regimen, cumulative dose, and dosing duration, the disease in question, the product characteristics, and the assay methodology used to detect both binding and neutralizing antibodies.

The route of administration. As Dr. Stein mentioned, it's also seen with interferons that seroconversion is more frequent with patients who receive interferon by the subcutaneous route compared to patients who receive IV infusion.

With respect to patient population, there doesn't appear to be any correlation between sex and age. There has been a suggestion that there is a correlation to ethnic origin, but the important point, obviously, here is immune status. If a patient is immunosuppressed, that is something that obviously must be considered when analyzing the numbers that we see.

The dosing regimen and cumulative dose, duration of treatment. In this particular study, what is seen, and what has been reported elsewhere, is that the higher cumulative dose is associated with a lower

neutralizing antibody formation. Here in the first treatment, the cumulative dose is between 504 million and 524 million units which translates to 2.5 milligrams. The percent neutralizing activity is 13 percent. The cumulative dose in the second two treatments are exactly the same. There is a slight difference in neutralizing antibody activity which may or may not be associated with the different regimen. There is not a great deal of data out there, but the one thing that has been purported by a number of individuals is that there was a correlation between the cumulative dose and the percent neutralizing antibody formation.

The disease itself. As you will see in a later slides, there are a number of indications for interferon ranging from hairy cell leukemia, hepatitis C, Kaposi's sarcoma. In general, the higher antibody titers are seen in patients with infectious diseases compared to cancer patients. This may have something to do with the fact that in a number of instances cancer patients are immunosuppressed to begin with, but direct comparisons are difficult due to the number of variables that we're discussing here: patient population, dosing regimen, and so forth.

With respect to product characteristics, in mouse models anyway, oxidation my increase immunogenicity.

The reason for this has been purported to be that oxidation leads to aggregation of the interferon resulting in interferon interferon aggregates or, depending upon the formulation of the interferon, if there's HSA present, leading to interferon HSA aggregates as well.

It has also been noted that contamination with certain altered forms may enhance immunogenicity. By altered forms, I'm referring to acetylated forms of interferon, and there are some altered forms of interferon which only contain -- like for example, an interferon alpha which has two disulfide bonds, an altered form might be an interferon which has only one disulfide bridge. Both have been shown to increase immunogenicity.

The assay methodology that's used. The frequency of antibody detection is higher with the immunoassay compared to the neutralization assay. This is because the immunoassay, whether it be an ELISA or IRMA or radioimmune assay, will detect both neutralizing antibodies and binding antibodies. The way things are done normally in a test, the patient's sera is screened using an in vitro assay like ELISA. If there are positives, then the patient's sera is put into a neutralization assay to determine the amount of neutralizing antibody that has been formed.

The next four slides are eight licensed

interferons. The first two on this slide are both recombinant interferon alpha. Intron-A is interferon alpha 2b. Roferon is interferon alpha 2a. They are both recombinant, as I said, made in E. coli. They differ only by one amino acid. The indications for Intron range from hairy cell leukemia, where 0 percent neutralizing antibody response is reported, to 13 percent for chronic hepatitis C. There are three different routes of administration for Intron, IM, SC, and IV, and only IM is used for Roferon.

Continuing with the interferon alphas, the first two, Alferon and Wellferon, are both natural products. One is leukocyte interferon and one is lymphoblastoid. Their indications are condyloma acuminata and chronic hepatitis C. There is no neutralizing antibody response for the leukocyte interferon. For Wellferon, which has just been recently licensed, a 6.7 percent neutralizing antibody response is reported. For Infergen, which is consensus interferon, which is indicated for chronic hepatitis and given subQ, there is no neutralizing antibody data for this, only binding antibody data, which is reported as being 15 percent.

The beta interferons, Betaseron and Avonex, interferon beta 1b and interferon beta 1a, both are indicated for the treatment of multiple sclerosis. The difference here that you see is in route of administration.

One is given subcutaneously. One is given IM. But there is a difference in neutralizing antibody response, with Betaseron giving 45 percent and Avonex 15 percent neutralizing antibody response.

Actimmune, the only licensed interferon gamma 1b, is indicated for the treatment of chronic granulomatous disease given subQ, and there is no antibody response here.

With respect to ongoing issues, treatment in the face of antibodies, obviously by definition, neutralizing antibodies neutralize the activity of interferon. Binding antibodies may affect or be associated with the bioavailability of the interferon. So, it's an issue that must be addressed.

In most studies that are done, there's a predetermined time that the patients on the study are going to be given interferon. They determined at the end of the study or through the study how much, if any, binding and/or neutralizing antibodies are formed.

One point of interest is a study that was done by Dr. Ron Styce and colleague at NCI a few years ago. He decided to take a look at the antibody response in patients who were on interferon therapy for a long term. He found that of 35 patients tested, 16 when they went into the study, patients were given interferon up to 2 weeks before entering the study. After that time, they were tested on a

weekly basis for both binding and neutralizing antibodies. 16 patients did not develop any antibody at all, and they remained so. 9 developed non-neutralizing antibody and lost it over a period of 14.5 months, and 10 patients who developed neutralizing antibody, 3 became negative, 5 developed non-neutralizing antibody, and 2 retained their neutralizing antibody. This is something that is very interesting because it was something that was not really looked into before. Patients were on a study for a particular length of time. It was decided how much antibody they were making, and that was it.

One other thing that was determined in this particular study is that the neutralizing antibodies that were formed were specific for the antibody that was given, which in this case was recombinant interferon alpha 2a, which leads into the next point.

