the cardiotoxicity, for want of a better term. The cardiotoxicity would probably come out of some of the cardiology -- you know, like the American College of Cardiologists. And as far as the vasculitis -- or I will put it in an even more global and broad -- immunotox group would probably come out of the American College of Rheumatology and maybe the American Academy of Allergy and Immunology. The clinical immunologists who deal with patients so affected, and they would be the ones then who would be able to get the clinical samples and translate the basic science. In fact, a lot of those people are already clinicians and scientists and would love to get involved with initiatives such as this.

CHAIRMAN DOULL: Okay. The third charge there had to do with the mechanism by which this group does its thing, whether they do workshops or publications or meetings or whatever. And I think that also ought to be flexible. You know, we should let those guys get together and figure out what is really needed is a big workshop to bring biologists and clinicians together or it needs to be a subgroup or

whatever. So we are saying to them, however you need to get the job done, here is what we have kind of defined as the job. And you guys go ahead and do it the best way you can. Resources, we can't deal with until we have some idea of what all of this is going to involve.

DR. ANDERSON: Mr. Chairman, there are a couple of other things that at some point I think we should do. It is 1:30 now and you may want to do something else. But number 2 says that there is a proposal to advise the membership. I guess that is the full committee. And the other is to endorse or reprioritize the target system. I would not like to see us just ignore that recommendation or that suggestion from the FDA. And maybe we should do something about that before we leave today.

CHAIRMAN DOULL: Yes. I think in terms of the full committee, they will probably ask us to tell them what we are doing the next time they have the Advisory Committee meeting. I think the reprioritization -- if we had made this so narrow that we got them off on the wrong track, then they would

need to reprioritize. Hopefully by having it broad, they won't need that. This committee will have a pretty good hold on where they need to go and would hopefully do that.

Okay. Any other concerns the Subcommittee has about this? I think in terms of -- we are pretty much focused on the kind of recommendation we would make for biomarkers and the committee and how this might happen. We need to get out there --

DR. CAVAGNARO: Do you want me to put a motion together or what?

CHAIRMAN DOULL: Yes, do we need a motion? Do we?

DR. MacGREGOR: Well, I think we need a clear statement if you are going to -- if we are going to endorse an expert group, I think we need a clear statement of the focus to go out with.

DR. CAVAGNARO: Okay, I'll try. I'd like to propose that we define the working group or the question, and the question is to better predict cardiotoxicity -- to identify biomarkers to better predict cardiotoxicity. If we want to say as related

to doxorubicin or whatever -- a certain class. I don't know. Frank, help me in terms of generalizing. Drug-induced cardiotoxicity. And that the working group to address this would be a multi-disciplinary working group. And that would --

CHAIRMAN DOULL: Yes, we could be more broad, Joy, and say biomarkers for tissue injury such as cardiotoxicity.

DR. CAVAGNARO: Well, you could. But then we might get other -- I am just thinking that it is going to take probably a year or more to get a working group together. And if you do that, then you will have such as. This is a prototype. So we could look at this as a prototype group. It could be that after the group gets going that something else is realized or even with some of the same players.

CHAIRMAN DOULL: Well, would you charge that group with proteomics concerns also?

DR. CAVAGNARO: Multidisciplinary.

CHAIRMAN DOULL: Okay.

DR. CAVAGNARO: The question is better predictors of drug-induced cardiotoxicity. And so

that would -- anything to bear. That would include toxicogenomics, proteomics, whatever technology. Because it is the question, not the answer to the question. You are putting forth the question. How can I better -- what is the most sensitive indicator than what we are currently doing?

CHAIRMAN DOULL: Okay. How does that sound?

DR. DEAN: Second. I think Joy is right. I think if we focus it on a particular toxicity at this point and get started, then we can come back later and look at other toxicities that are -- or data gaps that we need to focus on. But this would be the prototype for what we think we need to go forward.

CHAIRMAN DOULL: Okay. Why don't we go ahead with --

DR. MacGREGOR: Could I just raise -- I don't want to prolong this. But I guess we have another half hour of discussion time. Maybe I will just pose it to think. If we go with a very specific expert group such as cardiotox -- I agree. I think that is a very good idea. But I might reemphasize

what Joy said. Because of the nature of our subcommittee, we probably will only meet every three or four months at most. So we could consider whether we would want to go with two specific groups possibly if we wanted to go with specific groups. And the other one has been put on the table, which is vasculitis or more general immune response.

DR. ESSAYAN: Yes, immunotoxicity I think would be a --

CHAIRMAN DOULL: Okay. Let's go ahead and vote on the one she is talking about, the cardiac one. I hear a consensus for the committee to do that involving whatever it takes, Frank, to make that thing work. Then I guess what you are asking is whether we need a second one. And if we weave proteomics into whatever it is, then it would be there for both is what you are saying.

DR. MacGREGOR: Yes.

DR. ESSAYAN: And I think the issue here is that if we look at -- if we take a more bird'seye view of efforts in this area -- I mean we know hepatotoxicity is being dealt with by other groups.

Cardiotoxicity has been an unmet need, so we are there now. I think the other real issue here is immunotox. There are aspects that may be being touched by other initiatives, but I think starting with vasculitis and then being able to branch out from there would be a very good perspective to go after another unmet need area and also an area that has the potential for positive ramifications in a wide variety of clinical disease states. So I think it would be an important area to pursue.

CHAIRMAN DOULL: So we could -- you know, our notice would be the Nonclinical Subcommittee is thinking about forming a group to do this cardiac toxicity and a group to do immunotoxicity.

DR. CAVAGNARO: I don't think we want to use immunotoxicity.

DR. DEAN: I agree.

DR. ESSAYAN: What terminology would you prefer?

DR. CAVAGNARO: I would be very specific, vasculitis. Address an issue.

CHAIRMAN DOULL: Vasculitis?

DR. CAVAGNARO: I just think immunotox -- you are right, it is a sub. It could be a sub. But it is a universe of -- I mean, it would be really difficult to focus in on a particular icity within that, I think.

DR. DEAN: But Dave may be right. It may have an immune component, some of it anyway. But it would be better to keep it broad, I think.

DR. ESSAYAN: Okay, that is fine. I mean, my thing is I am actually thinking of immunotoxicity as the more broad umbrella term and that the vasculitis would be the initial objective of that group. I don't want to limit the group to a particular disease state or a particular histologic finding if there are other things that come up that need to be pursued.

DR. CAVAGNARO: Except it will focus the technologies a little bit, don't you think?

DR. ESSAYAN: Okay.

DR. CAVAGNARO: I mean certainly -- so it is another prototype. And then afterwards, you could do another part of immunotox. I just think if you cast

the net to immunotox, it is going to be harder to get the technologies refined enough to focus on the question of vasculitis.

DR. ESSAYAN: Point taken.

DR. DEAN: Can we hear from Frank again about the breadth of this problem of vasculitis? Because I would like some assurance it is just not the problem of the day. I mean, is it a broad general problem for the agency?

DR. SISTARE: If you look at sort of the number of drugs pulled off the market for vasculitis, you won't find any. So if you approach it from that problem or from that perspective, you will say it doesn't seem to be a problem. If you ask yourself how many sponsors have dropped development of a drug because of findings of vasculitis, I think you will see a bigger signal. If you ask the question, how many -- how much hair has been pulled out in meetings between sponsors and regulators over findings of vasculitis, or you could sort of flip a coin and in one hand stop development of the drug and in another hand allow development of the drug with the perception

that all review divisions are not equal, then it becomes a real problem to the agency. So it is a problem with a number of different dimensions. But if you ask the question, like I say, of how many drugs have been pulled from the market because of vasculitis, you won't find many. If you find about hepatotoxicity, you will find a lot more.

DR. DEAN: But doesn't that tell you we are screening -- we are currently doing a good job of eliminating compounds? Not putting them onto the market because we have identified that problem?

DR. SISTARE: Well, I could point to -- you are identifying the problem, but we don't know the clinical relevance of the problem. Okay? And we are allowing drugs to go forward with evidence of drug-induced vasculitis in animals, and we still don't know what the clinical relevance of that problem is. So it is sort of like it begs. It is begging for scientific knowledge. It is really saying -- you know, we sort of can be accused of sort of making arbitrary decisions. They are not arbitrary. But one could argue that they are not the most scientifically based. Because the

science just isn't there. And we want to become more scientifically based. And this is an area where we feel that void.

CHAIRMAN DOULL: Jay?

DR. GOODMAN: If the overarching issue is predicting from animals to people and this particular endpoint is not a problem in people, where would you propose getting the human material to make the correlation?

DR. SISTARE: Right now, again, we don't know if it is a problem in people. But there are drugs which have been approved which have shown preclinical vasculitic signs. So if we can establish biomarkers in these animal models where we can induce toxicity in a dose response fashion, and we can do that with a number of different compounds with different mechanisms of action or a different class and we see the same biomarkers and we can link it to its an endothelial cell product and we can show that. So we feel good. Like troponin. We get it out of a heart cell and we feel good that that is indicating cardiotoxicity. If we can show the signal we get out

of a proteomic approach or a SELDI approach or something like that as coming and derived from an endothelial cell which is damaged, so everything is in place. Now we go into the clinic -- if we go into the clinic and we find patients that have been treated with some of these drugs or they are on some of these drugs which we have approved and we don't see some of these signals, then maybe that is a good sort of way to address it. If on the other hand we find some patients, as Spencer has pointed out, that might be a little sensitive for some set of reasons to some of these things and there is some clinical sign of some -- some soft signs of vasculitis, maybe they will be flushed out. It is hard to say. I mean, I would say Theophylline is a drug which has been on the market for 40 or 50 years. If it was being developed today, we would be pulling our hair out not knowing whether to approve it or not because of some findings in chronic studies with rats. If the safety margin is right, maybe we will go forward with it. If not -- on the other hand, we have people with asthma that die and we don't always do autopsies and find out if they

have some sort of vasculitis going on.

