So even though proteins are very complex and we agreed, the first one, the lack of ability to understand their making is not equal to increased risk in my view. And, again, the reason why we know it is we demonstrated every day whenever we release a new lot of a protein. We are making something new, and guess what. It worked, and therefore, we obviously know how to characterize stuff pretty good.

Signature, I believe, could be a very strong -- I'm almost done -- very strong and unique additional set of tools for the arsenal for biocomparabilities, especially and definitely for every lot release. Anything that adds more to the way that we understand that molecule is definitely useful.

And finally, I think that the risk based product specific, which means, you know, it doesn't work for everything just like nothing in life works for everything, but the agency's approach on risk basis for all of its decision, I think, which again, I think the key would be extensive post marketing surveillance.

the last one is the non-science part, but you know, should satisfy basically all of the key needs.

(Applause.)

DR. CHERNEY: I think there's time for a couple of questions from the panel.

DR. CHANG: I was just wondering whether or not you need a highly purified -- do you need a very high purity of proteins to do your signature study?

DR. CHAIT: In our specific case, we do not. We actually work in formulated final dosage. It all depends on how we assay the protein because eventually the test comes down to do just a protein assay, and if you use, for example, tools like HPLC, if you can separate it from the excipients after you do the test, just to do the assay, you're fine, and we've done that for formulated products as well.

DR. CHANG: What can you recommend to establish your signature and the activity relationship?

DR. CHAIT: Normally the things that I think Robert said before, especially if you have microheterogeneous products, you get lots of samples, and you sort of run them all through the signatures, develop the signature, and in a statistical manner sort of put a ring around it, you know, that says all

of these in between, inside here are good.

If you can tell us that, and then you can also maybe establish degraded products, oxidize them, whatever it is reasonable. Stop the gun through stability and was shown using independent means to be less.

DR. EGAN: Could you comment a little bit just on the use of this technology for proteins that have maybe two, three, five, maybe ten percent protein impurities, and also the complexities that may arise when you have microheterogeneous materials? So you have this whole mix of various glycosylated proteins. If I understand, you know, what you're using here, you're looking at solvent accessibility of residues?

DR. CHAIT: Okay. Maybe I will -- yes. I cannot get into the depth, of course. Yes, we are looking at the outside, sir, of hydrogen Therox. with solvents that we custom make, if you wish. So if you have basically a population, then we'll get a population of signatures, but as I've seen it, there's a case specific issue, and we've done it for glycosylated product quite successfully. It doesn't mean it's going to work to the next one.

DR. EGAN: But if this population is

changing somewhat from batch to batch or manufacturer to manufacturer, how do you account for this?

DR. CHAIT: Simply the proof is in the pudding, and the pudding is simply to say can you put a bar around all natural variability that is not related to reduced potency, as well as those that we know that correspond to reduced potency, and our job when we design typically method development, we designed the solvent structure, which is to separate those, and that's really what we do, is we sort of tweak it until we get things that correspond to the functional aspect of the protein.

Will it work for every case? No.

DR. CHERNEY: We're going to have to move on to the next speaker.

DR. CHAIT: Thanks.

DR. CHERNEY: Okay. The next speaker will be Christopher Holloway.

DR. HOLLOWAY: I'd like to thank FDA for the opportunity to speak here. I shall be addressing the European perspective. At the same time I want to emphasize that I'm advocating necessarily the European perspective, but sharing experiences, obviously a sensible thing to do.

Just to clarify a point from this morning, there is actually a legal term for these products in Europe, not biosimilar. That's not used by any of the regulators. It's actually similar biological medicinal product, and you will find that in Commission Directive 2003/63, amending Directive 2001/83, Annex 1.3.4.

What I'm going to tell you about the European perspective may sound extremist. From certain parts of industry it seemed that way not only from the generic industry, but from originators, too.

At the same time I'd like to point out that there is actually a case of recombinant DNA product approved with a heavily abbreviated preclinical and clinical program, approved in 1999 in Europe and seems to have escaped the notice of many people, but it actually is a good case study which I won't be addressing here. Perhaps that's one for the important meeting early next year. It does show that this will have to depend on the complexity of a product, the approach that can be taken.

Now, in Europe we've been faced with a situation of many patents that have been expiring much sooner than they have in the U.S. So many companies

have turned their attention to Europe before doing so in the U.S. We have a formal scientific procedure at the European Medicines Agency, which has been used extensively by sponsors of follow-on biologics to clarify the kind of data that they would need to provide for their products, and I believe the consumption of stiff whiskeys after those scientific advice meetings soared considerably.

There is no Freedom of Information clearly on this topic of the scientific advice procedures. That's why forums such as this are so valuable even if certain information, of course, is proprietary and can't be discussed.

Now, from my perspective, I understand that the legislative procedural basis for these products will inevitably be different in the United States and in Europe and for that matter in other parts of the world, but surely the scientific basis for approval should be quite similar, and the only contentious point seems to be the amount of nonclinical and clinical testing that's required for approval.

And as we know, this sounds very simplistic. That will depend on the extent to which

similarity can be proven, and I will actually be rounding my talk off in a moment with a couple of case studies for you.

Now, the consistent doctrine for biological products, we're all very conversant with this. It was suggested before the lunch break that this is perhaps obsolete. I consider it to be far from obsolete, at least for the more complex biological products on the basis of experience.

The product is inexorably linked to a producing cell line, and this is the issue with creation of a new master cell bank. If you do it from the original producing cell line, it's far less potentially of a change than completely recreating a producing cell line.

The specifics of the manufacturing process, even the scale of manufacture, and I'll be bringing you an example of that, too, and particular manufacturing facilities. You would be surprised. I've seen the case where the length of a particular piece of tubing going into a fermenter could considerably affect the productivity and, therefore, the potential impurity profile.

Process changes in Europe traditionally

have required an extensive comparability exercise not limited to obviously drug product, which is primarily what the follow-on biologics sponsor will have; also drug substance and, most importantly of all, the comparison of in-process data, and the critical thing is not actually the data, but the impact of those data, and the impact of any differences.

The current guidelines are listed here. Uncomparability, they took an inordinate amount of time to finalize, and that possibly reflects in part the fact that the European authorities decided to incorporate the question of similar biological products in that guideline and contrast, of course, to the draft ICH guideline.

The one defines quality and the other defines nonclinical and clinical issues, and I'll be quoting a couple of sentences from those guidelines because I think they're very often misinterpreted. So I've highlighted some parts in red in a moment.

Now, the increasing font here defines the four cases which in Europe need to be considered for a comparability exercise for anybody's comparability exercise. A change with absolutely no impact on quality, that would include in-process data as the

simplest case.

A change would impact on in-process controls, but without impacting on drug substance and drug product specifications is the next more complex case, and already here we have the issue of can the follow-on biologic sponsor provide that information and so on and so forth. And the further down you get and the larger the form gets, the more over and above CMC data you're likely to have to provide.

Here are some direct quotations from the European comparability guidelines which should make this possibly rather extremist position quite clear. The first, the manufacturer, although possessing information on his own manufacturing process would normally not have access to all necessary information that could allow comparison in terms of quality without any other products already on the market. I would think that that's quite straightforward, whether you like it or not.

The second is the expression/vector system, production purification process, facility/equipment, analytical techniques may be different from other manufacturers. The extent of the difference cannot be evaluated by the second

applicant. Also true.

Comparison based on testing and characterization of drug substance and drug product is not sufficient to establish all aspects pertinent to the evaluation. Perhaps this ought to be qualified except for the very simplest of products. That would be a good amendment that would probably take about three years to get through the process if they wanted to make that.

I'm going to now turn to one or two case studies because they're perhaps the most useful. There are real case studies that I have neutralized and made as anonymous as possible so that intellectual property is protected. The first concerns a product which is approved in Europe but has never actually been submitted in the U.S.

I would ask you to look first of all at Process 2. This was a sequence from Process 1, 2, and 3, from the original approval through variations post marketing to improve the production, specifically improving the cell culture media. The third process was actually eliminating all mammalian materials.

Now, when the second process was submitted, the European authorities asked the company

to kindly update some of its analytical methods and see what else they could do to characterize.

At that point they discovered a new product related species at the level of about one percent. This is actually a post translational modification which has never been reported before. I wish I could tell you about it because it's exciting, but anyway, it's a post translational modification that has not been seen before, and it was present at one percent.

So the concern of the authorities was is this a change compared with way back when? Because the company had samples of drug substance frozen, from the original clinical trials 13 years before, they were able to force some of those out and show that that particular species was already present then. That you could not have done with drug product 13 years old.

Interestingly, actually moving to Process 3, this post translation modification has almost disappeared. The cells appear to be much happier in point of fact. Interesting though, the major isoform has shifted. Is 54 and 50 percent equivalent? If you just had one batch of each, you might think so, but if

you had a large number of batches, you'd find it's not, and that's the importance of enough batches.

And one point that we've always seen in applications from originators, because they've gone through clinical development ad nauseam, they have a long manufacturing history. If you're simply going to do a pharmacokinetic equivalent study, you may only have one batch, and os you make three, and that that's not a manufacturing history.

This is another interesting case. This is actually an impurity that's very, very difficult to measure, and the limited quantification of the assay is 100 ppm in drug substance. Now, 100 ppm can cover anything, of course, up to 99 ppm.

When the process was up scaled, the apparent levels in drug substance remained the same. If you look back though because of the up scaling, there was an increase in commission medium and only by studying this at each stage of downstream processing could it be found that actually measurable levels of Column 2 eluate showed that there was comparability. That information would not be available to the follow-on biologic sponsor.

This illustrates very quickly a case of

specificity of analytical methods. This is host cell protein in two different processes, and if you use Process A assay and Process B assay, the results are reversed using the products, and if you use a generic host cell protein assay, you get totally different results altogether.

So here this illustrates the next point that data are only relevant if you're using the same analytical method.

Case Study 4 is a cutie because this actually compares a follow-on biologic with a marketed product in which the company wanted to show that its level of dimer are the same as the originator. They appear to be on the face of it.

