cyclosporin in changing protein abundances in the kidney, which is the site of the specific toxicity that we wanted to investigate. The pathogenesis of this effect was unclear. But if we look at the protein pattern of kidneys of animals treated with cyclosporin, once again using these arrows, there is a protein here which is very strongly down-regulated. Its abundance decreases tremendously. And that protein turns out to be a protein called Calbindin 28kD, a calcium transport protein. And that can then be located using an antibody to the protein in a slide of kidney tissue and shown to occur in the cells of the tubular epithelium. In treated animals, the abundance of this protein is radically decreased, as expected from the results with proteomics. And we see these deposits of calcium salts in the tubules of the kidney, which is one of the signs of the pathology caused by this drug.

Now the real question was whether this is really related to the mechanism of action of the drug or is the side effect specific to cyclosporin A. This was investigated by Dr. Sandra Steiner, who

subsequently joined us from Novartis, and looked at with a specific assay for this protein. Not using the 2-D gels, but using that information to construct a specific assay. And the level of this protein in control kidneys is very strongly decreased by cyclosporin treatment and by treatment with the compound 506, which acts by a similar mechanism, but is not down-regulated by rapamycin or by an analogue of cyclosporin which is not immunosuppressant. Therefore, there is a very strong relationship between the effect on this protein and the action of a compound by the cyclosporin mechanism. And that leads one to be discouraged about the possibility of eliminating that toxicity while still retaining immunosuppression by the cyclosporin mechanism.

Lastly, let me show you an example of protein changes which involve covalent modifications of protein molecules caused by drug treatment. This occurs in a respective compound called methapyrilene, which used to be in Sominex and a wide variety of over-the-counter medications but was withdrawn in 1980, when it was determined to cause liver tumors in

100 percent of the rats to which it was administered. But once again, it is not overtly genotoxic.

However, this compound, when one looks at the protein patterns, causes very specific structural changes to proteins. I have highlighted here in red three specific proteins which each have three different isoforms. These are separated by a single charge unit difference in the structure of the protein molecule. And after treatment, there is a series of additional charged forms, which is indicative of modification of the protein by covalent addition of some group, which we infer to be a reactive metabolite of the drug itself. What is interesting is that all of the proteins that are modified in this way are mitochondrial proteins. In fact, mitochondrial matrix proteins. And that gives us a hint that in fact this drug is metabolized apparently to a reactive metabolite but inside mitochondria instead of in the endoplasmic reticulum, the microsomes, as is usually the case.

We went on to measure the level of this modification in a series of five systems -- the rat

and the mouse in vivo. And these numbers are the aggregate number of modifications per protein molecule in the mitochondria, negative because the shift is to the left. About one modification per molecule in the rat, much less in the mouse. And then looked at in vitro systems, hepatocyte systems, for the rat, the mouse and human. And the high prevalence of the modifications in the rat is correlated with the tumorigenicity there and the lack of tumorigenicity in these other systems. In the human system, epidemiological data indicates that there is no effect causing cancer in human liver. But the use of specific protein markers to relate together these in vivo and in vitro systems allows us to project utilization of these markers towards high throughput screening.

Let me conclude by giving you the bottom line from a different perspective. The use of proteomics to look for markers of drug mechanisms, both toxic and therapeutic, really works. I haven't shown you the examples of disease state markers, which we are pursuing mainly in human samples, but they also

work. We can find treatment effects having to do with both the therapeutic and toxic mechanisms quite readily. And in addition, by looking at many proteins, it turns out that we can both classify the mechanisms and disease states much more finely than is possible with single markers, but also detect them more sensitively because of the statistical power that is inherent in having a large number of markers which are measured instead of just one. And finally, the data bases of these drug markers and mechanisms are being built and will provide, we believe, a large number of potential markers for implementation through a range of different technologies going forward. Thank you.

CHAIRMAN DOULL: Thank you, Dr. Anderson. Does the Subcommittee have any questions? Why don't we go ahead and take our break. Why don't we try and hold it to ten minutes. Can we do that?

DR. CERNY: I want to remind the audience that we will be having a working lunch and that this may be a good time for you to secure some food in the meantime. And Dr. Farr, if he is present, if he could

identify himself to Nancy Chamberlain in the red coat. Thank you.

(Whereupon, at 10:45 a.m., off the record until 11:00 a.m.)

CHAIRMAN DOULL: Can we come to order? Okay, let's start up again. We are now going to hear about applications of SELDI to the protein biomarker discovery strategy.

DR. PETRICOIN: So it's always great talking after Leigh and Gordon with their beautiful data. I am envious. I am going to talk today a bit about what our group is doing at the FDA. We have a tissue proteomics initiative that is joint with the National Cancer Institute and specifically with Lance Liotta's laboratory and laboratory pathology. Most of our research revolves around the use of technology that was discovered or invented in Lance Liotta's laboratory looking specifically at disease progression biomarkers. But recently, we have made a foray with Frank Sistare in some work that we are doing at the Clinical Center looking at different technologies, not only for tissue biomarkers but for body fluid

biomarkers as well.

Cancer as a disease presents us with an opportunity on the research end to discover biomarkers early-on. As a disease, it presents us with a window really uniquely among diseases where for most solid tumors you have an opportunity to find markers at the earliest stages where premalignant lesions start occurring. This is especially true with prostate cancer, which is one of our most widely studied cancers in our initiative. Where for many years and perhaps decades, premalignant lesions will be apparent before you get full blown, frankly invasive cancer and metastasis.

And what we have been doing is studying or mimicking the five solid human tumors from the C-gap initiative -- breast, colon, ovary, lung and prostate -- and mimicking the technologies employed there using proteomic technologies in its place.

We have the luxury of using many different technologies concomitantly. So we are using 2-D gels, though nowhere near the high throughput nature that Large Scale and Oxford employ. We are also using SELDI

technology, which has been discussed earlier. So I am glad you have had a primer, at least, on that technology, since that is what I will be focusing on.

But we are also using array technologies -- antibody arrays, protein lysate arrays, algulents and calipers and a lab on chip technologies concomitantly. Going at it really with all guns to see if we can generate as much discovery as possible.

What we have been focusing on, however, is the input material. In our case, instead of cell lines or bulk tissue specimens, it has been solely using laser-capture microdissection as material for all of our proteomic analysis from tissue cells.

If one wants to study changes in cells involved in disease progression, you really need to think about it in the context of the cells in a three-dimensional tissue organ structure, especially with solid human tumors. In the past, if you wanted to study say the changes associated with epithelium surrounding a prostate or a breast gland, the entire section was lysed, homogenized and then studied through whatever biomolecular marker endpoints. What

we have decided to do is to use a technology whereby these cells of interest can be selectively procured directly from human tissue and studied in the absence of the rest of the surrounding tissue.

That is not to say that the surrounding tissue isn't important in the disease process. It is obvious that stromal cells and epithelial cells communicate with each other. But we would like to be able to study these events independently. And the LCM, the laser capture microdissecting tool, helps really to enhance the sensitivity of discovery. Because you are really enriching dramatically the ability to discover new biomarkers, either for therapeutic intervention or for imaging.

This technology is simple yet elegant in that using an inverted microscope, a laser enhances and energizes and ethylene vinyl acetate transfer film, which is put directly over a tissue section. It can be stained with any stain that you want from a histopathological standpoint. Once the laser hits this film, it actually activates it. It swells and it hits the cells directly below it and picks them up. This

is different than the Moment machine that the German group invented, and that is more of a laser oblation technique, where you get rid of all the cells of interest leaving the cells that you want behind. This technique is actually much more rapid, because you procure the cells that you want directly on the bottom of a cap that looks exactly like na Eppendorf tube cap. And you can then analyze it depending on the tools that you want to employ.

An example of this is shown here. This is normal prostate epithelium. The lumen is shown here. The diamond of the laser beam is shown here. This laser beam is from an old scope they have, and we have employed laser now with a single cell capture. We have a trade with Arcturus where we are actually working with organellic capture as well. So we are hopefully able to employ an even more specific capture.

This is the material that is transferred to the cap after dissection, leaving behind the stroma and gaining just the surrounding epithelium from this tissue specimen. You can now microdissect the entire disease range in these cancer tissues from normal to

premalignant to full tumor and employ whatever downstream strategies that you want to use.

As I said, we are working with a variety of technologies simultaneously. For today's discussion, I will show you data we generated from SELDI data and go and finish the talk with how we are employing SELDI specifically for use of body fluid biomarkers of toxicity.

As a generation of the kind of geometry we are working with with the SELDI chip, on the top is a tissue section of a stained human prostate specimen. I don't know if you can see it very well, but this is the laser capture microdissected cap with the microdissected tissue specimen showing as a stained hemotoxylneocin part here. The cells are lysed off the cap and applied to the SELDI chip.

As stated before in one of the previous talks, laser energy is applied to the specific region. And depending on the size of the protein and whether or not it is even able to be ionized, you generate a time of flight molecular weight profile that can now be analyzed.

As also was stated before, each of these circles that are shown in here contain a Bait surface that either is open-ended or you can apply an antibody of interest or even a purified membrane of interest or DNA molecule, or they come commercially available with different Bait capture surfaces such as copper, nickel, reverse phase C-18 type resins, that have been routinely employed in the past for protein purification.

I wanted to mention some things that I think specifically the SELDI has advantages over 2-D gels, and also speak to disadvantages of SELDI over 2-D gels. I think since we are employing these at the same time, we have a unique perspective about where and when these technologies should be employed, and I think they are very actually not competing but complimentary. Especially true for biomarker from body fluids. Most of the body fluid analysis that I will show you were the exact same ones that Gordon used on the 2-D gel. We employed the exact same set on SELDI. So the results can be compared. However, the sensitivity of SELDI may be greater depending on

the up front purification done in the 2-D gel. It is potentially theoretically in the atamolar range. It requires no secondary label, so there is no need to stain the gels with anything. It is extremely rapid. This is really part of a technology that I think holds great promise, as well as a small volume of sample needed. Reproducibly, we can get SELDI fingerprints in as little as five minutes from as little as a hundredth of a microliter of sample. And this is specifically, I think, prescient when you talk about getting samples from IRB, especially patient samples that might be hard to generate. One microliter of sample is enough to perform hundreds of experiments in our hands. And, therefore, reproducibility from a single amount of material that is extremely small is no problem.