So it is -- you are right. It is not an easy question. I think we may have opportunities where we are developing drugs that show vasculitis for some very clinical scenarios like cancer or AIDS or something like that. And we will be a little more bold in moving forward in clinical trials. And maybe some of these will be developed from NCI perspective, so it is in the public domain. It is not a pharmaceutical company. And maybe in a situation like that, we could get access to clinical samples. So there is a number of different ways maybe we could approach it. And I don't presume to speak for an expert working group. But those are just some possibilities.

CHAIRMAN DOULL: One thing the expert group could do -- you know, some of the Subcommittee has some concerns about the magnitude of that problem -- would be to look at it and say we aren't convinced that it is a problem that merits a big formal expert group and all that kind of activity. What we can say then is that this Subcommittee is considering the formation of expert or focus groups or whatever who

would be looking at cardiac toxicity or the use of biomarkers in cardiac toxicity and vasculitis. And then if it turns out that those are -- you know, the problem isn't sufficient, why then they would look at the problem and could so advise the Subcommittee.

DR. CAVAGNARO: But how do you get an expert working -- I guess I am concerned too. I mean, I think there should be a real problem in the clinic. I mean I think that just to help validate -- again, this is a prototype to see if it works. Because what we are asking is during the course of this a development of all these novel technologies to bring to the table. And if there are no clinical materials to be able to "help to validate" --

DR. SISTARE: Well, there are clinical scenarios of vasculitis. Dr. Essayan has pointed out. It may not be drug-induced vasculitis, but there are cases of clinical scenarios of vasculitis. There are also instances of drug-induced vasculitis, which is more of an immune causative mechanism as opposed to some of the other things we are dealing with where the immune may be reacting, but it is not an immune-

initiated -- it is not a deposition of an immune complex on the vascular bed or something like that. So there are a couple of manifestations. But what we might be able to glean from this is at the end of the day, there is an insult to the endothelial cell and there is a vascular injury, and the biomarker may be generated regardless of the mechanism if it is proximal to the actual toxicity. So there may be different mechanisms of it up-regulating some biomarker. It may be an immune mechanism, for example, with some of the clinical vascular disease that may result in generation of a biomarker that would be the same as a drug-induced biomarker. These are sort of unknown until we --

DR. CAVAGNARO: But if you start going into the different mechanisms and then there is no way that you are ever going to be able to compare SELDI with toxicogenomics or the proteomics. Because if the mechanisms -- you know, if you are not even on a similar pathway, what are you correlating?

DR. SISTARE: The endpoint is the histopathology. The histopathology is you have the

lesion. We want to be able to predict the onset of that lesion. Right now, we can't do it until we have a dead animal or -- I am not going to say the other thing. So what I am saying is if we look in the blood for something that results from some mechanism of endothelial cell injury -- if we can detect that, whether it be SELDI or whether it be 2-D gels or whether it be looking at circulating lymphocytes and gene expression changes as a sentinel. I don't know what the answer is going to be. But if we can do a better job at it than we are doing right now, which is we are not.

DR. CAVAGNARO: So are the companies that are running into these problems or these impasses, don't they have individual -- their own internal -- I mean, if these are drugs that they want to pursue, I would expect that they would have their own internal research programs to do this.

DR. SISTARE: What happens is they abandon development of the drug. I can point to a couple of places where they have abandoned development of the drug. It had promise. They had gotten into a certain

phase of clinical development and then they just dropped it. Because there is no biomarker.

DR. CAVAGNARO: Who is going to support them -- if they have dropped the drug, who is going to pay for this initiative to address these -- to get back to Jim's question in terms of supporting these initiatives? If they have made the corporate decision to drop the drug, are they going to --

DR. SISTARE: I think they have made a decision to drop the drug because the solution is bigger than any one company can solve. You know, we have heard from Malcolm where they have an issue about cardiotoxicity and was it a reflex action of the physiology or was it a drug-induced change. And they were able to solve that using a beta blocker and some really good thinking. And they were able to solve that because troponin was pretty far along and advanced. And they could go into the literature and point to some things. Vasculitis is nothing you can point to. So it is sort of a big problem. And there have been some efforts on the part of sponsors when review divisions have gone to them and said can you give us

a biomarker you monitor. There have been some feeble attempts to come up with something, but nothing that really held any water.

DR. CAVAGNARO: So you think we would be able to get these drugs that companies have dropped to do this research?

DR. SISTARE: We have done it once with the SKF 95654. So we have one example. There are some other drugs, which as I say are on the market where we can go to high levels and we can induce toxicity in some of our animal models. I would like to see more. I would like to see more chemicals so that we can -- you know, you kind of need a number of these entities. And in talking to some of the pharmaceutical companies that have dropped development of these drugs, it is sort of like, well, we can't let you have it right now. Ask us in six months. We may -- we are thinking maybe it will come back in another entity or something like that. So we are going to ask in six months. I have got it written down on my calendar.

DR. CAVAGNARO: Yes.

CHAIRMAN DOULL: I think what we are

saying -- the first question that group would look at is whether there is a problem and define that problem. If nothing else comes out of that but a good biomarker for endothelial damage in animals, that is useful. We don't have a good -- right now a good tox marker for that. We have to do some public comments at this point or we are going to run out of time. Let's see, I have -- the only one that I have been advised is Dr. Ralph Snodgrass, who wanted to make a public comment.

DR. SNODGRASS: First of all, I would like to thank Frank and the committee for giving us a chance to tell you about what we are doing to try to address some of these issues. To try to correlate in vitro data with clinical trial outcomes.

The approach that we have taken is to try to use an in vitro system that has as much biology as one can get into an in vitro assay. And the concept is to try to make it as meaningful to a broad range of biology as one can. Such as you can start to evaluate cell/cell interactions, cell/cell byproducts, metabolic byproducts, all within the same assay.

In this very brief presentation, I want to

show you our approach to try to do that and what we are setting up to develop. We are looking for pharmaceutical and academic collaborators to help in this process. But the system is really based on stem cell technology. The technology that allows very broad, complex interactive tissues. And the concept is to have an in vitro system that allows you to start to assess biological endproducts.

We actually think that there is a mechanism that we can calculate in vitro therapeutic index from this system. That is the dose that gives you what you expect to be your therapeutic outcome versus the dose that you see as a toxicity dose based on this in vitro assessment. And it is that spectrum or that differential that allows you to really start to prioritize drugs.

And where we see our utility being at this stage is in the early prioritization of drug leads. With a goal of identifying those biomarkers that allows one to prioritize drug leads and then take those biomarkers on with that drug as it develops.

We are developing a data base, a

retrospective data base of failed drugs and compounds that have well known toxicities. And part of this issue -- because we can do both human systems, rat systems and murine systems -- is to start to get an information data base that allows us to start to make assessments about understanding better the issues of species dependencies. One obvious application is trying to better predict the MTD for early clinical testing.

The system also is extremely powerful for starting to understand the relevance of genetic polymorphisms for drug responses. Because one can actually either select and/or engineer the system to have the spectrum of polymorphisms you want to test. You can actually have a system where you can look at how those polymorphisms impact drug responses. Furthermore, I should say that because it is a high throughput in vitro system, a system that allows you to look at drug/drug interactions in both human and animal systems, it is a very attractive possibility here.

The biology is such that one develops a

very complex developing structure that includes a wide variety of tissues, muscle, striated and cardiac and skeletal muscle, neuronal development, complete spectrum of blood development, a very complex organized tissue interactive system that allows you to get at very complex tissue/tissue interactions. Now the beauty of this is this allows us to not only go from a very complex differentiation system to various stages toward a monospecific type of system. And a good example of that is the hematopoietic system. One can take this all the way from fetal development, where the cells express fetal globins and the red cells are nucleated, all the way to the endstage adult mature cells. The system at the various stages expresses everything you can find in human bone marrow or peripheral blood.

So the concept is to use this developing biology system to start as a way of profiling drug responses and to evaluate how those drugs impact the development of these tissues. And again, this is all within the same assay. So we can evaluate the development of blood vessels. So, for example, with

the vasculitis, because this has a very strong endothelial component and a very early capillary development, this might be a very fruitful way of looking at those compounds that effect blood vessel endothelial development. But again, blood -- complete spectrum neuronal development. If you look at all the neurotransmitters that are being developed, essentially you can find any neurotransmitter population you want. And one of the goals of this that I will talk about is to go from this very complex tissue to, for example, a group or culture that actually is 80 percent neuronal to actually the endstage, where you can select for monospecific cells individually from this based on particular markers that you want to select for. So a very wide biology.

The concept then is to use this biology and to take advantage of the wealth of information that is already sitting on the shelves. In some situations, we can't get to it because they are drugs that have failed and it is that issue we have been talking about -- getting access to some of these failed drugs. And to identify what the profiles are of

these drugs in this system. And what we are doing at VistaGen is actually comparing and contrasting using the cyprogen proteomics profiles with the gene expression arrays that we are doing in collaboration with NIEHS. So we actually want to start to develop a system where we compare and contrast the value of these profiles we get from both the proteomics analysis and the cDNA analysis.

It is these profiles that we think provide the clinical link. The link between an in vitro system and the clinical outcome. And the way we see doing that is to put history to work. I mean, take advantage of retrospective studies in a complex series of drugs that have been known to show liver toxicity in human beings in preclinical studies and develop a surrogate marker set of those profiles, such that you can identify the markers that are characteristic of that class of drugs. And we are in the process of doing this with -- certainly the first collaboration with NIH is on liver tox. We have an ongoing collaboration to look at neuronal tox and to look at kidney tox and to actually compare now the profiles we

get out of this animal system and human system and compare it actually to human nephronal isolated cell lines.

So really to start to look at the comparison of the in vitro profiles that one gets from both animals and human systems. With the ultimate goal at least on the prioritization side is to have these internal reference standards, such that now when we get unknown compounds, the profiles of that then on a predictive basis can be slotted. Is it likely that that new compound will fall into any of these toxicity classes? And if so, one would deprioritize that for development.

Because of time, I won't show you the other side of this. One could as well talk about the efficacy pathways. Talk about the pathways that we know to be important in effective drugs and classify surrogate markers that actually are representative of efficacy pathways. So you can start to actually identify hits early on based on what pathways they begin to hit.