I would like to discuss this recombinant interferon alpha followed by natural interferon alpha.

Patients who became nonresponders after treatment with recombinant interferon alpha -- it has been found that when they were given a second course of natural interferon alpha, they became responders. This was also a study done with interferon alpha 2a and the natural interferon that was used was leukocyte interferon.

I think, given all the variables that have been

mentioned, it's obvious that standardization of assay methods is something that must strongly be considered. Obviously there are certain things here that we can control. One of the things that we can control is an assay method. Ever since antibodies to interferon was first described in 1981, the numbers in studies have varied tremendously, and people have been looking for a gold standard to use for assays both for binding antibodies and neutralizing antibodies for some time. This is something that's difficult, but it's something that really must be considered because, for example, even in a neutralization assay, all else being held constant, if the cell line in the cell/virus combination that's used in the assay is changed, the numbers can be affected dramatically.

The last issue is pegylated interferon. This is interferon with a polyethylene glycol moiety, chemical modification. It has been shown that addition of this moiety will increase the circulating half-life by two fold of the interferon in the system. It's also been suggested that this will decrease the immunogenicity of the interferon.

This particular area of study of anti-interferon antibodies has been going on, like I said, since they were first described. It's something that's continuing. But I believe, to reiterate, that

standardization is something that we really must look into further because the numbers that you saw -- it is a difficult thing to compare basically between the interferon alphas when we don't have a standardized assay method to consider.

I think I'll stop there. Thank you.

DR. SALOMON: Thank you.

I actually wanted to start by asking one question. You've already somewhat addressed it just in that last slide. When you get neutralizing antibodies against a biologic, one of the concerns I have is if in some patients we're giving a biologic that also is a natural product, we're just giving more. The interferon is actually a pretty good example, but there are others, of course, like the colony stimulating factors, GM-CSF, G-CSF, et cetera, erythropoietin.

So, in the case in which you're getting neutralizing anti-interferon antibodies, has anyone looked to see whether or not that is also neutralizing natural occurring interferon? Now, your last slide you did kind of give one piece of that answer. Right?

DR. BEKISZ: It has been shown that recombinant interferon alpha 2a and 2b in neutralization assays cross-react, but there is no cross-reactivity with natural interferon alphas in anything I've seen. This even comes

from our laboratory. We've done it ourselves and seen no cross-reactivity with natural interferon alpha.

DR. SALOMON: Yes, Dr. Broudy.

DR. BROUDY: I think the point that our Chairman was just making is a very important point because particularly in the area of the colony stimulating factors, for example, thrombopoietin in which you give an exogenous agent and then you get an exogenous agent which cross-reacts with the native thrombopoietin, then the patient's platelet counts drop. So, this is one of the other dangers, is not just that you will lose the biological activity of your administered product, but you then may drop out temporarily or long term the activity of your native protein. I guess that's one of the major concerns that I have for this.

DR. SALOMON: Dr. Zoon.

DR. ZOON: Just to follow up what Mr. Bekisz said, in our studies, looking at human lymphoblastoid interferon, we have done a study where we had obtained serum from the patients that were antibody positive after being treated with interferon alpha 2a or alpha 2b. What we looked at is the binding to the different interferon subtypes. As Mr. Bekisz said, there are over 13 functional genes, and we've been able to isolate 21 components of interferon alpha from a natural preparation.

If you look at the cross-reactivity of the antibody raised against the recombinant interferon against each of the subtypes using a binding assay, you will find that there's a differential binding to the different alpha subtypes, and depending on the patient's serum, you won't see the same pattern each time they bind. So, whatever the response is, it doesn't appear to be to a particular epitope on the interferon. In fact, when you look at the cross-reactivity, it seems to be somewhat patient-specific.

DR. SALOMON: But the data you have, Kathy, I guess could be interpreted saying that, indeed, you are getting antibodies that are cross-reactive with natural forms of the molecule.

DR. ZOON: Yes.

DR. SALOMON: And it could be different from patient to patient, but that won't do the patient any good. Right?

DR. ZOON: Right. Joe was primarily speaking about neutralizing activity, where I was talking about binding to ELISA plates. It is very difficult to see neutralization, especially if you're only doing a subfraction of a larger whole, even if some of them reacted. There may be some that neutralize some of the natural. It's just the sensitivity of that assay probably isn't enough to pick it up.

DR. BROUDY: Just one other question. You presented the data on the 35 patients that were treated with interferon and then followed sequentially.

DR. BEKISZ: Yes.

DR. BROUDY: And in many of them, their neutralizing antibodies went away or became non-neutralizing antibodies. But I guess I'd just like to make the point that what would you expect to happen if you rechallenge the patient then with the interferon. Would you like to comment on that? Or perhaps should I address this comment to you?

DR. SIEGEL: Those studies were in the face of continuing treatment.

DR. BROUDY: Oh, these were getting ongoing treatment. Oh, I thought you treated them and then watched what -- because I guess I would have expected that the titers would then go up again.

DR. SIEGEL: They went down.

DR. BEKISZ: Oh, I'm sorry. I mustn't have been clear. No, this was an ongoing treatment.

DR. SIEGEL: But another aspect of that question that we don't have a lot of data bearing is what might happen if you interrupted and restarted treatment. There are some data that might suggest -- and this is involved in one of the questions -- that interruption and

restarting treatment may raise concerns, or intermittent treatment may be more immunogenic than continuous chronic treatment.

DR. SALOMON: Dr. Champlin?

DR. CHAMPLIN: I was just going to comment that in a system with a lot of redundancy like interferons, if you neutralize one of your 14 interferons, you probably won't lose much, as opposed to a thrombopoietin where it's a single gain system, and you lose that and you have a major problem.