I am going to show you a couple of anecdotes where we have used SELDI from laser capture microdissected material because of its need for small volume and its need for a small amount of input to generate a protein profile. It is especially important from the laser capture standpoint, where potentially

cells such as premalignant lesions, for example, which are in some instances a very small percentage of the entire cellular burden, can now be looked at at a proteomic level. Whereas before, it would be virtually impossible to run a 2-D gel and generate anything but maybe the top five most abundant proteins because the amount of cellular input is so dramatically or vanishingly small. We are able to now look at disease progression from patient matched material. From the same tissue slide of normal, in this case PIN, premalignant prostate intraepithelial neoplasia cells and frankly invasive carcinoma cells. After laser capture microdissection, SELDI analysis can be employed. I have to tell you that all of the spectrum that we see here, we performed the microdissections 50 times, and I am showing you a representation of one of those after we did extensive sensitivity and reproducibility. So each one of these was performed 50 separate microdissections to demonstrate to ourselves that the spectrum generated was reproducible.

And what we found in this anecdotal case

was two proteins whose abundances seemed to flip-flop as you went from normal to the premalignant to the tumor case, and we also dissected the stroma next to the tumor in this instance. And as stated before, you can show this as a mass chromatogram or as a gel view where each one of these protein peaks are representative as a density on a Gray scale.

We looked at a variety of tumors. We actually looked at 30 different patients altogether. This ratio held up in 28 of 30. I show you as an example down here ratio analysis that we have employed using these two markers. And in a blinded study set of 25 other prostate cancers, we had a 95 percent success rate at calling the pathological state of the cell without seeing actually an histopathology based strictly on its SELDI profile.

I have to say, though, that as much as Cyphergen has tried, and I know that they are employing a Q-tough tag-on instrument right now and they have added some reflectron in their MALDI, it is impossible at this point to really get ID on these proteins reasonably without doing a tremendous amount

of chemical acrobatics. However, I don't know if I can convince you or if I want to convince you, but we are at least exploring the paradigm of who cares about protein identity right now. If one can reproducibly generate a protein fingerprint that you can apply some type of heuristic pattern algorithm to, it is possible to use this without ID specifically in the application as a biomarker -- as a tool to detect is a change occurring or not. Not necessarily what that change is. If what you want to do is to identify a protein such that you develop an antibody to it so that you can eventually capture it on some type of downstream assay, simply to ask whether or not it is there or not, another thought process might be why go through the work if you have a capture tool already that is detecting it.

However, beyond not being able to identify the proteins on the SELDI really rigorously, this also -- and I think this was mentioned briefly by the previous speaker -- this is non-quantitative, as is all MALDI. I don't care how much data you can show me to support it, if you talk to mass spectroscopists,

MALDI type data is not quantitative, especially when you talk about ion signal suppression around molecules. We can make no statements about the relative intensity and correlate that to quantitation. And so there is a limit to diagnostically how much you want to push the technology. But I am going to show you some examples where I think if you use some other technologies as well in conjunction with this, that may not be necessary.

I also want to just show anecdotally as well -- again, these are representations of many, many, many machinations and microdissections for all these different tumor types. These are all microdissected tumor epithelium. These are what we consider to be the boiler plate pattern of what these tumors represent reproducibly. We were able to have about a 75 percent success rate in calling a blinded study set using a variety of these different tumor types without knowing the pathology based on pattern recognition alignments with the SELDI profile.

This is the first piece of data that I wanted to start out the body fluid analysis on. What

we have done is we have had a large study set of 350 serums from Chile with men with prostate cancer whose PSA was greater than 10. So these were men with frankly metastatic prostate cancer. Out of that 300, I think there was about 125. The rest were normal male serums. We analyzed all those serums by SELDI. And using a tag-along heuristic fuzzy feature map, heuristic data mining analysis with a company called American Heuristics, who take the raw SELDI data directly as an ASCII file right from the machine and analyze it without any preconceived filtering. We were able to cluster much in the way that I guess Leigh was showing you the clustering of a lot of their fold expression data. A lot of that kind of data can be used for SELDI. And in this way, we actually found regions in the mass map that 95 percent of all the serums that were prostate cancer fell into this pink as the disease group. The healthy group had a pattern cluster that was unique to itself. We knew here that PSA was 100 percent discriminatory. So in this case, we missed 5 percent where PSA was over 10. But I think by continued refinement, we can get to a point

where you can run SELDI data without having to do any type of downstream analysis and let these self-learning algorithms tweeze apart the data rapidly.

Now I will show you a bit about how we did that. We initially performed experiments where we showed reproducible serum protein mass profiles. This is a representation of one of these. This is from a cancer patient. You go through a peak identification program that selects, depending on the signal to noise ratio of these peaks, which peaks are present. And then we perform ratio analysis of heuristically mined protein peaks that are occurring reproducibly in all the data sets. We were able to -- in this small example, we found four different ratios of proteins that seemed to vary independently of each other with disease. And by a combination of these ratios, we were able to call 9 out of the 350, 100 percent of these cases just as a representation. The predicted was here and the unblinded set -- we predicted them to be this. The cancer and prostate were normal and these in fact were the knowns. So we were quite happy with that.

This is an example of one of the other regions of heuristic analysis from the serum where we found where the other 5 percent that we didn't find in the first round fall into. So 95 percent of the cancers fell into this type of pattern. The other 5 percent fell into this type of pattern. And all 100 percent of all the normals were called and they didn't vary in this specific mass range.

So this heuristic data algorithm actually goes through the entire mass range every 5 daltons, even though the mass accuracy of the machine is nowhere near that. It doesn't matter. It goes for reproducible pattern recognition data over hundreds of samples and therefore curates out the noise by its self-learning tool. And eventually what you get down to is when you really distill all the data down to after hundreds of samples, you really find which regions are really varying, even down to this type of mass range. This is probably one protein.

So we also at the same time with Frank started to look at the samples from the rat serums that Gordon showed you, the 2-D gel data results on.

I will just show you a little bit of that data now. This was the result of all the controls. This is like a master boiler plate of a SELDI area that is just one snapshot region between 8,000 and 10,000 daltons. These are the proteins that were reproducibly detected. These are the proteins that were reproducibly detected with the SmithKline compound for vasculitis. And by doing pattern subtraction, you can find out which of these proteins are in 100 percent of the time SmithKline compound specific versus untreated.

This is the serum analysis of the doxorubicin-induced proteins. This is what you get reproducibly in this small mass range. At 24 hours, reversing with the phosphodiesterase inhibitor. You can ask -- you know, plus a combination of these two therapies, which of these go away. As Gordon said, many do and some don't. We looked specifically at the low mass range, that is below 8,000 daltons. Where SELDI in our hands and most MALDIs have its highest sensitivity obviously at the low mass range. As you increase the mass of the window, your sensitivity

dramatically diminishes. For example, you would be really unable to analyze IRB-2 expression in microdissected cells. It is impossible to see a protein of that size from tissue lysates without microdissecting, for example, millions of cells because of the size of the protein.

However, in this low mass range, especially from body fluids, we have more than enough. You can then do subtraction and show ICRF modulated dox-induced proteins or ICRF unmodulated dox-induced proteins. And so we at the same time, I think -- you do get similar types of data where you can find entities which are induced by the treatment and which disappear when you put a reversible inhibitor in. We have actually gone back now -- we just completed the data analysis where we looked at Frank's entire set and we can find, for example, in the vasculitis control or samples, detection of entities that occur very early and continue throughout the spectrum of samples as well.

And we end up with kind of what I am hoping for the future for the use of SELDI

specifically for these types of applications. I have on here tissue base, but this could be applied for body fluids as well. Where you can eventually potentially think about developing bar codes of normal protein expression, maybe pre-disease and diseased proteins expression. We are working actively with NASA on a microbioreactor, where we are actually treating ex-vivo tissue from surgery of the prostate with different compounds -- finasteride and even herseptin, and looking for changes in protein expression from microdissected tissue after treatment. You can query the conditioned media from this as well. But what you hope to find is treatments where there is reduced toxicity basically. Where you have efficacy with reduced toxicity. Much in the same way that we Leigh elegantly and his company is pointing out. There are ways that proteomics can do this now. And these technologies, although disparate in their means and non-redundant, are very complimentary to each other and I think warrant further discussion.

I just want to point out some of the people that really were behind the tissue end of this.

This is an old slide, so I don't have any of the up-front -- the biomarker from the body fluids on here. But Lance Liotta and Mike Buck's group and Bob Bonner have contributed mightily to this effort. Thank you.

CHAIRMAN DOULL: Thank you, Dr. Petricoin. Let's move on then to Dr. Farr.

DR. FARR: Well, thanks very much to Frank Sistare mostly and Jim MacGregor for inviting me to talk a little bit about some of the things that we are doing in the wild and rapidly evolving world of toxicogenomics. So I am going to give a little bit of background of what toxicogenomics is about and why there is some need to address some of these issues.

And if I speak loudly, can you hear me? I can't hold still and speak. We don't have a microphone? Well, we do have a microphone. This will be highly constrained. I don't know if I can talk with my arms tied behind my back. Anyway, the major goals of toxicogenomics are to use gene expression analysis to predict individual human hypersensitivity drugs. And I don't mean hypersensitivity in the traditional immunotox. We want to be able to predict individual

responses to drugs. Obviously, we think that we can use some of the tools of toxicogenomics to design better clinical trials, and I will give you some historical and monetary reason for why we want to do that. And then to understand how specific disease states or one medication can affect the toxicity of an additional medication in the same individual.