So that is just a very brief overview of

what we are trying to do. We are a new company. We are actually involved in collaborations, as I mentioned, with NIH and with a variety of academic groups. Cyphergen and VistaGen are working together to develop better proteomics systems, more refinement of the profiles and more understanding of what those profiles mean. We are in collaboration with Tripost to understand the issues around trying to identify in a very complex data set that has a high degree of variability because it is a biological system, what are the really critical surrogate markers that correlate those very complex profiles with the real clinical endpoints. And we are certainly looking and talking to a variety of pharmaceutical partners on getting access to compounds and collaborating on the compounds that they have found to fail, either in preclinical development or in the clinical development, with the goal of trying to identify markers that allows one to not make those same mistakes again. And we have faced the issue of getting access to those types of compounds that have failed. Thank you very much.

CHAIRMAN DOULL: Thank you, Dr. Snodgrass. Does the Subcommittee have any questions at all? Are there other -- yes, Gloria?

DR. ANDERSON: Yes. You said you have faced the issue of getting access to the compounds that have failed. What do you mean by that?

DR. SNODGRASS: It is very difficult to go to a pharmaceutical partner at this point and get ready access to compounds that are certainly in development. That is almost impossible. But even that, finding their willingness to give us compounds that they have actually terminated and put on the shelf. It is an issue of getting enough of these internal standards such that we can start to identify the surrogate markers. And I think that from our perspective, one of the tremendous benefits would be if there was a more industry-wide push to make those compounds available such that we could glean information from those to avoid or at least predict those type of mistakes in the future.

CHAIRMAN DOULL: You are talking about a data base of adverse effects. I think you and Dr.

Farr and me need to get together and consolidate some of our data bases and maybe put them on the Internet. Yes, Joy?

DR. CAVAGNARO: Probably around 20 years ago, I think there was a move to look at these embryonal stem cells for predicting in vitro teratology. Is there -- is that no longer being done? Or is there anything that you can gain from those studies with some of those compounds?

DR. SNODGRASS: The problem with those studies is they were ahead of their time, in the sense that they were using straight cytotoxicity essentially.

DR. CAVAGNARO: Right. Yes.

DR. SNODGRASS: Now the technology confluence has come together where you have the tools to real start to assay in a very complex way.

DR. CAVAGNARO: Oh, yes, sure. It is much more sophisticated.

DR. SNODGRASS: So they were just ahead of their time.

CHAIRMAN DOULL: They also had some

causality issues that were involved in all of that. You know, what is the real cause.

DR. SNODGRASS: Just to say a little bit about that. For teratology, one of the critical -- retinoic acid is well known for teratology. The biology of retinoic acid, at least in animal models, is exactly the same biology that it has in this system. So as a biology tool, it replicates exactly what one finds in animals in terms of the biology of the retinoic acid. Thank you very much.

CHAIRMAN DOULL: Are there any other public comments? Well, gee, that will help us time-wise. Are we caught up? We are almost on schedule, Jim.

DR. TENNANT: John, if I could -- just for a point of information. The NIEHS, as an outgrowth of a meeting that was held in November on biomarkers, involved FDA and some of the drug companies, drug safety assessment people and other organizations has led to the development of an RFA that was approved by the extramural council two weeks ago. So there will be an RFP announced fairly soon for program project

grants for preclinical drug safety assessment -- surrogate biomarkers for preclinical drug safety assessment.

CHAIRMAN DOULL: I might add that in the material which you sent to us, Jim, Greg Downing had put together some listing of some of the NIH -- some of their programs that were support kind of programs. Just so that it helps, I think, to identify sources of funding.

DR. MacGREGOR: Could I just ask a question about a previous discussion? I am somewhat unclear whether we did or did not endorse the vasculitis group.

DR. DEAN: Could I still voice reservation about this particular topic? I would like to be more convinced that this is not a maladies du jour, but that we really have strong evidence in the clinic. We have people in the clinic we could study and then go back to the animals and model. I hate to disagree with Frank. But I mean if you weigh this against hepatotoxicity or other things where we still poorly predict from animals, then this seems to in my mind

fall below the radar screen in terms of the potential problem.

One of the issues I would like to raise is that are we sure that the initiative that ILSI has with hepatotoxicity -- will they get into the validation or evaluation or comparison of methods? Because I know there is the workshop that people are talking about that is planned. But is that really a do work or is it a discuss the topic kind of a plan?

DR. SISTARE: The ILSI effort -- there is a lot of work being done. In the last meeting on February 29 and March 1, there was a real solidification of a strategy to move forward and the focus to look at the power of genomics. What it can do in terms of giving a fingerprint of toxicity. So there is a time dependency and a dose dependency looking in at target organs. Your question of whether there is going to be, again, sort of a multi-modal analysis. Clearly, the focus is on genomics. But like I say, I think in the nephrotox, we have successfully lobbied to look at other omic approaches, including NMR and protein analysis of other body fluids and that

kind of thing. Not necessarily target tissues, but accessible biofluids. So those will be incorporated.

The hepatotox, I am not on that breakout group. But the initial phase -- and it is not clear where the ILSI effort is going to end. They know where they are going to start. And at the end of the day, once they evaluate the data, the decision will be made I think at that point of how much further do they want to go on. So they may end up with just two drugs, clofibrate and methotrexate, and then say we succeeded, we are happy, and we are going to go home and we are all going to work independently. On the other hand, they may say we have succeeded, we would like to look at some more hepatotoxic drugs, and then they can sort of expand the portfolio. I don't know. I can't predict where it is going to go. So I don't know the answer to that.

DR. MacGREGOR: Maybe another point worth making is the current plan is those two drugs in multiple gene chip platforms. So no focus on assessable biomarkers.

DR. SISTARE: Yes. Not necessarily, but I

wouldn't rule it out. And I think if this committee would so suggest it -- I don't know if it is outside the domain of what they could or couldn't do -- but if they strongly suggested that we set up a group that dovetails and works very closely with ILSI and expand the whole other avenue. You know, assessable mononuclear cells or assessable other biofluids, proteomics or whatever. That could be an effort, and it would be a real conservation of resources and a tremendous opportunity to look at the same endpoint with a lot of different modalities. That makes perfect sense.

DR. DEAN: I would be much more comfortable with that approach. Because I know that a lot of that is a paper exercise. I submitted a paper on the immune aspects of liver injury. I mean, it is multifactorial. And it would lend itself to multiple approaches. So maybe that would be -- and it is a clear unmet need now in terms of the predictivity of the animal models for human liver injury. Maybe that is a better way to go.

CHAIRMAN DOULL: Yes, David?

DR. ESSAYAN: Well, I mean if that is the way the decisions are going, toward hepatotoxicity, that is one issue. But what I would raise pertaining to vasculitis -- and as I look around the table, there are fewer M.D.'s than Ph.D.'s, and as a clinical immunologist, board certified, who actually takes care of these patients, I'd like to add the thought that clinically vasculitis can be a challenge to identify. It falls on a continuum. The obvious dermal vasculitis where you have very classic Harrison's Textbook of Medicine type lesions, that is easy. But a patient who comes in with another illness and has some concomitant changes in mental status and you are trying to figure out whether this patient has CNS vasculitis, presents issues of diagnosis and issues of potential toxicities of therapies without being able to have an easy access to tissue, where I think biomarkers of vasculitis might be very beneficial. So there is an unmet medical need for that. And as far as having the clinical scope of the disease well laid out, I don't think the clinical scope is well laid out because I don't think the clinicians even have an

accurate idea of what they are dealing with. In fact, it would be my expectation that working from the animal data that we would be able to detect patients who may have epi phenomenon of vasculitis that indeed turn out to have it molecularly and then could have that proven histologically. We may be able to generate a whole new level of diagnosis and intervention in these patients that we don't even -- we can't even conceptualize at this point.

CHAIRMAN DOULL: Jim?

DR. MacGREGOR: With regard to the hepatotoxicity issue, I think we should also note that in addition to ILSI, FDA and PhRMA are jointly sponsoring a large workshop in the early fall -- I guess the second week of September or something like that -- on this issue. So in a way for us to spin out an expert group now might be duplicative of that effort which is going to bring together experts to ask what should we be doing in that area. That has been in the planning from the highest levels of both FDA and PhRMA. So from that perspective, it might be well to wait until after that workshop to decide what to do

in the hepatotox area. Not to say that I don't agree that it is a good idea to try to interface with other initiatives. I do think that is a good idea.

CHAIRMAN DOULL: Actually, at the last meeting we had the people who were from that ILSI initiative here. Denise Robinson, in fact, talked to us a little bit about what is going on with that committee you will recall. Jay?

DR. GOODMAN: Hearing what David just said makes me even more reluctant -- more reluctant to suggest that this committee become involved with the vasculitis issue. Not that it is not a real issue and not that it is not important. But my concern is that there just would not be the human material to make the appropriate correlations with.

CHAIRMAN DOULL: Yes. Let me bring up one point. And that is that this committee meets infrequently, and the problem is it is difficult for us to get anything going if we put everything on hold so to speak. So that I am reluctant --

DR. CAVAGNARO: I think we have made progress. I think we have made progress. We proposed

to have at least this cardiotox committee, which we are not going to have a subcommittee to --

CHAIRMAN DOULL: But what I am suggesting is we --

DR. CAVAGNARO: No. We were going to have a subcommittee to decide what we were going to do. But now at least we have something and we know what they are doing. So I think that is much progress. That is great.

CHAIRMAN DOULL: So the question then is vasculitis. Do we want to put that on hold or do we want it?

DR. ANDERSON: Honestly, I think that is the least we could do.

CHAIRMAN DOULL: I can't hear you, Gloria.

DR. ANDERSON: That is the second one under tier 1. And he must have had some reason for recommending this. And I would go for leaving it with the group.

CHAIRMAN DOULL: So moved?

DR. ANDERSON: Yes.

CHAIRMAN DOULL: So moved. Yes, Frank?

DR. SISTARE: I'm not sure about the rules and stuff here. But --

CHAIRMAN DOULL: We have no rules. And Kimberly isn't here, so we are not sure that we are not violating.