However, unknown to the follow-on biologic sponsor, the actual formulation, the formulations, by the way, are different in this case, and this is an important point. Even if the patent is expired on the drug substance, it doesn't mean the formulation patent is expired, and you may need to have something different to cover that.

It appears to be the same. However, we're comparing fresh data with product that has been on the market halfway through its shelf life, and in fact, if

they only knew the nature of the dimer in this case is actually different. But they're not aware of that.

Conclusions. It's difficult to conceive how more complex follow-on biologics could be based solely on comparability. There's not only the issue of therapeutic equivalence, but dose equivalence. If you're claiming therapeutic equivalence, surely you've got to demonstrate dose equivalence. You try doing the statistics on that. You come out with a very large number of patients.

I would say that the manufacturers of follow-on biologics already have a significant advantage in knowing where they're going. They know what indications, what posology, and so there's already an advantage there.

And from the European perspective at least, since you have new cell line, you have a new product, that is the legal position at the moment. So before going into studies in humans, you are required to do some nonclinical work.

Thank you for your attention.

(Applause.)

DR. CHERNEY: Thank you for the presentation, but I'm afraid we have no time for

questions now. So we'll go to the next speaker, who is Andy Jones.

DR. JONES: Good afternoon. My name is Andy Jones from Genentech. I'd like to thank the agency for organizing this session and raising some of the questions that are up for discussion and to which I will be addressing some of my comments if I can learn how to use this PC.

Okay. This slide presents a high level overview of where analytical tools provide information during the development of protein pharmaceuticals. This as, as we've heard, required whoever produces them, and each manufacturer will have their own production system and cell bank, whose consistency and safety must be established or -- excuse me.

During the process development these tools provide information on the behavior of product variance and impurities and allow the identification of critical process parameters, possibly even in-process testing, and limits to assure consistency of manufacturing.

In addition to allowing the selection of methods used for the control system, these tools provide one of most critical elements of the

information database developed by the innovator. That is the extended characterization of those batches which were actually used in clinical trials.

Unless the materials has been used in clinical trials, there is no link between the analytical data and its significance with respect to safety and efficacy. I think it's productive for today's discussion at least to divide these tools into two broad categories. Nonresolving methods do not have the necessary dynamic range or precision to be informative in such comparative analyses. They provide only ensemble average data about a sample and are not useful for comparing sameness at the level we are concerned with today.

Whether the context is the presence of low levels, of structurally altered forms of the product or of the impurities, they suffer from the criticism that absence of evidence is not evidence of absence, and in this context one might frame the question is this hGH or at all of the molecules in this test tube hGH?

So with that as a background, this slide illustrates two points. One is that -- I have to pick up a pointer here. I'm sorry for you guys over there.

I can only point at one.

This here shows the development of the antibody response in an animal model that was based on a clinical trial rather than vice versa being predictive. This is a met-hGH, which became Protropin before the process change, and you can see that before the process change we had a very robust antibody development response, and after the process change, we had a very blunted one that was comparable to the pituitary hGH that we evaluated in that model.

So it's clear that it's not related to the methionine itself because we could make a dramatic reduction in the antibody response, but to this day, we still don't know what we didn't know then.

And the second point I'd like to obviously make is that different processes produce different materials. I should also point out in relation to the previous slide that the CD specter of these preparations are all essentially identical. They were all 99.99 percent free of E. coli proteins, and that they were very low in aggregates. So we really don't know what caused this.

There is some speculation that you might hear about later, but I don't have time to go into it.

So clearly most of the tools that we apply and from which we get a lot of information for resolving methods, chromatography, electrophoresis, mass spectrometry, and so on, and these provide the very detailed information on structural and charge heterogeneity and are the main tools for both characterization and for use in the control system.

They add to the accumulation of knowledge about the process and the product, and result in continuous improvement in characterization capability to enable the identification and control of critical product quality attributes, the informed ability to select the most relevant and appropriate specific methods and conditions. And a follow-on manufacturer does not know what the innovator found was critical to measure.

So one of the questions related to promising technologies, I think. Capillary electrophoresis is one that is continuing to evolve, and we've also heard that heterogeneity is a natural property of glycoproteins.

The detailed characterization data that we developed suggested that there should be 41 different charge states in this molecule, and these profiles,

while appearing to be information rich, are certainly consistent with 41 different charge states. Each of those charge states may well contain thousands or tens of thousands of different chemical entities. So even this apparently information rich method cannot really provide much information about sameness.

However, those of you that could be able to see on the fly, and I will read it, it also contains pharmacokinetic data from human PK trials on these three different lots. They really look quite similar by this profile, and the area under the curve for one week in humans was 92, 64, and 56. It does not appear to be related to silalic acid content or to the appearance of this profile, and in fact, after a lot of heavy duty analytical characterization, we were able to establish that it was neither the terminal galactose nor the sialic acid content, but rather the terminal n-acetyl glucosamine content of this heavily N-linked glycosylated protein that was determining the PK properties.

And each set of three points in this figure was obtained from a separate human PK study. A follow-on manufacturer would also have a lot of characterization work and extensive clinical work

to identify this parameter as critical and to develop the justification of suitable specifications to assure consistency of pharmacokinetics.

So one of the other questions arose: what are the factors to consider when assessing the similarity of different protein products?

And I think this is an example where a case-by-case approach is the only sound one. It's the synthesis of clinical experience, process data, and analytical data, the three legs of the stool that Dr. Garnick provided this morning that allows the innovator to provide key parameters and select appropriate methods for use both in the control system and in comparability evaluations.

Even so clinical evaluation may be required to assess the consequences of a process change by an innovator even if only subtle changes are detected in the product.

So this is another example. I hope the panel appreciates all of these examples. This is an example of an antibody. We had just heard they have glycosylation in the FC domain, and in this case, this antibody had low levels of an unusual glycan known as Man5. This is materials that was used

extensively in Phase 3.

Several different analyses of the reference material derived from that process, and here are analyses of the qualification and conformance lots where the increased level from about four or five percent to about five or six or seven percent was noted.

this raised questions about pharmacokinetics, and even though a mouse PK bioequivalent study was performed and showed bioequivalence and all the different glycoforms were demonstrated to be cleared equally, a human PK study was also initiated and showed that these were bioequivalent.

One of the other discussion points for this process change was that there was the potential for things that we didn't know about that are changed in parallel with this thing that we did know about.

As I say, technologies are improving all the time. I don't know of any radically new ones on the horizon, but I think it's fair to say that the ones that we have continue to evolve, and although it doesn't show too clearly here, this is an early mass spectrum of the light chain of one other antibodies,

and there were some baseline noise peaks that we didn't really know how to interpret.

A couple of years later, with a better toy, more expensive, more sensitive and so on, we could clearly resolve this issue as a real peak from the noise in the baseline. Luckily we identified it as a glycated form of the antibody due to the presence of the glucose in the fermenter, but also that was nice to know that it's a natural phenomenon, and every one of us has glycated antibodies circulating.

So this was not a significant discovery in terms of safety, but an improvement in the technology.

One of the tests for product X, presumably anonymous, in the control system is cation exchange analysis, and at the IND phase, we knew there were two specific modifications, and the first method that we used resolved five peaks to allow us to track the proportions of those modifications.

As our experience grew and the chromatography column manufacturers' experience grew, we were able to resolve eight peaks which allowed us to track five modifications from a batch to batch perspective. We knew about these other two, but this particular method didn't track them for us.

After approval, yet another column was available and we were able to track all ten peaks from these seven modifications.

Now, I believe some of the early methodology has been published, but not the recent. So a follow-on might be misled if they followed the literature.

Just a couple of brief words about predicting safety and efficacy. My understanding, I think, is that the animal and human studies are required for evaluation of safety both at the known and the unknown components or variance. And this clinical experience comes from the product of a controlled, consistent process and appropriate analytical characterization.

Beyond the clinical trials, continued safety is insured by the validated consistency of the approved manufacturing process, the validation of the control system, and the reproducibility of the product itself.

For potency assays, I think we need to acknowledge the limitations of them. Often they only show some aspects of biological activity. Some of these molecules have activities that we do not

completely understand, and they may or may not be directly related to the mechanism of action, and I don't think anyone would agree that they measure efficacy in man.

If there's no acceptable pharmacodynamic marker or surrogate acceptable pharmacokinetics only, i.e., bioequivalence would not be sufficient to insure comparable efficacy, and that would need to be addressed in clinical trials.

So to summarize, from an analytical chemist perspective, analytical technology cannot bring knowledge of a protein product and its heterogeneity to the same state as for a small molecule or as I have grown to learn over my years at Genentech, you don't know what you don't know.

To follow that logic, we see that what the innovator doesn't know is not the same as what the follow-on manufacturer doesn't know. However, the innovator does have substantial clinical safety and efficacy data to address whether what remains unknown matters or not.

Thank you.

(Applause.)

DR. CHERNEY: Thank you.

I think there is time for some questions. So Andrew.

DR. CHANG: Well, in your presentation, you stated a follow-on manufacturer does not know what is critical to measure. Can you elaborate that how does the innovator to identify a critical quality attribute in a product?

DR. JONES: Mostly in the case where it's a critical parameter from the clinical trials or the preclinical data. We would find out something from analyzing the product and correlating it with in vitro or in vivo animal studies or even human trials.

The pharmacokinetics data I showed you was from extensive human trials where we identified the critical parameter. That's not to say there's always a critical parameter that fits that bill, but if the follow-on manufacturer doesn't have access to the innovator's data, they won't know.

DR. CHERNEY: Bill.

DR. EGAN: Yes, thank you.

I had a somewhat similar question to Andrew's, and you know, I appreciate very much the fact that we have no knowns and no non-knowns and the rest, as Mr. Rumsfeld said.

(Laughter.)

DR. EGAN: But we do have all of these things we don't know, and there are things that are very, very complex and complicated, and we keep finding new things as technology evolves.