So here are just some examples of compounds that were either completely withdrawn or restricted, either by regulatory restriction or self-imposed restriction from the market over the last several years. And actually this number is a vast underestimate. If you add in the lost direct cost of developing these drugs, certainly the lost opportunity costs, and then the market cap cost to the companies when one of their favorite drugs fail, this is probably off by two. We are talking easily a $15 billion cost when compounds get on the market and fail. And these compounds failed because of toxicity that was not discovered earlier on and toxicity generally in a small subpopulation of the patients who were taking them.

So here are some other sort of historical data. We looked at -- there are a little more than 600 compounds on the market in the U.S. today. And at least 500 of those have serious side effects in some individuals. In some cases, there are very rare idiosyncratic responses, but they are serious. And to the person that has that response, it is a serious response. It may only be 1 in 10,000. In some cases, it is 15 percent of the patient population. Approximately 400 of these compounds have known interactions with other drugs. And then at least 40 that we know of today show toxicity in very sizeable subpopulations, that is, greater than 1 percent of the patient population taking that drug experiences some meaningful side effect.

So here are some examples of compounds that cause serious side effects in subpopulations. A number of compounds -- I won't name companies -- on the market or until very recently on the market that are known in a subpopulation to cause liver toxicity. Here are some compounds that caused -- and these are broad categories, all different types of blood

toxicities, anemias and neutropenias and agranulocytosis, et cetera. So some of your favorite compounds here. And then I throw in the following, which may not be life threatening, but I would submit that it is not insignificant to those patients that suffer from this. I would call this sexual tox, if you will. A lot of the antidepressant -- the tricyclic antidepressants cause impotence. Well, if you are depressed and you become impotent, I don't think that helps your depression. But a lot of other different types of sexual dysfunctions. It is not an insignificant subpopulation that take those antidepressants that develop sexual tox. I think people would like to know. If you are going to suggest that I take Celerex, am I going to be one of the one percent that will become impotent as a function of that treatment? There are, of course, a number of other types of toxicological endpoints that are induced by compounds that are on the market in a subpopulation of people taking those. I just show these as some examples that are near and dear to our hearts, our livers in this case, or other things I

can't talk about.

Those are compounds that are on the market. They were approved. They are either on the market -- well, most of them are on the market. And this is harder to get at because the Freedom of Information doesn't allow us to get at compounds that have failed. But through lots and lots of discussions with a lot of different sources, none of which are in this room, these are the numbers of compounds that we can find that failed in the U.S. in late stage clinical trials in Stage 2 or Stage 3 clinical trials. So between 1994 and 1998, 119 positive results and about 85 negative or mixed results. These didn't get to the market. And about a third of these didn't get to the market because of some toxicity that was detected in Phase II and Phase III clinical trials that obviously wasn't indicated in earlier settings. There is an interesting paper coming out from ILSI on that.

So one of the most important problems for regulators and toxicologists in general is every discussion this morning had some terrific technology

and has presumed for the sake of time -- I know people are much more sophisticated than this -- but we presumed an average human being, of which there is no such thing. And so one of the most important problems or vexing problems for the industry is that we are not an inbred species, except for my relatives -- but most of them are not. I grew up in a very small town in the mountains of Arizona. You didn't even know there were such things. Anyway. And that the individual responses to toxic damage very dramatically as a function of an individual's genetic make-up, which is expressed in a meaningful sense either through -- clearly, the first stage is transcription. The second stage is protein expression. And then I know that everybody knows this, but of course there is modifications to proteins after that. It isn't just is the protein there. Is it meristalated or is it phosphorylated -- one of six sites for C-June, et cetera.

So we think that we can begin to deconvolute an individuals -- not an average human being -- but an individual's hypersensitivity. Again,

I am not speaking from an immunotox perspective. Hypersensitivity to a specific toxic stimuli by looking at patterns of gene expression that are relevant to that stimuli.

So what are the requirements for doing this? What we would like to be able to do, of course, is by patterns of gene expression identify when an individual, if they took Trovan, for example, might have to go in and have their liver replaced versus 95 percent of the people who took Trovan would have no problem at all. It is a great drug for those 95 percent of the people. The other 5 percent, it is a really awful drug. If we could identify those individuals in some relatively rapid way before they took the drug, that drug could stay on the market and serve people well that would not suffer from the adverse consequences. But the trick is, of course, how do we go about this in any meaningful way. And I am going to tell you where we are making some attempts in conjunction with several others to do this.

So what do we need to do this? Obviously, we need clinical samples that we can obtain easily.

Most people aren't willing to give small snippets of liver or kidney to figure this out. People are willing to give a couple of mls of blood. We also need to start with drugs that elicit toxicities in the blood. Not that that is from a pharmacological perspective the most important tox endpoint, but it is a good place to start, because we have access to those types of cells.

So where do we want to start? We want to start with drugs, of course, that elicit some kind of blood toxicity. And these are a list of drugs that elicit blood toxicity of one nature or another in large and very sizeable subpopulations. I believe it is methimazole that elicits I think it is agranulocytosis in about 15 percent of the people who take that drug. But all of these elicit some type of blood toxicity in a sizeable subpopulation. So you have to have that. You can't find patterns if there is only one in 10,000 individuals who respond poorly. You've got to start with samples you can get a hold of. You've got to start with drugs that cause toxicity in a sizeable subpopulation and with drugs

that are taken by a sizeable population to begin with.

The next step, though, is you've got to look at all genes that vary. And now I am talking about transcription at this level. I want to look at all genes that are differentially expressed between individuals or samples, if you will. It has to be an open-ended system. We don't know yet which genes to look at that are going to tell us whether an individual is going to develop neutropenia when they take Ganciclovir. So we have to start off by looking at all genes, an open-ended system. Not 2,000 proteins. Not 7,000 genes. But all genes that are differentially expressed. We obviously need biological samples from normal and hypersensitive individuals. We need treated and untreated cells from these same individuals, so we can push the treatments up to toxic concentrations, which we can't ethically do in humans, at least not prospectively. And then we obviously need samples from normal responders when treated with a compound. So those are some of the types of samples we start with. And the open system that we are using, and there are a number of open

systems -- when I say open systems, they measure all genes. We are using AFLP. And so when we derive these samples from treated leukocytes or from treated individuals, normal and insensitive or hypersensitive responders, then we use a technology called AFLP, and it allows us to quantitatively identify all genes or all transcripts that differ between any two samples. Now obviously if you look at two individuals, a lot of genes are going to vary simply as a function of height, sex, brown eyes versus blue eyes, et cetera. So you have to go through the three sets of samples I described a moment ago to pick out candidate sets of genes. And for the sake of time, I won't go through every single step. But we want to get to clearly all candidate genes that are most likely to represent genes associated with that hypersensitivity to the drug.

Let me just move ahead. So if we look at in vitro human cells or various types of white blood cells treated and untreated, we are going to get a candidate set of genes that vary. If we look at individuals treated and untreated, we are going to get

another set of candidate genes that vary as a function of treatment. It might be the efficacy treatment. And if we look at normal versus hypersensitive individuals, we are going to get another set of candidate genes. What we are looking for are the genes that are consistent across all of these types of treatments. The goal is not to have to do something like AFLP on every single individual. It is very expensive and takes a lot of sample. So what we need to do as quickly as possible is to get to candidate sets of genes that vary and that are causally related to that hypersensitivity.

We also then need a method for specifically measuring these candidate sets of genes. And so what we are doing then is we take these candidates and we synthesize arrays. Now again, arrays are not the ultimate answer because of the amount of RNA required. They are still laborious and there is a fair amount of art. Anybody who thinks they are going to run out to Walmart and buy an array synthesizer and scanner, all they need is then a couple of million dollars and three years to get it working, and they

will be up and running. They will be in good shape. Anyway, you want to synthesize arrays with all of the candidate genes that vary as potential indicators of that hypersensitivity. You want to do this now with several individuals who were normal and hypersensitive responders to the drug of interest with your candidate sets of genes. And the idea then is with the candidate sets of genes to find the subset that co-vary with the hypersensitivity status.

Now how do you make this clinically useful then. And I won't go into the details of this because Dr. Frederico Goodsaid, who will follow me, will talk about the tools. But what you need then is a way of looking at a very small sample set. Something where you can use .5 nanograms of RNA or thousands of cells. So we are codeveloping some tox cards. These are 96 wells, and each one is a one microliter RTPCR reaction center. And we can then put the candidate genes into each one of these wells. And again, Dr. Goodsaid will describe how we load this up and the actual techniques of doing this. But this is the kind of technology then that is very useful in the clinic. This is something

that with all due respect to Dr. Doull, even most M.D.'s could run.

That is the idea. We might be able to come up with these great theoretical ways of doing it. But if we can't get it into the clinic in a robust fashion with the appropriate informatics, then it is not going to do anybody any good.

So where are we at? So we have the open-ended system. We have the arrays and we have the cards. And let me show you where we are at actually with these cards. So the idea then is to develop these tox cards with the genes that are indicative of what one's hypersensitivity status will be around each drug, or it could be classes of drugs. So before one prescribes Ganciclovir or Celebrex or what have you, one would use one of these cards. It is really quite easy to then determine what is your status going to be.

So what is the current status? And again, Dr. Goodsaid will go over this in more detail. We have -- it is very easy to design these -- well, very easy in a $24 billion company like PE -- very easy for

them. We design the genes from all the upstream work, and we get them into the cards. It is very easy for them to get them into the cards. So it is easy for us to say, well why don't you put in this card. We need this gene, that gene and the other gene. And here is just some genes that we decided to look at and we did some in vitro experiments. And this is just showing duplicate experiments with 1X or 10X amount of RNA. The 1X was, as I say, .5 nanograms of RNA, very quantitative. But I will leave those sorts of discussions to Dr. Goodsaid.