DR. SALOMON: I had one question and that was what's an IRMA, an immunoradiometric assay.

DR. BEKISZ: Immunoradiometric assay.

DR. SALOMON: Yes. What is that?

DR. BEKISZ: It is, to the best of my knowledge, the antigen is -- like for example, in an ELISA, you would coat the plate with your antigen. You come back with the patient's sera, and instead of an ELISA where you would have an antigen to human IgG or human IgM, which is bound to an ELISA, this is tagged. This is hot with radionuclide, and it's just read that way.

DR. SALOMON: Oh, okay. I had this idea that it was like adding antibody to a cell culture and looking at a CTL effect.

DR. ZOON: It's radiolabeled NK-2 monoclonal

antibody.

I just want to make one other point. There's very little literature available in looking at the impact of neutralizing antibodies on patient treatment. There is one study by Peter von Wushoff where he was treating CML patients with interferon alpha 2b. The patients that developed high titer neutralizing antibodies had an abrogation of the biological response so that they didn't respond. When he switched them to natural interferon, they responded again. Thus again, while there might be some cross-reactivity with some of the natural forms, at least the ones that weren't neutralized were still able to maintain the biological response.

DR. SALOMON: I think that's a very good start to this afternoon's session. Just so the committee knows, I guess there's a balcony area, as usual, reserved down in the cafeteria downstairs or the restaurant downstairs. We'll be back at 1:30 for resuming the session. Thank you.

(Whereupon, at 12:41 p.m., the committee was recessed, to reconvene at 1:30 p.m., this same day.)

AFTERNOON SESSION

(1:45 p.m.)

DR. SALOMON: Well, welcome back, everybody, from lunch. What I'd like to do is start again.

I remind everybody we're doing topic III, Immune Reactions Against Therapeutic and Diagnostic Biological Products. I think we've got the discussion off to a pretty good start. Let's see if we can continue that momentum.

Dr. Rosenberg is our next speaker, and the title of her talk is Immunogenicity of Other Therapeutic Proteins which, as promised by Dr. Zoon, is all other proteins.

(Laughter.)

DR. ROSENBERG: Good afternoon, everyone. I've been asked, as you've heard, to present the spectrum of immunogenicity of other therapeutic proteins.

I encourage people to move to the front because of the encyclopedic nature of this presentation. Some of the slides are rather small.

So, before I begin this talk, I just want to introduce some caveats. I'm not going to talk about immune responses to cell and gene therapies. This will be a separate topic taken up in greater detail in subsequent presentations.

The other caveat is that with all of these discussions of immunogenicity, immunogenicity is really defined exclusively by detection of circulating antibody. And the sensitivity of such assays may be low.

So, with regard to the spectrum of immune responses to biological therapeutics, it ranges basically from no apparent immune response, again limited by the sensitivity of assays -- I, unfortunately, don't have a lot of information on assay sensitivity -- to generation of antibodies which are binding but not neutralizing, to generation of antibodies which are in fact neutralizing and, in fact, to generation of neutralizing antibodies that cross-react on the endogenous factor. The discussion just before lunch actually anticipated many of the issues that I'll discuss.

The detection of antibodies that cross-react on the endogenous factor is primarily determined by either detecting by a direct analysis taking the natural product and looking for binding in vitro or by effects in vivo, that is, the symptoms of a factor deficiency, of the cell loss, et cetera.

The non-antigen-specific immune type of responses that, for instance, we saw with stem cell factor and was presented in great detail will not be covered today.

So, factors that may impact on the detection of immunogenicity: basically the type and sensitivity of the assay. So, for instance, assessment of serum antibody, which is what is almost universally performed, is not necessarily as sensitive as an ELISPOT or a plaque-type forming assay. In some studies from our Center, the ELISPOT assay was shown to be 200-fold more sensitive in detecting antibodies than a serum assessment.

One questions whether assessment of T helper cell activity may be of use and will it be more sensitive while retaining specificity. I think that with regard to that, because T-cells are necessary in inducing immune responses, in inducing antibody responses, one may actually be able to detect by, for instance, cytokine release assays the activation of T-cells. So, in this regard, although T-cells and B-cells here shown by an APC here with surface immunoglobulin, basically collaboration is required between T-cells and B-cells to produce at least an IgG response.

However, T-cells and B-cells see very different portions or parts of a biological therapeutic, and this has very interesting implications for their contributions to an immune response. So, the surface immunoglobulin receptor on B-cells can see portions of the native protein, so portions of the intact biological agent. Whereas, T-cells, as shown by the green peptide fragment that's encased

within the MHC molecule here, only see peptide fragments of the biological therapeutic; so that whereas B-cells are then theoretically able to neutralize the activity of a therapeutic protein either by direct binding through its surface immunoglobulin or through the generation of antibodies, T-cells, which see the peptide fragments are not able to do so.

However, an interesting implication of the specificity and the recognition requirements for T-cells is that T-cells may actually participate further in generation of immune responses by destroying cells that take up the biological therapeutic and process and present peptides in association with cell MHC. I think this hasn't been looked for, and I think it is of interest. So, whether this occurs by receptor mediated endocytosis of a cytokine molecule or just by other mechanisms, cells that are targeted by a biological therapeutic may be susceptible to destruction by T-cells.

