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AT

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

NONCLINICAL STUDIES SUBCOMMITTEE

OF THE ADVISORY COMMITTEE FOR PHARMACEUTICAL SCIENCE

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MILLER REPORTING COMPANY, INC.
507 C Street, N.E.
Washington, D.C. 20002
(202) 546-6666

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PARTICIPANTS

John Doull, M.D., Ph.D., Chairperson
Kimberly Littleton Topper, Executive Secretary

ACADEMIC REPRESENTATIVE

John Doull, M.D., Ph.D.

CONSUMER REPRESENTATIVE

Gloria L. Anderson, Ph.D.

INDUSTRY REPRESENTATIVES

Joy Cavagnaro, Ph.D.
Jack H. Dean, Ph.D.
Jack Reynolds, D.V.M.

FDA REPRESENTATIVES

David M. Essayan, M.D.
James MacGregor, Ph.D.

C O N T E N T S

P R O C E E D I N G S

Call to Order

DR. DOULL: I would like to welcome you all to the Nonclinical Studies Subcommittee of the Advisory Committee for Pharmaceutical Sciences. If you have looked at the agenda, I think you will agree that this could be a very exciting meeting and, hopefully, a very productive meeting.

However, before we get to the meeting, we have to take care of some housekeeping duties. I will turn it over to Kimberly to handle those.

Conflict of Interest

MS. TOPPER: The following announcement addresses the issue of conflict of interest with regard to this meeting and is made a part of the record to preclude even the appearance of such at this meeting

Based on the submitted agenda for the meeting and all financial interests reported by committee participants, it has been determined that all interest in firms regulated by the Center for Drug Evaluation and Research which have been reported by participants present no potential for an appearance of conflict of interest at this meeting with the following exceptions.

Since the issues to be discussed by the committee at this meeting will not have a unique impact on any particular firm or product, but, rather, may have widespread

implications with respect to an entire class of products, in accordance with 18 USC 208(b), each participant has been granted a waiver which permits them to participate in today's discussions.

A copy of these waiver statements may be obtained by submitting a written request to the agency's Freedom of Information Office, Room 12A-30 of the Parklawn Building. In the event that the discussions involve any other products or firms not already on the agenda for which an FDA participant has a financial interest, the participants are aware of the need to exclude themselves from such involvement and their exclusion will be noted for the record.

With respect to all other participants, we ask them, in the interest of fairness, that they address any current or previous financial involvement with any firm whose products they may wish to comment upon.

Thank you.

For those of you who have not been here and used these microphones before, if the red ring is on, then, you are live, the black button is what you push to turn it on and off. You only have to touch it once, and you don't have to hold it down.

DR. DOULL: Thank you, Kimberly.

Before we start, why don't we go around the room

and identify ourselves, so we know who each of us is.

Jim.

DR. MacGREGOR: I am Jim MacGregor. I am the Director of the Office of Testing and Research at the FDA Center for Drugs.

DR. REYNOLDS: I am Jack Reynolds. I am with the Pfizer Corporation, and I represent the trade organization PhRMA on this committee.

DR. DOULL: I am John Doull. I am a clinical toxicologist from KU Med and a member of the Advisory Committee for Pharmaceutical Science.

DR. CAVAGNARO: My name is Joy Cavagnaro. I am President of Access Bio, and I represent Bio Organization.

DR. DEAN: I am Jack Dean. I am the National Director for Preclinical Development for Sanofi Synthelabo, and I represent the Advisory Committee on Pharmaceutical Sciences.

DR. ANDERSON: I am Gloria Anderson, Callaway Professor of Chemistry at Morris Brown College in Atlanta. I am a member of the Pharmaceutical Sciences Committee and I am the consumer representative.

DR. DOULL: We are going to start off this morning by hearing from Dr. MacGregor, who is going to introduce this and give us a talk about FDA objectives.

Dr. MacGregor.

Introduction and FDA Objectives

James MacGregor, Ph.D.

DR. MacGREGOR: Thank you. What I am intending to do is to set the stage for today's discussion by addressing the FDA expectations for this subcommittee of the Advisory Committee for Pharmaceutical Sciences, and also as part of setting the stage, I would like to spend a few minutes just talking about the scientific background related to pharmaceutical development and lay out some of the issues that I think FDA is facing in terms of new science and bringing new science into our day-to-day operations at the FDA, and how we hope that this subcommittee can help us focus on the important scientific issues and to address those issues that warrant particular attention.

[Slide.]

Just to begin first, very briefly, with the functions and objectives in a general sense, and then I will come back in a more specific sense at the end. Our hope is that this subcommittee can provide us at the FDA with advice on improved scientific approaches to drug development in general, drug development in regulation, and this is a function that has been served by this advisory committee for a long time, the full advisory committee.

It is an advisory committee on pharmaceutical science, and a whole range of scientific issues have

routinely come before the committee, and they have fed back to us at FDA recommendations and advice.

Why form a new subcommittee? Our objective here really is to extend that previous function of the full committee in a way that focuses it in the nonclinical studies area, the scientific issues that are facing us in the nonclinical studies area, and to also play a role in recommending to us and actually facilitating areas where it makes sense to collaborate with our stakeholders and develop collaborative approaches to these scientific issues.

So, we are hoping that in addition to just providing us with advice, that this subcommittee will be able to play a role in actually facilitating scientific collaborations that lead us to advance the science and keep us, at FDA, abreast with scientific developments in this area.

[Slide.]

I think everyone is aware that science is moving incredibly rapidly, and I think we are in an era where we are faced with unprecedented opportunities in the area of science in general.

On this slide, I have just illustrated some of the areas that I think are making a major difference in the area of pharmaceutical development. Genomics and proteomics technologies, information technology, high throughput

technologies, advances in mechanistic knowledge of biological processes, understanding of the therapeutic areas related to cancer, inflammation, cell signalling, tissue damage, and so on, have advanced really dramatically in the last decade or two.

Artificial intelligence, imaging technologies, all of these areas are making, and will continue to make, I think an accelerated impact on the drug development process, and we at FDA need to be aware of what is happening in these areas, we need to keep abreast of them, and we need to bring this information and technology into our processes at the FDA.

[Slide.]

Now, just to focus a little more specifically, I have just picked out one example from this list, which is the area of the safety assessment, just to give some more detailed examples of some things that I think are happening in the safety assessment area and issues which I think we should be addressing at the FDA, and I hope I will set the stage for some of the issues that we will be discussing in more detail later today, in areas where I hope this subcommittee can help us in addressing these critical areas.

[Slide.]

One area in the safety arena that is getting lots of attention these days are biomarkers and the potential for

new biomarkers of tissue damage. This is one of the topics we will address today, we will hear about in more detail, and I thought I would just set the stage by setting out the biomarkers that are currently used to assess general systemic toxicity.

I think these fall into a small number of general classes, which I would call markers of cellular integrity, tissue constituents that are released upon damage to cells, such as AST and ALT, and so on; markers of homeostasis, things that we can measure that tell you that either cellular or tissue systems are functioning as they should be to maintain homeostasis. These would be measurements of things like BUN, creatinine, electrolytes, and so on.

Of course, pathology, morphologic evidence of damage, and, in a general sense, host defense responses, and, in general, the host defense responses that we are currently using as markers of toxicity are things like host defense cell infiltration into tissues and pathologic response to damage in tissues and cell systems.

Then, of course, there are some other general markers, such as how the animal is growing, behaving, and so on.

[Slide.]

Now, this slide really illustrates the same thing, just rearranged into the way we actually do safety

assessment in the nonclinical arena. We make measurements in clinical chemistry and hematology, and these include the type of markers that I just enumerated.

We do histopathologic evaluations, and these really include both the morphologic change in tissues, as well as the host defense cell responses in tissues, and then we do some special testing which, by and large, I think is not going to be our initial focus, but as we proceed with this subcommittee we may get into some of these other special areas, but I think in early discussions we decided that we should try to stay focused and address a relatively small number of areas where we could initially focus and see how the committee work, and our focus we thought should be in the area of general systemic toxicology in the safety arena.

[Slide.]

I think there are a tremendous number of opportunities for improved approaches to safety assessment. This slide summarizes some of those opportunities. I think we have the potential to develop new classes of damage, specific responses molecular responses to specific classes of macromolecular damage in cells.

We have the opportunity to extend the technology of host defense cell reactions by using our current knowledge and technology for identifying cell signallings,

cell surface markers to identify specific sets of cells, and so on, to refine the classic pathologic approach with some of the new biochemical markers that could be applied.

I will give an example of how I think there is an opportunity to reexamine the current marker set for integrity and homeostasis, and to consider whether there are better, more tissue-specific general markers for integrity and homeostasis that could be used.

We know a lot about the cell death process now, and there is the potential there already you can go out and buy kits to measure cell apoptosis in various ways, and we know a lot about that process which presents the potential for new markers of cell death processes.