And appreciating all of this even a simple case of growth hormone and the immunogenicity, which I guess the factors for that are still unresolved, what maybe you could comment on because I'm having a hard time with is yet we do make changes and we have comparability protocols, and sometimes we see changes, and how do we proceed? Do we always need to go into the clinic?

DR. JONES: No, I think that's where the data up and down the process as an example from the previous speaker where one can make some scientific judgments, not, you know, absolute truths from above, but best common science and best science, which I think is what we're going for on the basis of what was observed with previous lots from a process and how this new process differs, wherever it differs, and in some cases we have seen some even quite subtle changes where additional clinical evaluation was required.

So I think the status quo is that -- and

as Dr. Garnick relayed -- we are slowly learning not to make changes to the process.

DR. CHERNEY: Thank you.

Our next speaker will be Vytautas Naktinis.

DR. NAKTINIS: Good afternoon, everyone. First I would like to thank FDA for providing this opportunity to speak about the work, the company I used to work for, spent so many years to come to the results I'm going to discuss.

So I will build to my presentations, which are coming one after another, on a simple case study basis, and it will be two follow-on products that are reflected in these two presentations.

I use terminology "follow-on" unfortunately because I submitted my presentation before deadline, and after that my colleagues, associates actually came to an apparently better terminology, biopharmaceutical generics. So all right. So what can I do?

I have to, before proceeding, I have to tell that the presentations I'm going to do are made on behalf of SICOR, a member of TEVA Group, a company I used to work just recently, and these data are based

on experience in developing, manufacturing, and marketing follow-on by a pharmaceutical for more than a decade.

So let's jump into the story. We believe that current analytical techniques are powerful in characterization of protein therapeutics, and that provides a tool to reduce the safety and efficacy studies required for follow-on protein products.

These techniques, however, have to be available to demonstrate comparability within the specified limits established for innovator products and to demonstrate that the follow-on protein exhibits the same protein structure, the same physicochemical properties, the same biological properties, and comparable profile of impurities, and all of that has to be done in molecule to molecule comparison such that we identify differences, if any, which could potentially impact on clinical safety or therapeutic equivalence.

So now finally, I'm going straight into the data to demonstrate comparability based on current analytical technologies of follow-on product with innovator product.

It is established by regulators and

industry where in comparison business you do step by step. First you have to demonstrate the primary structure is identical, and I think "identical" is the right word once you speak about primary structure.

A plethora of methods used to demonstrate that and complexity of results made up from all the analysis allows you to make conclusion about identity. So let's go.

Mass spectrometry. In this case I demonstrate electrospraying mass spectrometry of three batches of follow-on product. They are in blue, brown and green, and innovator's product is in red. Also theoretical mass of this product is indicated, and you see masses calculated from this spectacle experiment. You see that agreement identity of the two products is unbelievably high, and standard deviation, of course, as has to be our days is below half delta.

So basically you can pick up on delta difference using this technique. Everyone is aware, I suppose, in the audience about power of this techniques. So simply speaking one may avoid doing anything else after having these results. Nevertheless, tradition is tradition and we go to peptide mapping.

Peptide map in this case is demonstrated, and hopefully you'll see it, yes.

three batches of follow-on product and one batch of reference product. This is Staphylococcus aureus peptide mass, which of course each peptide peak is subjected orthogonally to mass spectrometry, identification to cover 100 percent of a sequence, amino acid sequence of a protein.

Sine we have mass spectrometry rate, a lot of the question is why do we need N-terminal amino acid sequencing. So nevertheless we do, and we demonstrate it that five different batches of this product, follow-on product, completely matched theoretically expected structure for filgrastim.

Reverse phase HPLC, a powerful technique which serves dual purposes. There's identity, and you can see -- how does the pointer work?

So the principal peak, of course, you demonstrate follow-on innovator products, actually ofthe same hydrophobicity. If you go into a window with magnification of the results, you can see also the impurity profile of a few lots of follow-on product and innovator product. Maybe I'll come later to that, to speak about impurity profile.

You have to demonstrate that aggregation state of your protein is exactly the same as innovator's product, and you do that usually by size exclusion chromatography. So the principal peak, of course, demonstrated monomeric state is the same, but of course, much more interesting is to look carefully into magnified enhanced resolution of window where dimers and aggregates could be picked up.

And clearly you can see that both products, follow-on and innovator products, exhibit very similar profile in terms of dimerization and aggregations.

More subtle techniques are analytical ultracentrifugation could be employed to fairly decipher aggregation level.

So next step is, of course, to prove as confirmational elements of the general tertiary structure is similar of both products. Instead of employing a classic technique in a secondary structure determination CD, we like in our company second order fluorescence emission spectroscopy. Let's have a look briefly how does it look.

So if you put the simple fluorescence emission spectra, you have a spectra which is

literally informative, but if you apply mathematic apparatus to derive second order data, you start seeing quite interesting peaks, and if you follow red and blue lines, you see that is an innovator and follow-on products, they can overlap perfectly.

Just to prove that method is sensitive enough to detect subtle differences in confirmation, we artificially made them fold intermediate of this molecule, and you see green line definitely shows you significant difference.

On what principle is this method based? It reflects subtle differences of exposure of tryptophan and tyrosine, amino acid residues to subtle changes in solvent exposure. So if something changed within the molecule, you pick up it immediately using this technique.

So let's go in the critical part of this story, that security profile. So I will be showing now you a series of classic methods which apply to determine impurity profile, again, size exclusion chromatography, a few batches of our product and one batch of innovator, and you see aggregation level is very low, inconsistent.

Reverse phase HPLC, a method which allows

you to determine versus a product related impurities essentially. Again, the same had to have comparison demonstrates the follow-on product is consistent with what originator product has to offer.

Maybe I skip this. this is two slides which shows that the current but simple analytical techniques or SDS-PAGE are capable to pick up an artifact sometimes occurring with specific proteins, and you are applying analytical techniques and terminal analysis can easily decipher what are these artifacts forms, but I don't go into this.

So let's finish with potency. Yes, potency is a special topic, and we speak about it, how follow-on developed gets around potency and system first an how does it compare, but there are lucky cases, and G-CSF is one of the lucky cases. There are international reference reparations. So you can compare your product, your biosystem, well to originator's products, and you see the data is convincingly proving the potency is the same.

Now, process related impurities, host cell protein. Again, maybe I conclude on the side and comment that this particular level of process related impurities is quite sufficient in proving that this is

okay. Everything, the product and the process, it's saying it was cell DNA.

So to conclude this part of the presentation, I could tell that in our opinion the G-CSF follow-on product case study demonstrates a physicochemical and bioassay data are extremely informative to assess the comparability of the follow-on protein product to that of the innovator product.

And I would move straight to the second presentation, which is, in fact, a twin copy of a previous one. However, the case study will be interferon alpha-2b. So I skip introductory slides, and I go again into the proof of identity of principal component.

Mass spectrometry, a little bit different approach, different apparatus. Nevertheless, data again convincingly tells you that both follow-on product, innovator product, and a mixture of the two 50-50 percent shows you the same mass of a molecule. Basically you can stop here proving identity.

Nevertheless peptide mapping, and here we come to another wonderful feature. Follow-on developer has to harvest in Europe at least, in some cases in U.S. For some simple recombinant proteins we

have reference standards. This time it's a chemical reference standard from Pharmacopeia Committee of European Union. And, as a matter of fact, it comes from the innovator substance. So this is a quite friendly situation, which allows us to unequivocally demonstrate identity of the active ingredient.

Again, orthogonally, we apply mass spectrometry to each peak to cover 100 percent of amino acid sequence.

Interesting. This time I show you manual amide degradation picture and terminal analysis, few amino acids only, four, and what is important to demonstrate here.

I don't know. Do you see in this green window a little tiny peak which is magnified here? And you may follow a red line, which is our product, follow-on product, and the blue line is innovator product, and the peak labeled M is methionine.

So what does this particular thing tell? The innovator's product, up to ten percent of first amino acid is artifactual, not processed methionine. So it's nothing wrong in that because innovator fully demonstrated safety and efficacy of his product, but my point is here that current simple analytical

techniques allow you to pick up these quite subtle differences.

Okay. Let's move further. Again, following this same approach, we demonstrated that, and the monomatic state of the molecule is the same. You have to concentrate on this particular thing because in this case we use finished formulation of innovator which has a lot more excipients. So follow on this.

If you go HPLC reverse face mode, again, the same approach, that to have comparison of our product with innovator's product, mixture one-to-one shows the same hydrophobicity.

The same story we go to secondary structure. I am getting bored with myself telling the same story, but the point is here that techniques we have today, they are applicable to many simple proteins. The question is how much up we can go in complexity.

A little bit more time here is spent on the bioassay. Interferon has a pleiotropic activity, and two classic bioassays used to measure these activities until vital assay in blue and antiliferated assay in yellow. Again, simple visual comparison

demonstrates these two products are indistinguishable in that sense.

Purity characterization. Again, harvesting under that condition in Europe, they have a chemical reference substance coming from innovator in that case; also have European monograph. They can simply visually present impurity profile on SDS-PAGE gel with this follow-on product and with this innovator's product essentially. You see they're comparable.

The same on iso focusing analysis, which is dedicated to pick up charged isomeric forms, again, you see identical picture. Related proteins analysis.

What is interesting here, please have a look at the X axis. It's up to 30 different batches here, and that is very important because many times we already heard today that the follow-on developer comes with one batch of his one and one batch of originator product, makes comparison, draws conclusion that could lead to the trouble.

This is not a case for follow-on developer who does business in the right way, and that is the case. We use numerous batches of our own and numerous batches of originator as well.

So, again, I'm showing process related impurities, and I conclude in the same way as I concluded in G-CSF story. We believe that this case study demonstrates that physicochemical and bioassay data in case of interferon alpha-2b at least is extremely informative to assess the comparability of the follow-on protein product to that of innovator product.

