is working on that.

DR. GREEN: You mentioned the word "formulation" and its importance, but could you expand on what you mean in terms of how critically it is in terms of assay performance, the one formulation from another?

DR. FRYKLUND: In assay? I'm sorry. I didn't -- you mean?

DR. GREEN: Well, you highlighted the importance of formulation.

DR. FRYKLUND: Yes. Formulation is really to keep the proteins stable over its shelf life, I would say, and make it palatable to the patients. So you have a sort of -- you have a tripper role because you have to put the protein in a form that's going to be stable. You have to have it in a form that it won't hurt the patient when you give it, and it has got to be stable on the shelf for up to two years. Otherwise it's very difficult to get your manufacturing people to accept that possibility.

So you really have three things you have to think about. In the end you end up with a glorious compromise of sort of trying to adjust freeze drying conditions, the pH and isotonicity suitable for the

patient and the shelf life. You have to balance all of those together.

DR. GREEN: So do you see any relationship between formulation and any assay performance or bioassay performance?

DR. FRYKLUND: I would say bioassays are very difficult, at least in the case of human growth hormone. We have done bioassays on totally insoluble protein formulations which we've suspended, and the animals grew very nicely. So that wasn't a good test.

But in our standard in-house release assays, we would know what to look for there. We would look for aggregates and deamidation, and then, of course, the host cell proteins.

DR. ORLOFF: Can you address a little bit for the specific example of growth hormone because that's where your experience is what is the difference between the application of analytic techniques, structural analytical techniques, for example, to batch-to-batch comparisons within a product line as part of, you know, quality control and manufacturing and the kind of techniques that would be applied across different products?

What's the difference there? And I guess

specifically, what assurance do we have when these techniques are applied to batch-to-batch quality control of clinical comparability after manufacturing changes for your product?

DR. FRYKLUND: When we have introduced manufacturing changes, we've tried to keep the final product specification the same. We've tried to adhere to that, and I think it's fair to say that everyone has the same experience that the in-house, internal requirements for reproducibility are pretty much tied to the limits that we would set in the public domain. So we're not getting like 80 to 120 percent spread. We're much closer to that.

Every assay is chosen with a purpose in mind. It's not sort of taken, oh, I must have this, that and the other. We have a sort of checklist and we tick them off.

We've chosen the assays to show, for example, deamidation because that's important for shelf life, to show polymer formation because we know that about antigenicity and host cell proteins, perhaps not done on final product anymore, but they would be done on drug product.

So we've chosen a panel of assays to suit

the issues that growth hormone presents us with, the way that it behaves in our hands.

DR. ORLOFF: Can I just have one follow-up question? Do you think that whether they be follow-ons or whatever you want to call it or for innovator products, do you think that immunogenicity assays and functional assays in the clinical trials should be used to establish a shelf life for protein products?

DR. FRYKLUND: No, I don't think so. I mean, if you've done a number of stability studies with different formulations and different temperatures, exposures, and different containers, you learn a lot about the way your protein behaves. So you know that a certain formulation will give you a short half-life and another one will be --

DR. ORLOFF: But that's a structural half-life. That's not a functional half-life.

DR. FRYKLUND: No, but we also know that, yes, in the case of growth hormone the activity is difficult actually because the molecule is actually active, even if it's in fragments. So we wouldn't expose our patients to fragments because then we'd be worried about immunogenicity. So it's a little bit difficult to answer your question properly in a

defined way.

DR. ORLOFF: Okay. Thank you.

DR. FRYKLUND: Because we have to make a choice. We don't want fragments there because we don't think this is the way the product should look, but from the activity point of view, it's probably okay. You may get antibodies formed.

MS. BROWN: Any other questions?

DR. BURNS: You made a lot of definitive statements in your slides. For example, on the question are there new technologies that hold promise for helping to characterize the proteins, you answered no, not yet.

I mean, do you think that perhaps things should be considered on a case-by-case basis and there might be situations?

DR. FRYKLUND: I was thinking really that, I mean, a lot of the techniques that we've used were developed, I mean, not yesterday. They're actually old technologies, if you like. They have a decade or so behind them.