We have learned a lot about cancer, mutational damage. We know how to measure mutations in vivo, and so on, so there is another whole area for new markers to supplement the carcinogenesis area.

As I have said, I think we have already made the decision not to address this area initially. Noninvasive technologies, I have mentioned the whole genomics area has given us the ability to develop new transgenic models with humanized characteristics, and then cell culture technology.

So, there is an enormous range of opportunities, and the question is with the limited resources that we have, what should we be doing about these opportunities.

[Slide.]

Now, as I said, one that has really gotten a lot of attention, one we will discuss is inducible biomarkers, and on this slide I have just summarized what I think we have learned, and I think what we have learned in the last decade or two is that all living organisms have actually evolved systems to protect and repair the major macromolecular systems in the cell, and that these defense systems are often inducible and that, in fact, functional molecules in general tend to evolve from function to repair evolutionarily.

These key defense systems are obviously important because they are rigorous conserved across organisms, and so I think understanding these systems has the potential to provide a new generation of surrogate markers for monitoring damage.

So, the question is what should we be doing, how should we be addressing this issue.

[Slide.]

This is just an example of one class, the molecular chaperones and proteosomes that are highly conserved, that play a normal physiological role in many biological processes, in making proteins, assuring that they are folded, exporting them, degrading them when they are not properly conformed after they have been made, antigen

presentation, and so on, and then we have learned that these have subevolved into defensive mechanisms, so that when proteins are damaged by a whole variety of different mechanisms, that these classes of molecules can be induced and degrade the damaged proteins, so they can be gotten rid of. So that is just one example of a potential biomarker for a class of damaged generalized protein confirmational damage.

[Slide.]

There are lots of these known. There are analogies that have to do with not only protein structure, but DNA integrity, redox balance within the cell, generation of reactive oxygen species within the cell, and a lot is known about the biochemistry of these pathways, and they converge in some common ways through growth controlled genes that could be measured, that could monitor even a wider range of types of damage.

So, the question is can these be used, how should they be used, what should we, as an agency, be doing to consider the science and bring the science into our day-to-day regulatory activities.

[Slide.]

Now, there has been lots of emphasis--and we will hear a bit about this as the day goes on--about gene chip technologies. You can do lots of nifty things. You can

make chips that contain a large fraction of the known human genes. You can label them with probes that allow you to tell whether individual genes are up-regulated or down-regulated, and you can accordingly make them change color, so that in this example, for example, an up-regulated gene will turn red, those that are more or less at normal homeostasis will stay yellow, and down-regulated turn green.

You can do these things in chips in very large arrays, and you can look for patterns that tell you that, oh, yes, a whole range of, for example, DNA damage response genes have been turned on, and so it looks like there is a characteristic fingerprint related to DNA damage, for example, in this particular case.

[Slide.]

If you think down the road a little bit, about making measurements and using biomarkers in animals and extending that into clinical trials, you realize that you are not going to be able to go in and get the DNA out of a lot of tissues that we are interested in, in clinical trials, so you need to have other ways of measuring biomarkers, and there is a whole parallel proteomics technology.

This is just one example of many different ways in which specific proteins can be measured in high throughput ways and fingerprinted analogously to the example of the

gene chip.

[Slide.]

Now, I think for all these new for all these new biomarkers, before they could come into a regulatory practice, there are things that we need to define, and this is where I think this committee plays a role - what are the scientific issues and what the scientific definitions that we have to have in this area before we can consider bringing these things into regulatory practice, and I think we need to define the relationship of these endpoints to health, how they relate to outcomes in established assays.

In the case of laboratory models, we need to understand how they relate to mechanisms and things that happen in man, and we need to know something about the statistics of the process in a particular assay, how reproducible is it, can people in different labs get the same answers, how sensitive are they, and so on.

So, all these issues need to be resolved scientifically before we can consider regulatory applications.

Now, let me pause now and I will say this again at the end, it is not the focus of this committee to focus on those regulatory applications, but on the scientific opportunities to define the systems in a way that then the appropriate regulatory bodies within the center can consider

bringing them into regulatory processes.

So, the idea here is to focus on the science, things that we need to know to set the stage, so that regulatory recommendations eventually can be made and fed into the appropriate committees within the FDA, to then move forward in the regulatory area.

There are established committees that do that and that have that function, so the idea is that the scientific output that would come out of these activities would go into those regulatory committees eventually.

[Slide.]

As I have already said, we need to think even in the nonclinical I think about eventual application in human studies, and there are certain restrictions on biomarkers. I think one of the major issues in safety assessment that has been an issue for many, many years is often it is unclear how to relate the animal findings to the human outcome.

It is a major question. You learn a lot about mechanism response and damage in the animal model, and often it is difficult to determine how quantitatively different the animal is from the human. So, we need what I call "bridging," markers of bridging technologies that allow us to bridge the mechanistic information that we know in animals to the human, and so in developing new classes of

biomarkers and new technologies, we need to focus on those technologies that allow us to make that bridge, so that we can measure the same thing in laboratory models and then make key measurements in the clinic, so we can link those two and make use of the mechanistic information that we have in the laboratory models.

So, we need to think about markers that may be up-regulated and secreted, so they could be sampled in blood or plasma, or things that leak from cells that can be measured in an accessible tissue compartment, or perhaps the use of imaging technologies to, in some way, perhaps label up-regulated markers that may be linked to cells or on cell surfaces that are not immediately accessible.

I think in planning what you are going to do in developing biomarkers, you need to think about those ultimate human applications.

[Slide.]

I have already mentioned, and I am not going to dwell on this, but now I am just going to give some examples of what I have already defined as general classes of biomarkers. So, I talked about inducible responses, which could be measured either by genomic or proteomic technologies.

[Slide.]

As I have said, we know a lot about the pathology

process. We understand a lot about the chemical signals that have to do with response to cell and tissue damage, and the signaling to host defense cells, and we can use surface markers to identify those host defense cells, and this opens the potential to extend conventional pathology with the use of the molecular markers that can allow us to measure these signals and to better fingerprint the types of host defense responses that are going on within tissues.

[Slide.]

I think, as I said, there is also an opportunity to think about better markers of cell integrity and homeostasis. To think about the development of science, just to put things in perspective, I would say around the 1940s was kind of the time when modern biochemistry was developed.

Krebs, for example, was born in 1900. By the 1940s, we pretty much understood the basic biochemical pathways, and right about that time is when the conventional biomarkers of cell integrity were chosen and put into practice.

By 1950, AST, ALT, alkaline phosphatase, all these markers of integrity were in place and they haven't changed for 50 years or so. What we know now about cell biochemistry I think gives us an opportunity to address this issue and ask the question are there more sensitive and more

tissue-specific kinds of markers that we could be thinking about using.

[Slide.]

Well, one you will hear about a little later, that Frank Sistare will talk about, is marker of cardiac toxicity, the cardiac troponins which we have been working on in our own laboratories, which hold the potential for fulfilling these criteria, perhaps being more specific to cardiac tissue and more sensitive than the conventional biomarker creatine kinases that have been used as markers historically for cardiac damage.

Frank is going to talk about this a little bit, so I think I will skip over those slides of the actual responses to cardiac troponins, but suffice it to say that we have done some background work in one class of agents in rodents to shows that this model is, in fact, a useful marker of tissue pathology in doxorubicin-treatment, and the question is how general and sensitive is it for different types of damage in different classes of agents, and I think Frank is going to address this in a little more detail.

[Slide.]

So, now to come back to the committee, I think we can see that there is a tremendous scientific opportunity sitting out there, and the question is what should we, as an agency, be doing and how should we focus our limited

resources among the many opportunities that are out there.

This is what we hope that this subcommittee can do for us. We hope this subcommittee will consider these areas, will identify and recommend to us focus areas where we should be concentrating, and then we hope that we can actually move forward in a proactive way, perhaps through--and we would like also to discuss and hear recommendations about this--but in our early discussion, the idea was that we would move forward in areas where we would focus by forming more specific expert working groups, by identifying experts in focus areas where we would focus and to form expert groups using an open process where we solicited nominations from the public through the Federal Register, from the people that are on our committee, the committee itself, the agency, industry trade groups, professional societies, and so on, and then through the committee, selected working groups to focus in these areas, and then charge those groups with the definition of specific scientific endeavors which would have outputs, such as workshops and reports, and so on, that could help to move the field forward.

So, the idea would be that the subcommittee would focus as a steering committee to these collaborative projects, identify the appropriate experts, charge them and monitor the progress of the expert groups to help us focus

in the appropriate opportunity areas.

[Slide.]

Now, the collaborators that we currently have involved include representatives from CDER and CBER, representatives from two major industry organizations, PhRMA an BIO, our academic representative is Jay Goodman, who is the current President of the Society of Toxicology.