And now a few general conclusions. So if you have in your pocket just analytical techniques and you think you can make follow-on products successfully, that's a good dream, but that's not enough. So what do you have to have in addition?

This, or actually what do you have to exploit in addition?

First, we have a situation that we really know industry samples for simple biotherapeutics. We do know. Even they are not written, but from public domain, from compendial monographs, from analyzing innovator product, you can draw quite good understanding of what particular impurity you are allowed to be on the safe side.

Government study which was presented by Dr. Suzanne Sensabaugh at that actually has exactly

the same point to tell. Different impurities present in that product derived from different process. However, all of them are at the same level. She didn't demonstrate that. Allows you to make conclusion that these products are equally safe and efficacious.

Okay. The second thing, you never rely on the comparability data as it is already on single batch of yours and single batch of innovator's, and that is normal situation if you assume the fact that no follow-on product will be designed, developed, manufactured, approved in one day. Unfortunately this is the process of a few years at least, very, very least.

So in those few years you have opportunity to collect samples of different batches of innovators. So you have to do statistical analysis based on different batches, both yours and innovators.

All right. I'll pick up and sort of mention one more thing which helps innovators or follow-on producers to live quite easily. That is various right of international reference standards, reference reparations. They are both for chemical purposes, chemical analysis purposes available and for

biological assay analysis purposes.

So combination of all these factors, I believe, actually underscores, underlies the power of current analytical technique as essential tool in reducing and moving forward follow-on biopharmaceuticals.

Thank you for your attention.

(Applause.)

DR. CHERNEY: Thank you.

I believe we have some time for questions, and I'd like to ask a question. Given that we've been hearing about the difficulty in predicting immunogenicity with physicochemical tests, in fact, interferon alpha, sometimes products have immune responses that neutralize that effect.

What would you say would be something that would be useful to show in terms of a follow-on exercise in that regard?

DR. NAKTINIS: That's a good question, and very likely would tell since the product is known so well in the market for not many years, we know mistakes that were done in that case of product, and we are learning from our mistakes. They are, of course, our own mistakes.

So the simplest case to mention would be the story with covalent adduct formation between excipient human SER albumin and interferon alpha molecule itself. So that was just missed originally by innovator and turned to be that this particular adduct is quite immunogenic. So that is easily detectable by single analytical techniques if you know the story.

So I think that not exhaustive answer, but partial answer to your question.

DR. CHERNEY: But if you don't know the story of what's inducing the immunogenic responses to products, and there are present, then what approach should we take?

DR. NAKTINIS: If you don't know what you're looking for, actually you will never find. That's --

(Laughter.)

DR. NAKTINIS: But to make it serious, I would come back to the industry standards again or industry trends, what we know today. We know that we have to look at aggregation as number one cost perhaps of immunogenicity. So, therefore, I mention today that simple size exclusion HPLC currently is really

not sufficient. That's clear, but there are other techniques, analytical ultrasonification because beloved instrument of FDA our days for aggregation measurement, and that's quite informative instrument.

So you get that. So basically you do what can you do today to the current trends of industry standards. You cannot do more.

DR. CHERNEY: You could do an immunogenicity study.

(Laughter.)

DR. NAKTINIS: And we have perfect examples, exhaustive studies at clinical level unfortunately missed to detect that, what you're asking for.

So the question again, but I would like my colleagues who are expert in immunogenicity will answer that study later tomorrow.

DR. CHERNEY: Bill.

DR. EGAN: Yeah. If I can just concentrate on the physicochemical characterization, you seem to rely very, very heavily on the use of fluorescence emission spectroscopy or the second derivative of that curve for the determination of the three dimensional structure of the molecules.

Could you comment on the sensitivity of that method and, moreover, its ability to detect in this folded forums, and the reason I ask about in this folded forums is because I think, you know, the fluorescence is dependent on whether it's in, you know, an aqueous or nonaqueous environment, the tryptophans and tyrosine residues.

DR. NAKTINIS: Okay. On the picture I stressed one simple thing. On the picture I showed you before in case of G-CSF, we put actually more of an mount of misfolded, artificially misfolded variant of G-CSF to demonstrate what is the technique capable to do.

But as in any analytical exercise, you cannot rely on one technique alone to answer the question. Our approach is if you have hydrophobicity identical, which is proved by reverse phase HPLC; if you have monomeric state identical to the innovator product, if you have primary structure demonstrated already, and if you have this fluorescence spectra indication of identical confirmational elements, you can draw with a very big assurance conclusion that the higher order structure confirmation of that molecule is okay. That's my answer.

You can come to CD, and we will spend hours or days talking how informative is CD.

DR. CHERNEY: I think Steve.

DR. MOORE: Yes. What criteria would you use in determining how many lots or batches of innovator's product and/or age of product in comparing the uniformity of your product to the innovator's product?

DR. NAKTINIS: I cannot give you straight answer because it happens historically. You develop your process and you pick up these lots which are available in the market or there is a need for your comparison exercise.

But what you have particular assurance, when you look retroactively to your data and you compare all of your data derived from different lots of innovator product, then you can draw a conclusion about what variability was there and, well, basically what statistics is about.

But today we don't use premeditated, master plan sort of pick up so many batches from so many, you know, years.

DR. CHERNEY: Okay, and last question with Andrew.

DR. CHANG: Okay, in our Federal notice announcement for this meeting under characterization section, the first question is what is the probability of current analytical technology to adequately characterize protein products. Now, I want to ask you whether or not current probability of analytical technology is adequate to characterize a product that may have hundreds or thousands of the impurity in their product.

DR. NAKTINIS: Okay. This is critical question because it's on a borderline with philosophy because we will be discussing what is adequate, and we, again, could spend days and days in discussing that, but fortunately, I believe, I have to address again to this fact that today we know industry standards or I call it industry trends about quality.

We know what in general for this type of products specifications could be or are for each particular impurity, and I didn't talk today, and I didn't have time for that, to demonstrate that, of course, we assess how our product is comparable with innovator in stability study, in stress stability studies, in accelerated stability studies, and we are lucky to demonstrate maybe because molecules are that

simple that impurities, product related impurities which occur during such studies also actually are similar if not identical based on molecular structure identification.

So it's perhaps to answer that.

DR. CHERNEY: Okay. Thank you.

I think we have to move on to our next speaker.

DR. NAKTINIS: Thanks.

DR. CHERNEY: Thank you.

The next speaker is Walter Hauck.

DR. HAUCK: Good afternoon. I'm a consultant to the United States pharmacopeia, and I am speaking on their behalf.

I'm normally hard to hear. So can you hear me okay? No? Boy, it really is resonating up here. Okay. Is this better? Well, let's hope so.

So the USP in a very broad stroke at least, a major part of their effort is devoted toward setting product standards for therapeutic ingredients or dosage forms to assure purity, strength and quality, and what I'm going to do today is summarize for you what the USP is going in the areas relevant to the topics of today's meeting.

Okay. So I'll cover the following as shown here. I'm going to start with really sort of a snapshot. It's really no surprise that this is an ongoing process. This is a snapshot from a couple of months ago where things stood in terms of the monographs. So the monographs can be either for the active ingredient or for a dosage form, and while the number is small relative to the total number of monographs in the red book, it is certainly a growing and increasing, substantially increasing aspect of their activity, and the numbers are there for you.

I'm really going to hit this part very quickly because you can read faster than I can talk. Within the USP book, there are chapters some of which they call information chapter, the above the 1,000 chapters in the lingo of the pharmacopeia. These are for information only, and there's a process in place and ongoing to establish information chapters on methods and techniques relevant to equivalence of biological products.

There's a couple of them listed here. This lists some more, and some of the ones that are in process, and of course, then there's the ICH documents, and the Q5 documents as well.

The other thing I wanted to mention as relevant to our topic today is that one of the general chapters is Chapter 111 on design and analysis of biological assays. You have certainly been hearing about bioassays today at various times. This is an interesting chapter for someone who has been working off and on in the statistical issues of bioassays since the mid-'70s. I mean the chapter predates my involvement in that, and it's very interesting. It's full of lots of good stuff, and it's very useful, but if you're not doing whole animal assays, its usefulness is a bit dated.

So there's a major effort ongoing with the group that's been formed by USP representing both the FDA academia and industry, a fairly large group that is completely rewriting this chapter. So this is really kind of a heads up to let you know that that is coming.

We've been presenting about this at various and sundry meetings just to kind of keep everybody informed on what's happening and getting input on that.

Now, every five years the USP holds what they call a convention. This is a rather large

meeting with representatives from academia, industry, other pharmacopeias and regulatory agencies both in this country and around the world.

And out of the last -- I can never pronounce the word they use -- the last five-year convention from 2000, there was a Resolution 2 that came out of that convention urging the USP -- well, again, you can read it -- but essentially to address the issue of equivalence of complex active ingredients.

Now, you'll notice that in the resolutions it mentions botanicals and dietary supplements. In practice thought the work that has been done in this area is related to just the proteins. The botanicals and dietary supplements have not been addressed.

Based on that resolution, USP began a process, really substantially began at the end of 2002 when it convened an expert panel, again, representing academia, industry, and the FDA with experts in statistics, chemistry, biology, the full set of things, looking at the product and the methods, the analytical methods.

You see the sequence of activities there. The most substantial part of that was at the end of

last year. We held a public meeting in which there was a substantial presentation by this expert panel, by others, and a lot of very useful discussion, I think, which has been put together into a publication which is being reviewed at this time for publication. So hopefully that will be out shortly for all of you to see.

Now, we mentioned that we are addressing the science of equivalence here, and the science really addressed three aspects. It was addressing the types of complex actives. It was addressing the different types of analytical methods, and it was addressing the science of establishing equivalence. Everybody in this room probably is more qualified than me to address. I will just say as a statistician with the equivalent side of things, in looking at what is a key aspect of establishing equivalence, which is summarized there as the difference/sameness issue.

And one of the things that has happened over really since mid-'80s, early to mid-'80s is there is finally developed good agreement that when we're talking about equivalence what we need to do in statistical language is set up what's called the alternative hypothesis as similarity.