DR. ESSAYAN: Igor will stop you if you do the wrong thing.

DR. SISTARE: I think we might be able to glean something if we ask people in the audience, who I know are representing a number of the drug developers. Is this fair to do?

DR. ANDERSON: Mr. Chairman, there is a motion on the floor, I think.

DR. SISTARE: You can't do that?

CHAIRMAN DOULL: I don't know. Can we?

DR. ANDERSON: There is a motion on the floor.

DR. CERNY: The Chair is allowed to ask for anything that the Chair wants to do. So the Chair -- Kimberly isn't here, so I can say this. If you want to get some input from folks outside, you can sort of say this is your open public hearing all over again.

DR. ANDERSON: Mr. Chairman, are you finished with my motion?

CHAIRMAN DOULL: No, I heard your motion. I guess what I thought we were saying before was we could give the group the authority to explore the issue of vasculitis.

DR. ANDERSON: Yes.

CHAIRMAN DOULL: If it turns out there wasn't a problem, then they would say we don't think that justifies a big huge effort and forget about it. But now what I hear you saying is maybe we shouldn't even touch that.

DR. DEAN: I will reverse my position. That is a reasonable approach.

CHAIRMAN DOULL: Okay, then let's deal with Gloria's motion, which is that we include vasculitis as a topic for a focus group.

DR. ESSAYAN: Second.

CHAIRMAN DOULL: Second.

DR. DEAN: For feasibility?

CHAIRMAN DOULL: For feasibility. We are going to reword -- so it will appear in the

announcement appropriately. Okay, Jay?

DR. GOODMAN: Who do you propose for this particular focus group?

CHAIRMAN DOULL: You mean in terms of members?

DR. GOODMAN: That is right?

CHAIRMAN DOULL: We are going to do it with that same procedure. We are going to say in some notice someplace that the Subcommittee is thinking about establishing focus groups to look at problems, one of which would be biomarkers for cardiac damage. Another might be biomarkers for vasculitis. And that we welcome suggestions from all the appropriate groups as to who should be on these committees.

DR. GOODMAN: If you form a focus group like that, I think it would be composed of people -- I think -- who would tend to be in favor of this as opposed to people who would volunteer saying, no, I am not interested. So I think if you are going to form the focus group --

CHAIRMAN DOULL: That is pretty likely, isn't it.

DR. GOODMAN: Then I think it really means moving ahead with this.

CHAIRMAN DOULL: Yes. But I think this committee has the option, of course, to balance out all those folks with a list of people that we think would give good solid argument.

DR. DEAN: Well, I could volunteer to be on the focus group to counterbalance.

CHAIRMAN DOULL: Well, I think we can rely on the judgment of the people that would be involved in this to keep us out of trouble. Okay, we are about ready, I guess, to move on --

DR. GOODMAN: Excuse me, are we voting?

CHAIRMAN DOULL: Oh, well, I thought that was consensus. Do you want to vote? All those in favor, raise your hand.

(Vote taken.)

CHAIRMAN DOULL: That does it. We did it. Okay, are there any other comments from the public? Yes, Steve? Dr. Farr, why don't you use that microphone right there.

DR. FARR: I have no particular penchant

for one disease or the other, so I am not arguing for vasculitis or appendicitis or pancreatitis or anything like that. I would only recommend as someone who has spent a number of years trying to understand the relationship between these molecular endpoints or at least some molecular endpoints and important and specific toxic manifestations, that if at the same time you are, if you will, validate -- I know it is a loaded word -- new technology and find new biomarkers for an endpoint, it is a whole lot easier if you pick a quantifiable, readily identifiable, you've got it or you don't got it endpoint. Again, I don't have any particular interest in vasculitis or any of the other ones. That might be more difficult. That is the reason a lot of people look at peroxisome proliferation. It is not really relevant for humans we don't believe, but you can count the damn things. That is something to think about.

CHAIRMAN DOULL: Good point. I am glad to hear you say that peroxisome proliferation has nothing to do with people. It just kills rats. Yes?

DR. APOSTILU: Alex Apostilu, toxicology

consultant. I was wondering -- I heard different approaches that you can follow and they seem all to be very good and wise. Except they start from different points and I don't know where you are going to meet altogether. I was wondering if the group would make its work easier if you put a list of criteria to what should be chosen and what the strategy would be. Like, for instance, epidemiology, clinical significance, information availability, clinical and preclinical, feasibility, expertise ability, cost, et cetera. And certainly it is better -- you can make a better list if you get altogether. That just was an example, and I was wondering if that makes it easier for you to end up with.

CHAIRMAN DOULL: Yes. We had some input in fact in the last meeting that I think would be helpful to -- we can include that in the statement about these are the kinds of things that we are thinking about in terms of criteria for these groups to look at.

DR. ANDERSON: I would certainly hope you would include the objectives that we are trying to achieve that we are always given each time. So that

the people who will be advising us will understand what we are trying to achieve.

CHAIRMAN DOULL: That is a good idea. And we need to do that, Jim. The things you have in one of your slides need to be part of that announcement.

One other thing I guess which we need to clarify is if the -- if we send out this announcement and get suggestions about people who could contribute to this effort and so on, then this committee really kind of has the responsibility for appointing those people, I gather, or advising somebody that these are our recommendations as to who would be on this task force. So we need a mechanism to get that done. And rather than have a full scale meeting where we all get together and talk about these names again, we are thinking maybe we could do this by phone. And I don't know whether it is even legal. So I guess we would have to explore that. But if it is feasible, let's not have to have a full scale meeting just to agree on who these experts would be. And you guys can explore that and let us know how we get that done.

I guess we are pretty much on time, right?

So we will get into the other area that we are going to be talking about.

DR. GILL: Excuse me, Mr. Chair, while we are on the other subject of soliciting nominations.

CHAIRMAN DOULL: Right.

DR. GILL: Do you know when you will be putting that notice out, and will you try to do that before your May meeting?

CHAIRMAN DOULL: Yes. My thought was we need to put together this notice, and I guess -- does it have to go in the Federal Register? I guess it does. So we would put together some notice to be in the Federal Register, and I don't know how long it takes before all that happens. How long, Jim, would it be before we could put together some kind of a --

DR. MacGREGOR: I don't know the answer exactly. Do you know, Igor, how long does it take to get a Federal Register notice out?

DR. CERNY: Yes, usually it takes probably a few months. First you have to write it up and then you have to get it printed up. So it takes a few months.

CHAIRMAN DOULL: So we are going to do this with measured deliberation.

DR. MacGREGOR: One other thing that was discussed that I might just mention, because I think it would be a good idea to do. It was that in addition to announcing in the Federal Register, we would actually send directed letters to known parties of interest such as the disciplinary societies involved in these areas as well as the participating organizations like PhRMA and BIO and people from various universities in fact are involved. Ask them to solicit.

CHAIRMAN DOULL: Yes. I think since it is clear what we want to do and that we in fact are going to do it, I think those of us in the room here should begin to convey this to our associates and people that might have good suggestions and so on. Because we want to move ahead on this. There is no sense in sitting around for a year or two.

DR. MacGREGOR: I think the solicitations can go out from FDA. So for those of you that are on the committee, if you have places that you would like

letters to be directed, if you let either me or Dave Morley know that, we will arrange for that.

CHAIRMAN DOULL: Okay. The other issue that we need to talk about is PET scanning and its use as a biomarker or its use in nonclinical uses in evaluating drugs. And we are going to start off with Dr. Cherry. He is going to talk about micro-PET experiences with small animals.

DR. COLLINS: Dr. Cherry is on the faculty of the Pharmacology Department at UCLA, and he is also the Associate Director of the Crump Imaging Institute at UCLA. He is also part of the leadership of the Society of Nuclear Imaging and Drug Development. And at our last meeting in December, we heard from one of the past Presidents, Dr. Richard Frank. So, Dr. Cherry?

DR. CHERRY: Thank you very much for the invitation to speak here. First of all, let me tell you what I am not. Although I am a faculty member in the Department of Pharmacology at UCLA, I know very little about pharmacology. My background is actually in imaging physics, and I have mainly been involved in

developing imaging technology. So I just wanted to give you that disclaimer before we start so you have some idea as to what kind of questions I might be able to answer and what kind of questions I might not be able to answer.

So what I am hoping to do today is just to quickly run through the fundamentals of PET, just very quickly to remind you how it all works. And what I really want to focus on today is the use of PET for small animal imaging. And recent developments in technology that have allowed us to start to use PET scanning in mouse and rat models of human disease. I will show you some of the applications that we have been using the system for at UCLA in the last two to three years. And then I will close up with some comments about where PET imaging in the area of small animals may be going in the future.

So here is the one physics slide in the whole talk. I just remind you as to how PET works. We use radionuclides that are positron emitting. As the name implies, that means when they decay, the emit a positron. The positron is the anti-particle to the

electron. When matter and anti-matter meet up, they annihilate. They turn their mass into energy. This is a great example of Einstein's $E=MC^2$ equation. You turn the mass M into energy E. And the energy comes out in the form of high energy gamma rays. And these gamma rays have an energy of 511 KeV. That is about a factor of 10 higher than diagnostic x-rays, to give you an idea of the energies we are talking about. So this is very penetrating radiation. So it easily gets out of the body. And then we can detect these gamma rays externally. The most important point is that the two gamma rays are always emitted 180 degrees apart. So we have a ring of detectors externally around the subject and we detect gamma rays on opposite sides simultaneously in time, and we know that the decay took place somewhere along this line.

We collect many millions of events in a typical PET scan from these decaying nuclei, and then we can reconstruct using mathematical methods cross sectional images which reflect the concentration of the radionuclide in tissue. And the reason that is of interest is that usually we have tagged a compound of

biological relevance with this radionuclide, and so we can thus infer the regional concentration in absolute units of the labeled compound that we are actually interested in.

There are many different positron emitting radionuclides available to us. Here is a partial listing. The ones most frequently used are the top four here. You will notice we have positron emitting radionuclides of carbon, nitrogen and oxygen. This is wonderful because these elements are obviously abundant in molecules of biological relevance. So that means that we can often directly substitute the stable atom with a positron emitting one and track the natural compound in vivo.