He is a member of the subcommittee and unfortunately, he wasn't able to attend today. We have discussed, because of the large focus at NIH now in the biomarker area, that we really should bring into the subcommittee a representative from NIH to give us a link to their activities in the development of biomarker, and, of course, they are involved in imaging and many other areas, as well, where we ought to have linkages.

So, our current thinking is we probably would bring in to the subcommittee an NIH representation to essentially represent the public institution constituency here.

[Slide.]

To get again back to the objectives, to restate in just a little bit more detail what I opened with, I think the objectives for this subcommittee are to recommend approaches and mechanisms that would improve the nonclinical information for effective drug development, the predictivity

of nonclinical tests for human outcomes, and improve the linkage between nonclinical and clinical studies, and to also serve this facilitating role to facilitate collaborative approaches to advancing the scientific basis of drug development and regulation.

[Slide.]

Now, just to provide a little bit of history, this subcommittee actually met at the end of August. It was not a public meeting, it was an organizational meeting to discuss the value and how it might work, and so on.

This committee actually began with the technical committee for the collaboration for drug development improvement, which is an activity that has been discussed before the full advisory committee for a number of years, and that group discussed how this committee might operate, whether it was a good idea to initiate such an activity, and define how it should go about structuring itself.

The concepts then were presented at a full public meeting of the Advisory Committee for Pharmaceutical Science on September 24th. The ACPS endorsed this concept, so that led to the scheduling of this meeting, which is the first formal meeting of the subcommittee, and then we hope that we can move forward by discussing and selecting initial focus areas, and then to set out a mechanism for forming these expert groups, and then through these expert groups, to

identify actual collaborators and resources to actually engage in collaborative science.

That is the conclusion of my remarks, and I hope sets the stage for the discussions to follow.

DR. DOULL: Thank you, Jim.

Dr. MacGregor has given us our marching orders, and it is a pretty formidable charge. I am sure many of you have questions and comments. Our plan is to hold all those until the end of the presentations this morning, and then we will have our discussion section at that time.

We move then next to a discussion of the industry perspective of this. We are fortunate to have Dr. Reynolds with us. Dr. Reynolds is a key organizer and formatter of this whole concept and was a key player in its implementation.

We are delighted to have you here, Jack. He will give us the industry perspective.

Industry Perspective

Jack Reynolds, DVM

DR. REYNOLDS: Thank you, John. I am very happy to be here.

I don't have any prepared remarks, but I just kind of wanted to set the stage where I see this committee activity going. Many of us know the rapidly escalating cost of drugs, and I think the diseases that we are trying to

treat are in part responsible for that. They are complex diseases with complex endpoints, but for many of us, this long and very expensive process frequently ends with safety concerns around the drug, either it is not approvable or it has difficulty in the marketplace, or it has very restrictive labeling that in many cases keeps this from patients that need that.

We do know that there is a tremendous wave of innovation primarily in the area of genomics/proteomics, but also in computer technology and instrumentation, and I think that with these waves of innovation, most of us see the tremendous commercial potential of these, and because of that commercial potential, a lot of these technologies and the assessment of utility of these technologies is really being driven by industry. So, I think that provides unique opportunities for FDA to partner with industry here, which they are doing.

But I think most of us know, and it is relatively intuitive, that these technologies have been utilized and really at the cutting edge in clinical trials for determining efficacy endpoints, defining both disease states and responses of patients to these drugs, and as Jim I think pointed out very well, there has been very little evolution of these technologies or new ways in which we can assess safety of our drugs, either preclinically and even

clinically.

I think that a part of the focus on safety really is the heightened awareness of, and the heightened concern of, both patients, regulators, and even industry on the safety of our medications.

So, I think again this committee, partnering with industry, academia, and other stakeholders, really can help us seize the opportunity to improve, not only the efficacy endpoints, but also improve those safety endpoints, and I think not just safety endpoints in clinical trials, but because the drug development process is so complicated, it lasts for a long time in most cases, and very expensive, I think these technologies have a real opportunity to help us make decisions around the best of the most appropriate drug for a specific indication, business decisions, but also even medical and safety decisions.

Because most of what we do in the regulated pharmaceutical industry has issues where we use the data to support our claims of safety for these compounds, there is some requirement for us to establish the validity. We need to document the data. It needs to be reviewed, it has to be demonstrated that it is repeatable, and we have to demonstrate that we have been thorough in our search for the safety and safety kinds of endpoints.

So, I think what we want to do in this committee,

as Jim again very well pointed out, is to help us evolve the scientific underpinnings that will help us make more astute decisions around the risk management of new drugs, and I think that partnering is an excellent way to do that.

I personally, in my own experience, have seen the value of partnering with regulatory agencies, and I think this will be but yet another example of a win/win situation.

So, Mr. Chair, I am very happy to let me say these few words. I appreciate it very much.

DR. DOULL: Thank you, Jack. As you point out, we seem to have made more progress in the efficacy end than in the tox end, and perhaps that is the balance we need to seek.

We move, then, into a description of these new technologies, and we are going to start with Dr. Collins, who is going to talk about the PET scan.

Positron Emission Tomography Imaging

Jerry Collins, Ph.D.

DR. COLLINS: Thank you, Mr. Chairman.

[Slide.]

Our Laboratory of Clinical Pharmacology is one of the units within Dr. MacGregor's Office of Testing and Research. John Strong and I direct this unit and we have a team of five scientists.

As those of you who have heard our presentations

at other meetings of the full committee know, our largest project is actually drug metabolism as related to drug interactions, and our recent experience in positron emission tomography is a new endeavor for us. Clearly, with the small size of our group and our newness to this field, the only way that we can make substantial contributions is through collaborative efforts, and so the whole theme of this subcommittee meeting of looking for a partnership between the academic, government, and pharmaceutical industry sectors is sort the lifeblood of our opportunity to continue to contribute in this area.

I would like to acknowledge our current collaborators, various units at the National Institutes of Health and their academic centers. Without their support and funding, we wouldn't have even got off the ground.

With those preliminary comments in mind, as Dr. MacGregor said, in keeping with the charter of this subcommittee in general, and the spirit of this enterprise, I am not here in this presentation to deal with regulatory issues that surround positron emission tomography. I am certainly not going to be discussing any specific products, not the kinds of claims for clinical utility that might be made, and I am not an authority on GMP or chemistry issues.

I think what we are all very excited about is the science underlying the field of positron emission tomography

and the way that it can ultimately serve as underpinning for regulatory decisions will play out in those arenas, but in order to develop and understand good therapeutic products, we need to focus on the science of imaging.

For those members of the audience who don't have a copy of my handout, it will be available at the table in the hallway, and if they run out, you can just leave your address with one of the FDA staff. I would be happy to E-mail a copy to you.

[Slide.]

What are the scientific issues? Well, you can't do any imaging at all unless you have a satisfactory probe of the function that you want to look at, and so the number one issue I think for nonclinical studies is more emphasis on defining the characteristics of a good PET imaging probe.

The nuclear physics are pretty immutable. There is not much we can do about the half-life of carbon 11 or 18 fluorine or the other isotopes. We know a lot about targets for drug development as a result of our discovery programs for therapeutics. What we need to do is to figure out a way to join together the nuclear physics with the targets in a way that provides information about whether the drug is impacting the target or not. We need to know a lot about the metabolism distribution, the localization of potential candidate PET imagine probes. I would say that is the

number one gap right now in terms of helping this technology penetrate further into our consciousness in therapeutic development.

Once we figure out what the characteristics are, what we need, it is a pretty complicated process in the small size of my organization or anybody's organization, it is difficult to imagine that all the different kinds of expertise are available.

Nuclear physicists and their associated cyclotrons and other fancy equipment, all the way through the spectrum of nuclear medicine physicians, PET imagers, and clinical interpretation, and in between, perhaps the most underserved discipline at the moment is pharmacologists who can make the link between their colleagues who are doing drug development and colleagues that are doing imaging.

A lot of fundamental questions we are still stumbling through and it is unlikely that the most efficient way is for everybody to do that by themselves independently, and not share their experiences. It seems very attractive to pick some common projects for a consortium and let people pool their experience.

[Slide.]

Although our organization is new to the research field of positron emission tomography, we don't think that we are starting exactly from ground zero without any

relevant past experience at all. In fact, one of the most attractive things to us about positron emission tomographic imaging is to view it as an extension of pharmacokinetics and pharmacodynamics.

Those are topics that have long been within the domain of the parent committee, the Advisory Committee on Pharmaceutical Science, and, in fact, I think it is an understatement to say that FDA and CDER have had a historic interest in the development and application of pharmacokinetic and pharmacodynamic PK/PD tools.

In fact, it would be fair to say that for many decades, long before I was there, FDA has been a leader in promoting and developing pharmacokinetics and pharmacodynamics as a tool for drug development.

So, even though we are new, we are confident that at least we understand the orientation of where these tools fit into the larger picture of drug development, particularly understanding PK and PD.

[Slide.]