We are now able to -- and this is based upon a number of cytokines -- we are now able to look at -- so the previous example was from human in vitro cells. This example is from blood samples -- unprocessed blood samples, other than we spin them down and take off the buffy coat. And this is looking at all the leukocytes. So we are now able to take a very small amount of blood, a couple of mls, and actually look at gene expression. And here are a number of individuals. Here is an individual, by the way, who has asthma -- very severe asthma. And those

of you who are asthmatologists, IL-4 I am told is a very good indicator of asthma sensitivity. So this individual is off the chart, about 400-fold higher IL-4 levels than anybody else. And we can now -- so the third point is you've got to be able to take white blood cells and grow them up and then treat them with the compound that interests you. So now we are doing that, although this is not a very interesting compound. We treated cells with PHA in this case and we are looking at treated and untreated cells with PHA. So we isolate a few cells, grow them up, treat them and measure the gene expression of the genes that interest us. And again, we derive these genes from the upstream process I described before.

So that is where we are at today. And we are now ready to start taking clinical samples. So if anybody has drugs that affect a subpopulation, we need clinical samples. And I am very appreciative that Jim MacGregor and Frank Sistare think that it is worthwhile, at least knowing what Phase I is doing in this regard. I don't want to talk about Phase I. I am talking about where toxicogenomics is going.

Because it is going there and we would like to work with you versus in complete ignorance of your interests and concerns. So thank you very much.

CHAIRMAN DOULL: Thank you. I don't really have any problem with running that chip thing. The problem I have is saying that all those toxicity that were associated with those drugs are in fact due to gene --

DR. FARR: Are due to what? Differential gene expression?

CHAIRMAN DOULL: Yes, your gene thing.

DR. FARR: Well, I think you could turn the question around and you could ask are there any differential states that a cell can entertain without having differential gene expression. And I think other than necrosis, probably not. I don't know of any meaningful toxicity that is not only accompanied by but predicated on differential gene expression.

CHAIRMAN DOULL: I guess that is the issue. I am not sure I am convinced of that yet. But I will think about it. Let's go ahead and hear from Dr. Goodsaid.

DR. GOODSAID: Good morning. My name is Frederico Goodsaid, and I am a senior staff scientist at the PCR R&D Business Unit of PE Biosystems. And my talk today is very closely linked to what Dr. Farr has just described. I am only going to go into the nuts and bolts of what you can do with the Taq Man cards, in particular the Taq Man cytokine and tox cards.

So I am going to talk a little bit about the test procedure, the specifications and the validation results that you can get. I will make a point of trying to see how this methodology is unique in the accuracy and the precision of the information that is obtainable in terms of gene expression.

So the test procedure, as Dr. Farr described, requires RNA isolation, cDNA generation -- at least our initial validation of the method is being done with cDNA. In fact, in the long run, we should be able to go with one step RT-PCR. But it is conceivable that we could just go from RNA isolation straight to RT-PCR, card loading and sealing, thermal cycling and data analysis. the overall time required for steps 3, 4 and 5 is one hour and 40 minutes. And

we expect that that will be reduced to about half an hour over the next year or so.

I am going to show some video that describes how the card actually works -- the hands-on requirements for the card. I have a couple of samples that are here. One is open. It shows the card with the 96 wells. And that card is loaded by having it evacuated and having the sample of cDNA go straight to all 96 wells in that card.

The first card that we launched back on February 15 is the human cytokine card. And it has the polycarbonate body here as well as the fill consumable for filling the card. It is run on the 7700 instrument. Let me go back here a second. The 7700 instrument, which has been around now for about three years. And the procedure by which this is done is shown over the next few seconds. You are going to see that we have in the case of the cytokine card 24 cytokine assays that have been loaded in quadruplicate. So we have four replicates. And each well, which is a one microliter well, contains the cytokine target as well as the 18S endogenous control

primers and probes. So you get results for quantitative Taq Man PCR in each well normalized to the endogenous control. That is how you fill the card. You evacuate the card and the sample flows into the card very quickly. The Taq Man reaction is generated in the card, and at the end of that reaction, an hour and forty minutes or so, the results are analyzed on the 7700 software and the relative quantification of gene expression is obtained from that.

The way in which the card is actually used is shown in somewhat more detail here. First of all, the card represents a reduction of something like 50-fold in the amount of sample that is needed relative to 96 well plates, which is the technology we have today. And the way that you load the card is really simple. Because there is no need for any accurate pipetting. You just have an excess of over 250 microliters of total reaction mixture, and that is all you need. While on a plate you have a very rather cumbersome usage.

The way in which you actually run the card on the 7700 is by having it going to an optical

fixture, such as is shown here. And on the 7700 instrument, you have the software required for the analysis. This will be a very brief show of how the actual analysis works. You load your card with your sample, which has been sealed. You turn on the instrument to do thermal cycling. It is 35 cycles that take about an hour and 40 minutes. And you start the run. In about an hour and 40 minutes, you get your data back, which has not just endpoints, which is essentially what you get from hybridization results. But the more important piece of data is the quantitative PCR result. And this is what we use for our gene expression measurements. What we determine is the CT, which is the value of each of these curves of the quantitative PCR curves that crosses that threshold -- the intersection between each of the quantitative PCR curves and the threshold. Both for the individual targets that you saw before as well as for the endogenous control that you are seeing here. That data is then exported to the Relative Quantification Software, and that software gives you the relative quantification of gene expression

results. So you have your untreated plate here, for example, and then all the treated ones here. And you calculate that and it gives you the gene expression results.

So that is how this works. That is where we are at in terms of this technology today. And I am going to go on now showing a little bit of the information about what kind of data we are actually being able to get from here. Both from what Dr. Farr showed as well as from the slides that I have just shown you here, you look at those semi-log plots for gene expression and you see numbers of hundreds or thousands or ten thousands. When you look at chip data or when you look at any kind of hybridization result, gene expression numbers usually die off by about 100-fold. That is considered extremely high. In any kind of endpoint method, you will have that kind of a constraint.

So one of the first messages that we have here is that we have a dynamic range with the Taq Man card which is far wider than anything that you can get with the hybridization method. And the result of that

is that we are measuring gene expression levels that are over a range that just hasn't been reported before. The dynamic range that we are seeing here is on the order of 1, 2, 3, 4, 5, 6 logs -- 5 logs at least. You can -- this is the absolute range here, but then you can get a relative value obtained with this range that essentially doubles the dynamic range that you actually report. And this is just showing for IL-6 on the cytokine card the kind of validation you can do for the expected versus actual CT values. That is the actual quantification that you measure matches very well the expected value over a five log range.

In terms of fold discrimination, I talked about a 5 log range. And as far as that 5 log range, how can you be sure that two results are different from each other? Well, we have done this in a couple of different ways. In this graph, I think you can see a pretty nice show of what we are talking about. For six standard deviations for a 99.6 percent confidence level, we would say that two results are different if they are four-fold apart from each other. This is,

again, operating at that confidence level and trying to really look at what a true -- the true dynamic range of the gene expression results is.

This is work that was done at DNAx, by Rene Diwall Malifeat. This is the cytokine gene expression levels in peripheral blood monocytes. And in this case, we are looking at different kinds of effectors. And you can see the kinds of numbers that are obtained here. In doing quantitative PCR and in getting results for the rates rather than for the endpoints, what we are getting here is induction rates of 1,000 fold, 10,000 fold and beyond. In the case of PBMC activation, you can see some targets that are really way, way up in the induction rates you get.

At Source Precision Medicine, they have also been looking at other kinds of inductors that they are not telling us about. But the numbers are what we found really interesting for the gene expression levels. Again, you can go up to about 10,000 fold and beyond that.

In liver cell cultures, not surprisingly cytokines are not exactly bound to be very much

induced. And just as a test, we looked at Phase I molecular toxicology at the effect of actinomycin here. And all the levels for all the targets here in the case of the cytokines were under 10-fold for induction.

This was actually shown by Spencer before, and we can see here some targets that are induced and some targets that are actually suppressed in the case of mitomycin C.

And methane methylsulfanate produces this pattern in the case of liver cells. Again, some targets for the toxicology card now. This is not the cytokine card, but the toxicology card. Some targets go up and some targets are suppressed.

Also, the other part that I wanted to show is that we can see potentiation. We can see dose response in these studies -- a pretty nice dose response for several of these targets. In this case, we have carnustine, which only affected significantly two targets.

This is what Spencer already went into. I won't cover. The work that we have started to do now

is to try to do the obvious. I mean, these cards are very easy to use. These cards give you accurate and precise measurements of what the actual gene expression levels are. So the next step would be let's see what we can do by studying toxicity in blood, and that is the work that we are currently developing with Dr. Farr and Phase I toxicology. Thank you very much.

CHAIRMAN DOULL: Does the Subcommittee have any questions about either of these? Thank you, Dr. Goodsaid.

DR. GOODSAID: Thank you.

CHAIRMAN DOULL: Okay. At this point, we have heard the discussion of the proteomic biomarkers, and Frank is going to help us focus in on some possibilities for expert groups. Frank?

DR. SISTARE: Well, with this morning's speakers, I guess the point we were trying to nail home is that with the technology platforms that have been described here this morning -- and this is, I think, a pretty good picture of not everything that is out there, but the power -- the examples of the power of what is out there and how the timing, I think, is

right to put together an initiative like what we are proposing here.

What I hope we succeeded getting at is showing what can be done and what is being done in this area. So like I said this morning, the key of what we are proposing to focus in on is biomarkers that are accessible -- relatively easily accessible and that are measurable. Hypothesis of any working groups that would be established to promote and proceed in this area would be that optimized panels of toxicity biomarkers do exist, and they exist in accessible body fluids, whether it be plasma, urine or circulating leukocytes, that we are not presently routinely measuring.

A little bit of locker humor took place during the break. We were a bunch of guys in the rest room commenting that a lot of our biomarkers were being sent to the local sanitary commission during the break. And in fact the comment was made that maybe we were sending a lot of Nobel Prizes down the drain. And I have heard other colleagues say that. At the end of the day when an experiment is done, you look at what

you can measure and then everything else just kind of goes down the drain. And there is a lot of hidden secrets in what you dispose of.