Now, I'm not saying quality because, as you know, a statistician deals with variability. So we don't show things are exactly equal, but we can show things and try to establish similarity, which is, of course, what's used now for bioequivalence for noncomplex actives.

And one of the things that comes out of that once you accept or realize or understand that we're talking about trying to establish similarity, then you can immediately come basically down to that final bullet up there, and that final bullet, boy, that captures -- and I could talk for a couple of hours just on that because that's really the key aspect of all of this, and it's capturing both a lot of information and really capturing both stuff that is simple and stuff that is difficult.

I think the simple part of it is the recognition that we really have or, as I said over the last two decades or so, really established the structure, that is, the statistical structure and the sort of, if you will, philosophical structure for addressing equivalence. So that's really the simple part.

I mean, we have that in place. We know

how to think about things, but then there's the difficult part. There's the what's the question questions to a certain extent, and that is if you're going to show equivalence, what do you need to compare, first of all, and there's certainly been substantial discussion on that today, and then how close is close enough?

I mean, the vernacular language is the goal posts. So what replaces the AD-125 that was used for pharmacokinetic equivalence, there's no reason to stay with that. So how similar is similar enough?

And that is always the tough question for any equivalence problem. The fact that we're talking about complex actives doesn't change that. I mean, you'd be dealing with a clinical problem and have exactly the same thing. It's consistently the most difficult question.

And I'm not going to spend tim eon this one, but when we talk about what could be looked at to assess equivalence, there's a large laundry list. It's clear that probably for most complex actives, we're probably going to be more interested in what's called pharmaceutical equivalence than in pharmacokinetic equivalence, although it was

interesting today to see how useful bioequivalent studies were proving to some folks.

Okay, and last point, I want to mention that there is another aspect of the product, standard activities of the USP. they do have reference standards, reference standards have a variety of uses. They can be for the substance. They can be for the method. they can be for ancillary materials or they could be for reagents, and I think that I've also been hearing today that reference standards would seem to have a use in some of the activities we're talking about to provide, you know, a reference stability, if you will, in some of the activities that we're doing.

That's it. On time.

(Applause.)

DR. CHERNEY: Thank you.

Any questions from the FDA panel?

No questions. The next speaker will be Jacob Hartman.

DR. HARTMAN: Okay. Good afternoon, and thank you. My name is Jacob Hartman, and I am representing Bio-Technology General (Israel), which is a wholly owned subsidiary of Savient Pharmaceuticals. We develop and produce innovative as well as follow-

on proteins.

In my talk, I will briefly touch on manufacturing and the characterization issues. We claim that the strong scientific background and technological know-how are key factors in interpreting manufacture. The different manufacturing processes can be used to successfully in comparable protein products, and we contend that the characteristic of the follow-on protein are maintained so long as a scientifically sound process design is used in its manufacture.

Scientifically sound process design is a well controlled and reproducible, robust process with demonstratable, systematic removal of false process and product related impurities, which results in homogeneous, biologically active protein product, well characterized by predefined, biological potency from ecological, biochemical, and physical parameters, and of course, stable, active pharmaceutical ingredient and formulated finished product.

This table describes manufacturing of recombinant proteins and E. coli, an established source for many pharmaceutical's protein for almost a quarter of a decade. I listed several parameters and

the alternatives used by the industry for innovative products, as well as the follow-on products, and you can see that various strengths of E. coli were used with different expression systems, and of course, the cellular localization of the protein is usually in the inclusion bodies, but the virus proposals were used as either with the methionine terminus, of course, all of them. To begin with sometimes the methionine is removed by the internal aminopeptidases. Sometimes you need to remove it externally.

Of course, the solution in the falling of the protein varies, and sometimes you would like to add chemical modification, reversible chemical modifications in order to allow proper processing of the protein. This can be also achieved by enzymatic treatment. As I mentioned before, aminopeptidase to remove the extra methionine, and sometimes endopeptidases and proteases to release a certain bridging peptide.

Of course the purification strips are complex, and each producer will use its own purification outlines. What adds, of course, are the standards if they are available. The WHO usually provides standards for potency. The USP and European

authorities provide the standards for biochemical characterization, and of course, there's always the competitor's material to do the comparable studies.

In the follow-on slide, I will show some comparative research results that were carried out with the gene. First I'll demonstrate the identity of several insulin, recombinant human insulin produced by several manufacturers using different sources, and then I'll give you some examples about interferon alpha.

In this slide, I hope it's visible. You can see peptide mapping of several insulin preparations. The first one is the European standard, some produced by us and two others produced by Novo and Eli Lilly. You can see that the identity is complete. Of course, you can apply more sophisticated measures in order to characterize each of the peaks.

Anyway, you can see the insulin which is relatively a simple molecule, but one of the most complex in the industry is identical by processes that are utilizing different choices and different processes.

The same, I move to interferon alpha again. This peptide mapping shows identity of our

interferon alpha compared to the European standard.

The next slide demonstrates a physical as well as a biological activities of this material by the immunoprecipitation assay. Antibodies raised against the innovator product are shown to immunoprecipitate in a dose response manner. IntronA is our interferon, and of course, when you measure the remaining activity in the supernatant, you see, of course, the opposite results. The more antibody you add, you lose completely the activity and gradually there is increased depending on the amount of antibodies added.

Again, another activity assay of interferon alpha which demonstrates antiproliferative activity on Daudi cells compared to the innovator. You get essentially the same response.

In summary, our expense shows that the implementation of the validated science based technology, coupled with confirmation of product characteristics and specifications, guarantees comparability of the follow-on protein to an already approved protein pharmaceutical product.

Thank you.

(Applause.)

DR. CHERNEY: Thank you.

Any questions from the panel? Lawrence.

DR. YU: This afternoon we learned that no only manufacturing processes, but also the scale of the manufacturer, the scale of the same manufacturer could have impact on product quality.

For example, the previous speaker showed that there is a fivefold difference in the imputed level before scale-up and after the commercial batch compared to pilot in the commercial batches. I wonder what is the industry doing to insure the products before and after scale have the same quality, same safety, and the same efficacy, particularly, the same immunogenicity.

Thank you.

DR. HARTMAN: Well, of course, you control your process at each step, and the impurity profile is determined by the industry standards, and your aim is to reduce and to get rid of the impurities as much as you can.

So I don't think that any adverse effects were demonstrated in 25 years of using proteins derived from E. coli even though that sometimes antigenicity -- well, antibody formation was

demonstrated.

But in any case, if you control your impurities, as mentioned previously, to a level that is acceptable in the industry, and of course, during this 25 years' specifications and limits were established in relating into impurities derived from E. coli, and you are controlling your process each step, I believe it is satisfactory.

DR. CHERNEY: Bill.

DR. EGAN: Is the immunogenicity of the two products, innovator and follow-on, are they the same?

So if you look at the antibodies that are produced by both of the proteins?

DR. HARTMAN: Well, I'm not aware that any antibodies are produced based on the cases that we developed and analyzed, which is growth hormone, insulin.

I don't think any antibody to a significant level that cause any critical consequences were detected.

DR. EGAN: Well, not necessarily human antibodies, but in animal models.

DR. HARTMAN: Oh, in animal models you

will expect the antibody formation, of course.

DR. EGAN: Yeah, and they ought to be the same for both products.

DR. YU: Or equivalent.

DR. HARTMAN: Why would you want to analyze for immunogenicity in animal models? By definition you'll get antibodies.

DR. EGAN: Yeah, if they're the same products, I would expect the same antibodies and the same ability to cross-absorb them just to look for differences.

DR. WEBBER: Any others?

DR. CHERNEY: Yeah, we heard from one of the speakers that, you know -- and I'm paraphrasing -- that frequently you don't see what you don't know to look for. That obviously has been a problem for people who are trying to do comparability exercises because sometimes they miss something because they didn't know it was there, and that level of not knowing what to look for is perhaps at a higher level when you haven't had the history there.

So how do we solve this issue?

DR. HARTMAN: Well, I believe that meticulous characterization will help. You know, if

you work within the standards of the industry and try to improve and characterize your product as much as possible, of course, you'll save later on some problems in the latter stages of development.

So if you are sure that you're working within industry standards, I don't think you should expect any --

DR. CHERNEY: Would you suggest using robust orthogonal methods to look at the same type of feature? I don't see it sometimes in the presentations that we have, but then when people talk, they say, "Well, we would do this other assay and this." Is that part of your --

DR. HARTMAN: No, my point is that you will have to do more characterization in the analytical testing of your material and perhaps in comparison to the innovators, unless there are preclinical and clinical studies. So the impact will be on the characterization.

DR. CHERNEY: Okay. Thank you.

DR. HARTMAN: Thank you.

The final speaker for this session will be Charles DiLiberti.

MR. DiLIBERTI: First of all, Id' like to

thank FDA for offering me the opportunity to speak, and I'd like to thank the audience in advance for bearing with me as everyone I'm sure is anxious to get to break. I'll do my best to get through this quickly.

Charles or Charlie DiLiberti, Vice President of Scientific Affairs at Barr Labs.

Things have changed. There have been tremendous advances over the past couple of decades in terms of how proteins are characterized and the analytical methods available to us. One of the biggest issues in the past has been that chemical methods had an upper limit for precise analysis of perhaps a couple of thousand Daltons, and to go beyond that, we had to resort to less precise biologically based analytical methods which resulted in the characterization that is somewhat less robust than what we currently have available to us today.

Also in the past, because of the analytical methods available , the analytical variability was often on the same order of magnitude as the batch-to-batch process variability for the product.

So as a result, in order to give us a

sense of better assurance of the product, we sought to control the process very rigorously and also possibly to look at clinical studies to demonstrate comparability.

Nowadays we have much more powerful methodology, and the shining star here is really high resolution mass spec. We can routinely assess the covalent structure for proteins over 100 kilo Daltons. We can routinely detect changes at or below one Dalton, and we also have very sensitive analytical methods to detect higher order changes and structures.