When we talk about applications of positron emission tomography specifically or noninvasive functional imaging more generally, it is really helpful to split these concepts of pharmacokinetics and pharmacodynamics, although I have to tell you, having spent 20 years of my career trying to convince audiences like this that there is a real

difference between pharmacokinetics and pharmacodynamics, some of it really blurs into semantics when we are talking about noninvasive functional imaging and PET imaging in particular.

The distinction between what is really kinetics and what is really dynamics blurs, but I don't think people came to this meeting this morning for semantic discussion of the classification of PK and PD. It is what can be done with these tools that give us information that facilitates drug development.

But nonetheless, if we think of kinetics or PK as drug delivery to the target, one of the major things, major opportunities that I personally see in this area is the ability to assess delivery, particularly modulators or delivery.

The Human Genome Project has identified transporters as one of the major classes of proteins in the human genome. Perhaps up to 20 percent of the genes that we have are related to transporting something around in the body including xenobiotics, such as drugs.

There are a number of programs, very expensive, very active, very prolific to develop modulators of these transport systems. In the oncology area, it is thought that resistance to anticancer drugs is mediated in solid tumors by efflux pumps, so that as soon as the drug gets near the

target, the tumor cell, the tumor has a mechanism for pumping it out very fast and keeping the concentrations at the target very low, making them ineffective and actually promoting the development of further resistance mechanisms.

There is no way that you can study that process in vivo by looking at our traditional tools of plasma sampling and urine sampling. That is not where the action is. In fact, so long as the delivery of drugs is a reversible process to tissues, then, the things like area under the curve and even half-life will be unaffected by the presence or absence of a functioning efflux pump at a target site in a tumor.

So, we need a tool to focus directly on drug delivery at the target of interest, not an integrated global assessment of what is happening, such as we get from plasma concentration, but we have got to look right at the target, and we have to see what is happening there. That is what I would call pharmacokinetics, assessing whether the drug actually gets there.

Just because a drug gets to the tumor doesn't mean that it is going to kill it, but for sure, if the drug never gets to the target or gets pushed away as soon as it is there, there is no way that it is going to be effective, and the sooner we find that out, the sooner we can consider alternative therapy or the sooner we can implement

strategies for attempting to modulate this PK issue and for assessing whether it's actually working.

The second major category is looking for the drug impact on the target, pharmacodynamics, dynamics, or I think many people would say function, how we change the function of some particular target, and what are the targets that could possibly be imaged with something like positron emission tomography.

Well, enzymes have always been critical targets for drug development. Usually, we want to inhibit a particular enzyme that is over-expressed or that contributes in some way to the development of the ultimate pathology.

How do we know if we have inhibited the enzyme? There is no way that we know how we inhibited the enzyme by looking at plasma concentration of the drug that we administered. We have got to look at the enzyme where it is.

Oh, it's real handy if the enzyme happens to be in a red cell or a white cell, and you can take a blood sample and look at it, or it's otherwise somehow on the surface and accessible, but most enzyme targets aren't there, and we need a tool again for looking at the enzyme.

Receptors are probably the number one target for all drugs. I am thinking of receptors generally, and receptors are in very inconvenient places, like in the

middle of the cranium, deep inside solid tissues. There is no way that we can assess what is happening at the receptor even though we spent millions of dollars in our discovery program developing the perfect receptor-based assay to optimize, to get a lead compound in the clinic, and then can you imagine running a clinical program without having any idea whether you occupied that receptor, whether you changed its endogenous function, and yet, that is what we do in the absence of the ability to look directly at the receptor.

The same thing is true for processes, such as blood flow and more globally for things like energetics.

Try not to have an entire talk which is taken up by words and thoughts and speculation. Let's look at what has actually been done, what are the precedents for using positron emission tomography, particularly as it relates to nonclinical issues, what kinds of things do you have to know at the nonclinical level before you are comfortable to approach human testing that involves positron emission tomography.

[Slide.]

Well, this is an interesting study that was published in Cancer Research just a few months ago, and it is looking at rats that are implanted with human-derived tumors of two types. There is the parent line, which is called by the wonderful name GLC4, and then a subline has

been developed that over-expresses one of these transport pumps, the p-glycoprotein pump that is so effective at lowering concentrations of anticancer drugs in many tumors.

If you look at the panel on the left, what has happened is the investigators have injected a positron emission probe labeled with carbon-11 verapamil. In vitro studies of cells in culture have indicated that the verapamil, although it has what we think a lot about its other effects on channels in the body, is also an exquisitely good substrate for p-glycoprotein, and so these authors have suggested its use for probing drug delivery and drug impact at the level of the tumor.

So, if you look at the panel on the left, after giving a dose of carbon-11 verapamil, it appears that the lower tumor, there is a tumor in both flank regions of this rat, and in the upper region, the parent line, shows up very well. The dark spot indicates that the carbon-11 verapamil has localized in that tumor. That tumor has a very low expression of the efflux pump.

On the other hand, in the lower flank region, you can barely see any uptake of carbon-11 verapamil as a probe for p-glycoprotein, because it has all been pumped out. It's not that it didn't get there, it's that the tumor was very efficient at getting rid of it.

So, from a functional standpoint, you would think

this rat only had one tumor rather than two.

In the same rat, an hour later, an injection of cyclosporin was given. Cyclosporin is known to inhibit the efflux pump, P-gp or MDR, and so a second injection of carbon-11 verapamil is given because the half-life of carbon-11 is only 20 minutes, you can rapidly do sequential studies.

Most people think of a 20-minute half-life as a tremendous logistic headache, and it surely is, but the other side of the coin is before the biological system has changed, you can sequentially probe different intervention strategies, so the headache is converted into an opportunity.

So, Panel B is the exact same rat, the exact same tumors in the rat, looking at a second injection of carbon-11 verapamil after it has been modulated by a dose of cyclosporin, and all of a sudden, there is a couple of dramatic changes. It is the exact same dose of carbon-11 verapamil, yet, that lower tumor in the lower flank is now quite visible, because the efflux pump has been blocked.

In addition to drug delivery to the tumor, the rest of the body isn't just sitting there being unaffected. In fact, the largest change is seen in the head area because the blood-brain barrier, which has many mechanisms and many strategies for keeping xenobiotics one, one of them is a

very high expression of the p-glycoprotein or MDR, and when you inhibit that with the intention of treating a tumor, be concerned that you are also putting a lot more drug into the brain area.

So, if your target is in the brain, it's a win/win situation. If your toxicity is in the brain, that could be a major concern. You can find that out presumably without putting patients at particular risk by using tracer doses and by looking at the impact at the tracer level.

Nonetheless, every impact, every modulation, in addition to its positive spin, also has a potential negative spin. Beautiful study done preclinically in rats. There are some differences between the protein in humans and in rats. They have to be taken into account as this proceeds forward, but at least it is a proof of concept or proof of principle.

[Slide.]

What other kinds of applications are there? Well, I think we need to really be clear about the kinds of questions we are going to ask in the clinic, so that when we are at the preclinical stage or the nonclinical stage, we can develop probes that have characteristics that will help us answer these questions.

If you haven't done your homework preclinically, it is far too late to think about these kinds of questions

after you have started your clinical trials. So, in the pharmacodynamic domain, what we want to know is the same three questions that we have always wanted to know about therapeutics, did this treatment that we have invested millions of dollars in, that we put out press releases explaining our hopes for a potential benefit to patients, did this treatment even impact at all the target that we had chosen in our preclinical screening. It may actually be a terrific drug for some other reason, but when we are testing the hypothesis that our screening system picks targets and picks drugs that impact on the target, we need to look at that.

So, the first question we have always been interested in is that if we have an enzyme inhibitor, does this particular new therapeutic inhibit the enzyme. Our focus is on the drug, not on the probe. The probe in this case is a tool to help us understand the therapeutic.

Secondly, in an item of tremendous concern here at the agency, we are always encouraging sponsors to get the dose right. What is the minimum dose that you have to give the people to get the desired therapeutic benefit, what is the maximum dose that you can give to people before the side effect profile starts to overwhelm you.

We can answer those questions with clinical observations. We can answer those questions with very

simple, but enormously large, cumbersome, complicated and long-running clinical trials if we have no window into the fine structure of the targets. We can still get answers to those questions, but if we had a tool to use early in development, to pick one or more likely two doses that are likely to be where we want to be, then, that would really help those incredibly expensive Phase III trials that are in the A directed for the therapeutic.

Again, the imaging probe is just a tool to help us get the dose right for the unlabeled drug or therapeutic. Finally, what is the interval between doses? Well, of course, the marketing department at pharmaceutical companies always has the answer to that. It is once a day, because many studies have shown that once a day is the most convenient for the patient and the easiest to have four-color ads in medical journals, but it is not always the right answer.