What we are proposing here is that there are ways to get at those hidden secrets, and that there are panels of biomarkers which are measurable and can reliably herald the onset of drug-induced, system specific damage prior to the visible morbidity or prior to any significant, irreversible, insidious damage.

So our objective again in the formation of any working group to define -- to proceed in any particular area that we may prioritize is to define those biomarkers with improved ability to profile a prioritized set of system specific damage endpoints covering a variety of mechanisms and drug classes. As Malcolm York pointed out in his presentation with his troponin measurement and some of his other measurements, he spoke to issues of sensitivity and specificity. And he is really eluding to the ability of that particular assay system to specifically measure sensitively and specifically that particular

endpoint. But in order to get a comfort level up with the biomarkers that we may propose that would be useful, there are a whole lot of drugs and toxicities and physiological manipulations which will have to be introduced, I think, before we can really get comfortable to accept a lot of these endpoints into routine use. And that is really the task that we have once we discover.

So at the last meeting in December, I proposed several priority areas from our vantage point. And I was tasked to go back to our regulatory colleagues, our review colleagues. We have a subcommittee in pharmacology and toxicology that is focused on research initiatives. And I tasked this committee with helping to prioritize and bring some feedback back to this committee in terms of what they viewed as some priority areas. This is one perspective that I am going to share with you. You have heard other perspectives.

There is the ILSI perspective where a poll was taken. In terms of the application of genomics and risk assessment, ILSI came back with we need to

better predict hepatotoxicity, nephrotoxicity and genotoxicity better. So that was that perspective in terms of priorities and that is where that group is going. So ask a set of people and you will get a set of answers.

I am going to bring back to you what I was charged to do, and that is the committee came up with what I would say are three tiers of priorities. And there is a lot of reasons for these priorities. Some scientific, some regulatory, some political for lack of a better term.

In tier 1, the message that was sent back was, yes indeed, we would like to see this resolution of the usage of troponin as a good biomarker for drug-induced cardiotoxicity. We get into situations where we propose the use to sponsors and the sponsors are uncomfortable in many instances using it and introducing it at certain stages of drug development. They need to know is it specific for cardiac-specific drug-induced damage. Do you see it when you get renal dialysis patients? They get troponin increases and some suggested that that may be an issue. What

happens when you get a decrease in blood pressure or a person faints? Are you going to get troponin? These are all things that have to be resolved. And to be fair, these are legitimate issues that need to be.

They also propose, and this is really not something that we have even touched upon this morning, that a consensus approach to this resolution of prolongation -- drug-induced prolongation of the QTC interval or drug-induced torsades is something that maybe, if we set up an expert working group that is versatile enough in cardiotoxicity, another group could be established to sort of prioritize an approach to resolving this particular issue, which is a regulatory nightmare essentially right now. I would like the opportunity to edit and change that term when the minutes are actually finalized.

Drug-induced vasculitis. The need for biomarkers was endorsed resoundingly from this committee there. Again, we get ourselves into situations with sponsors where we are trying to unravel the significance or the relevance of tox findings in animals with this very insidious but

potentially very deadly toxicity.

Also, in the discussion with vasculitis, the committee encouraged growth into biomarkers of what I would call immune system activation. In a sense -- and this is really why I asked Spencer and Frederico to come and talk about what can be done with peripheral blood leukocytes. Can we use those as sentinels of deeper bed tissue injury? Can they report to us that something else is going on where histopathology may be our only way right now to get at. So the feeling is that as we strategize to get at issues of drug-induced vasculitis, can we take advantage of these technologies to tell us that something else is "wrong in Denmark"?

Tier 2 -- there was still a strong feeling that neurotoxicity keeps coming up time and time again. And the question was raised, and it is really nothing I don't think anyone has addressed -- is can peripheral neurotox damage be picked up by some sort of plasma markers? Can we pick up central neurotox damage by looking at some of these proteomic techniques to look at cerebral spinal fluid, and maybe

some of our colleagues in the proteomics tech field could help address this. But it is really -- I don't know. I don't know the answer to this. But they felt impelled to get this on the table. And this also could be coordinated very carefully with non-invasive imaging initiatives that may also come in terms of this afternoon's discussion.

Hepatotoxicity, again mentioned. The concern there was that there is an upcoming FDA/PhRMA conference to address hepatotoxicity in a very sort of high level way. And that it might be politically prudent to sort of await the completion of that particular conference. But this certainly is something of great interest. And also the message being that there are a lot of other initiatives going on in the field of hepatotoxicity. The ILSI initiative, for example. And this is something I think the committee can also endorse. That there be a very careful coordination of all these efforts. A lot of mileage can be gained if we very carefully coordinate all these ongoing initiatives at the same time.

And the third tier -- I mentioned to you

before biomarkers using accessible tissues. We don't usually think of skin as an accessible tissue, but clearly small biopsies can be taken. The question being can we use early changes that occur in skin as a predictor of carcinogenicity.

The thinking there is -- this is a different technology than anything else we are talking about right now. And we are trying to extrapolate a very complex biology. Early changes that occur. I kind of made this point in my December meeting. Changes that occur on day 1 trying to predict what is going to happen 6, 7 or 12 months or 2 years down the road in your best animal models. And there is efforts -- there are efforts underway to look at and develop alternate models for this very complex biology using tumor endpoints. So maybe biomarkers may not be the best thing to do at this particular point in time. Although we are continuing our research in skin biomarkers, and there are several centers within the FDA that are working together with us on that and invite collaboration in that mode. But maybe it might not be ready for a consortium type event at this

point.

In terms of renal toxicity, again something they would like to see more progress on. But again, the coordination with ongoing ILSI initiative in this area is a message again to send. I know several other members in the audience together with myself are part of the breakout group in the ILSI initiative to look at genomics for assessing risk. And the renal tox group has devised a very elegant procedure strategy to look at the power of the microarray or gene chip and gene expression changes. And we have argued successfully to collect body fluids as a part of that effort that could be analyzed using some of these other technologies. And that is kind of the thing we are saying here. That we don't have to all work individually and reproduce and replicate and waste valuable animals. We can really work together in a concerted effort to get a lot of multi-parametric endpoints out of a single study without compromising study design.

Also mention is made that we are all aware of another collaboration going on through the Imperial

College in England where other body fluids are being assessed using NMR. And again, that is not a technology we have even discussed this morning. But there are these other technologies, and our hope is that with the formation of expert working groups in particular areas that we decide, all of those body fluids could be gathered and collaborating and leveraging with these other groups operating simultaneously, we can get a whole lot of data and these other endpoints as well.

So what is the proposal then to this subcommittee? We are proposing that you endorse the formation of expert working groups charged with the task of deriving specific implementation strategies. That you advise on the make-up of those expert working groups, what the membership should be, the experts that you guys and ladies are aware of that should be part and parcel of these initiatives. And help facilitate the formation of these expert working groups.

And take a look at what I have proposed in terms of the priority areas that we are getting back

from a PHARM tox regulatory initiative. And either endorse, for example, the tier 1 approach that we have proposed, or if you can bring members from the audience or if there are other perspectives from experts on the table -- why something on tier 2 should be moved up to tier 1 and why something on tier 1 should be moved back to tier 2. That is the last challenge I guess I make to the group.

I made a few notes, and I always have trouble reading my notes because I don't have bifocals because I don't need them because I am not old yet. But I will struggle as I try to read what I have written here.

The technology that Dr. Petricoin introduced in terms of being able to use microdissection and to be able to deal with very, very, very small sample sizes. And Dr. York described as well as Dr. Petricoin the use of SELDI to get at those very small sample sizes where you need like a one microliter extract of protein. If you let your imagination fly and you think, okay -- you think about bridging some of the stuff that Spencer and Frederico

talked about where you can use peripheral blood leukocytes. Well, rather than a microdissection, what if you use a cell sorter and you spin out certain subpopulations of cells. You don't need a lot. If you are going to look at message expression, by the same token you could look at protein expression and subfractions. The design, I think, is -- the key is to ask what is the best question? There is all these technologies and we can coordinate and we can look at a lot of endpoints all at the same time. The key is to get the right question.

Once you have decided on the right question, you need the right tools. Some of the best tools reside in the Pharmaceutical houses, where they have some of the best drugs that can induce some of the most blatant toxicities that haven't been developed because of their toxicities. We have to do a lot of this in our animal models to start out with. As Spencer pointed out, there are a lot of drugs which are on the market that are inducing toxicities. But this is a filtered set of drugs. These are drugs that have passed and gotten through the agency, so

they are not so toxic, as toxic or as many problems as there may be with them. As he said, it is a low percentage of patients. So they may not be the best things to start out with to study. I think we need some really good paradigms. The SKF compound is an example. Here is a drug that causes a very profound and reproducible vasculitis that we can do some really good toxicology with. That is only one. We are struggling to find a second or a third to sort of validate sensitivity and specificity. We need other drugs that are brought to the table. So I think we really need a partnership to really make this whole thing work.

There is a whole lot of other thoughts here, but I think I will leave on that note. So I invite good lively discussion and a clear charge from the Subcommittee on how to move forward here.

CHAIRMAN DOULL: Thank you, Frank. Well, I think you all heard those marching orders. So the task for the committee then is to begin to zero in on what we heard this meeting and the last meeting in terms of biomarkers that could be useful.

I think Frank made an important point there. He said you have to distinguish between the things you can measure. We have heard about some very elegant things that you can measure that are really gee whiz in a sense. Very impressive, but are those the kind of things we want to really focus on and push ahead on, or do we want to look for things that are more developed and have more probability of in fact being useful to do either toxicity analysis or efficacy or whatever. So we need to discuss then.

Let me mention one other thing. When Greg opened up the discussion, he defined biomarkers very broadly. As a matter of fact, you could include imaging in what -- in the NIH definition of biomarkers. But we have decided, I think, in the last meeting that for our purposes, it is useful at least to separate biomarkers from imaging. Because the techniques are somewhat different. So we will focus on biomarkers, the things we have been talking about. And hopefully this afternoon we can do the imaging in the same sort of fashion.

So what are your thoughts about

biomarkers? Joy?