Nowadays most of the methods are chemistry based and consequently analytical variability tends to be much less than batch-to-batch or intra-process variability. And as a result, many in vivo studies might not be necessary.

The product is not the process. The old product is the process theme implied that the process could somehow impact safety and efficacy without being detectable analytically. But if this were really true, then we could never justify the use of comparability protocols for process or formulation changes.

The reality of the matter is that current

analytical tools are capable of discerning not only clinically significant product changes, but even minute batch-to-batch changes.

This schematic is intended to show three different methods by which we can assess product equivalence: analytical characterization on the top, verifying that the product is from the same process, which is in the middle; and comparative clinical studies, which is at the bottom.

I'd like to actually start in the middle with the gray bar. This bar represents the batch-to-batch variability inherent in a biological process. We can see from this that we could have various processes that vary around this range and would still be deemed clinically equivalent. I think this is very nicely demonstrated by Suzanne Sensabaugh's presentation on hGH.

We had six different products, each with a different range of variability, and it would be virtually impossible to determine that any of them was different from one another in a clinical study.

So really the process would have to vary considerably outside the clinical equivalence range in order to come up with a clinically distinguishable

product.

Now, on the other side, we have the small error associated with analytical variability, and we know that this is very small because we are able, in fact, to distinguish very readily the characteristic signature of each individual batch within a process. We can determine that analytically.

So what is the best measure of product equivalence? I think the best measure is obviously the sharpest tool we have, which is analytical characterization. It's the most precise.

The least precise method is to try to demonstrate equivalence via clinical studies. We're really interested in the comparative characterization to demonstrate pharmaceutical equivalence between two products. And the same characterization principles ought to apply equally to both justification of process changes, as well as to justify the introduction of a generic product through pharmaceutical equivalence.

And just because the innovative product may not be as thoroughly characterized as we would like should not in any way impede the approval of a generic product.

As Carole Ben-Maimon this morning so eloquently pointed out, there really is a continuum of products. There's no clear distinction between the complexity of biological and small molecule products. There are simple and complex versions of both.

There's also no magic about the biological source. We've been dealing with complex fermented antibiotics and other naturally derived products for decades. No magic about peptide bonds. They're well understood.

So really why should we have a different procedure for characterizing proteins versus conventional pharmaceuticals? It boils down to two main reasons.

Number one, some proteins are large enough to have the potential to misfold and also aggregate. So we need to in our characterization address secondary and higher order structure and aggregation. That's no real problem.

And, secondly, the size and chemistry of protein molecules does require some different types of methodology from small molecules.

In characterizing proteins, current technology really does enable comprehensive

comparative characterization of virtually all therapeutic products. Pharmaceutical equivalence may be concluded on comparative characterization, and really the process form which a product is derived may be irrelevant. Really the only relevance of the process is that it's under control. As long as you can show analytically that one product is the same as another, that should be sufficient.

Characterization does justify an abbreviated approval process, and the extent of the clinical program ought to be inversely related to the extent of characterization, and many protein products can be characterized so thoroughly as to eliminate the need altogether for clinical studies.

No one size for characterization fits all. Each product requires its own particular strategy, although typically we would apply a comprehensive array of sensitive and selective analytical methods to multiple batches of both test and reference products. So this is really a comparison of the test process and multiple batches from that versus the reference process and multiple batches of that.

We typically apply a wide array of orthogonal methods so that virtually every observable

property is probed, and we're not going to go through the list in detail, but it's certainly comprehensive.

We need to look at these results from the characterization collectively, and when taken all together, the analytical results represent a highly selective and sensitive fingerprint of each product or process.

We need to show that the scope of variation seen with the test product or process is comparable to that of the reference. Imagine a test product with each batch representing a fingerprint, and the reference product with its own collection of fingerprints making sure that the spaces outlined by each array of fingerprints is comparable.

Again, the process is not really germane to the comparison because two different processes, as we've seen earlier, can yield therapeutically equivalent products.

To answer the question that was posed for this meeting, what is the capability of current analytical technology to adequately characterize protein products? It's really excellent. We can with certainty completely elucidate the covalent structure of products. Particularly powerful is peptide mapping

with high resolution mass spec, as was so beautifully demonstrated by the earlier speaker, Dr. Naktinis.

We also have sensitive methods for comparing higher order structure, and this is a fingerprinting kind of technique so that we can make sure that the two products are, indeed, equivalent with respect to higher order structure.

We also have very sensitive and selective methods for measuring impurities, and ultimately we compare products using multiple orthogonal analytical methods to a high level of assurance that the two products or processes are pharmaceutically equivalent.

Are there new technologies that hold promise for helping to characterize proteins? I think the answer is yes, but I'd like to start off by saying that the technologies that we have in hand are already adequate, more than adequate to characterize comprehensively the vast majority of proteins available, but future advances I would say that hold promise would involve multi-dimensional methods because they have greater information content, and I think even more importantly, we need to look at methods for analyzing the data, and there are some very powerful tools for analyzing multivariate,

complex data sets.

For example, there's a whole branch of chemistry called chemometrics which -- I'll be done quickly.

(Laughter.)

MR. DiLIBERTI: -- which allows reduction in drilling down from complex data to drill down to identify those variables that are really critical to issues like immunogenicity and other critical safety parameters.

Is it possible to accurately predict safety and efficacy from analytical studies? I think the better question to ask is: is it possible to insure that two products have evaluatent safety and efficacy from analytical studies?

And I think, once again, the answer is yes. Modern analytical methods are much more sensitive indicators of product changes than are clinical studies, and the tools that we have already, if we look at the information content in the analytical data sets, that already contains all of the information that we need to know about safety and efficacy. It's a matter of how to analyze the data, and that's beyond the scope of this conference.

Conclusions. I think the same characterization strategies should be applied to both justify and process changes, in other words, comparability protocols, as well as to show pharmaceutical equivalence between generic and reference products.

Modern comparative analytical characterization really may eliminate the need for many in vivo studies on later products using the same chemical entity, and right now there's no scientific reason to delay the approval of safe, effective, generic protein therapeutic products. The technology and strategies are already in use and in practice with comparability protocols.

Thank you.

(Applause.)

DR. CHERNEY: Yes, we have time for a couple of questions, I hope. Andrew.

DR. CHANG: For the follow-on company, what factors do you use to guide you for the process directly?

MR. DiLIBERTI: The reference product ought to be the target. So one needs to analyze multiple batches of the reference product with the

wide array of techniques that I've said, and looking at how the reference product varies with respect to each one of the variables. That gives us a range within which to operate on the test product, and we can develop our test product to match that range.

DR. CHANG: But do you have access to the intermediate that the innovator company has? Is that necessary for your product?

MR. DiLIBERTI: I don't think that's really necessary. I think that the characteristics of the finished product really define the safety and efficacy of that product, and that's all really open for us to evaluate through thorough characterization of multiple lots reference product and our own product.

DR. CHANG: And that leads to my second question. How are you going to identify structure and safety relationship?

MR. DiLIBERTI: If we fall within the same pattern of results as the reference product, that already demonstrates it. Now, if our product has significant differences from the reference product, that's a different story. I'm not really addressing that.

DR. CHERNEY: I have one question. You were equating that precision of the assays that we know are pretty precise.

MR. DiLIBERTI: Yes.

DR. CHERNEY: But that's only one aspect of this. Are the assets capable of predicting the safety and efficacy of the product?

And we've certainly seen questions about the ability of physicochemical tests to predict immunogenicity. So would you suggest that your data that all of the product attributes are within the innovator's product, that there is no need for any additional types of clinical or nonclinical studies?

MR. DiLIBERTI: I think it's important to note that we are talking about comparative safety. We're not trying to predict the safety of a new chemical entity that has never before been studied. We're comparing two products. If the variability of one product is consistent with the variability of another product and the match is excellent on all accounts with every one of these wide array of methods, then I think it's safe to assume that the products are, indeed, equivalent with respect to safety and efficacy.

DR. CHERNEY: But I think that was the issue, is that in some products we've made those determinations, and then in terms of immune responses the products turned out to be different and some products not as safe.

MR. DiLIBERTI: I think that historical examples where differences or potentially immunogenic differences have been failed to be detected are the result of not applying the full array of technology that we had today, and had we applied that, I think we would have seen it in the past.

DR. CHERNEY: Okay. Thank you very much.

MR. DiLIBERTI: Thank you.

(Applause.)

DR. CHERNEY: And I think this concludes this session. We will take a break.

DR. WEBBER: Okay. We will take a 15 minute break. Actually we'll reconvene at ten to four for the potency and surrogates for safety and efficacy.

(Whereupon, the foregoing matter went off the record at 3:36 p.m. and went back on the record at 3:53 p.m.)

DR. WEBBER: Okay. I'd like to get

everybody back into their seats and reconvene so that we can start the potency and surrogates of safety and efficacy session.

This session has two speakers, but we'll I'm sure be filled with stimulating information, and we should have some time for questions as well.

For starters we'll bring up the questions that we have, and introducing the panel -- these are the wrong questions, huh? Yes, they are the wrong questions, aren't they. You have the questions, right? I'm not going to go searching for them here. Apparently they're linked to the wrong slide, but we'll have each of the panelists, as usual, introduce themselves, and then this panel is being led by Janice Brown, and then we'll jump into the speakers.

Thank you.

MS. BROWN: Hi. My name is Janice Brown. I'm a chemistry reviewer in the office of new drug chemistry.

DR. ORLOFF: I'm David Orloff. I'm the Director of the Division of Metabolic and Endocrine Drug Products in CDER.

DR. SWANN: My name is Patrick Swann. I'm the Acting Deputy Director of the Division of

Monoclonal Antibodies in the Office of Biotechnology Products.

DR. GREEN: Dave Green, Office of New Drugs, Pharmacology and Toxicology.

DR. BURNS: And I'm Drusilla Burns, the Chief of the Laboratory of Respiratory and Special Pathogens in CBER.