Sometimes we use plasma pharmacokinetics to try to get this answer. Sometimes we are really disappointed that the half-life of the drug in plasma is one or two or three hours, and we think that means that we have to give the drug continuously or four times a day or very frequently, but the answer to the interval of how often to give the dose is not how fast it disappears from the plasma, but how long it takes for its effect to wear off at the target site, and

unless we are monitoring the target site, we have very imperfect tools, such as plasma kinetics, to do that.

If you have nothing else, then, certainly plasma kinetics are an excellent guide to at least the minimum dose interval, but it may really be too cumbersome and slow down the development and really have proven retrospectively to have been unnecessary.

[Slide.]

The second example that I want to share with you of how this technology has been used is actually from a human study, but I would like you to look at this human study through your nonclinical viewpoint, that is, to see what it was about the results that were obtained in humans that was really set up and prepared by excellent preclinical and nonclinical development program.

This is a study that is not new, it is not a couple months old. This study is more than six years old. It was published by Joanna Fowler and her colleagues at the Brookhaven National Laboratory at Stony Brook on Long Island, published in Neurology in 1993.

The target for this particular drug Lazabamide is monoamine oxidase Type B. Of course, the main place that the target is located, well, it is expressed in many places in the body including platelets. People have tried to use platelets as surrogates for brain activity, but platelets

just aren't the same as brain, and there are many reasons why it is not.

So, what the goal of this study was, was to see whether a potentially reversible monoamine oxidase Type B inhibitor was actually impacting that target at its primary site, which is located deep within the cranial vault of homo sapiens.

So, these images are from patients or actually a healthy volunteer in this case, looking at MAO-B enzyme activity. Well, how do you do that? Well, you need a probe for that enzyme. It's a well characterized enzyme. In fact, one of the therapeutic agents Selegeline or Deprenyl is known to be a mechanism-based, irreversible binder and inactivator of that enzyme.

So, by labeling Deprenyl with carbon-11, and injecting that in a tracer dose, the places where Selegeline sticks to tissue are places where, through the enzyme, it has been irreversibly bound to its target.

So, in the upper lefthand panel, you have the baseline distribution of MAO type B activity in the brain, and the color scale, which is more or less conventional, although you have to be a little careful about that, is red or white is the hottest, following by green, followed by blue, followed by darker purple, and so forth.

So, you can clearly see in the central part of

that picture where the majority of the activity is, but you can also see activity elsewhere. That is just a baseline plot.

In the upper right panel is a repeat injection while the volunteer was on a dose of 25 milligrams of Lazabamide twice a day, and what has happened. There is no red left, almost all the green is gone. More than 80 percent of the enzyme activity has been inhibited at this dose of 25 milligrams twice a day.

So, we have learned something about a particular dose and a particular schedule. Let's explore around both dose and time. In the lower lefthand corner, the dose was doubled, the 50 milligrams twice a day, and if there were a little tiny candle that was still burning in the brain that represented MAO type B activity, that candle was completely snuffed out, and there is no observable MAO type B activity.

Now, that does not tell you that you have a great therapeutic. What that tells you is that for your particular goal at the target level, you know something about the shape of the dose response curve, and if you go much higher than these doses that are studied, the probability is that you are only going to buy more toxicity, and not more efficacy, because you have already got essentially complete inhibition.

How long does it last? This was intended to be a

reversible inhibitor of the enzyme. Well, after 36 hours being drug-free, this volunteer's image of MAO-B, as shown in the lower righthand corner, is identical to what it was at baseline.

So, the half-life of pharmacologic effect is relatively short. This is indeed a reversible inhibitor of the enzyme and it would appear that twice a day, which is what was done in this clinical study, is actually an appropriate time interval. Maybe you could get away with once day, but you would get substantial recovery every day of enzyme activity, and you have to decide whether it is worth the convenience of once a day in return for the down side of having to give larger doses for the same pharmacologic activity.

This is an example of a study that answered all three questions from the viewpoint of the therapeutic, not the probe. It says that the therapeutic does impact the target, it gives us information about the dose response curve, and it gives us information about how to design pivotal clinical studies that have appropriate dose intervals. It doesn't tell us whether the drug works, just tells us that we have got a good trial design that tests the question of whether it does work.

[Slide.]

So, our hypothesis is that this kind of technology

of noninvasive imaging can make a difference. It can change how we develop therapies, and it can also help us in selecting therapies for individuals.

[Slide.]

Be clear about hypothesis testing, though. All we are doing here is seeing whether the drug impacts the target, whether it is an enzyme or receptor, or whatever. We are largely in the semantic domain called biomarkers. That is what the role is in drug development for this kind of probe.

The more important question in terms of licensing and ultimate approval of therapeutics is clinical benefit. It is not likely that biomarkers by themselves have a role in determining quality of life or increased survival. Controlled clinical trials with or without surrogate endpoints is a different area, a more sophisticated area, a more validated area I should say, than biomarkers.

I think where the opportunities lie here, although there are obviously opportunities at all areas of the spectrum, the real opportunities apply early in dose selection and dose interval selection at the level of biomarkers.

[Slide.]

There are far more opportunities than there are examples. I, in the interest of time, trimmed my collection

of other people's examples, and telling you about some of the things that we are doing in our laboratory, but it is almost an unmined area in terms of things that need to be done, but unfortunately, there is also a lot of work to be done, and that is where we need to think about some collaborative ventures.

[Slide.]

As you scan the literature for positron emission tomography, nine and a half out of every ten articles will be related to the application of fluorodeoxyglucose. As the probe, it's an overall probe of cellular energetics. It has been outstanding at jumpstarting the field, capturing people's attention, getting this launched, but certainly there is more to PET than FDG, and that more is a lot of work at looking at target systems that we have and figuring out how to design probes from them.

So, if the opportunities are there, yeah, they will still be here in 10 years, but I am certainly not interested in waiting a long time until our small lab has the ability to do one or two things, and I am very excited about discussions with this subcommittee about ways that consortia might be developed.

[Slide.]

Again, if we think about the development of a probe for imaging as complementary to the development of a

therapeutic, of building and feeding on the knowledge base that is already there, I think we will be far better off than if we expect somehow, when we start human trials, some clever person will all of a sudden think of an excellent probe. That is not going to happen. You have to lay the nonclinical groundwork in order to harvest the benefit once you reach the clinical stage.

So, the question for discussion after the break or during the committee's discussion period a little bit later this morning, is what specific ways can this potential consortium of academic, industry, and government labs work together to facilitate the nonclinical aspects of PET imaging probe development, and I look forward to joining with you folks in that discussion.

DR. DOULL: Thank you, Dr. Collins. I guess we are going to have to stop telling the students that kinetics is what the body does to the drug, and dynamics is what the drug does to the body. It is more complicated. But that wasn't bad, it lasted us quite a while.

We are going to move on now to Dr. Frank's discussion. Dr. Frank is from Sanofi, and he is going to continue the discussion of the PET scan.

I might just point out that Dr. Frank has to leave and therefore we will entertain questions and comments, and so on, dealing with his talk immediately after his

presentation.

Dr. Frank.

Positron Emission Tomography Imaging

Richard Frank, M.D., Ph.D.

[Slide.]

DR. FRANK: Mr. Chairman and ladies and gentlemen of the subcommittee, I am very grateful for this opportunity to speak and I would like to express my gratitude also to the many contributors of the slides I will be using for the presentation today. Some of those contributors, in fact, are in the audience today and might help me in answering any specific questions that you may have about the slides.

I do have a large number of slides and therefore I plan to move fairly quickly, and in that regard I would like to tell you a little something about myself. I grew up in Missouri, and we have horses. A friend of mine and I went to the blacksmith who was making horseshoes. He pulled one out of the fire and threw it on the sand to cool. My friend picked it up and put it right back again, and the blacksmith got a little smile on his face and said, "hot, ain't it." My friend said, "No," he said, "It don't take me long to look at a horseshoe."

Well, we won't spend very much time for any of these slides either. I have assumed a fairly high level of sophistication in the audience.

[Slide.]

As the Chairman said, I am a clinical pharmacologist with Sanofi-Synthelabo. I am also immediate past President of the Society of Nuclear Imaging and Drug Development.

[Slide.]

This is an outline of the presentation. I would like to begin by telling you a little bit about how I think PET can enable our work in drug development. I will give you a couple of examples in toxicology and pharmacology, which are fairly well worked out.

I will spend a certain amount of time, then, explicating the potential of some new tracers. I will discuss a little bit some validation issues, and finally summarize, and especially as regards the validation, but also the other topics, I think the speakers who have preceded me have done an excellent job of laying the groundwork, so my job should be quite a big easier.

[Slide.]

I see positron emission tomography in particular, and noninvasive imaging in general, as a relatively new opportunity which is a due to the coalescence of a number of factors. In particular, the receptor/mechanism-based development of drugs will only become more and more the case with the advent of genomics, and I will just skip right down

to the bottom, the regulatory flexibility is also a very important factor, and I think the sort of work in collaboration with the FDA, of which this subcommittee meeting today is perhaps a very good example, is very important in drug development.