DR. CAVAGNARO: I think what was also pointed out was to focus on what is the question. Okay? So I guess that is the first -- what is -- do we want to be more sensitive? Do we want to look at earlier endpoints? Are we looking at screening versus mechanistic or diagnostic?

CHAIRMAN DOULL: Right.

DR. CAVAGNARO: You know, for any of these. And then before the implementation, is there -- I mean, we have heard a number of technologies, as you mentioned. Is there an effort to do what some speakers did and that is highlight the pluses and minuses of each of the technologies and give an overall sense of what I refer to as prioritizing innovations. I mean, there is much out there, and I think that that is what most people have the most difficulty with. Which do I select or which techniques? And is that an initiative before -- I mean certainly the expert working group is endorsed. But prior to the implementation strategies, what -- is there going to be an overview of the available

technologies? I mean we have had speakers here and there is clearly logs of various companies and individual investigators working on similar strategies.So is there going to be an effort to do that as well?

CHAIRMAN DOULL: Yes. That clearly is the issue. I think in -- if you look at what the recommendations are, the recommendations have to do with the troponin or the cardiac assay and the vasculitis. We have heard this time and at the last meeting fairly good plus and minuses for each of those two techniques with some risk/benefit kind of estimation for that. I think as you move on down, I am not sure about whether we have really had all the plus minus spelled out for them.

DR. CAVAGNARO: Well, I see that as two points. One is whether or not -- one is a technique issue or one is a tools issue and one is whether or not measuring to quantum, which isn't measured routinely, is useful. So I guess I see it very distinct. One is endorsing the use of troponin as another analyte to measure -- or more sensitive to

measure cardiotoxicity. The other thing is to worry about genomics or proteomics or some other approaches. Is that -- Jim?

DR. MacGREGOR: Well, I think an important element of the discussion should be -- and one of the speakers made this point, I forget whether it was Gordon or Leigh in their talks that a lot of what we heard today I would say is in the realm of discovery technologies that are very powerful for identifying specific new biomarkers. And that in most cases there is probably another phase of inexpensive implementation technologies. So I think we need to think about our strategy in terms of the limited resources that we have at FDA of entering into collaborations. What is the most fruitful way for us to enter into this. And I think if I can paraphrase part of Frank's recommendation, it was that perhaps you could do a little bit of both by focusing on the specific biomarkers that we have studied for toxicities of current regulatory interest and that keeping those in mind and doing specific work on the known biomarkers to bring them into practical use. At

the same time, you can use some of these discovery technologies to look at the same tissues and perhaps compare other potential candidates for biomarkers with the known ones at the same time. So I think we need to -- I don't know how the Chair wants to address that in the discussion. But I think it is important to not mix all the technologies together. You have to kind of define the objective.

CHAIRMAN DOULL: Right. We may lose sight of what we are doing. David?

DR. ESSAYAN: Yes, I agree with that somewhat more rational approach. I think what we are seeing here is that a lot of this is two-edged sword. The biomarkers as I am looking at them are going to be a function of both the subpopulation of cells that need to be identified and a reliable assessment of the physiologic form of that biomarker, be it a gene form or the protein form.

I like being able to go after something that we know a little bit more about as part of process validation and immediate relevance to the work that we are trying to do now, and then have the more

discovery technologies follow that. I mean, with all due respect to Chip, I think his comment was something to the effect of the actual identification may be irrelevant and the footprint is what we need to be looking at now. And that is fine for the discovery phase, but the next phase is actually going to be the identification of that target. I think a lot of the peripheral blood approaches are fine to develop, but I think also that a lot of the cells of relevance in particular disease states are not actually going to be represented in the peripheral blood, and we may do better off going after the target tissues initially in order to identify what we may be able to see under optimal conditions in the peripheral blood after we know what we are looking for.

CHAIRMAN DOULL: I guess with Jim's point, limited research is the question of how much blue sky can you really do or how much difficult issue. Ray?

DR. TENNANT: Having not been present for the previous meeting, I hope I can legitimately ask some naive questions. In taking the title of non-clinical studies, this would imply to me that this

deals with the intrinsic properties of drugs, either for efficacy or safety. And either enhancing or supplementing the failures of existing nonclinical methods of telling us efficacy or particularly safety of drugs. And can biomarkers then be used within that arena, truly nonclinically. This is, I believe, in many ways much different than the issue of identifying adverse reactions. I mean, I think Spencer outlined very well what I see as an anti-parallel to the nonclinical. It means trying to identify biomarkers for those rare individuals. I mean, if the nonclinical methodologies are good, then the majority of individuals will not suffer from exposure. It is only the rare individual. And the problem of trying to use a biomarker then to identify the rare individual who is going to be adversely affected it seems to me to be a separate strategy. That is not a statement but really a question.

CHAIRMAN DOULL: I think the hope is that if one could identify things that would be useful in the nonclinical stage, they might be things, for example, that would be useful in identifying

toxicology before you even got to that stage. And hopefully then would also be useful once you went to humans and were looking for the first signs or those sensitive individuals that might express that reaction. I think last time we heard that there is a potential problem in toxicology. When they start using combinatorial chemistry and high flow-thru and all that, they are going to have hundreds of drugs, for example, that will need to have some kind of an evaluation. And if we use the traditional approaches of toxic -- acute, subchronic and what have you -- you know, we are never going to get that done. So we need methodology that will help us screen large numbers of compounds and make some kind of prediction, and it would be nice if the test that did that screening could also then be used clinically when those things get to that stage and would be highly predictive in the sense that they would be useful in animals and that would carry over into humans.

What we are hearing about the recommendations, at least from the FDA people, is they have some evidence for that carryover for some of

those things. Others, they are not sure about yet. Gloria?

DR. ANDERSON: I have in my notes three proposals that we presented. The first one doesn't deal with which one of those we decide on. I have a question about the specific implementation strategies that you have in your first proposal, the last three that you listed. You said one of the proposals is to endorse the formulation of the expert working group to derive specific implementation strategies. Would you elaborate on that a little bit?

DR. MacGREGOR: I think the concept that was discussed when the Subcommittee was formed was how -- what should be the structure by which FDA goes about pulling in the appropriate expertise to help make these decisions. And the concept that I was trying to go over in the beginning of my introduction was the role of this committee in identifying the important topic areas and moving to subgroups of more informed technical individuals to get down to the specific levels. So I think that is what you are asking about. What is the mechanism of this committee

versus expert groups. And I think in my mind the function of this committee is to identify the key areas that we should be pursuing and then to pull together the appropriate experts to address those specific areas. And then those experts would report back to this committee with their recommendations.

DR. ANDERSON: That is part of it. I think what is not clear to me is if I were to say, yes, I endorse the EWG to derive specific implementation strategies, I am not sure what you mean by implementation strategies. That is -- is it how we go about doing it based on the areas that are selected or what?

DR. MacGREGOR: Yes, exactly. Implementation strategy. I mean, we already at FDA are involved in some collaborations and we are trying to approach both of these areas. And the question then -- well, then what you are referring to is what would the expert group do is they would look at these areas and presumably they would include people who would collaborate on projects that would help us to work together to get these answers through collaborative

working groups. And I think part of this that I think is premature at this meeting but that we will have to get to eventually will be if resources are necessary that don't exist, how would resource issues be handled. I think that is a subsequent issue, but perhaps the expert groups could ultimately address that question as well. In other words, they could develop a scientific plan and say this is what you need to do to have better markers of cardiac toxicity, and here is how we think we could get the resources to do what we think needs to be done. They could come back with those kinds of recommendations.

DR. ANDERSON: I think you just answered my question. You see, the third one down here says endorse or reprioritize the target toxicities or specific toxicities. And that is a list you gave the level I and level II, tier I and tier II.

DR. MacGREGOR: No, that was Frank.

DR. ANDERSON: Okay. My question I guess is are we forming the group to implement strategies before we accept the levels.

CHAIRMAN DOULL: No, no. I think the first

thing is to get some idea about the kind of focus groups that we think should be formed. Because last time or previously, you folks have talked about the mechanics for forming the committee. You know, going to societies and making advertisements and so on. So the mechanism for that has kind of been laid out already. But what we need to do is to decide what are the areas we would like to develop focus panels for.

DR. ANDERSON: Yes. I don't have any problem if we form the group. The problem I have is that I am not sure what they are implementing, because I don't think the committee has recommended anything. Am I the only person who has a problem?

CHAIRMAN DOULL: No. Jack?

DR. DEAN: John, going back to the statements you made and the objectives that Jim had on his slide, it strikes me that this is a little bit broad. Because on the first objective, I think you are talking about how do we improve high throughput screening by introducing in vitro toxicologic methods, gene arrays, et cetera, that then improve the process of selecting better compounds. The second part of

the animal toxicity only predicts about -- the compounds that succeed through the animal safety evaluation and then get into man, the predictive value is less than 50 percent that we will then see an adverse effect in man that we could have predicted from the animal data. So the other approach then is are there better biomarkers between the linking man and the animal that we don't have today or are not using today. And if we were able to improve that area, then those could then be used earlier in the selection process. But I think there is a problem in putting all of this new technology in at the compound selection area stage. Because I think what we may do is kill a lot of very important drugs because they produce some effect in one of these test systems, yet we don't fully understand what the test system means relative to man.

I have probably made a very convoluted argument, but which do we want to focus on?

DR. MacGREGOR: I think it is clear that we at FDA don't have the problem of selecting those initial compounds. That is really an industry issue.

And I think that the -- you know, my first slide of objectives was a very general set of objectives for this Subcommittee over time and was intended to convey the interest really of all parties in the collaboration. And it is my personal view that the technologies that are going to be useful to us in regulating better and having a better linkage between clinical outcome, those same technologies are going to be useful to you in making those discovery issues. And the question is what is the common ground? If we are going to have collaborations, what is the best thing to pursue initially that is going to benefit all parties that are collaborating.

DR. DEAN: It seems to me the most viable linkage is to link the animal experience and the human experience with more sensitive biomarkers. I think that would be most helpful to both or all the communities or all the stakeholders.