With regard to the second point, the detection of cross-reactivity on endogenous factor, by looking for effects in vivo, I think it was mentioned here that this in large part depends on the extent to which the biological activity mediated by that factor is mediated by other endogenous factors. So, in the case of interferon alphas where you have maybe 21 species, the loss of 1 is not going

to necessarily be detected by just looking in vivo. In the case of other molecules, which are either primary or solely responsible for maintenance of a cellular level, the loss of that will be picked up very rapidly by assessment of activity in vivo.

So, many factors that impact on immunogenicity have been discussed and I won't repeat them. Clearly in the products I'm going to show you, the immunologic status of the patient population is something which appears to be critical, at least in some cases.

A factor that hasn't been mentioned or has been briefly touched on is that of the immunomodulatory properties of the agent. So, agents that are in general immunostimulatory may actually up-regulate immune responses to themselves; whereas, those that are down-regulatory may actually be able to down-modulate an immune response to itself. This is something that I think you'll see in the subsequent discussion.

So, I'll start with thrombolytics. Thrombolytics are of particular interest with regard to immunogenicity because of their very widespread use in immunocompetent populations: acute myocardial infarction, deep vein thrombosis, et cetera.

So, with regard to this, we have three products. The top line is urokinase, which is a natural

human product produced by a human kidney cell line. In this case, no antibodies have been reported.

Again, the format I show here involves the production cell and, of course, the production cell is important because of glycosylation patterns, whether the antibodies were seen in preclinical models, whether binding antibody was appreciated, whether neutralizing antibody was observed, and whether you see cross-reactivity on the native protein, and as a further measure of immunogenicity, looking for allergic reactions which may indicate hypersensitivity or IgE mediated phenomena.

So, urokinase, no binding antibodies have been reported, and allergic reactions are rare.

Similarly for tPA, which is a recombinant human product produced in CHO cells, a mammalian cell line. So, the glycosylation pattern should approximate that of primates. Antibodies were seen in animals, but the frequency of binding antibodies in a large number of patients was very low. It's .18 percent. And no neutralizing activity was observed.

One thing I should point in addition is that although binding antibodies may not be detected in a neutralizing assay, neutralizing assays usually involve the inhibition of proliferation of a factor-dependent cell line by dilutions of patient sera. At least in theory, in vivo

binding antibodies may have the capacity to eliminate your biological therapeutic by Fc receptor mediated uptake and destruction in the reticuloendothelial system.

So, for tPA basically we don't see a high level of immune responses generated. Allergic reactions were rare.

The third thrombolytic is streptokinase, and that's a bacterial product produced in group C strep. In this case, there are variable levels of anti-streptokinase antibodies present in individuals as a result of a recent strep infection. So, in this case there are a lot of allergic reactions that have been noticed on infusion, 1 to 4 percent, and some anaphylactic and anaphylactoid responses. The manufacturer cautions against readministration within a period of 5 days to 12 months of either administration of streptokinase or the development of a strep infection.

So, in terms of the licensed hematopoietic growth factors, we have erythropoietin, G-CSF, and GM-CSF. The immunogenicity issue was of increasing importance with regard to these products because both erythropoietin and G-CSF are increasingly being used off label in healthy human populations. So, I think it's critical for us to be able to look and see precisely what studies have been done in normal volunteers. In fact, with regard to G-CSF, there

were two recent NIH/FDA joint conferences that were concerned with the effects of this agent in normal volunteers in the setting of allogeneic bone marrow transplantation or peripheral stem cell transplantation.

So, the first one, erythropoietin. It's recombinant human. It's produced in CHO cells. Antibodies were observed in preclinical animal models. However, no antibodies were reported in either binding or neutralizing assays, and allergic reactions are rare.

G-CSF, produced in E. coli and therefore not glycosylated, unlike the human homologue, nonetheless was also not reported to elicit antibodies, and allergic reactions were rare.

GM-CSF, which is a recombinant human product produced in yeast and used in the treatment of AML and bone marrow transplantation, elicited antibodies, about 2 percent, and these antibodies were neutralizing. Whether they cross-reacted on native protein, the endogenous protein, is unknown because clearly the activity on granulocytes could be compensated for by G-CSF, the activity on monocytes could be compensated for by M-CSF. I would have to go back and check whether direct binding activity was observed. In addition, allergic reactions were reported.

Now, GM-CSF has a lot of immunomodulatory

activity as well which is worth considering in that it is a very potent activator of dendritic cells, which are the professional antigen presenting cells in vivo. Whether this is a factor in the elicitation of antibodies to this is not known. Also, the glycosylation patterns mediated by yeast are quite distinct from that of mammalians, and whether that's a contributory factor is also unknown.

We come now to the investigational hematopoietic growth factors, and what I've shown here are three of the thrombopoietic growth factors: the dual cmpl receptor agonist and IL-3 receptor agonist, the peg-MGDF, and the recombinant TPO product. These are all recombinant products. The first two are produced in E. coli, the last one produced in CHO cells.

Of great interest with these molecules, as has already been mentioned, is their immunogenicity so that antibody was observed in preclinical animal models and non-human primates. Unlike most of the situations we see, where the animal response is restricted to the xeno components, the reactivity here actually cross-reacted on the endogenous TPO of these animals. This is probably due to the fact that there is very high sequence homology among all species. It's over 80 percent in the business end of the molecule in mice to man and higher in non-human primates. So, this was surprising.

These agents were used to treat oncology patients and in one study a group of normal volunteers. They were all injected subcutaneous.

Of interest here, on the screening antibody or the binding antibody was that this was found to be a highly sensitive assay, and it was positive in some subjects prior to administration of the agent. This was the case for actually more than one of these studies. So, it either indicates that the screening antibody assay is highly insensitive, or it indicates that in fact in either normal or oncology patients there is a certain percentage of persons who have extant antibodies to TPO. The implications of that are rather interesting to consider but not the subject for a conversation here.