But what's still lacking is the secondary and tertiary structure, and these subtle changes in hydration and things like that. The surface of the

molecule, we don't really know enough about what's going on on the surface.

DR. BURNS: But do you think that you might have the assays that tell you enough that you could be assured about the product would be efficacious? And I'm saying this may be a case-by-case situation.

DR. FRYKLUND: I think you have to take it case by case. I'm sure you do, but that's what I would like to have, is more assays that show what the protein looks like as a protein in 3D space surrounded by its hydration sphere.

MS. BROWN: I've got one last question. You talked in your characterization slides about a dynamic interaction between the process developers and the analysts, the different types of characterization analysis to pick up some of these impurities like the trisulfide and the glycosylated variants.

Well, a follow-on manufacturer would also have a process developer, and they would also have to characterize it. So could you clarify the difference between what you do and what they would do?

DR. FRYKLUND: No, I think it's probably the same. I think experience teaches you that you

shouldn't ignore anything that turns up unexpectedly, and if you're a follow-on, you don't know whether that little blip on the shoulder of the peak is something that shouldn't be there or should be there, and I think that that's the key. It's the amount of experience.

And everyone learns it over time. I mean there were people talking today that have been working for 14 years that are basically in the same category as we are. They're not follow-ons anymore. They're doing the same sort of thing as everyone else has been doing.

So I think it's a question of experience and knowing that you can't ignore little blips, that they may be very, very important.

DR. ORLOFF: Can I ask one more question? It relates to some of the things you said about immunogenicity.

It sounded to me as though you were proposing that a follow-on protein, however it might be approved or whatever the basis for approval was, because of these issues related perhaps to experience, perhaps to non-comparability in manufacturing, the manufacturer should be required to monitor its

immunogenicity in post marketing for some period of time, and I just want to know why. What's the rationale there when we don't monitor in any formal sense through, for example, controlled trials or registries immunogenicity of innovator products that have been changed over time with regard to process?

DR. FRYKLUND: No, that's correct. I was thinking more of different patient groups as well because in case of Somatonorm, we were misled actually for more than a year because we decided to test. The first Phase II trials were done in patients that were already were almost at the end of their therapy. They were on their last years. We couldn't do anything dreadful to them. They had already sort of achieved their final height.

And they did fine. There were no antibodies produced, and then we went back into naive patients, and lo and behold, there were antibodies there. So there was a difference in those two patient categories. So I think that's something that is not obvious when you do your clinical trials. that you made within one subsection of patients, which is simple to get hold of, but then when you go up broader, you may see things happening that you

wouldn't know about.

So that's my point, and I should have mentioned it in my talk. I'm sorry. I forgot about it.

MS. BROWN: Is there any more questions from the panelists?

DR. WEBBER: Thank you.

(Applause.)

MS. BROWN: Our next presenter is Dr. Patricia Weber. Could you state your title and your affiliation?

DR. WEBER: Yes. My name is Pat Weber. I am Chief Scientific Officer of ExSAR Corporation, and I would like to thank the FDA and all of you for allowing us to tell you about our hydrogen/deuterium exchange technology that is capable of characterizing proteins at high resolution in solution.

Basically there are three methods to obtain high resolution information about protein structure. They are the well characterized X-ray crystallography and nuclear magnetic resonance methods, and the latest tool to emerge on the scene is a mass spec-based method to look at protein structure at high resolution.

This method is uniquely suited for the application to biopharmaceuticals because it can be applied to sample samples of unlabeled protein in solution.

So I would like to just address one question today, and that is: does this method provide reliable information on protein confirmation? Because this is really what we want to know. Do the two protein samples have the same confirmation in solution?

And in trying to address this question, I will also give you an idea of what kind of structural information is provided by the hydrogen/deuterium exchange method.

This slide shows a little schematic of a protein structure there on the left. All proteins have amides. The amide is the linkage between the different amino acids. So basically there's one backbone amid for every amino acid in the structure.

The second thing that all proteins have is they have tertiary structure. So they have folded domains, and it's this folding that's responsible for the biological activity.

As a natural consequence of the fold of

the protein, the amide protons are exchanging with the protons in water. The exchange rates vary and reflect the protein structure in solution, and we can capture these exchange events by incubating the protein in deuterated water, and that's really what our technology depends on.