CHAIRMAN DOULL: Let me make a suggestion that the Agency in essence is suggesting that the focus groups that are ready for prime time, so to speak, would be one that was focused in the general

area of cardiac toxicity and one that is in the area of vasculitis. And there is some linkage between the animal data and clinical data and so on in those two areas. We could think about those two as focus groups that we could identify. Then we could think about one that is a little more blue sky -- what you are saying, Jack. That hopefully we might be able to get some tox links with some clinical links or open up some new areas. And I guess would proteomics be that kind of area? We have heard some -- well, let me ask first, how many of these focus groups can we have? Is there any limit?

DR. MacGREGOR: Could I just make one clarification? I think Frank can correct me if I am wrong. But you said that the Agency recommendation was to focus on cardiovascular. I think that is not quite what Frank said. That is Frank went to the Pharm Tox Policy Committee and said from your perspective, where do you think we should be focusing. And there are several different perspectives, as Frank pointed out. There already are scientific collaborations through ILSI. We already have internal

research programs in both CDER and CBER that are taking certain directions. The Pharm Tox Policy Committee has certain regulatory issues and demands. And what we are really asking this committee is to think about all those things and come to a focus for one or two initial groups where we can get this process going. Not to try to cover the whole picture, but what are likely to be the, say, two groups or at most three groups I would personally say initially to get those expert groups started to facilitate some of these things.

CHAIRMAN DOULL: Right. In fact, we asked Frank to do that. We asked him to bring your recommendations and you went to your groups and did that. And, of course, last time we pointed out there are a lot of other activities. We have heard about all the ILSI activities and so on. So we don't have to do it all. Other groups will be involved in it. Our lunch is here.

DR. GOODMAN: In terms of the cardiac toxicity, there is one aspect that if I understood it right I find confusing. And that is these QT changes.

Because I thought it was also said that it was questionable as to what the significance of these QT changes are. If indeed I am correct and that is the significance of this is questionable, I think that is the last thing this committee should touch. We should only be involved in looking for biomarkers of toxicities that are real.

DR. MacGREGOR: Frank, do you want to comment on that?

DR. SISTARE: The last thing I want to do is to get into a debate about when a QT interval change is significant. But the only reason I bring that to the table was thinking about biomarkers in a very broad sense and not as a molecular entity, but a signal, an electrical signal that you can get from a heart, a non-invasive sort of image of electrical activity if you want to call it that. The committee felt that there are approaches, for example, being taken by our colleagues across the ocean, feeling for example that a rabbit Percingee cell model is the way to go to really predict whether a drug is going to have this or not. In the States, the statement that

was made to me -- and I am not an expert in this field -- was that maybe we are not quite as organized in our thought process here, and that maybe we could use the advice of this committee to establish a working group that could also develop -- maybe an expert working group to outline an experimental approach to delineate what is the best way. Now if the committee feels that we are not quite ready to do that yet, that is their take-home.

DR. GOODMAN: Again, I think in line with what you have said, Jim, that we really ought to focus on one or two or three where there is a chance of hitting a homerun and not solve the problem -- not try to touch the problem of whether some change is indeed a toxicity.

DR. ANDERSON: Just quickly, that was precisely my point. The proposal number one says implementation. I think if it is revised to reflect what that gentleman said, it would take care of what we want. I don't think we can decide here today all of the things that -- or where we think we should go with it. The proposal number one says implementation,

which obviously follows some decision about what you are going to do. My concern was that it does not allow for refinement of the idea before we get to the implementation stage. And what he just said, I think if that is included in here, at least to me it would be acceptable.

CHAIRMAN DOULL: Okay. Why don't we take five minutes to get lunch and give this a little thought. Then we can think about which proposals we want to move toward.

DR. CERNY: I'd like to remind all the presenters who have presented today if they could get FDA their electronic copies of their slides to Kimberly Topper. Her card is over there in the corner. So that the electronic copies can be put on dockets. Thank you.

(Whereupon, at 12:41 p.m., the meeting was adjourned for lunch to reconvene this same day at 1:04 p.m.)

A-F-T-E-R-N-O-O-N S-E-S-S-I-O-N

(1:04 p.m.)

CHAIRMAN DOULL: I think we might as well go back to this charge that the Subcommittee has, which is to implement this first step, which is to get some focus groups set up. I think what I hear you all saying is that you are a little reluctant to develop or to recommend a focus group which is fairly narrow. We have talked about one for troponin, vasculitis and so on. We could probably recommend a focus group that is somewhat more general and allow that group then to consider the kind of biomarkers or the area of biomarkers that they felt would be most useful to explore. Keeping in mind that there are already these efforts by ILSI to look at hepatic something or other and nephrology sort of things.

In talking to Gloria and Jack and other members, one possibility would be to look -- if they have a focus group that would really kind of focus in on the proteomics area in general. Looking at the gel techniques and the SELDI and all the things that we have heard about in that area, and kind of sort

through that and perhaps pick out from that either a specific area or even with subgroups or whatever some way that they could move ahead in that area to look for biomarkers that would be useful in the nonclinical areas. Does that sound like a reasonable thing?

And the other one then, in order to incorporate the tissue-specific kind of damage that results in injury that you have a biomarker for, rather than making it focused in heart, which the troponin would do, or skeletal muscle or vasculitis or whatever, we could just leave it at that. That you should be looking at tissue specific biomarkers which will be helpful, both in the toxicologic and perhaps in the clinical things.

And then I guess what I am suggesting is maybe then that this group could spend some time looking at the available biomarkers in those areas and develop a recommendation that would focus in one. It is possible, for example, that some might say we think cardiac biomarkers are the way to go and that would be the focus. It makes it a little more difficult to put the committee together, because you have to get

somebody who knows about cardiac biomarkers -- or biomarkers of cardiac damage versus biomarkers of some other kind of tissue damage.

Well, I guess we will have to formulate the recommendation, but I think what I heard you say, Gloria, was that it should be not too focused to begin with, because we ought to allow the experts to put together something. You know, we don't want to put those guys in a straightjacket in terms of what it is that this focus group is going to do. We want them to have sufficient flexibility so they can move in whatever direction is most likely to be highly profitable to everyone that is concerned about these areas. Is that what I hear the Subcommittee saying?

DR. ANDERSON: I was endorsing the formation of the expert working group that would eventually derive specific implementation strategies. But the caveat was that what is missing in this recommendation is that it doesn't allow them the opportunity to review what we have and recommend to us more specific areas in which to work. I think that is what you were --

CHAIRMAN DOULL: I think that is what we are saying to them. You know, you guys need to get together and focus in those areas and bring to us some kind of recommendations. They only problem -- you know, it is probably easier to make recommendations in the proteomics area -- for that group to get together and in the tissue specific damage area rather than just -- you know, we can say the committee doesn't feel comfortable with forming focus groups, you guys, and get a subcommittee to form focus groups. We can do better than that. And from what we have heard, we are talking about a focus group that would deal in proteomics and one that would deal in tissue injury and probably one that would deal in imaging after we talk about that this afternoon. So that would be fairly specific. And within those groups then, they could begin to put together a more specific recommendation. Yes, Ray?

DR. TENNANT: John, I guess I would like to direct a question to Frank. In terms of the vasculitis problem, is this a focus because preclinical methods, surrogates, rodents and so on

have not prospectively recognized this? Is this principally an adverse reaction that has occurred once the drug is in the clinic? Just I would like to understand the basis for what you want to achieve.

CHAIRMAN DOULL: It has occurred. We have got a bunch of those drugs.

DR. SISTARE: The reason that I put vasculitis on the table is because in our regulatory setting we see examples of animal studies where a deep bed vascular injury occurs in the absence of any sort of clinical pathology signal. So the first thing you see as you cut open the animal is you see hemorrhage or vascular injury and that kind of thing. And the question then comes up of, okay, what blood level did occur? Are there any other metabolites that you don't see in human, et cetera? So now they want to go into clinical trials with this and so what safety margin do you put on it? A safety margin of 10. Because there is no way of knowing when it is beginning to occur in the clinic.

So then you look at the class of drugs which this has been seen in, and then you say, gosh,

we have some drugs on the market in this same class. Are we doing something clinically right now that we are not aware of? And we don't really know. So can you tease it out of the epidemiology? Can you look for increased incidence of thromboembolism or cerebral vascular accidents or something like this. And it is a very difficult thing to tease out. So the answer to that is right now it is stymieing the development of certain drugs. Which if it is totally irrelevant to the clinical situation, then one could argue we are keeping good drugs from getting into the marketplace. If it is extremely relevant but we are operating at low doses of these drugs which are inducing sort of a low level toxicity which may accumulate over time, maybe we have a public health issue on our hands and we are unaware of it. So we are hoping that if we have some sort of accessible biomarker that we can go from the animal study into the clinic, we can assess the relevance of it.

And I would like to -- since I am given the opportunity to speak -- I would just like to point out that with all these technologies, I don't want to

make the initiative a focus on a technology. I want the -- my proposal is for the Subcommittee to endorse a program or a project or a consortia area. Here is a problem. Here is an issue. Here is a question we are struggling with. The expert working groups, I think, should be charged with delineating what technologies to bring to answer that question. But what I am saying in this set of presentations is that there are some problematic areas here. And again, this is one because -- I put vasculitis on the table because it is one that I am privy to or I know of. The industry may say, given the opportunity, that hepatotoxicity is something that they are very concerned with and would like to be able to go from an animal study into the clinic and be able to predict better. That may be something that they bring to the table. And they have with the ILSI initiative.

So all I am saying is with all these kinds of different thought processes and all these different perspectives of the regulator and of the industry and of scientists in general, let's prioritize. Let's try to focus in on a project or on a problem, and then

charge an expert working group. You guys figure out -- not you guys, I mean the expert working group -- you guys figure out how you want to answer the question. My point in orchestrating the presentations this morning was to show that these are feasible. These are doable. And you can get a multi-parametric assessment by looking at a number of different endpoints in this same study design. We don't know which one is going to be the one that helps you, but all of these you can go from an animal into man with. They are all accessible. That is the common theme behind all these presentations this morning.