Finally, I will just focus on the development of new tracers. As Dr. Collins said, it is too late if we wait until we get into Phase II and then realize we are not sure whether the drug is getting to the target, so the notion of beginning this work in the nonclinical stages is a very important for one for me as a clinical pharmacologist.

[Slide.]

Just to make it clear what is unique about PET and also magnetic resonance imaging, which you will be hearing about a little bit later, is it really carries us into a new realm, whereas, some of the older, more established imaging technologies gave us a view to anatomy and perhaps physiology, such as gallbladder contraction, these new technologies permit us to examine metabolic and molecular, as well as functional or, as you see in the handout, I have used the word "cognitive" functions in the body.

Therefore, these imaging technologies enable us to look at functions in the body which previously have been inaccessible, at least in the living, breathing human.

[Slide.]

Dr. Fischman at Harvard has made clear what are the four main areas in which PET can help us - tissue metabolism, tissue blood flow, tissue kinetics, and ligand-receptor interaction.

You see a common theme here is that we are looking at the level of the tissue, and not at the central compartment as regards kinetics.

[Slide.]

There are some advantages inherent to PET which are shared to some extent with other imaging technologies, but PET is uniquely well qualified for your purposes today, and that is, that PET is quantifiable and can be expressed in familiar units, such as milligrams per milliliter.

There is possible an exact attenuation correction because of the physics of the energy in positron emitters. The resolution can be achieved to the level of millimeters, and 3-dimensional images can be created.

Number 4 is perhaps one of the most important aspects which is relatively unique to PET, and that is the isotopes that we use, that are incorporated into the tracers, include carbo-11, oxygen-15, and nitrogen-13, as well as fluorine and iodine and others, and therefore it is possible to label literally any organic molecule, any drug, any endogenous substance, and therefore it is really quite powerful.

PET allows repeat measures because of the short half-life of the compound. This is actually a great advantage. It does create some logistical difficulties in terms of the chemistry that has to go rather quickly in order that we still have some isotope when we are done with the chemistry and ready to inject, but, in fact, the possibility of giving repeat injections over a short period of time is really quite powerful.

Another factor is because these can be tracer doses, we have minimal perturbation of the system, and it may be the injection of a tracer actually perturbs the system being measured less than a magnetic field, for example, in magnetic resonance imaging.

Then, finally, and perhaps most important to the pharmaceutical industry and to the FDA, is that because we are able to label the endogenous molecules or the drugs, the actual drug itself which is to be administered, we have perfect mechanistic relevance, and these parameters, the imaging parameters can be correlated with gold standard clinical instruments.

[Slide.]

We have already about the importance of bridging, and I would just like to emphasize, I will try to give some examples to support this, we can bridge from in vitro to ex vivo, ex vivo to in vivo, then, in vivo, rodent or primate

to the human, and then ultimately, among the clinical phases of drug development. As a clinical pharmacologist, I am most interested in translating from the nonclinical, from the animals into the human, and then providing to my Phase II colleagues relevant information about dose ranging and duration of effect.

I would just like to emphasize on other thing that Jerry Collins was talking about. We can see the clinical development in two phases. The first phase naturally should be to confirm the mechanism of action of the drug and to do comparative clinical pharmacology in which we confirm that the drug does the same thing in the humans that it did in the animals, and that action in the animals comprise the basis for the decision to develop the drug.

If we can, in Phase I, confirm that that drug is doing the same thing in the humans that it did in the animals, there is a great deal of value to that, and it is then a second step to confirm a correlation between that mechanism of action and the disease itself.

[Slide.]

This is to emphasize the possibility of bridging from in vitro to in vivo. This is a typical phosphor imaging plate, and the case that I would like to make is that positron emitters actually generate gamma radiation.

The physics of this are that the positron will

encounter an electron and annihilate, and will emit at exactly 180 degrees coincident radiation and 511 kiloelectron volts, and therefore this is in the gamma range and it can be used then in phosphor imaging plates.

What is in this petri dish, therefore, can be ex vivo tissue sample after the administration of drug, it can be tissue culture, or, in fact, it can be cell clusters, such are commonly used today in oncology experiments.

[Slide.]

Just to summarize, then, with this little cartoon, basically, about 25 years ago, in the pharmaceutical industry, we were correlating drug effect with the dose administered. We then learned about central compartment kinetics and over the past 25 years or so, we found a great deal of value in correlating drug effect with exposure to drug in the central compartment, both for toxicology and for pharmacology, and PET now gives us the potential to go to true clinical pharmacology, which is to correlate the drug effect with the concentration at the site of action. I will give you an example of that.

[Slide.]

This is a study conducted at the Hammersmith Hospital and involved ziprasidone, which blocks postsynaptic D2 receptors, and 11C-raclopride is the tracer for those D2 receptors.

[Slide.]

This slide, you will have to focus on carefully. I lifted it from an article, a publication. On the x axis, we have the time post-dose, and images were taken from 4 hours to 36 hours after the does. On the y axis, we have the occupancy of the receptors.

What we can see here is a curve defining, not the central compartment kinetics, but the actual occupancy of the targeted receptor. So, in a very small number of subjects, in fact only 7 subjects in this case, it was possible to define the time course of binding of the 40 milligram dose.

It is easy for you to understand that if I chose a particular time on this curve, I could then conduct a similar study using different doses of ziprasidone and I could define the dose range, as well.

[Slide.]

This is an example now in a rodent, a mouse in fact, a 30-gram animal, which has relatively small striata, and using a recently developed microPET imaging scanner, it is possible to use a dopamine transporter agent, WIN-35,428, and we can image the striatum in a mouse, which as you can easily imagine is relatively small, and the resolution therefore is quite excellent.

[Slide.]

We can also do whole body imaging, whether FDG or with another tracer.

[Slide.]

This is an example now going from rodent to human. This is the same compound that WIN-35,428 imaging the striatum in the human, and you can see that the signal-to-noise is really quite excellent. Therefore, using this agent, it is possible to do studies in which, in the human, I used virtually the identical protocol design as was used in the rodent in order to investigate drug action.

[Slide.]

I would like to expand a little bit so long as we are on the topic of dopamine transporters and the striatum, and this is my toxicology example for today, and so I will dwell on this a little bit. There are a couple more slides to go with this.

The MPTP story is a toxicology story, but you may also think of it a quality control story. The chap who did this was trying to make a drug of abuse in his garage and unfortunately, he made a neurotoxin instead, and therefore a number of people suffered neurotoxicity as a result and bilateral Parkinson syndrome, and people died in fact.

On postmortem, they found that the striatum had suffered damage, but it wasn't clear what was the mechanism, and, in fact, now in a noninvasive way, we can administer

MPTP to the primate having imaged the dopamine transporter before administration, and then after a unilateral administration of MPTP, we can demonstrate in the living primate the destruction of the striatum.

[Slide.]

This is an example, not in the primate, but in the rat. On the lefthand side, we have the WIN-35,428 compound again, imaging the striatum, the dopamine transporters in the striatum. This is pre-administration. This is not MPTP, this is 6-hydroxydopamine model, but it is essentially the same lesion.

So, unilateral administration in the internal carotid artery has resulted in a lesion, a unilateral lesion.

On the righthand pair of panels, we have imaging by raclopride, which is a postsynaptic D2 receptor, and you can acutely there is actually an increase in the amount of dopaminergic activity.

So, it is possible to look at more than one aspect of the striatum.

[Slide.]

In fact, I have listed here on the slide that pitopride [?] should be over here where it belongs in the column under PET, but using this range of tracers, it is possible to look at the postsynaptic D2 receptors, the

vesicular transporter. It is possible to look at the activity of dopamine decarboxylase using fluoradopa, and I am also going to show some more recent examples with fluoro-meta-tyrosine, and we can measure also, as I have mentioned already, the dopamine transporter, so it is possible to look at all aspects of the dopaminergic neurotransmission.

[Slide.]

This is with fluoro-meta-tyrosine. In this model, the primate was administered MPTP unilaterally initially, and that has caused the unilateral lesion here, and I should mention that contralateral and ipsolateral in this case refers to the side on which the injection was made, on the side on which this Parkinson syndrome has occurred.

So, the initial injection, ipsolateral, destruction of the striatum and then there is administration over a period of time systemically intravenous administration in order to cause destruction also of the contralateral side. Therefore, this has proven the utility of this model, this MPT administration model as a model for Parkinson's disease.

So, here we have an example of something which began as a toxicology example and has now migrated to the development of a model for the studying of disease and therefore also the treatment of that disease.

[Slide.]

Just as one more example, gene therapy, as you know, is burgeoning and in order to study the effect of the gene, it is possible to link the intended gene to a marker gene, that marker being something which can then be imaged, and therefore, in a noninvasive way we can determine whether the gene therapy was successful and where in the body it is successful and also the duration of that activity.