So the question is: well, what use is this information? How is this useful in this application?

Well, the first thing that this information will tell you is whether or not the protein is folded. This is because in unfolded regions, the amide exchange rates only vary 50 to 100-fold, and this variation simply reflects nearest neighbor effects in extended polypeptides.

But what's interesting is that in the folded domain these exchange rates can vary up to eight orders of magnitude. So this gives us a very sensitive signature of the protein confirmation.

This slide just tells you a little bit about how we actually determine H/D exchange rates. On the left you can see our fully protonated protein. We can incubate it for a defined period of time in D2O. We can stop the reaction, degrade the protein by

proteolysis, analyze each one of those fragments, and here you can see I've shown very schematically that the difference in molecular weight for those peptides can be measured by mass spec. So we can look at the level of deuteration in every peptide.

And then by looking at the deuterium incorporation as a function of time, we can get aneuterium build-up curve. We can determine the rate of deuterium incorporation for those peptide segments in the structure.

And as you can see at the bottom of this slide, this information can be distributed over the entire protein sequence, and if you have a portion of the protein that you are particularly interested in, we can tune the resolution of the technology to the level of interest in a specific part of the protein.

So I wanted to make the point that the method is highly tunable to answer individual questions about a specific protein.

One of the first things we did at ExSAR was to see whether this mass spec-based technology could give us comparable exchange rates to those determined by protein NMR, and this slide here is a color coating of the stabilization of the folded

protein as measured by H/D exchange, and similar coloring you can see exists for the samples at both pHes and by mass spec and by NMR.

This slide also shows an interesting point that for NMR based analyses, only certain amides are available in the NMR time window. This is not true in the mass spec experiment because we look at the entire protein.

So what we have been doing at ExSAR is we've been looking at protein drug targets and seeing how their H/D exchange behavior changes as a function of ligand binding, and this slide here just is a depiction of the data that we've presented to one of our clients showing how sensitive the method is to differences in the chemical structure of the small molecule.

And you can see we've got different gradations of blue for regions that are stabilized by ligand binding and different in some red regions where a ligand will increase the rate of hydrogen/deuterium exchange.

In the course of doing these experiments, we were amazed at how sensitive this method is to the environment that the protein is in and also to changes

in the protein structure in terms of the covalent structure.

So that led us to come here and let you know that this technique exists and show you how sensitive it actually is. So what I've tried to do in the next couple of slides is find data that we have that can address some of the issues related to comparability of protein structure.

So the first one is a comparison of protein constructs. This is one where our client wanted to know if the truncated version of the protein retained the same structure, and throughout these discussions I will be using these deuterium build-up curves as methods to show that the structure is either the same or different.

And here you can see three curves that tell you that some regions of the protein are the same, but others are different. So this was a greater surprise to our to our collaborator, that truncation of the protein really destabilized it as evidenced by increases in H/D exchange.

A second question is, well, how reproducible are these experiments, and here I offer some data from a study that we did where we looked at

the same protein four or five times in the course of a month. So these were doing repetitive ligand binding assays, but we would run the unliganded form of the protein as a control, and here you can see that we have very good reproducibility throughout the protein structure, as evidenced by the fact that the deuterium build-up curves are basically the same.

Another question that has arisen today deals with different forms of the protein, and here I don't really have exactly, I think, the kind of experiment you would like to see conducted, but we did have various versions of a protein with and without certain tags, and also one version from the mouse.

And here, again, you can see that all of these protein samples really displayed very, very similar structures. Again, you can see that the deuterium build=up curves are very similar basically throughout the protein, and each one of these panels shows a specific peptide derived from the protein.

So in summary, I would like to say that the information provided by H/D exchange will give you the rates throughout the entire protein at defined segments, and it's a very sensitive measure of the

protein structure in solution.

And is the method suited for assessment of structural comparability of biopharmaceuticals? I think the answer is definitively yes because it is widely applicable. We've seen that it is tremendously sensitive, and we know that it is also tunable for the questions at hand.

So, again, thank you, and I'll entertain some questions.

(Applause.)