Fluorine 18 is also very useful. It has a somewhat longer half-life. You will notice the first three here have pretty short half-lives, ranging from two minutes up to 20 minutes. Fluorine 18 is closer to 2 hours. That gives the chemists a bit more time to actually synthesize labeled compounds so you can get into somewhat more complex chemistry with Fluorine 18.

There are also a bunch of longer-lived radionuclides, and also some that are generator produced, which means they have long-lived parents. So you have a generator that sits in your institution constantly decaying into the positron emitting radionuclides so you have a readily available source. These short-lived ones at the top here generally have to be produced by an on-site biomedical cyclotron.

The real strength of PET lies in the chemistry. The fact that we can label just about any compound of biological interest that you might be wanting to study. This is just a very small partial listing of some of the compounds that have been synthesized with positron emitting tags, and it shows the different biological processes we can measure, going all the way from very simple things like blood flow, tissue profusion to substrate metabolism, both glucose metabolism and oxygen metabolism, protein synthesis, enzyme activity. Of interest here, of course, is being able to track drugs in vivo. And then we can look at neurotransmitters. And more recently we have been developing assays for measuring

gene expression in vivo by PET.

Another key point is because radioactive assays are very sensitive, we can image very low mass levels of these compounds. So we are really not perturbing the biological system we are trying to study. These are truly tracer kinetic experiments.

Now PET for humans has been around for some 25 years now, being refined over that time. We now in the human get beautiful images. On the right side here, these are cross sections through the human brain going from the top here down towards the bottom of the brain using a glucose analog, fluoridioxi glucose. So you are essentially looking at patterns of glucose metabolism in these images. Darker areas here represent areas of increased glucose metabolism. This is a typical whole body human PET scanner, and there are now a fairly large number of these distributed throughout the world. And particularly as PET is finding more of a clinical role in recent years, there has really been quite a large proliferation in the number of PET centers for human imaging.

I just wanted to emphasize that PET, of

course, is a kinetic technique. We are not taking static snapshots of what is going on. We are actually watching distribution of radio-labeled tracers over time. This shows a single slice through the brain. As a function of time following the injection of Fluorine 18 labeled fluorodopa. This is a precursor for dopamine. So what you are seeing is initially delivery through the vascular system. You see high uptake in the vessels here. You see it being distributed into the brain over time. And then gradually you are getting specific uptake in the striation as you would expect.

And then typically what we would do is we would go in and analyze so-called time activity curves that show the time course of the labeled compound in different structures. We can then create models that relate these time activity curves to specific parameters of interest. For example here, the conversion of fluorodopa into fluorodopamine. And we can actually measure these rate constants. And this is typically the way in PET we would go about measuring the rate of a specific biological process.

And again it is important to emphasize that we can do this quantitatively.

So that is the introduction to PET. But what I really want to focus on today is the ability now to start to use PET in small animal models. And first of all, just to set the motivation for this, although I suspect I am somewhat preaching to the converted here. Of course the reason we would like to move PET into small animal imaging is to give us a way for studying these animal models in vivo. Because we all know that in vivo is often not the same as in vitro. These non-invasive imaging technologies are non-destructive. That means we can study the same animal repeatedly. It means that each animal can serve as its own individual control. Imaging technologies generally allow you to survey the entire animal very efficiently. There is the possibility of rapid in vivo screening. I know that rapid screening and high throughput screening has been mentioned I think in every talk today, so I had to mention it as well. People often think that imaging is not a high throughput modality. But I want to try to show you at

the end of the talk that that may not be true.

Probably one of the most important things in the context of this kind of meeting is that imaging provides you with a bridge from animal studies up into human studies. And of course we hope that ultimately we can show that imaging can lead to making better decisions about drug candidates sooner.

Now there is a bewildering array of different imaging technologies available to you. These are some of the major technologies on this slide that have been and are being used in small animal imaging today. You will notice that most of the traditional medical imaging technologies such as x-ray, CT, MRI, ultrasound and PET/SPECT, all those technologies have not been developed for small animal imaging or are in the process of being refined for small animal imaging. From the other end, the optical folks who are used to looking at cell culture and tissue sections have moved their technologies up into in vivo imaging as well, at least in the mouse using bioluminescent probes and infrared contrast agents. So this gives you now a broad spectrum of techniques that can address

questions at different levels.

I think the big advantage of the medical imaging modalities is that you can do the same experiments that you do in an animal in a human. That is often difficult with the optical technologies because light doesn't travel very well through tissue, at least not through large amounts of tissue. And depending on what you are interested in, you can move from very high resolution anatomical techniques to techniques like PET that give you information on molecular function and metabolism.

So in terms of choosing which imaging technique, there is no one answer to which imaging technique is best. It is a question of what you are asking, what you want to see and what you want to measure. So you really have to define that. You have to define the spacial scale that you want to measure things on, the temporal scale, the sensitivity you require. And of course it does to a certain extent depend on availability as well.

So at UCLA we have been focused in the last four or five years on trying to adapt a human PET

technology and really push it for small animal imaging. Our goals were several-fold. First of all, to dramatically improve the spacial resolution. Because a lot of people think that PET images in humans are pretty fuzzy, and now we are going to try and take this down to a mouse. So we have got to really improve resolution.

Another major thing that people think about PET is that it is very expensive. And clinical scanners, it is true, tend to range in the $2 million kind of price tag. So we wanted to drop that cost by at least an order of magnitude in trying to develop these systems. We also wanted to make them compact and user friendly, the kind of thing that a biologist could actually use in their lab.

Now we are not -- we haven't achieved all those goals yet, but we have taken, I think, the first steps in those directions. So this is the micro-PET scanner, which my lab developed and built three years ago. It has been in routine use by many different biologists at UCLA for just over two years. We have done something like 2,000 animal studies in that time.

So we have got a lot of experience now in knowing some of the questions that it can address and some of the questions that it cannot address. I will tell you demand for time on this machine is just incredible. We are also entering into a number of pilot studies with pharmaceutical companies to test some ideas of how you might use this kind of device in the drug development process.

Now although I am going to show you data from our system at UCLA, I want to make you aware that we are not the only people doing this. There is a large worldwide effort in developing these small animal PET scanners. I have listed here most of the other efforts that I am aware of that have really resulted in practical imaging devices so far. Just to make you aware that we are not doing this in isolation.

Now this slide is here to illustrate the improvements we have made in spacial resolution with these small animal PET scanners. This is actually a test object that has been imaged. But on the right, you see the image you get on one of your $2 million

clinical PET scanners of this test object. Then we have moved it into our micro-PET scanner and imaged the same object with the same amount of radioactivity in it, and you can clearly see the vastly improved definition. It is about an order of magnitude improvement volumetrically. And in fact the volumetric resolution here is about 6 microliters. So that is about where we are with PET imaging technology today.

So let me show you a little bit about some of the applications and how it is being used and show you some images to give you a sense of what you can and can't see. These are whole body studies in the rat. They don't come out too well here because of the brightness of the projection. But just to show you that we can do whole body surveys in rats and mice to look at the distribution of radio-labeled compounds. In this case it is fluoridioxi glucose again. So we are looking at glucose metabolism. You see the heart here very clearly and the brain here. These are the herdarian glands, these two very intense areas here and then excretion into the bladder. So this is an

overall survey in an animal to show biodistribution.

We now home in on some of the specific organs in the rat. We will start out with some -- I will show you rat studies first and then we will move into the mouse in a few moments. So these are cross sectional images at the level of the heart in the rate. Again, we are looking at glucose metabolism. You see the left ventricle very nicely outlined here. These are not gated studies. These images are acquired while the heart is beating. So you see clearly illumination of the myocardium. In the next slide I can show you how we actually go about using that in our animal models.

These are three studies in the same rat done in the same afternoon, and we are using ammonia labeled with N13 as our tracer. And this gives us a measure of myocardial profusion. And you see different views of the heart here. Short axis images, long axis, vertical long axis and horizontal long axis. And these plots on the bottom are the so-called polar maps, where we have taken the three-dimensional cone shape of the heart and squashed it down onto a 2-D

plane, so you can visualize the information from the entire heart in a single image. And you can see in the baseline condition in this rat, we have fairly uniform profusion in the heart. You see a nice ring for the cross sections through the myocardium here.

We then occlude one of the coronary arteries in the rat. You see reduced blood flow to that region of the heart. Again, you see that area very clearly on the polar map here. And then in this case, we release the occlusion fairly promptly and blood flow recovered in that region essentially to normal levels. So now we have a model where we can study the same rat repeatedly over time during the course of intervention.

Moving to the rat brain, that gets a lot more challenging. The size of the structures are a lot smaller. And obviously here is where the limited resolution of PET is going to start to come into play, and we have to consider what kinds of questions we can realistically address with this kind of tool.

On the bottom row here are coronal sections through the rat head, images obtained by PET,

non-invasive images. And then we excised the brain at the end of the study and did autoradiography. Just so you can compare what we can see in the images.

So in the PET images, we can clearly see the cortical rim in the rat brain. We can identify the thalamus quite nicely and we can see the striata. So we can see the major structures in the brain. But obviously we are never going to approach the very fine detail that you can see with invasive techniques such as autoradiography.

Now so far I have shown you a lot of pretty pictures, and it is very easy to impress people with pictures. But a key point of PET is that it is a quantitative tool. And we really need to validate that we can quantitatively measure things in these small animal models if it is really going to be a useful tool for biologists and potentially for the pharmaceutical industry.

So we have been validating a number of different applications, and I am just going to show you one example here. But this is the same model that you saw on the previous slide. So looking at glucose

metabolism in the rat brain and comparing it with the gold standard, which is 2-D oxyglucose and autoradiography based on the Socoloff method. And you can see here the correlation between the autoradiographic results and the quantitative PET results are extremely good. And here in individual structures again. You see a slight underestimation in the PET measures, and that is to be expected because we have a lot worse spacial resolution, and that leads to a slight underestimation of the glucose metabolism in small structures. But nonetheless, we really have an excellent correlation, and you would have really no concerns in terms of using micro-PET, at least in the structures that I outlined on the previous slide, for quantitative and non-invasive measures.