[Slide.]

Now, this is a slide I have lifted from an earlier presentation. It is a little bit hard to read, so I will help you. Regarding surrogate endpoints, it lists three main categories. It can either be a complete failure as a surrogate endpoint, it can be a partial success or a complete success.

Just as one example of a failure, it may be that we are measuring the intended action of the drug, but that action of the drug is not really connected to the disease therapy, successful therapy, and therefore, the biomarker may have been useful. We may have correctly measured the mechanism of action of the drug, but as a surrogate endpoint it failed because that biomarker is not relevant to the disease process.

If I go all the way to the other end of the spectrum, it may be there is a perfect one to one correlation, that there is only one mechanism of action.

That mechanism of action is relevant to the disease, and if we measured accurately, we have a perfect reflection of the potential efficacy of the drug, but there is a category beyond that, and the category beyond that is one in which the efficacy of the drug cannot adequately be measured by the existing clinical instruments. I will give you an example of that.

[Slide.]

I am referring now to Deprenyl or Selegeline. Jerry Collins had mentioned this, as well. Selegeline is intended for treatment of Parkinson's disease, and the question was whether this is only symptomatic therapy or whether instead it is actually modifying the disease. This is a big difference in terms of labeling, whether symptomatic or disease modification.

So, for lack of imaging prior to the availability of this technique, the researchers decided that they would administer the drug for a period of time, measure the improvement of symptoms, withdraw the drug, and then after the drug had disappeared from the central compartment, if the symptom improvement continued, they would assume that there was a disease modification benefit to Selegeline.

They did the study. They washed out the Selegeline and found that the symptom improvement continued for almost two months, but gradually, after two months, the

symptoms returned to what they had been.

Now, the explanation for this can be understood from noninvasive imaging. The fact is Selegeline is an irreversible inhibitor of MAO-B, and in fact, then, the time for return of symptoms, which was about two months, is directly related to the synthesis of new MAO-B, and that synthesis of new MAO-B could be measured using labeled Deprenyl. So, using the classical instruments led people to the wrong conclusion, that this may be disease modification.

There is another point here, and that is, the site of action is actually in the brain, and that is where we should be looking for the duration of effect of the drug, not in the central compartment.

[Slide.]

I would like to talk a little bit about an MAO-A inhibitor which has been developed by our colleagues at Synthelabo. As you can see from this slide, Befloxatone is selective for MAO-A.

As you can see from this slide, it binds in regions of the brain where we expect high concentrations of MAO-A. The cerebellum is a nonspecific area of the brain, and so it is used as a baseline, and you can see once again that kinetics in the brain are quite different than kinetics in plasma.

[Slide.]

Moreover, in this slide, we have administered the Befloxatone at the label, and you see the uptake as expected, and then at this time point, there was injected a large dose of Befloxatone, and we find that depending on the dose of the Befloxatone injected at this point, 0.02 milligrams per kilogram, up to 0.4 milligrams per kilogram, we find displacement of that tracer, and therefore, the performance of this tracer is quite good and reversible.

[Slide.]

The specificity was confirmed by administering an MAO-A inhibitor, moclobemide, and finding complete displacement, and administering an MAO-B inhibitor, and finding that it did not displace any of the drug.

So, now what we have is the possibility of using Deprenyl to measure MAO-B activity, to use Befloxatone to measure MAO-A activity, and therefore, the situation is not unlike what we encountered with atypical antipsychotics.

[Slide.]

As you may recall, clozipene came along and we couldn't figure out why it was efficacious at such low doses when it didn't give the same D2 binding as haloperidol and others, and the answer was that it binds also serotonergic receptors. This provided feedback from the clinic into the preclinical area for drug development, so we now knew that we could look for atypical antipsychotics defined as a

balance between the D2 and the 5HD2 antagonist.

[Slide.]

This cartoon shows the effect of amphetamines to block the dopamine transporter that I was talking about earlier, and the net effect of blocking the transporter is to keep more dopamine in the synapse, and therefore, that dopamine competes with the administered 11C-raclopride for binding at the postsynaptic D2 receptors, and we get less of a signal when we administer amphetamine.

The important fact here is we are now measuring the downstream effect of the administration of a drug which acts upstream.

[Slide.]

You can see from this slide, which Bill Ackerman has given to me, that we have administered 11C-raclopride and found the typical curve of binding, as would be expected, with bright spots in the striatum.

We then administered amphetamine at this point. It caused the expected blockade of the dopamine transporter, increased the dopamine in the synapse, and displaced raclopride from the D2 centers. Therefore, we have effectively measured the downstream effect of amphetamine.

[Slide.]

We carried this model one step further using instead ketamine. We can find that not only can we assess

the effect in the postsynaptic D2 receptor with the 11C-raclopride, but it is possible in the living, breathing human to correlate this also with the advent of schizophrenic symptoms. Therefore, you can see that the development of clinical models using this noninvasive imaging has really a very great potential.

[Slide.]

To summarize what I have said up to now then, I think that PET really satisfies all the major criteria for clinical pharmacology tools. It is noninvasive, minimizes risk, and also minimizes the perturbation of the system.

Because of the short half-life, it is permitted to repeat assessments within subject, and this helps to control variability, reduce the number of subjects in the trials.

We get objective results and it helps to minimize the bias of subjective assessments. It is specific and sensitive, it is relatively inexpensive although any single scan may seem to you to cost more than sending off a blood sample. In fact, if we can do this in small numbers of subjects and get accurate data very quickly, it is obviously very efficient.

The mechanistic relevance is quite important, and having results quickly is also quite important.

[Slide.]

Now, I would like to move to an area that is a

little more speculative because these tracers are not fully validated, but it will give you an idea of the breadth and scope of the potential for PET. I will use an example of androgens.

[Slide.]

In this case, 5a-dihydrotestosterones are labeled in a couple different positions, and it is possible then to label the prostate in the rabbit.

[Slide.]

Perhaps even more useful, this is a baboon, and we can see not only the prostate, but also the bulbourethral gland and the corpus spongiosum. So, clearly, androgen receptor-positive tissues can be imaged by this.

Those androgen receptor-positive tissues may include also cancer tissues, and therefore, we can determine the receptor positivity of tumors without the need to take a biopsy.

[Slide.]

This example is a little more complicated, so I will spend just a moment on it. We have two different complexes of copper. PTSM will be taken up and retained in normoxic cells, and ATSM will be taken up and retained in hypoxic cells.

Therefore, if we administer the two, we will find the normoxic tissues will light up with the one, and the

hypoxic tissues in the other, but, in fact, these can be administered simultaneously because we can use two different isotopes of copper.

[Slide.]

The one isotope, copper-60, has a shorter half-life and 100 percent of its radiation is beta or positron emission. Therefore, because of its shorter half-life, the image, an early image after the injection of these two, will be dominated by the copper-60, which is complex with what will be taken up into the normoxic tissue.

The longer half-life compound, the copper-64, then binds to the complex which is taken up into the hypoxic cells, will dominated the later images.

Therefore, in the same tissue, in the same experiment, you can get an early image showing the normoxic tissue, a later image showing the hypoxic tissue.

[Slide.]

The hypoxic also is important in cardiac tissue, and this is a similar experiment in which there is an artificial model of ischemic in the apex, and you can see that the binding of the hypoxic tracer greater than in the normoxic tissues.

[Slide.]

Now, just a qualitatively different situation here. The carbon-11 label and the hydroxyphenylalanine is

put in two different positions. In the one position, it is metabolized off by the action of dopamine decarboxylase, in the other situations it is retained, and therefore, we can get an estimate of enzymatic activity by comparing the results of these two scans.

[Slide.]

So, the general point that I would like to make as a clinical pharmacologist is that there is a continuum of value added possible with PET, and if we begin in the nonclinical, the preclinical development as a biomarker, then, that same marker will be available as a mechanistic intermediate for me to use in Phase I.

It can be used also perhaps as confirmatory evidence, not so much as a surrogate marker, but instead, to contribute to the assessment of efficacy and perhaps achieve the desired result in a single Phase III study rather than requiring two, Phase III studies, and then finally, they do have potential as surrogate markers.

[Slide.]

I think that PET also has the potential to get early into the human, and this would be a great leap forward in terms of drug development. If were able to screen our drugs on the basis of clinical data rather than nonclinical data, the obvious relevance of the correct species would benefit us a great deal.

[Slide.]

I think that in order for this progress from the biomarker in the nonclinical stages to the mechanistic intermediate, and so on, in the later stages of clinical trials requires a great deal of validation work, and this may be an area in which the subcommittee can facilitate clinical studies quite dramatically.

I won't dwell on all these issues, it is quite a long list, but the main point is there is really a great deal to be done in the validation of a marker before it can effectively be used in multicenter Phase II and Phase III studies.

[Slide.]