DR. SWANN: I have a question. Your second to last slide showed a comparison of a variety of proteins, HisTag, human HisTag mouse, but I guess the conclusion from that, there was no structural difference between these proteins; is that right?

DR. WEBER: Right. In the areas, right, that we analyzed, we saw that basically the structure was the same.

DR. SWANN: Is there a high degree of homology between the murine and human form in this particular --

DR. WEBER: Yes. The extent of homology is very high. It's about 98 percent.

DR. SWANN: And I guess perhaps it may be

useful to some of the people here would be to the extent this method would be stability indicated, for example, if you were to stress this protein in any way, do you have any data long those lines?

DR. WEBER: Well, we do have data for the two different pHes of human growth hormone, and you can see that the structure is different in that way. So we could clearly look at pH dependent structural properties. We could look at H/D exchange.

So what I would envision in the end is that you could develop a series of stress tests for the protein and then analyze the samples using H/D exchange, and I think this is very much like the analogy of like when you go to your cardiologist. They don't have you sit in front of the television to see the health of your heart. You actually undergo some sort of stress test.

And so this is exactly the same idea that I think you could apply using H/D exchange.

DR. SWANN: Can I have just one last question?

Do how would one go about determining the significance of the difference that you saw, if you did see that structure was not comparable?

DR. WEBER: Let's see. The significance, well --

DR. SWANN: From the safety/efficacy standpoint.

DR. WEBER: Right. It's very interesting because in order for the exchange event to occur, many times this involves breaking hydrogen bonds in the structure, and you know that there are fluctuations in the protein structure normally, and that's how you can measure -- I mean, that's why H/D exchange occurs.

So you could understand by looking at these data to what extent the protein had to change in order for the exchange even to occur. So I think that this method is widely used in the protein characterization field to understand folding, to understand denaturation, to understand changes in activity.

So it's well established what it takes to change the H/D exchange pattern, but what is new here is the high throughput, automated technology that we've developed to use this technology at high resolution.

MS. BROWN: Could you tell me how this technique performs in like a mixed sample where you've

got like a heterogeneous proteins? You may have some wholesale proteins in there, and particularly like in human growth hormone where the damidated variant contributes significantly to the total amount.

DR. WEBER: Right. Actually there are a few parts to that question. One is what level of protein purity do we need to simply do the analysis. I think the protein purity has to be relatively high, but on the other hand biopharmaceutical must be relatively high in purity.

The other has to do with could you detect altered peptides, and you would detect here that the peptides were different because you would start the analysis with a certain protein, and if you next batch was different, then that peptide would be missing from your analysis.

So the answer is, yes, you would probably pick up heterogeneity in the protein sample, and what's interesting here is that you would find out where those sites were located in the structure, again, because of this high resolution application.

MS. BROWN: Are there any more questions from the panelists?

Thank you.

DR. WEBER: Oh, you're welcome.

DR. WEBBER: Okay. Well, this has been one heck of a full day I'd have to say. We got a lot of information, and I'd like to thank certainly everyone who volunteered to come and bring their information to us. I think it has been valuable for everybody in the agency who is here to hear it, members of the panel, members of the audience.

I think it is probably certainly valuable to all of the folks who came just to hear the presentations as well.

I guess for tomorrow we will be starting at 8:00 a.m. rather than 8:30 just to give you forewarning about that, and we will be listening tomorrow to presentations with regard to immunogenicity, which I know from the talks today is a very hot topic and one that will be a great deal of interest to all, as well as the clinical and preclinical aspects of evaluation of what currently we're still calling follow-on biologics or follow-on biotech products.

And I'd certainly like to thank the audience as well for participation.

One other thing I should mention is that

for tomorrow the schedule is not completely full. So hopefully if there is time at the end of the day, we'll be able to open the floor to comments from the audience, questions, as well, for speakers.

As you know, this format here is primarily or essentially to gather information for the agency, not for us to disseminate information. So I certainly would keep that in mind, that questions should be directed as opposed to the panelists.

And with that, I thank you very much.

(Whereupon, at 4:55 p.m., the meeting was adjourned, to reconvene at 8:00 a.m., Wednesday, September 15, 2004.)