With regard to the neutralizing antibody in the first trial with the dual cmpl IL-3 receptor agonist, there was a report of neutralizing antibody. This was, however, reported as weakly positive. Neutralizing antibodies were observed in the oncology patients in the peg-MGDF study and were present at higher levels in the normal volunteers, so about threefold higher in the normal volunteers than in the oncology patients. In the full-length TPO molecule, there was also a report of a neutralizing antibody which was read as potentially neutralizing in that it was of low titer, it was transient, and actually is considered partially

neutralizing.

Again of great interest is the fact that clinical findings of thrombocytopenia were observed in many of these studies, and the thrombocytopenia was not of necessarily short duration. No allergic reactions were seen to these products.

So, I believe this is the worst nightmare scenario where you generate an antibody response that is fully capable of cross-reacting on the endogenous factor. It was picked up because of the loss of platelets. And what these data really do indicate is that TPO is the primary, if not sole, agent that's responsible for levels of platelets. One would have expected that if IL-11, for instance, were important in regulating levels of platelets, that that would have compensated, but it did not.

Turning to licensed interleukins, we have two, IL-2 and IL-11, both recombinant human products produced in E. coli. Both elicited antibodies in preclinical models. Both used in an oncology setting. The fascinating aspect here is with IL-2. Binding or screening antibodies were observed in an extremely high percentage, 66 to 74 percent of patients in the renal cell carcinoma and melanoma trials. In those particular trials, none of those antibodies proved to be neutralizing.

In a different study, 1 out of 106 patients

manifested a neutralizing antibody which did cross-react on native protein. Apparently there were no perceived effects on this patient in vivo.

Again, IL-2 is one of many factors which can regulate T-cells. IL-4, IL-10, IL-15, for instance, perhaps compensate in this regard.

In addition, allergic reactions have been reported, and of greater interest is the fact that administration of IL-2 in some cases led to the exacerbation or the initiation of autoimmune or inflammatory disorders, and hypersensitivity responses were also seen in combination with other agents. I think this speaks to the high percentage of antibodies here and the initiation of autoimmune disease speaks to the immunomodulatory properties of this molecule.

In contrast, IL-11, which is basically a down-modulatory cytokine in immune responses, there were antibodies that were picked up in the binding assay, 1 percent, but none of them were neutralizing. Just some minor local allergic responses were seen.

So, I think here it may be an example where the immunomodulatory properties of these cytokines figure into the immune response against a therapeutic protein.

Looking at the earlier investigational interleukins, I have limited data here. However, with

regard to IL-1 alpha, a pro-inflammatory cytokine, no neutralizing antibodies were reported, nor for IL-3 or the IL-3/GM-CSF fusion protein.

With regard to the more recent trials using interleukins that figure very prominently in T-cell activation, we see some surprises here. So, IL-4, which is the prototypic B-cell stimulating factor which also stimulates T-cells, has been tested. It's a recombinant human product that's made in E. coli. It was administered subQ. So, basically it seemed to have all the necessaries for generating an immune response to itself. However, no binding antibodies were observed and certainly no neutralizing antibodies, and no allergic responses were observed for a factor whose administration or whose activity skews to IgE mediated responses. So, this was a surprise and it may reflect the sensitivity of the assay. It may reflect the dosing schedule. This may be the subject of further inquiry.

Again, another surprise was with regard to IL-12 which is a potent immune activator. IL-12 in this case is a recombinant human product produced in CHO cells, but injection of this product failed to produce antibodies that were perceived in the binding assay.

So, a few surprises here that don't quite fit what the expected activities are.

One product that's approved for wound healing is platelet-derived growth factor. It's a recombinant human product produced in yeast. It has been used to treat diabetic foot ulcers and the route of administration is topical. 2 of 475 patients showed some activity in the binding assay, but in this case the nonspecific binding could not be excluded. No neutralizing antibodies were perceived in the use of this product.

So, within our office and our Center, we have agents for which we expect preexisting immunity. This was touched on earlier. I mentioned streptokinase is one. In addition, we have an IL-2 diphtheria toxin conjugate molecule which is recombinant produced in E. coli and used to treat cutaneous T-cell lymphoma. In the binding assay, 39 percent of persons prior to study had antibodies to diphtheria toxin, and by the end of the third course of treatment, virtually 100 percent of patients had antibodies to diphtheria toxin, measurable ones anyway.

What's of interest here is that by the end of treatment, 50 percent of patients had antibodies detectable to IL-2. Given the dosage here, one wonders whether or not the diphtheria toxin acted as an adjuvant to elicit immunity to IL-2. The neutralizing antibody certainly is neutralizing for diphtheria toxin, and that made a difference in terms of the pharmacodynamics and kinetics in

vivo. However, it's not clear whether or not there were neutralizing antibodies to IL-2. There's a 2 percent incidence of allergic reactions.

The other product that is of bacterial origin is asparaginase used to treat acute ALL. It's a bacterial product produced in E. coli. Although there were few details -- in fact, no details -- provided in the package insert on the incidence of antibody, nonetheless the very high level of allergic reactions, 3 to 73 percent and really tending on the higher side, indicate that such antibodies must in fact be present. And this is with the first course of therapy. In fact, the manufacturer has a scheme for skin testing and desensitization should skin tests be positive prior to therapy.