MS. BROWN: Well, the goal of this session is to answer the questions that were presented in the Federal Register. We're having some AV difficulties, but the question is: what factors should be considered regarding the bioactivity and potency assays used for comparing two products?

And the second question is: what is the role of an in vitro and in vivo assay for use as surrogates in establishing safety and efficacy?

Our first speaker is going to be talking on numerous topics that span some of the other sessions. So her talk will be 30 minutes.

Dr. Fryklund. Could you please state your name and your affiliation?

DR. FRYKLUND: Thank you.

Madam Chairman, members of the panel, I would like to thank the FDA very much for inviting me

to be here to speak on this very interesting workshop, scientific considerations related to developing follow-on protein products.

And as you already heard, I intend to address the questions that were posed by the FDA and answer them in turn, illustrate them by actual examples of company hands-on experience of manufacturing protein pharmaceuticals. And I think you'll see that all of my topics are actually relevant for the questions of potency and surrogates, as I continue on.

And I would like to make a comment. There have been people talking about simple and complex proteins during the day. Some of the ones I'm going to talk about at face value are simple ones, but I think you will see that they are not quite so simple and need a lot of hard work and investigation to understand what exactly is going on.

Also, I would like to make a point that most of the examples I'm going to give you are rather trivial in nature, but actually had quite major impact on safety and efficacy.

Many people have discussed this part of what aspects of the manufacturing process determine

the characteristics of a protein product, and you've all heard about the organism DNA sequence, the plasma construct, and I would like to make a point about the strain.

The strain actually can be quite important because it can determine the amount of proteolytic enzymes present which would affect the impurities present and the product itself, and fermentation conditions we have talked about, harvesting, the selectivity and the specificity of the isolation and purification steps.

And also I am going to talk a little bit about formulation, excipients and preservatives filling lyophilization and container/closure, and my point is that they all are determining the characteristics of the product.

My first example is a trisulfide impurity we found in Genotropin, which is human growth hormone which has been on the market first produced by Pharmacia and now by Pfizer since 1987, and you will see on the slide here my little diagram of the lower half of the molecule and the C terminal between residues, 182 to 189.

And my example is intended to show you how

a change in molecular weight of 32 by the addition of only one sulfur atom in one single disulfide bond can actually have a major impact on both the primary and the secondary structure of the protein. And I think it doesn't need much understanding to understand if you put a bit sulfur atom in that lower disulfide bond, it's going to affect the secondary and the tertiary structure of the molecule.

It also turns out to be a very stable bond. So once it's there, it's there and it doesn't go away.

We found this by chance when we were running a new hydrophobic interaction chromatography step, and we assumed it was a variant that had lost one of its amino terminal amino acids because we already knew one that had lost its phenylalanine, and that was affecting its hydrophobicity. So we assumed this was the same, but in fact, it wasn't the case.

Once we found it there, we of course could remove it by manipulating the elution conditions in one of the chromatographic steps, and we also were able to show that it was produced during harvesting conditions. So we were able to reduce the amount of product found there.

It was a long way to find out what it was. The tryptic peptide that contained this extra molecular weight of 32 had a methionine in it. So the immediate assumption was that its methionine sulfur and with two oxygen atoms which, of course, everyone knows has up to 32, but mass spec showed that it wasn't two oxygens. It was one sulfur, and our peptide chemists synthesized the tryptic peptide with an extra sulfur atom in there and were able to show that it was exactly the same as the naturally occurring tryptic peptide we found in Genotropin, and that way we were able to prove that it really was a trisulfide variant.

And I think this brings me on to my topic. People have argued this back and forth during the day, but I maintain that the process significantly finds the product because the process, the harvesting process put the trisulfide in part of product. We were able to remove it, but not until we understand where it came from.

One of the other questions was what aspects, what parts of the manufacturing process should the agency focus on when assessing similarity between products, and I think you can't choose one or

the other. You have to choose all parts because all can in their own way individually affect the final product.

And even if you have access to the final product you can analyze it backwards and forwards and in and out with all of the assays at your command. It doesn't tell you how that protein actually got there and what it was subjected to en route, and that's actually a very important thing because it can determine stability in the end.

So some of the things I haven't talked about like things like hold steps for intermediates. Proteins like growth hormone, they're hydrophobic. They tend to aggregate if you put them in the wrong conditions standing around too long.

If you handle them too harshly during filling steps, they'll precipitate on you, and in lyophilization you can get aggregation and deamidation forming.

And then, of course, the container and the closure can also affect the product, and the container geometry also can affect the product and the way it freeze dries.

One of the products we worked on which is

no longer on the market, but it's called Groliberin, which is a growth hormone releasing hormone. It was a 29 amino acid peptide, and we were just investigating some new HPLC steps. This was way back, and we had identified a monomer, dimer and polymer on the chromatogram, and we found batches coming out of production that had high levels of polymer, so we thought.

It turned out it wasn't polymer. It was solids leaching out of the filling lines and solids of plasticized, they're softeners as you know, they are plasticized, and you certainly don't want them in your product. So it wasn't polymer. It was a contaminant from the filling equipment.

And this is just to raise your awareness that things may not be what they seem to be, and even if you're 90 percent or everyone is convinced that it's a polymer, it may not be, and you need to be really sure before you go on and say that there's something wrong with the freeze drying step. In fact, it was something wrong with the filling equipment.

We also found polymer formed in our methionine growth hormone, Somatonorm, somatrem, and we found a polymer formed. It was perhaps about one

percent, which shouldn't perhaps worry you really, but we knew in the naturally occurring product, Crescormon, there was hardly any measurable level of polymer. So we knew polymer was not good for human growth hormone. It tended to be antigenic, and the reason it was there was there was a new stopper that we had introduced into the process. It had different geometry, and freeze drying time, the drying time in the lyophilizer had been reduced from 18 hours to about five, which, again, wasn't obvious, and it meant we had to go back and do a lot of work.

And, again, my point is that that process defined the product and needed changing and improving.

The second part was characterization of proteins, and I would say that current analytical technology has really no capability at all without tailoring to the manufacturing process at issue because there are all of these assays out there, but they don't mean anything unless you have been able to adapt them and who that they actually could be used on the product you're working on.

And you really need an ongoing and synergistic and a mutually reinforcing collaboration between the process developers and the analysts, and

it's going to be a dynamic interchange. This is where the persons express it. It can be actually quit acerbic at times because the analysts say, well, the process developers know what they're doing, and the process developers say they're too fussy.

But the best way is to have a really good interaction between these two group of very different types of people, and the corollary to that, of course, is you have to develop proprietary assays based on current technology, but they should be developed concomitantly with your manufacturing process so that you can measure the appropriate product and host cell related impurities, as well as container derived contaminants.

And these assays and reagents, of course, are not in the pharmacopeia. They are not on the open market. You can't get them off the shelf. They are not in the public domain generally. So some of the might be, but not in obvious places.

In fact, to go back to the trisulfide example, I was quite surprised two years ago when I talked about it at an FDA meeting when a lot of the people there had never heard about it, although it's in the literature.

So this cannot be stated more than enough that your proprietary assays are not generally available, and they are developed to the process that you have developed yourself.

And as I said, the trisulfide impurity was found in a high resolution hydrophobic interaction chromatography method. That was actually designed for another purpose, not for looking for trisulfides, and it was really by chance that we found out what it was.

As I say, in the labs at the time we thought it was a terminal proteolytic cleavage minus two amino acids.

So my point here is that you need experienced analysts, and you need this dynamic interchange between your process developers and your analysts ongoing all the time.

And the second question was characterization, are there other new technologies that hold promise? And I would contend, no, not yet, although there have been very significant advances because we still can't get down to these complex profiles.

And I know we have heard this afternoon about fluorescent spectroscopy and comparing bands and

so on, but I would contend that what we're looking for is something much more subtle than that, and again, no technology is any use in itself without process specific application and development, and you need this reciprocity of process and technique, and this isn't really the same as for small molecules, as people have said already today.

And I have another example. We worked many years ago on yeast-derived IGF-1, and we found again by change because a band appeared in a hydrotropic interaction exchange gel, and you can see it was about half of the product, and we assumed it was mismatched, disulfide interchange mismatch, but it turned out to be glycosylated.

And of course, everyone know that IGF-1 is not glycosylated normally. this was a de novo glycosylation are residue Threonine 29, a de novo O glycosylation, two mannose residues, and this wasn't obvious, through, of course, when you go back and start reading the O glycosylation literature, Threonine at position 28 was sort of encourage -- sorry -- Proline at residue 28 would sort of make the Threonine at rescue 29 more prone to be glycosylated if it was going to be glycosylated.

But my point is that there were just two mannose residues, and they were very minor, but you can see there was a major change in chromatographic position because of that, and it needed advanced NMR spectroscopy and analysis to understand what was going on there, and once having found it, we tried to get rid of it, but we were very unsuccessful there. So we have to live with that, and that led to a huge loss in yield.

Another question that was asked was what factors including quality attributes, impurity profiles and changes in the process should be considered when assessing similarity, and I think we have heard plenty today say that quality attributes and impurity profiles and changes in the process have to all be considered, and they are dependent on the manufacturing process, and minor changes can unwittingly cause a critical parameter to be exceeded by column overloading, and I would like to give an example of that.

This was an example from our Somatonorm base, and it was a minor change in a washing procedure of a filter made in the manufacturing plant, and they hadn't thought it was any point telling anyone. They

thought it was just simple change. It was identified as an extra bound in the final product, and it was traced back to an overload of a column at the very start of the process introduced by this change in washing procedure which allowed the filter to filter a little bit faster.

And as you can see, it's there tagging on the back side in the first ion exchange column on the front of the growth hormone peak and the second, and you can see no way will it be removed unless you know it's there and you can adjust to that.

And this look trivial, and it was trivial, but it actually had a major impact on our production capacity for a while, and a lifetime of people spent times analyzing it.