CHAIRMAN DOULL: Yes, I think, Frank, that is what the Subcommittee is attempting to say. It is that if you look in the area of proteomics, for example, at the various things that are available in there, it should be possible to sort out the ones that are going to give you the biggest bang for the buck. What you really do -- in the list that Farr gave us this morning, for example -- all those drugs that have adverse effects, you want to know if you can how you could sort that out. But there is a whole score of

drugs which are sitting on the shelf which haven't been introduced because of those same problems. A lot of those might in fact be better if we could identify with biomarker techniques somehow which one of those was better than what is already there. Which should help. That is the whole basis of what we are doing. That is therapeutics. Yes, Jim?

DR. MacGREGOR: I could just maybe add to and reinforce a little bit what Frank said. I think what Frank is saying really is perhaps that the focus of the group could be the development of assessable tissue specific biomarkers by whatever technology, and then we would have to think about what kind of people should come on to that. But you could go to our sources of experts, the societies and make announcements, et cetera, the kind of mechanism we have talked about. And even define it that broadly. And my guess is you would end up with a large focus on proteomics because proteins are probably the major class of assessable biomarkers rather than specifically limiting it to a technology.

And you could even go further than that as

a committee and say well let's try to put some emphasis on the particular classes of toxicity that are of current regulatory concern. So that you get some people on the group that are focused in those areas.

CHAIRMAN DOULL: Yes. I think one thing the committee has to do, of course, is be aware of what else is going on in the field like the ILSI things. Yes, David?

DR. ESSAYAN: I think that clinical focus is actually going to be important to maintain as Frank has put it. Because that is going to help constitute those expert groups, and it is going to help focus the limited resources, however limited they may be, in areas where we stand the best chance of getting the maximum amount of information quickly and with the least expenditure in personnel time and resource.

CHAIRMAN DOULL: Jack?

DR. DEAN: John, if the committee would agree, it would be nice to focus on a couple of toxicities or target organs. Because in the past life, I remember spending some time on a CDC committee

that had the broad charge of biomarkers. And it was costed with a bunch of experts from different target organ disciplines. And the problem is everyone spent a lot of time saying why his target organ was more important than everyone else's. I think if cardiotoxicity is a significant issue or vasculitis or hepatotoxicity that we are not predicting well, then it would be nice to focus it on in the broad sense not what should be done but that those should be the focus areas. And the nice thing about your suggestion about the proteomics is that they could track in parallel looking at what are the proteins that are induced by liver injury as opposed to the issue of are there already tests out there like troponin and others that have some relevance today to just see how sensitive they are. So that is another way to think about trying to focus it a little bit.

CHAIRMAN DOULL: Yes. Those committees in fact might start off jointly. You know, defining areas, which might be helpful.

DR. DEAN: But it might be nice to think about whether we could focus on a couple of target

organ systems that we don't think are well served by the current methods.

CHAIRMAN DOULL: Yes. Certainly, we could give some examples in there which would illustrate what we have in mind without really binding them to those examples. Yes, Ray?

DR. TENNANT: I was just going to ask whether -- I understand the utility of the proteomic approach. But the microarray approach is a rapidly evolving discovery methodology. I don't see in my mind why a limitation or why a preference toward the proteomic. I mean, I think they represent distinct but equally potentially valuable approaches.

CHAIRMAN DOULL: My feeling is that is a little more blue sky than the genomic. I don't know. Maybe that is isn't so. Joy?

DR. CAVAGNARO: I think I would agree. And I wouldn't separate the groups. I will make the recommendation that I made last meeting, and that is there is a problem in the agencies clearly. It is significant enough that the limited resource dollars that are available within CDER and FDA are focusing on

this issue. And let's say it is troponin as a biomarker. We know now that ILSI is doing hepatotoxicity and renal toxicity and genotoxicity. So it allows us not to duplicate. So we have a finding. It is being used in the clinic. The problem is sponsors are not voluntarily using it. But that could be a recommendation.

My concern about separating the toolmakers from the clinician is that you will have a group of -- what we have heard is that the people who are developing the technologies don't have the samples to validate them. So they have to be in the same group. I don't see separate committees. I see the clinicians with the preclinical people and the toolmakers and the -- you know, working together. Because that is what has happened. Everybody is working independently, and I think we are not leveraging. So if there was an opportunity for this working group to have expertise in proteomics and gene chips and the clinicians that are treating the patients, what they are seeing, then you have the opportunity. If you don't like troponin, like you said, it could be that during the course of

asking the question, which is the clinical outcome -- how do we better predict that clinical outcome -- some other marker might trump it. And then what we have done is we have -- this is the question. And I fundamentally believe that the focus should be on predicting, better predicting, potential human toxicity.

CHAIRMAN DOULL: Yes, which -- you know, the first charge is to endorse something, whether it is one or two committees or whatever. The second charge is to figure out how to get people for those. And I think you all went through that, Gloria, didn't you, before? You talked about going to professional societies and asking for recommendations. Going to the agencies and getting -- there are a whole variety of things one can do once you announce the idea that you want to do something to bring in the right kind of people. And I guess the skill with which that is done has a lot to do with how well the committee does their job. But in a sense, that is something that we don't do. We don't select a bunch of people. We give some methodology to get those committees or groups

activated, right? And you've already talked about methodology for doing that somewhat.

DR. ANDERSON: Yes, at some point we did.

DR. MacGREGOR: Well, I think some of the details of that methodology have to be worked out. But I think in our initial discussion, which happened at the organizational conceptual meeting for this Subcommittee, was that all these different mechanisms might be used to bring in nominees and the advisory committee system can open up a public docket to receive all these nominations from the different sources. But then at some point somebody is going to have to create a balanced committee from the recommendations. And the initial thought was that that would be this Subcommittee. That this would be the oversight committee that would pull out of those nominations and form the committee.

CHAIRMAN DOULL: I don't really see any great problem. That seems reasonable. You know, that you would create the document, solicit good people from all the different sources that would know that, and then this committee could be involved helpfully in

formulating that committee. David?

DR. ESSAYAN: I think what I am interpreting Joy as saying is a multidisciplinary problem oriented approach is really going to be the best way to go because it will allow play within the field, but it will bring together all the components. I think an important part of this, which I was discussing with some people over lunch, is going to be that there is going to be in all likelihood no one discipline -- proteomics versus genomics versus whatever other omic technologies we have been discussing. It is going to be that a certain one is good in a certain application. And a lot of what will come out of these studies is going to be that sort of comparative study, where you can side-by-side a set of samples and figure out what the optimal use for each of the technologies is and the optimal context.

CHAIRMAN DOULL: True. But the problem you have is what Jack says. You know, if you get one expert from each of the different areas, it is hard to get the committee to really get the job done in a sense. And I don't know how we are going to do that.

I guess it involves some skill in getting the names and selecting the committee so it is balanced and has an opportunity to do that by area, if you will. Jim? Jay?

DR. GOODMAN: I agree with what Joy was saying in terms of focus being in terms of what is happening in people and animal studies as more predictive. The issue I think we also need to address is the question of which people are we talking about and are we talking about samples in people or people who already have a particular illness, et cetera. The thing I think that certainly should be stayed away from -- and nobody has said it -- but we certainly should stay away from any inclination to try to press for some of these new technologies as sort of fishing expeditions for compounds currently under development. I think nobody has said it. That would be very, very wrong and I think that is something we should really stay away from.

DR. DEAN: Jay, I think there is very little chance any of us would offer our new compounds.

CHAIRMAN DOULL: Well, I -- we need to go

back and review that procedure, I guess, that you all talked about formerly of how you might get people for this. Certainly, we need to ask NIH. They are very excited and interested in this. NIEHS probably has -- there will be biomarkers in transgenics, Ray, that will need to be included. And the professional societies, the ILSI groups that are already functioning. So there are a lot of people that hopefully would have good suggestions as to how this committee might be put together and formulated. So the next step really would be having agreed that we are going to endorse a focus group or two focus groups if it looks like that is more appropriate. And that we then will proceed with recruiting experts to sit on those focus groups that will deal with some of the things that we have identified.

DR. DEAN: Mr. Chairman, could we just advertise for the expertise we want in the Federal Registry? Would that be an easy way to --

CHAIRMAN DOULL: That is part of it. When you open a docket, don't you do that? You open a docket and that means then that there is something in

the Federal Register that says --

DR. DEAN: Calling for expertise?

DR. MacGREGOR: Well, you have to put out a Federal Register notice, and then you can announce. They send it in and there is a mechanism for receiving that. And they can receive recommendations from any source that comes in.

DR. DEAN: Including self-nomination?

DR. MacGREGOR: Self-nomination, nominations from collaborator groups, individuals, the public, anything.

DR. DEAN: Okay.

CHAIRMAN DOULL: But we are going to go beyond that. We are going to go out there and grab a few people by the neck and say, hey, you need to help with this. It is important. If you just sit around and wait for people to come, you are still waiting when the millennium comes.

DR. CAVAGNARO: But it is real important when we specify the expertise, I think, to make sure you have translators in that mix. What I mean is that there are many societies that have their own experts

and they talk the same language. But it doesn't advance what we are trying to do. Because what you need to do is translate the molecular biology and the proteomics to people who are actually -- so, I don't know how you advertise for that expertise, but I just want to make sure that we are not going to be just getting individuals who know very much about their particular tools or sciences, et cetera, and can't communicate once -- because I really fundamentally think that this is an opportunity to get a dialogue between true experts and then to translate that into something.

DR. ANDERSON: I think he said it when he said problem solving. If we can keep them focused on the fact that we are trying to solve a problem or some problems, that might help that.

CHAIRMAN DOULL: Actually, we have had some people in these last two meetings who meet, I think, your criteria. I have felt that we have had some really good people to translate some of these issues. David?

DR. ESSAYAN: Yes. The translators for