The peg-asparaginase product whereby you get pegylation actually appears to decrease the incidence of these allergic reactions, 11 to 12 percent, at least in non-sensitized patients. The effects of pegylation on this product and on others may be to down regulate the immune responses. However, in other circumstances, pegylation has the potential to increase immunogenicity, and this will be something that we would like to look into further.

I wanted to point out to you that we have some agents that are administered over a protracted period of time in patients. Among these are, of course, epoietin in

the setting of renal failure.

One I haven't mentioned before is DNAse which is used to treat cystic fibrosis and it's administered by an inhalational route. Binding antibodies were reported in 2 to 4 percent, and we don't have any information as to whether these had neutralizing activity. This I think would be of great interest in persons who have to take this perhaps for the rest of their lives. There is a certain incidence of allergic reactions manifested as rash, 3 percent in this case.

G-CSF is chronically administered to patients with severe chronic neutropenia. Again, the incidence of antibodies -- I'm not sure how well it's been studied in that particular patient population.

That about wraps up the encyclopedic tour of the other biological therapeutics. I think we've identified clear areas for further follow-up. Particularly worrisome are the cross-reactive kinds of responses that we've seen with regard to TPO that led to thrombocytopenia in some instances. I think the guidance from the committee would be of great help with regard to what is needed to prevent this in the future.

Thank you.

DR. SALOMON: Thank you very much, Amy.

I had one question. What does NA exactly mean

in these?

DR. ROSENBERG: I'm sorry. It's not applicable.

DR. SALOMON: So, I don't understand. For example, if you've got interleukin-3 or interleukin-1 alpha, why would cross-reactivity on the native protein not be applicable?

DR. ROSENBERG: No. It would be applicable, but we don't see anything in terms of neutralizing activity. So, with regard to that, the likelihood of cross-reacting on the native protein is virtually nil. So, binding antibodies most commonly are specific to unique determinants as a result of the production; whereas, neutralizing really seem to hit the business end of the molecule.

DR. SALOMON: I mean, there wasn't any data on binding antibodies, so I was getting a little confused.

DR. ROSENBERG: Yes. No, I'm sorry. I was limited in part as to what I could retrieve.

DR. SALOMON: I think you did a great job. There's a lot of data here.

DR. ROSENBERG: Well, thank you.

DR. SALOMON: I was just trying to get a handle on it.

Yes.

DR. MILLER: Can you comment on the IL-2 diphtheria toxin drug and comment on the clinical effects of the neutralizing antibody? My understanding, looking at it, is that by the time you're done with the course of therapy, there's such a high level. But looking at the data, there's no evidence that there's a continued response. Well, there's a response, but whether or not you need all those courses. Is there any data about why you should keep giving that drug with 90 percent neutralization?

DR. ROSENBERG: I'm not as familiar with this as I'd like to be, but one thing that is mentioned in the package insert is that the administration in patients who did manifest antibodies didn't seem to correlate with treatment effect. So, whatever effect it had on the tumor itself did not correlate with the presence of antibodies. That I could glean.

But your question is a good one and I don't really have the answer to that.

DR. KEEGAN: Dr. Miller?

DR. ROSENBERG: Oh, Dr. Keegan may have a better answer for you.

DR. MILLER: As you continue treating -- when you stop in a T-cell disease, your clinical response keeps going and going and going often if it's going to respond.

It can takes month after the treatment is done before the final plaques resolve. So, I was just wondering from a utility standpoint about any -- I mean, I think that's something we're going to have to discuss.

DR. KEEGAN: I'm sorry, Dr. Miller. There was a question that was addressed as an area that we didn't have much information on at the time that this was presented before the advisory committee, and because of the lack of information, it was recommended and it has been made into a post-marketing commitment for the company to further investigate the impact of additional cycles/optimal duration of therapy relationships between antibody responses.

DR. SIEGEL: You've touched upon a problem that has somewhat vexed us in this area. I was thinking, as some of the questions came about, about what is the clinical significance, which is that in the chronic diseases or diseases which require chronic therapy, what we've seen -- and this has occurred on more than one occasion -- is, of course, the antibodies are generally, with some exceptions, as we've heard about, not there at present and may occur a few months into therapy. If we're talking about an imaging agent, we may immediately know there are antibodies there. You can get immediate feedback whether the drug is still working or not. But a lot of

biologic response modifying agents -- the time course of efficacy is not closely related to or the relationship is unknown of the time course of therapy and of having active drug on board to the response.

So, if you're treating an immunomodulator to treat arthritis and you treat for three months and the arthritis is doing fine and antibodies come up and you see that the arthritis is still doing fine a half year later, you don't necessarily know that the drug is still active or whether three months of therapy is enough to give nine months of response. So, it's often the case with these drugs that knowing whether an antibody response has a clinical impact is not so easy to determine.

DR. O'FALLON: Until you can get to the point where you know the specificity is near 100 percent, however, you don't know whether you've got true antibody present or you're just dealing with a measurement that is detecting an awful lot of false positives. What you just described as one possible explanation, another possible explanation is all those people were not really positive antibody responders after all. It was just a very poor assay.

DR. SIEGEL: Yes, in some cases that could be the case.

Often we have good information about

specificity, and in some of these cases, we'll see pharmacokinetic changes so we know that antibodies are doing something, but we don't know what the clinical implications are. So, the half-life of the molecule will be shortened. We know that they're binding in vitro, but we don't know whether that has any meaning because again the association between the half-life and the pharmacokinetics and the efficacy are not well known.

Specificity is an important issue and a lot of companies have expressed some concern, I think appropriately, that assays may be picking up non-specific responses. The concern, of course, is sometimes you can technically get around this, but usually there's a drug tradeoff between specificity and sensitivity.