We picked it up, of course, because it resorted in our final product analysis. It was a band that shouldn't have been there. It may be very faint and resolute, and we finally tracked it down to a host cell contaminant that actually wasn't the whole protein. It was a proteolytic fragment of a protein. This will make life even more complicated, but there you are. That was an example of a trivial change in a filter washing procedure.

The next question was characterization. Is it possible to predict safety and efficacy from analytical studies? And I would say, no, it is not possible to predict safety and efficacy, although we know an awful lot. We know, for example, that aggregates of growth hormone are known to be antigenic. We know that product related or host cell impurities could be antigenic or excipit, changed activity. And we also know that breakdown products may either retain, lose or gain activity.

But you really only know that by bitter experience and having been there and found them and gone back and evaluated them. They're not in the textbooks. They're not in the pharmacopias. They're not out there in the public domain.

And I would like to go back to the de novo O-glycosylated IGF-1 at Threonine 29. You'll see here on the little diagram at the side there's a double peak there. There's the IGF-1, which is not glycosylated, and there is glycosylated, which is the black circles, and you'll see that that doesn't bind so much to the high molecular weight fraction in serum.

And this was found to be the case, that

these two mannose glycosylation at one amino acid actually changes totally the binding characteristics of IGF-1 to its binding protein. So instead of forming a nice complex and being taken out of circulation so to say, you've got all of this free O-glycosylated IGF-1 in circulation which you wouldn't have known about unless you had gone back and done the analysis.

And one of the side effects of IGF-1 is hypoglycemia, which can be life threatening. So this is actually a trivial change, a trivial thing we observe, but actually could have a life threatening potential because you could be dosing your patient incorrectly, giving them 50 percent free IGF-1 if you didn't know about this glycosylated form.

Fortunately we found that out before we got into man, but again, this was a trivial thing, but has a major effect on safety.

And you wouldn't have predicted this because your receptor binding activity of this particular product was as wild type. There was no difference. It was only when we looked at the binding to the complex that we saw the difference.

We also know that deamidated growth

hormone is active, but it is actually a degraded byproduct, and it has a shorter shelf life because that's what is on the way to breaking down totally.

And I always like to make a point that deamidation is not one process. There are many residues on a protein that get deamidated. They usually occur in a specific sequence. Some of them actually form isopeptide bonds, which will tweak the protein backbone, and I think it's very important to understand which sites are deamidated, what is actually going on there.

And they could be bioactive, but I think in general, manufacturers would like to have the amounts down very, very low because we don't know what happens over time. They could potentially be antigenic if you're tweaking the backbone, and if, of course, you combine it with some of the other changes that we've observed, you may mean perhaps deamidated polymer is more antigenic, for example.

And, again, my thesis is we cannot predict immunogenicity.

And how and what extent should it be evaluated? And I think it has to be mandatory to evaluate immunogenicity. We can't predict it, an we

also know that the follow-on product can have neither the identical manufacturing procedure nor the formulation, nor in most cases the container and the closure, and the product and process related degradants and host cell contaminants can be immunogenic or become immunogenic by adjuvant action.

And there's a generic product out for review now called Omnitrope, and it was found to be immunogenic, and this is amazing. It's 20 years on. they've had access to all of the fancy technologies available, but this actually is antigenic as Somatonorm was in the early days, in 1983.

So I would contend that it's not a question of going out and reading the literature. You need to know a little bit more than that, and the company says its host cell contaminants. Whether it's the same host cell contaminant, as we recognized in Somatonorm all those years ago, I couldn't say, and we have no way of knowing, but I would just say immunogenicity is impossible to predict even if you know it should be there and you may be looking for it. Obviously this could be missed.

And I think you should test early in animal species and primates, at least for a lot of

human proteins, a good species to test in. You have to do your Phase II trials with more than one production scale lot, and I mean a decent scale as well, and of course, you need post marketing surveillance as well.

And that just brings me back to my question of Omnitrope. That was one batch that was found to be immunogenic. Obviously it's an uncontrolled process if you don't know what else could be there and why it was really antigenic.

And host cell contaminants, this again sounds very trivial, and people say that there is state of the art, and there's industry standards. I would like to ask what industry standard.

The host cell protein is determined by the strain you're using and the organism you're using, and you need to adapt it and tailor it to the process you have developed. Otherwise how will you know what you're trying to measure? And how can you know what you're trying to evaluate?

And we know from our early experience that we were down to parts per million before we were during that we had removed host cell contaminants of any import.

So I would say that you need more than one production batch to be sure, and I would like to go back to my old example of Somatonorm again. This is antibody titer, this anti-E. coli on the Y axis, anti-growth hormone on the X axis, and it's a log scale, and the samples have been taken at different time points, 1.5, three, and six months treatment.

And you can see that there's a linear correlation between anti-E. coli and anti-growth hormone, and obviously you can see when we reduce the amount of E. coli, we also reduce the antigenicity of human growth hormone. Somehow we had an adjuvant effect there which we couldn't identify. We think we know what the culprit was, but we're not 100 percent sure, but anyway, there was the proof in the clinical study. We could get anti-E. coli titers down. In parallel, anti-hGH were dropping.

And I would again say that in answer to this question, can you eliminate animal or human studies, I would say that they need to be mandatory because you don't know. You can't be sure what problems the follow-on process will come up with, and perhaps when you have been able to show that you have manufacture reproducibility and linked safe clinical

use, perhaps you can reduce the amount of tests.

You also need a large number of patient years depending on the treatment time, and more is required for the chronic treatment.

And I would also like to add that there should be no additional risk to patients because drug quality standards must be maintained. If you've had safe products out on the market there for 14 or 15 years, why would you think it was justifiable to introduce a product that was not safe and had more risk?

And I think it is unethical to subject patients to any incremental risks when safe and efficacious protein biologicals have been available for many years. So there should be no more risk involved.

There was also a question of potency and surrogate, and we have talked about bioassays, and bioassays suffer from high intra-assay and interassay variability, and they're not precise enough to even compare products, let alone even show they are similar or identical.

And I would say that you can only show loss of potency in a growth hormone bioassay if you

have lost about 50 percent, and then you would know anyway from other assays you were doing that there was something seriously wrong.

And supposing you saw a difference in bioactivity. How would you evaluate it? More important, I would say that a minor loss of activity can actually result in poor long-term clinical effects, especially if you're treating quietly.

So my point is that bioassay's lack position and reproducibility, and my example here is from our database. We now have 40,000 patients that were treated with Genotropin over the years, and this shows height velocity in centimeters per year and mean does in units per kilogram per week.

And you can see the scattergram, and this, of course, is Genotropic, but the point I'm trying to make is that if you give a substandard product, a follow-on biological that's not quite up to standard, but it's sort of 90 percent, what you're going to actually do to that person who has been treated, that child, you're going to give them substandard treatment, and they're going to lose out. They're going to lose two or three centimeters per year over their lifetime of treat, which is six years.

So that's head and shoulders, and I think that again is not justifiable if you already have therapy on the market, which is proven and safe.

So minor loss of activity should be looked out for because it has long-term consequences, especially with these drugs that have been used for long-term therapy.

And I think there was a speaker earlier today that said that product were identical. Actually in our database they're not. There are some that are substandard, and they show up as the bottom of that scattergram.

And I would say again that there is no role for surrogates in lieu of clinical data. Bioassays are not precise enough, whether they're in vivo or in vitro, and if you want to do long-term safety and efficacy testing, you have to choose a species which is not going to product antibodies to your product because that will invalidate totally anything you're trying to show, and I think those should be mandatory.

And finally, it was a question of terminology, and that has been discussed as we say ad nauseam today, but I think follow-on can give the

suggestion of comparability which may not be the case. I think second generation is fine, and I would just like to summarize that I think as of today, as it was 20 years ago, the process does significantly define the product.

In characterization, experience is key, and not taking the easy way out, but is it really polymer? Is it really not something else? Are we really 100 percent sure?

We know that immunogenicity cannot be predicted, although we know a lot more than we did perhaps in the early '80s, and for both preclinical and clinical you need a comprehensive package, and there are no surrogates for clinical trials, especially given that you had products on the market for 14 or 15 years before the follow-on products are going to come on which have been proven safe and in many thousand of patient-years have been shown they were safe.

And finally, the terminology follow-on may suggest a degree of comparability which does not exist.

Thank you.

(Applause.)

MS. BROWN: You talked about a bioassay that's not precise enough, but when you use a bioassay, after you do extensive physicochemical characterization and you increase the number of independent measurements to increase the precision of the assay, do you think that a bioassay at that point would be more useful?

DR. FRYKLUND: I don't know. Not in the bioassays that I've been involved in, which is the rat bioassay and some cell based assays. They're really not precise enough. There's too much variability unless you can -- I don't know -- perhaps if you could run two or 300 animals and run the same sample many, many times over, maybe you could do fancy statistics and show there was a difference. I don't know. We haven't really done -- we did that in the early days. We were trying to look for differences between various products and various degradative forms, and we weren't really able to show that.

With regard to IGF-1, which is the surrogate parameter for growth hormone, those levels are also a little bit imprecise because the normal range is sort of spread, and the assay comes out plus or minus ten percent. So if you're always on the

bottom, is it to the right or should be in the middle or where should you be?

So I think that they're very imprecise. Perhaps a really nice receptor assay would do it for you if you could really analyze it and you were really sure that the receptor was functioning as it does in vivo, which is another problem.

I mean, you have BioCore assays where you can measure a receptor, but it's actually soluble receptor. It's not sitting in the membrane. It's not really doing its signal transduction.

MS. BROWN: So you feel that in addition to physicochemical characterization you would need a clinically relevant bioassay?

DR. FRYKLUND: Yes, I think so. I mean, if you go back to enzymology in the old days, like 30 years ago when people really did enzymology properly and they worked out all of the enzymes and the constants and substrate inhibition and everything in enzyme pathways, if the same thing was done in signal transduction, which is all enzymes, it's all kinases; they're enzymes; maybe we could have a very tidy assay that would measure binding and activation of enzymes.

I don't know, but as far as I know, no one