This is example as to how our group is using this at UCLA. This is a model of traumatic brain injury in the rat. This is the same rat studied four different times and these are at three different levels in the brain -- coronal sections again. And so here is the baseline scan. Fluid percussion injury was then applied to the left side of the brain. You

see two days after the injury -- you see a reduction in glucose metabolism here. And then you can monitor subsequent recovery of that function, metabolic function, over time. So this emphasizes the fact that we can study the same animal repeatedly and use each animal as its own control.

Another example of its use in the rat brain. This is now looking at the dopaphenergic system. And this is showing two different compounds. One which binds the dopamine transporter. And here is the control study. You see the left and right striata nicely. And the other compound which binds to the D-2 receptor. And again, you see the two striata very clearly in the control condition. We then took this rat and it was lesioned with 6-hydroxydopamine unilaterally, and you see the loss of the signal for the compound that binds the dopamine transporter. And interestingly, a slight increase in the signal for the D-2 receptor binding compound. So, again, an example using multiple different PET traces in the same animal in two different experimental conditions.

Now moving down to the mouse, things get

even more challenging. You might think that the mouse brain is far beyond what PET imaging could ever address. But if you have a very specific probe, for example that probe for the dopamine transporter, even in the mouse brain we are able to obtain quantitative data. Here you see a cross sectional image of the mo use brain showing the left and right striata clearly separated. We can get time activity curves showing the specific binding in the striata and non-specific clearance from the cerebellum. So if you are asking the right question with the right probe, we even have access to the mouse brain by PET methods.

Moving into a cancer application, again to emphasize the fact that PET is a whole body technique. We can image the entire mouse in a single setting. This is looking at an antibody against a tumor here. And interestingly, this is labeled with a much longer-lived positron emitter. So this scan is actually done 12 hours after injection. The half-life of Copper 64 itself is about 12 hours. So this is the kind of study you can do without the need for an on-site cyclotron. The volume of this tumor, by the way, was

about 100 mg. And to give you an idea of the total amount of activity that is in the mouse when this image was taken, we injected 70 and we scanned 12 hours later, at which time there was actually less than 30 microcuries in the entire mouse. So we are getting fairly high quality images with very tiny amounts of radiation.

A big effort in our institute at UCLA has been to develop methods to assay gene expression using PET. That is reported gene expression. We have been trying to develop methods similar to those that are conventionally used with green fluorescent protein as a reporter gene. Unfortunately, there is no reporter gene that spontaneously will emit x-rays or gamma rays for you. So we have to have a kind of two-step approach where as our reporter gene, rather than green fluorescent protein, we use something that will produce a protein product that will trap one of our PET-labeled probes. In this case, the reporter gene is the gene for HSV1-tk, and then we come in with a probe which is fluorinated ganciclovire, which is a good substrate for that enzyme. And here you see an

example of one experiment. We delivered the HSV1-tk gene using an anti-viral vector through a tail vein injection for reasons that I don't have time to go into. The majority of the virus ends up going to the liver when you inject the IV into a mouse. So you would expect now that this gene would be expressed in the liver. We also did a control study on the left side here, where we introduced the control gene in the same way. And indeed you can see that we are getting a specific signal from the liver in this mouse and not in this mouse. And we have gone on to show that we can quantitatively assay over a reasonable dynamic range the levels of messenger RNA for HSV1-tk.

Now coming to the specific question of drug development and how you might use PET methods in small animal studies, there is a number of different strategies. Probably the most obvious one but maybe not the most useful one is direct radio-labeling of the drug itself. The problem there is that the radio-labeling process is a bit of a bottleneck. It often takes many months of a radiochemists time to figure out the synthetic pathway for a labeled compound and

to figure out how to do that quickly enough given the half-life of the radio-labeled probe. Although there are now groups that are looking at ways to rapidly produce large numbers of related compounds labeled with carbon-11 or Fluorine 18.

Probably more useful is to look at binding competition studies, where you already have a PET ligand for a specific target that you are interested in studying with your candidate drugs. And then we can look at displacement type studies using that kind of approach.

And then, of course, is the idea of using surrogate markers, things like blood flow and glucose metabolism, which we can readily measure with PET. There is huge literature on that already.

So to summarize what I think are some of the major advantages and disadvantages of PET. The advantages, of course, are that we can measure a very wide range of different biological processes. We have very high chemical sensitivity that we can measure down into the nanomolar to picomolar range, and that we get whole animal by distribution and kinetics from

a single scan.

The disadvantages are still, compared with some technologies, are our spacial resolution is still fairly coarse. It is at the mm type level. Our temporal resolution tends to be at the tens of seconds to minutes type level. There is this issue of how you get the radio-labeled compounds. And another issue that I would like to turn to briefly is the fact that we have very little anatomical information, particularly when we have very specific probes that home in on one specific tissue region. You look at these images and you see these blobs of color, and you really don't know where it is localized. So if you go to the next slide, something that our group has been working on a lot in the last couple of years is trying to combine PET with other imaging modalities. In this case, MRI, and trying to build a PET scanner that will go inside an animal MRI scanner so you can do both PET functional and molecular imaging with PET and anatomical imaging with MR simultaneously.

Now this is challenging to do because of the very high magnetic field inside MRI scanners. But

nevertheless, we have built this tiny little prototype to prove feasibility. This is the little PET scanner here, and it is connected by a lot of optical fibers to all the electronics. And here you see it in a test set-up actually inside a clinical MRI scanner. Here is the little PET scanner. And then we can put a rat inside the PET scanner, and on the next slide you can see what I think are the first ever simultaneous images acquired with PET and MRI at exactly the same time. Fluorodioxi glucose glucose metabolism scan through the rat's head and the anatomical MR image. This is the brain here. The brain on the PET scan is here. Not fantastic quality images, but the first proof of principle that you might be able to combine multiple imaging modalities together to give you more information.

Now really looking way into the future and thinking about how we can really use these technologies in a much higher throughput manner than we are currently doing. Typically right now when we do a PET scan, it involves a team of several people, and we may scan for two or three hours and then there

is all the data analysis. We have got this fairly refined at UCLA now and we routinely do this. As I said, we do something like 1,000 studies a year. But still there is the thought of can we really push this to much higher throughput so we could maybe have an in vivo screen for drug candidates where we might use surrogate markers such as blood flow or glucose metabolism that are easily measured by PET and where we can get the PET images in just a few minutes and combine that with anatomical imaging, maybe with CT or with MR. And therefore get three-dimensional, anatomical, and functional information on the whole mouse at a rate of something like 10 to 20 mice per hour. I think the imaging challenge is not actually the difficult challenge here. I think we can build a machine to do this. The difficult thing is what you do with all that data once you've got it. Because it is not very helpful to the biologists or somebody working in the drug industry for me to hand them several hundred gigabytes of data of mouse images. We have got to find sophisticated ways to sift through all that data and extract the information that is

actually relevant and of interest.

So I would like to finish by acknowledging a huge team of people who contributed in one way or another to the data I have just shown you. And I would like to say that I think small animal PET imaging is now a reality. There are several scanners around. There are several commercial companies now starting to develop animal PET systems. I think by the end of this current year, we will see something like 10 to 15 animal PET systems in the United States, probably growing to 20 or 30 by the following year. I think PET can address certain questions, but you do have to bear in mind that it does have resolution limitations. And you do have to be clever about how you do the chemistry so that you can actually do fairly large numbers of studies and keep the costs down. But I think bearing in mind those limitations, there is a huge amount that you can potentially do with this technology. Thank you.

CHAIRMAN DOULL: Thank you. Questions? Is that 3-dimensional? Can you do 3-dimensional?

DR. CHERRY: Yes. The data is acquired as

a volume. And obviously it is difficult to show here. But, yes, you have volumetric data. You can slice it and look at it any way you want.

DR. COLLINS: While the cables for the next projector are being set up, I might remind you that Greg Downing in his lead-off talk this morning mentioned that many of the institutes at NIH have identified imaging as a funding priority. I'd say NCI in particular or the Cancer Institute has made an enormous effort, both in terms of money. They have announced programs in upwards of $100 million investment. The next speaker is Dr. Tatum. And they have also symbolically created a biomedical imaging program within their Division of Cancer Treatment and Diagnosis. And fortunately, we have a representative from that program, Dr. James Tatum, who will tell you about some of the initiatives that are ongoing there. I think anyone who has doubts about the infrastructure being available in this high technology area should be at least convinced that the NIH is doing its part in droves to raise the level of infrastructure across the country. We are still in the hook-up mode.

The other thing is there is a tremendous irony in having our meeting today on March 9. Because six weeks ago, the NCI announced the funding of something like six or eight of these small animal imagers that you just saw described by Dr. Cherry. And their principal investigators meeting is today in St. Louis. So, again, any concern that these machines -- they are highly specialized. There isn't one on every benchtop. But at least you are beginning to see the diffusion of them throughout the research community.

DR. TATUM: First of all, let me thank you for the invitation to come and talk about our program. It is a relatively new program. If I ever get the slides, I can actually tell you a little bit more about it.

The Biomedical Imaging Program actually really has been in existence just for a few years. And now we have grown to a fairly sizeable part of the vision on cancer treatment and diagnosis. And we now have about 14 full-time FTE's and we have a number of other part-time individuals in the group. And we are

now looking for more space as we begin to grow.

Let's really get started now. Let me talk about the Biomedical Imaging Program at the National Cancer Institute which I am a part of. Our program actually started, as it should, with a set of vision and mission statements about what the imaging role is in health in general, but particularly in cancer. Our vision is that imaging sciences are essential to understanding biologic systems, controlling disease and enhancing health. And the more we learn about microsystems and microenvironments, we really believe in in vivo imaging and therefore an assessment in the intact organism is very important.

Our mission is to promote and support outstanding basic translational, which is one of the big coin words this day, and clinical research and imaging sciences. And since we are part of the NCI, we are particularly interested in how they interact with the challenges of cancer, although we know a number of the processes that we are looking at particularly these days cross over many different disease processes including cardiac disease, connective tissue diseases

and others.