DR. O'FALLON: Of course, there is. It's the way it works.

DR. SIEGEL: In general, to investigate these problems, our preference is to cast the broader net and to look at sensitive assays and then try to investigate, when we see something, whether it has any clinical meaningfulness.

DR. CHAMPLIN: In this example of the IL-2 diphtheria toxin, it could be antibodies to the diphtheria toxin won't matter at all since the IL-2 is what targets the conjugate to the cell and is internalized and the toxin

may still be active. So, you could actually test that in vitro without even needing clinical data to see if, in the presence of antibody, the agent would still kill cells.

DR. MILLER: But the clearance of the protein --

DR. CHAMPLIN: Clearance, yes, could change.

DR. KEEGAN: I don't know which members from this committee were actually participants in the ODAC, but at least some were. As you know, Dr. Vose, there was very clear data showing that the pharmacokinetic impact of the antibodies was very significant. There were great alterations. Dr. Sausville was there as well, as I recall. So, these were likely not false positive results, but were results that impacted at least upon the pharmacokinetic effects of the profile of the product.

DR. SALOMON: An interesting direction right now in cell biology is probably pertinent to raise here, and that is, there's been an assumption for a long time that a lot of these factors -- growth factors, for example, chemokines for another example -- act primarily as soluble molecules. However, a lot of new data suggests that that's actually probably not true at all, and that most of these factors are being presented. They're being presented by extracellular matrix molecules. They're being presented by charged moieties like heparin groups on cell surfaces.

There's data for TNF. There's data for IL-8. There's data for STF-1. There are a lot of results out there suggesting this is a factor.

So, measuring soluble concentrations or suggesting that antibodies enhance clearance may actually not be measuring the biologically relevant presentation of these molecules in the body. That may be why you see that they disappear, you can't detect them any longer in plasma or serum assays, yet they're functional.

DR. ROSENBERG: Yes. Fred Finkelman's IL-4-11b-11 conjugate is of interest in that regard, and that's an antibody complex to IL-4 in which he sees actually more prolonged activity because of the release. So, that's another one where that might figure.

DR. SAUSVILLE: I would simply say that in reference to the IL-2 diphtheria toxin, Dr. Keegan mentioned that in that particular case the need for more studies was clearly part of the post-marketing activities. But I think for each one of these products, though, the issues are so particular to each one that I think we're going to have to really think about these biologic issues that you raised in designing an assessment of these issues that are never, I think, going to be modeled very well in preclinical or early phase trials.

DR. SALOMON: A point well taken.

I'm sorry. Yes, please.

DR. CHAMPLIN: One last comment. I recall six months or so ago there was an article about PIXI, an IL-3/GM-CSF conjugate, whereas both of the independent drugs did not elicit antibodies, the conjugate actually did, which limited the ability to deliver repeated dosing. I think Dan Longo was the author, if I recall.

DR. ROSENBERG: We have to revisit the package --

DR. CHAMPLIN: And it raised the concept that even if the component agents are not immunogenic, when you make a conjugate of it, the binding factor perhaps was the target for an immune response.

DR. ROSENBERG: Yes. The data I have regarding PIXI was early and limited and should be revisited.

DR. SALOMON: Well, I think someone earlier -- I believe it was Dr. Stein -- had mentioned the idea that there may be aggregations after oxidation. No. But anyway, aggregation, oxidation, and that you could even present it with human serum albumen, HSA, in the preparations. So, the idea that these sort of aggregates could act almost like adjuvants is an interesting thing to bring up.

DR. ROSENBERG: Yes, it's of interest in that IL-2 is actually aggregated; the therapeutic IL-2 is

aggregated. That may in part explain its --

DR. SALOMON: Well, a lot of molecules, including peptides at high concentrations such as you give trying to reduce the volume you're giving to patients, will end up aggregating even though once -- the concentrations they would normally be in a biological fluid with loss of protein, there would be no aggregation, but in a normal saline or water, as you're injecting it in the patient or during the preparation, they're aggregated.

Thank you very much, Amy.

Then the last speaker before we get into the discussion of the questions in earnest is Dr. William Schwieterman, and he'll present a clinical perspective.

DR. SCHWIETERMAN: Thank you very much, Dr. Salomon.

Actually I only have a handful of slides because I think the other speakers nicely covered many of the issues relevant to the clinical issues and that the committee has brought up a number of the issues already that I think bear some discussion. It was quite clear early on in the organization of this session here for this particular committee that there were many, many issues that could possibly be discussed. As Dr. Zoon mentioned in her introductory talk, it's very clear that we will need to come back to this committee periodically to discuss these

various issues. As Dr. Sausville rightly pointed out, many of the issues don't lend themselves to general discussion since they get down to the particulars of the product, the assay, the indication, the issue at hand.

So, what I'd like to do here today is simply describe in some broad brush strokes some of the issues that I think we felt were particularly important, whether because they were more common, more practical, practically oriented or more clinically relevant to some of the issues that are happening now, and then get right into the questions with the idea that we'll focus on some of these more pertinent areas, but that if others on the committee want to bring up other points of discussion with regard to clinical assays, with regard to treating through therapies, with regard to many of the things that we talked about in some of the telephone conversations earlier -- Dr. Salomon brought up today again about what to do when you see assays; how do you appropriately characterize what's relevant or not with regard to whether that has an adverse effect on either the safety or efficacy of a product -- that we can have that sort of a discussion.

So, with that, I'll simply start by saying that we have four questions listed for the committee in two different broad areas: three with regard to product development, and one with regard to product labeling. The

three that are involved with product development involve a question, again a general sort of question, on preclinical issues. We have a question on assay development. Many of you understand the issues. We've had something of a discussion already about this. And the issue of repeat administration is something that I think that we would very much like input from this committee on given that many of the biological therapies that are now being brought to us, and they're even being approved, involve the treatment of chronic conditions with chronic therapies.

And then we'll end the formal discussion with our final question about product labeling. This question essentially revolves around the issue that Dr. Auchincloss and others were talking about earlier this morning about the nature of comparing data and what ought or ought not go into the label for the label to become maximally informative for patient and caregiver alike.

With that, I'll simply go through now what I think are many of the issues we've discussed. Again, this is a partial list. I think it's clear that animal data often are non-predictive. This is an issue not only for immunogenicity but for many other aspects of product development, but certainly one that's relevant for this discussion.

Secondly, PK and PD measures are very often

helpful in phase I. We've seen examples already of the kind of dissociations, whether it's in the initial volume of distribution, whether it's in serial measurements of PK/PD profiles after repeat administration. PK/PD often allow you your first glimpse into what the actual effects of an immunogenic antibody might have, and they play a particularly helpful role early in product development.

Concomitant immunosuppressives is something we touched upon earlier and something that actually as a clinician in the FDA that we have to deal with on a fairly regular basis. Really there are two issues surrounding this.

One is what are the considerations about immunogenicity when the product itself is immunosuppressive? You give an agent, it suppresses the immune response, and thereby is effective hopefully but also self-tolerizing in the sense that it dissipates any antibody responses that occur in the short term. What are the implications of that for product development? We'll get into this with repeat administration and so forth.

And secondly, I guess the second issue here is what about sponsors that want to pursue product development with combination therapy with other immunosuppressives? It has been a rule of thumb that we have that we like to adequately characterize these products with regard to their

immunogenicity so as not to default to a combination therapy that may not be necessary, in other words, to get some data. But certainly there are judgment calls as to when and when not to ask for monotherapy when you could suppress the immunogenicity otherwise.

The second slide has to do with the one I talked about earlier, the repeat administration study. There are obvious questions here, but the durability of safety and efficacy. I listed here two examples. I think there are several more you could easily think of, but the two that come up very often are should you require or ask for or think about readministration studies when you anticipate that there are going to be intermittent courses of therapy, whether it's a single therapy, for example, with a diagnostic agent, whether it's a chronic disease and the patient is going to be on that treatment for a long duration and therefore very likely to have certain periods of time when they would not be on that therapy for whatever reason, whether it's their own compliance with the product, whether there are drug holidays, for other reasons, et cetera. When do we start asking sponsors to evaluate these likelihoods in the future given that there are implications for starting and stopping therapy with regard to immunogenicity and therefore the safety and efficacy of the drug profile in the long term?

The second point I brought up here is obviously something we discussed already. Neutralization of endogenous function is a real concern when you're giving biologic therapies. Perhaps it's one of the obvious distinguishing features of biological therapies from some other drug moieties. We've heard some examples already today of when this actually occurred. And there are other considerations with regard to idiotypic networks, and Dr. Salomon brought up with autoimmune disease and so forth, that are related to this particular phenomenon. Biological therapies are very often ones that might actually involve risks with regard to these sorts of things, and I think that this committee has accurately pointed out some of these risks.

The third and final slide on the product development issues -- again, it's a partial list of things -- has do to with question 2. The first item does. Mainly, we very often have requests to companies to pursue improved assay development for their products. An example is Enbrel for phase IV. Immunex has been quite forthcoming in wanting to pursue a better characterization of their antibody profile, and we believe that this is something that is probably not likely to be the first time, as products come to market, that we attempt to get better handles on the assays and so forth.

And we have a question to this committee, a general sort of question, on what they think ought to be proper recommendations in this regard since, obviously, it's very important that we get accurate information on this, particularly with chronic therapies.

The second point is something again that's not necessarily specific to immunogenicity. Phase IV studies often help complete the safety database in a number of areas. But I think it's safe to say that immunogenicity is something that we often look for in phase IV, just given the sheer numbers, ask for the other adverse events, and so forth. Dr. Schaible again has pointed out that Centocor has experienced some post-marketing AEs, but they're actively pursuing studies of this. I think of this as something that is again not going to be specific for any one product, but something that's likely to continue again in the future.

I'll just simply finalize the product development issues by saying that registries of studies of readministration may be helpful since, obviously, it's important to collect these data on these particular areas.

Two more slides on product labeling and promotion, and these all revolve around question 4, which I'll get to in a second. Let me just make some basic points.

Immunogenicity data is required for the package insert. It's the nature of the data that we need and how it's presented that really is at issue.

The location in the package insert varies somewhat. It very often ends up in the safety/adverse events section. Sometimes there are labels where the antibody data, immunogenicity data is in the clinical trials description section. I guess the question to the committee, is this relevant, is this important? Ought there to be a format that we use and so forth? What ought to be the disclaimers? Again, this is coming up in question 4.

The third point I just want to mention here is that we consider it important that the label include information sometimes on other kinds of therapies, other kinds of diagnostic tools that might be adversely affected. This is a generic question. If there's going to be a cross-reactivity of a particular kind of isotype or other kind of class effect that might somehow influence other therapies or other diagnostic tools, I think that that's something that needs to be considered.

Finally, but certainly not least, is the topic that started out today's discussion for this particular topic, and that is the comparison of data between products is difficult given the things that we've discussed today,