As I said before, we are in the Division of Cancer Treatment and Diagnosis, which is made up of basically five sections, many of which you are quite familiar with. Just until recently, we were the Diagnostic Imaging Program and changed into the Biomedical Imaging Program, and I think it was a representation of clearly understanding from the beginning that probes, drugs and contrast agents and those kinds of things were such a large part of our initiatives that were going on.

The program is actually made up of four branches, and they really don't work separately. Because we know in imaging there is so much crossover that we kind of almost work in a matrix type of organization. But basically we have a Diagnostic Imaging Branch, which currently is headed by Dr. Ed Staub, who is also the Director of Radiology of the Clinical Center at the current time.

A Molecular Imaging Branch, which is primarily where I work, and the chief is John Hoffman, who came to us from Emory not long ago. Very well

known in the PET world. The Image Guided Therapy Branch currently is not filled. So Dr. Sullivan, our Associate Director, is in fact overseeing that particular branch. And Imaging Technology and Development Branch, which is actually headed by Dr. Larry Clark, who recently joined us from Florida. So we have a fairly good complement except for the Image Guided Therapy Branch at the current time.

One of our very first interactions has been very much with the Cancer Therapy Evaluation Program or CTEP. And one of the things I did when I first came here was to actually begin to sit in on the protocol reviews so that there was more active imaging input. And of course more and more, imaging is a part of the endpoints you are looking for in therapy evaluation, going all the way back to basic anatomical measurements, but more and more these days to looking at functional parameters. So this has been a very interesting and mutually beneficial arrangement that has been going on between the two of us.

In addition, we are now going into, and I am going to talk about at the end, an idea that we are

working on currently about facilitating imaging drug development, particularly with relation to some of the regulatory barriers that some people see with the Developmental Therapeutics Program, which is one program in which they have actually done this with therapeutic drugs. The RAD program, if anyone is familiar with it. is the one that we are kind of patterning after at the present time.

We are now also beginning to work more closely with the Radiation Research Program, particularly in microenvironments, which is very interesting to us both from an imaging and a therapy standpoint and with the Cancer Diagnosis Program as we move more into the screening area. So the interactions are really growing and the whole group is beginning to work together quite nicely.

NIH funding for imaging -- and this is kind of a screwy slide. It is kind of complex. If you look at the red, that is basically kind of across institutes or whatever where imaging is used. And you can just see what the growth has been in the millions of dollars, now approaching about $350 million and

actually may exceed that in FY99 when we get the numbers in. But imaging is a big part of almost all the institutes and many of the programs and many of the grants that actually go out.

If you look at that blue one that says NIH-RAD, what that is is trying to figure out where dollars have gone with respect to correlations with departments of radiology. And in some cases, that is not a very clear distinction. Sometimes it is in basic research and sometimes it is in radiation oncology. And sometimes the designation isn't clear. But you can see there has been a very slow but progressive growth in that particular area, now in the range of about $120 to $130 million.

The gold bars are just the BIP, that is our portfolio, which I will talk about a little bit more. And actually is growing now at a somewhat increasing rate as we begin to move and get better staffed.

I thought it would be interesting just to look at our research portfolio at the current time. And there is a mixture of things in here. These are

recent programs. And one of the largest at the top you will notice is ACRIN, which is the American College of Radiology, cooperative group trials, which basically was a bidded trial and the ACR won it. And primarily their initiative is looking more at Phase III studies, at informatics and data-based type studies. So there is a series of things coming forward that actually I think will give a better scientific or evidence base to some of our radiology procedures that are used or imaging procedures in general. It is not exclusive to radiology by any stretch of the imagination.

Another RFA that was in 1998 was Developmental Application of Imaging and Therapeutic Studies, an area that we are extremely interested in. And it was mentioned just a minute ago, and in fact there will be more of these. It is the Small Animal Imaging Programs or SAIRPS as we call them. There are five of those at about $15.3 million. And these are basically to build labs where we do have some of the things you have seen before utilized in animal models. And maybe particularly applicable to the mouse models that we see growing up. So there is a major effort in

this particular area.

Other programs -- planning grants for the Molecular and Cellular Imaging Centers, ICMIC's. And again, we are looking now at ways to image at the molecular and cellular level and producing expert centers or resource centers of this type. There is two pieces to that. There was the planning and now we have in fact a number of these centers in place and there will be more of these. An extensive amount of money has actually been put into this.

The 1999 RFA for Imaging Guided Therapy in Prostate Cancer that came out of some of the prostate initiatives. It is a phased innovation award. We also have the Diagnostic Imaging Guided Therapy SBR STR initiative as well in the prostate. More programs -- and you can see some of these are in the pay line and some of them actually have been funded. Developmental and testing of digital mammography displays. Of course, that is an area right now -- we have now got one approved digital mammography system out there. Exploratory developmental grants for diagnostic cancer imaging, R21's, innovative technology awards,

innovative technologies for molecular analysis and cancer, bioengineering research partnerships, research grants and novel technology contracts. So you can see kind of a broad portfolio there.

We also have a number of collaborative programs. I mentioned our relationship with CTEP, actually which is really quite ongoing and working together. We are in the protocol review, so we are involved with the contracts or the U10's. You can also see that we are in the pediatric brain tumor consortium clinical trials. RFA's for new centers are going out. And a new program that actually we are looking at right now is RFP's for Phase I and preclinical trials for imaging -- specifically for imaging probes. Those things that may in fact already be at an IND stage, but they are lingering to look for a place to go for their first Phase I and II clinical evaluations.

This is the graph I showed you before. Just to show you where we are, FY96 through FY99. And right now the portfolio is about $83 million in the current year, and it looks for all purposes that we

will grow a little bit further. So this is the initiative that we put forward in basically biomolecular imaging.

So that is kind of the portfolio -- kind of the money part that we have been putting out and the initiatives that we have been putting forward. The other thing that has been quite active is particularly an attempt to do interdisciplinary meetings and workshops. And I am just going to talk about two that I was intimately involved with, but there have been several in nature.

The first one was one that we held out in Jackson, Wyoming, called Imaging and 20/20 back in September of last year. And this was kind of a novel concept because what we tried to bring together were basic scientists, particularly chemists, combinatorial chemists, translational people, and then real imagers, people who worked in the labs, and tried to put them all together for a week and get them to cross-fertilize and understand what the challenges were on both sides and where maybe we could come forward. This meeting actually is going to be held now every

two years. And we are also building an interactive Website to continue discussion among the parties that are involved in this. It was a nice place and it was a great meeting. We actually got written up in Science and another long article in I believe it was the Engineering and Science Journal.

Another one that we just held recently was an angiogenesis imaging methodology meeting. This is a good example of where we had a challenge from the clinical trials people about how to use imaging appropriately as an endpoint for some of the new anti-angiogenesis therapies. And there really wasn't a good direction. So what we did is we used the Bethesda conference concept and pulled together teams. One team for CT, one team for ultrasound, one for MR and one for PET and nuclear. We had the teams basically do a research of the literature and come bring us up to state. And then when we met, we all got to take a pot shot at each other and argue about whose technology was best or if any of them were. And in fact we will publish the proceedings from this. And what they did was such great work, they basically each

wrote a chapter that brings us up to the state of the art.

What came out of that meeting though that was very interesting is that for every one of those modalities, it was clear that what we really needed to get to where we wanted to be was a new probe or contrast agent. And in some cases, those are very close to approval but have not been approved. In some cases, they are in fact further away, particularly when we get to probe type contrast agents for example.

So kind of based on that or building on that concept, it came pretty clear to us -- and some of us had been in drug development before -- that one of the problems we had is that there are probably a number of good imaging agents out there -- probes, contrast agents, whatever -- but that for various reasons they can't be brought forward even to the point of an IND. In some cases, it may be a commercial company and they feel that the market doesn't substantiate the risk. In other cases, it may be a basic researcher or it may be an academic investigator, and they simply don't understand the

process or they don't have the resources to bring forward the steps that they need to do this.

So we are coming up with an idea. This is not an approved idea yet. It has not been funded, but it is getting close. A program called DCIDE, which is Development of Clinical Imaging Drugs and Enhancers. And the purpose of this is to facilitate preclinical development of promising imaging enhancers or contrast agents and molecular probes, all related to imaging. And it could be multi-modality. It doesn't have to be one or the other. And we are particularly interested in how they might fit into future trials for agents that we might be using. The process is very similar to what is now called the Ray Process, that we call for proposals twice a year and that they be written. We would be looking for things that in the beginning had been synthesized and characterized reasonably well and in which there are steps that needed to be put in place. We would do an outside review and an inside review to match resources for those things that were scored high by the outside review.

These are kind of the basic steps in

preclinical drug development, probably somewhat simplified or significantly simplified. But if you look, we would be trying to work somewhere in the synthesis characterization and documentation and would be providing the rest of the steps or any missing steps to try to get to an IND so these could then be put into a probe library.

Now the types of things we are doing -- there is a couple of pieces that are kind of unique. One is on there, dosimetry, which can be actually done, however, with pharmakinetics. But in addition, we need to do something called imaging feasibility. And this is not a classic part, obviously, of therapeutic drug development. And the purposes of this particular component will be to look at how feasible the probe or imaging agent is in an appropriate model. If it is a tumor model, so let's say a mouse model or rate model, or in fact if it is just a contrast agent, it may be in something that is not carrying tumor. And we want to determine the things such as timing, usable dose range, imaging modality characteristics, before investing further in this particular probe with

toxicology and those types of things.

Another piece is that we know that a subset of what we will be dealing with will be radio-labeled. And so another unique aspect would be a radio-labeling program. And that could be something that is a great ligand and a radio label makes sense and we will take it from that point. It could be something someone has synthesized but has a complex labeling procedure that needs to be simplified or expedited for clinical trials. It may be building sufficient bulk. It may be a PET tracer. So this actually gets to be quite a challenge.

What we are proposing is radio-labeling centers, and they would have the potential for doing PET, even if it has not been perfected by the investigators, or to optimize labeling and distribution if necessary.

The last piece is that what we are planning to do is these agents are identified and they go through and do a successful IND and that they go into a translational probe library. And the reason we want to do that is obviously to allow them access to

further studies. Clinical trials and more importantly for the development proof of principle piece, that they could also be utilized in animal studies even though they had had approval at the level of an IND.

Phase I and Phase II, what I talked about before project would fit also into the end of this as things could move from the library into the next step in Phase I and II.

This is just some of the places basically that could feed into this just in what we know of the various programs that exist within NCI, where drugs might be utilized in this aspect for further investigation, for use of surrogate endpoints. So it is a myriad of things. In there is included the Phase I and II BIP trials and the CTEP Phase I and II contracts.

I want to end up by saying that one of the things that we are really interested in, which is a real challenge and a real problem and going to be really difficult in the regulatory scheme and that we are interested in is targeted probes that produce highly specific imaging and do integrated therapy. I

use ultrasound as an example because it is really nice for targeted imaging and therapy delivery. I know many of you have probably seen these types of things. But basically nanobubbles can be now tagged quite nicely, so that they can interact with ligands. And so you can get very specific localization. But what is really neat is you can also take the same bubbles, place them inside of a packet, and you can basically deliver a targeted drug to a site. So basically number one, you would have the bubbles or the tag going there. You would see where they were going and then you could actually, by using the ultrasound, cause the disruption by a change in the bubble architecture and deliver specifically to the site. And this has been done in animal studies and is being investigated very vigorously by a number of different companies as a very unique way. What is nifty about it is it integrates the whole thing together. But when I step back from the other side and think about the approval process for doing this, I kind of get a shudder at the same time. So it will be complex. It has great potential. And this is the futuristic things we are

looking at with a lot of different tag compounds. It doesn't have to be this. Nuclear is another one. And we now have tag MR agents that are very specific and these types of things. So that is a ways off, but shows tremendous potential.

So I think I have given you a pretty big quick but reasonably thorough overview of some of the things that are going on at NCI in this particular program. We have some really good people there. They come mostly from academia, but they are still very energetic and they are really inquisitive about how we use imaging in this form. So I am very optimistic. We are very enthusiastic and we have a lot of fun. Thank you.

CHAIRMAN DOULL: Thank you. Questions for Dr. Tatum?

DR. TATUM: Everybody wants to get out of here.

CHAIRMAN DOULL: I guess then we are ready, Jerry, to talk about the proposal.

DR. COLLINS: We will do our obligatory change of notebooks now. When I was here speaking

with you last December, I divided the potential areas into two parts. One was application -- could we have the lights on? Unlike Dr. Sistare, I do have bifocals. But I have to be able to read. There are applications related to drug delivery or pharmacokinetics, and I spoke about efflux pumps the last time, and I included some additional background material in your briefing packets here. So I won't take your time this afternoon and redo that stuff.

What I will talk about is a pharmacodynamic or drug impact on the target application. And a particular field which I want to describe, shown in the next slide, is that of the potential for imaging proliferation or DNA synthesis. In our view among the FDA staff, this is a cross-cutting biological process that has a number of potential applications. So it is attractive in that standpoint. In the preclinical domain, it is one of the universal tissue responses to injuries. And in the clinical domain, as you just heard from Dr. Tatum, there is a lot of activity going on in terms of imaging tumors. So the possibility of doing

simultaneous linkages from the preclinical or nonclinical domain into the clinical domain is not just a possibility. It is very much a funded reality and the infrastructure is there and seems to be getting better all the time.

Furthermore, we did consult with the NCI in terms of trying to avoid overlap. And as Dr. Tatum mentioned, they have a large effort in many areas including angiogenesis. They are actually funding individual RO1 grants in the area of DNA proliferation, but they don't have a program per se to develop and to compare them.

How do you do proliferation or DNA synthesis? Well, in our lab when we are looking at proliferation in cell culture, we add tritiated thymidine to the wells and look at how much tritium is incorporated in the DNA. The same thing is done in animal studies with either tritiated thymidine or Carbon 14 thymidine. In human beings, neither of those tracers is an imaging agent. So we substitute a variety of things into the thymidine to try to get imaging. We try to replicate in vivo in people what

has been done in cell culture and in animals. So there is various labels for thymidine that can be used and thymidine analogues as well, some of which are also used in the laboratory.

The horse has already left the barn. There already have been some clinical studies done using carbon 11 thymidine for imaging. It was first proposed almost 30 years ago. Over the last decade, a number of studies in different tumors have been conducted and reported in the literature. I will just show you one example of that. But I would just make the point that in terms of being able to make some links between preclinical studies and clinical studies, we have both tracks riding simultaneously.

Unfortunately, there is a problem with thymidine. The body really likes to metabolize thymidine. The body has never seen a thymidine molecule that it doesn't like to metabolize. And it metabolizes it rapidly to thiamine and then all the way down to bicarbonate and CO_2. PET imaging is a total radioactivity technique. And so if you have a tremendous amount of catabolism going on, then your

background noise is very high, and that limits ultimately the kinds of studies that you can do. So a number of laboratories, including our own at FDA, have searched for alternatives to endogenous thymidine, and what we are looking for is things that aren't catabolized but still follow the same anabolic pathway as thymidine itself.

These are some of the structures that have been reported as being used. Thymidine itself is the second one from the left. Tony Shields and his collaborators have put a fluorine in place of the hydroxyl at the bottom of the sugar, a compound three prime fluorothymidine FLT. Our lab and others have reported putting a fluorine in the two prime up or error position in the sugar, and that molecule is called FMAU. And then finally to your far right, FIAU. Iodine has about the same molecular radius as a methyl group does. And by putting an iodine in place of the methyl, you open up the possibility of a variety of iodine isotopes which could be used for imaging.

Our lab and some others have shown in cell culture one of the first primary nonclinical screens

for whether you have a potentially good imaging agent. That FMAU indeed follows all of the anabolic pathways of non-fluorinated thymidine. It readily enters cells. It is phosphorylated. It is incorporated in the DNA. In our lab, we take the DNA out and we digest it and we do HPLC and we verify the chemical identity of the molecule in DNA by HPLC.

These are three examples of different thymidine and analog compounds that have been successfully used. The first one in the upper left is work done by the group at University of Washington in Seattle and also at Wayne State in Detroit published about a year-and-a-half ago in Journal of Nuclear Medicine looking at carbon 11 thymidine itself. Even though in the year 2000, all groups have essentially moved on from carbon 11 thymidine as being an impractical agent, nonetheless, this image shows you the potential of thymidine and its analogues for imaging proliferation or DNA synthesis. The large arrow at the top shows an enormous primary lung tumor. You don't need something as expensive and complicated as PET imaging to know that this patient has a large

tumor. What you do find out in a quantitative way from this picture is that the areas of white and red are the highest areas of uptake of radioactivity in this tumor, followed by yellow and followed by green, blue and purple, and you can actually quantitate the radioactivity that you are imaging in those particular locations.

DNA synthesis is also ongoing in normal tissues, and the arrow in the lower part of the picture shows a red area, a hot spot, in the vertebral space indicating synthesis of marrow at that location. It begins to give you some idea of the advantages and disadvantages of trying to look and the possibilities in looking for thymidine.

Over to the right is a picture very similar conceptually to one that Dr. Cherry showed for gene expression experiments looking at thymidine kinase again as a reporter gene. Thymidine is the substrate for thymidine kinase. If tumors are transvected with thymidine kinase, then they will convert a lot of the substrate and trap it to thymidine monophosphate and ultimately in the DNA.

And the important thing about the picture on the right in direct contrast to the pictures that Dr. Cherry showed, this was taken in a human imager and the resolution is very poor. And the ability to find anatomical landmarks is almost non-existent. Someone had to trace a diagram of the rat around it. The kinds of studies that are being done starting today with more animal imagers out there are going to be more like the ones that Dr. Cherry showed, and you will see fewer of these crude pictures. But nonetheless, it is enough to show proof of principle for individual tracing probes.

And finally, the image at the bottom shows the tracer fluorine 18 FLT, again done by Tony Shields, now at Wayne State University in Detroit. And it shows that the bone marrow in a normal dog is a very active site of DNA synthesis. You have an excellent imaging of the spine. And somewhat a little bit of a surprise for those who don't do canine studies that the epithelial tissues in the nasopharynx are very active, which of course is one of the characteristics of the canine mammalian species. So it

shows up as trapping FLT as well.

So I think the overall message is that thymidine and its analogues are addressing a cross-cutting issue and area in which a lot of people are working in. What is my recommendation for this group to consider? In forming an expert working group, I think it should be limited to PET imaging for many of the reasons that went around the table. If we try to make it so broad that it includes MRI and optical imaging, then the various disciplines will understandably lobby for their subspecialty, and perhaps in subsequent meetings of this committee, if this is successful, then separate working groups could be formed down the line.

My own recommendation is this expert working group should consider proliferation probes for all the reasons that I have described this afternoon. But of course, the whole idea of getting an expert working group together is to get the best professional judgment of those folks.

I think the opportunity is never going to be better. The infrastructure at NIH is out there. So

investigators can join in. This is a doable project. The technology, although it may seem incredibly fancy and it is, it is here today. There is no new piece of nuclear physics equipment that has to be invented in order to be done. What we need is an emphasis on the appropriate pharmacologic targets. And it is an opportunity to move ahead. Thank you.

CHAIRMAN DOULL: Let's be sure that last slide gets in our notebook. Thank you, Jim. Well, the proposal then is to go ahead with PET/SCAN as a tool. Last time -- at the last meeting, you will recall Dr. Johnson from Duke talked to us about MRI pretty extensively. And we haven't said anything about that today. But what you are saying essentially is you think PET/SCAN is further along in terms of possibilities than MRI?

DR. COLLINS: Maybe Jim should comment. I don't think you want the individual imaging modality advocates to be pitted against each other. At this time within the FDA staff, we are ready to commit to this project and to work on it. All of us are very excited within OTR at the potential for MRI. But