This is a longer list still. These are less absolutely necessary, but certainly contribute to the utility of the marker in Phase II and Phase III. These are in your handout, so I won't dwell on them, but once again, these validation issues are something that perhaps the subcommittee could facilitate.

[Slide.]

Just as an example of the validation situation, we are all accustomed to thinking of the validation of an assay, are we measuring accurately what we set out to measure, but when we get the correct answer to that question, we are not there yet. We are not there yet in

terms of clinical studies, and this is a good example.

This first reference is to the approval of PET scan using FDG for diagnostic procedures in cancer, and despite the fact that significant additional clinical data in these two publications, still it is necessary.

Tony Shields is working with CETP and SWOG to actually validate the use of FDG to assess the success of therapy, and that is different than actually determining whether or not it has diagnostic utility.

[Slide.]

The study is relatively simple. PET imaging before and after a series of treatments, known positive treatments, chemotherapy, plus or minus the PET scan, and the obvious downstream objective is to correlate the PET results with the efficacy outcomes in order to determine whether the eventual efficacy, which comes months or perhaps even years later, whether that could have been predicted within a few weeks of the completion of the course of therapy by looking at FDG.

So, this answer will be coming.

[Slide.]

There are some disadvantages to PET. Some people don't like radiation exposure. That is a time worn issue that we are all familiar with, I won't dwell on it.

The time to develop new tracers, if I am asked as

a clinical pharmacologist to help determine whether the drug is crossing the blood-brain barrier during Phase II, it is too late, because I have to then spend a year or so developing the tracer, but if instead we have begun the development of that tracer in the nonclinical stages, then, the possibility of the utility in clinical studies is huge.

The validation, I have just mentioned. This is another area, Item No. 4, in which the subcommittee may be able to facilitate things, certainly the NIH can, and that is the infrastructure required.

I would like to emphasize not so much the hardware and the software, but the training and experience, because if the industry does actually take up these imaging technologies, there will very soon be a shortage of the intellect necessary to make a go of this.

Somehow we need to train physicians in the research disciplines, and we need to train researchers in the clinical disciplines, as well as a bit of physics, in order to really optimize the use of these new possible methods.

In that regard, to address the training issue, the Society of Nuclear Imaging and Drug Development, in conjunction with Lehigh University, will be putting on a teleconference, Distance Learning they call it, and we will have four, 2 1/2-hour sessions on the fundamentals of PET,

as well as some case studies. People will attend by videoconference in their local institution. The FDA have agreed to participate. The Continuing Education Department is on-board with that. Also, the pharmaceutical industry and academicians can participate in this in their local videoconference facilities.

It is planned for the 20th and 21st of March, and you can find details on our web site.

[Slide.]

Finally, the issues for industry and academia, and in this case the subcommittee, as well, include the obvious proprietary concerns. I think the industry is delighted to participate in this, but they do have legitimate proprietary concerns that need to be addressed by way of technology transfer.

There will be potential confusion of research use with diagnostics, and I think each of the other speakers have addressed that tissue today. We are not talking about approval of tracers for diagnostic use, we are talking about development of those tracers to study the action of drugs.

To a certain extent there is a lack of a common lexicon, and I think that would be a common starting point for any subcommittee to make sure we are all using the same words to mean the same things, but there is another issue which I think we will always struggle with, and that is, if

we are doing cutting edge science, if we are breaking new frontiers to crack the diseases which remain, then, it is inevitable that we will be in unchartered or unvalidated waters, and somehow we have to find a balance between the amount of validation necessary for these results to be acceptable to the reviewers at FDA, and yet, on the other hand, truly be on the cutting edge of developing new medicines.

There is the inevitable fear of guidelines and labeling, so I think the subcommittee could quite logically keep in mind that anything you write down on paper, the regulatory departments will be reading and thinking, oh, no, there is another element, another hurdle to approval, so we have to be a little sensitive to that.

There is the inevitable interaction with the development plan. If we propose that a method be developed in parallel with what is already a fairly expensive and time-consuming drug development program, then, the sponsor may decide the additional time and money required to develop the methodology is not warranted since otherwise they still have to do the full clinical trials program.

Then, obviously, we have to assess carefully the value added as measured against logistics and the costs.

With that, I would like to conclude and thank Mr. Chairman and the subcommittee again for this opportunity to

speak.

DR. DOULL: Thank you, Dr. Frank.

As I indicated, Dr. Frank has to leave, and so we are going to allow questions and comments at this point from the subcommittee.

Does the subcommittee have questions, comments for Dr. Frank? Jack.

DR. DEAN: Richard, what is the lead time in developing these probes on average?

DR. FRANK: The lead time depends primarily on the chemistry. If the chemistry is relatively straightforward, meaning that we can incorporate the label at a late stage in the synthesis of the compound, then, within a year we can have a tracer into the human, in fact.

The amount of time to fully validate, then, of course, depends on what the application will be. If the chemistry labeling is more complicated, in fact, there are some compounds that we will eventually give up on, but that chemistry could take a year or a year and a half in itself.

So, I would say at the near term, from the decision to start to getting into the human, around a year, and, in fact, Merck has recently achieved that.

DR. REYNOLDS: Two questions around validation. Are there particular things that one could do as guiding principles or practices to facilitate validation of some of

these PET probes, but also I guess keeping in mind what you emphasized, the need to keep pushing the envelope here, so are there principles or concepts we could think of?

The second question is what group do you think we could best partner with here to advance PET and other aspects here of imaging?

DR. FRANK: Well, to answer the first question, in fact, the Europeans already are working in a bit of a consortium although it is limited to academicians, to try to define standard ways of using WAY-106-35 to characterize the 5HT1A receptor.

The problem there has been that each of the institutions use their own image reconstruction software, their own time collection, scan durations, and so on, and so they would publish on ostensibly the same issue, and yet there were so many methodologic differences that there were more than one possible explanation for differences in results.

So, at one level it is possible for the academicians to simply get together and agree that this specific activity should be used, this duration of scan should be used, and this particular image reconstruction should be used, and so on, and despite the fact, just as another example, despite the plethora of FDG studies, it certainly is the case there is more than one way to

reconstruct images even from FDG, and that may need to be defined by disease basis. Image reconstruction for cancer might be best done one way, whereas, for flow studies in the brain, perhaps it would be done a little differently.

So, I think simply to nail down a consistent methodology would be the logical first step, and then whether you needed validation guidelines or not to go forward from there, I think is something for this committee to work out what that would be.

To answer your question about with whom should you partner, the Society of Nuclear Imaging and Drug Development, our mission statement, as you will see in the brochure, is very similar to the objectives for this particular subcommittee. I am sure we would be thrilled at the opportunity to continue our participation.

Beyond that the stakeholders are going to be the industry and the academicians who currently represent the reservoir of the hardware, software, and intellect at this point.

Is that the answer to your question?

DR. REYNOLDS: Thank you very much.

DR. DOULL: Dr. MacGregor.

DR. MacGREGOR: Unfortunately, you will be leaving before the end of the day, but I hope by the end of the way we will be able to come back to the general question of

where should FDA be focusing at this point in time considering the many different opportunities and the limitations of our resources.

So, since you won't be here at the end of the day, I wonder if you have any thoughts on that. Considering the broad range of opportunities that now are available to us in the nonclinical area, is PET one of those areas where we should be focusing our resources, and specifically, what areas within that do you think are the most fertile to be addressed at this point in time?

DR. FRANK: Well, I will resist the temptation to mention specific diseases or specific toxic effects, but the general approach, I think in toxicology, is the same that we take in the clinic for efficacy, and that is, first, define what is the clinical question, and if the clinical question is easily answered by drawing a blood sample or by measuring heart rate, then, this is not something for which we need a noninvasive technique like PET.

If, on the other hand, there are unanswered clinical questions, or in the case of toxicology, unanswered safety issues in animals, then, these are examples of something we should develop the technology for.

To just expand on that a little bit further, I can easily imagine a situation in which a toxic effect is identified in the animals which is both serious and

irreversible. In this case, the FDA may be not very anxious to permit the clinical development to begin, and there would either be a clinical hold or the sponsor wouldn't submit in the first place.

If, on the other hand, the very great sensitivity and the noninvasive nature of PET would allow us to detect the beginnings of that toxic effect, if we understood the mechanism of action of the toxicity, if we are able to detect that in its subclinical stage and therefore terminate the dosing when we early detect that in the human, it might be possible actually to study a drug in a very important disease purely because we are able to detect that toxicity before it becomes unacceptable and irreversible.

Is that the sort of question you are asking?

DR. DOULL: I think Dr. Frank has given us an excellent start here. He outlined Fischman's things that PET can do and the advantages as he sees them, and others see them, of this technique, and he, I think in his concluding slides, laid out pretty much what we will be talking about later in the day.

I might just mention one thing, and that was the Deprenyl studies. You mentioned the studies with Deprenyl as separating out the dynamic versus the kinetic effects of that agent, and pointed out that because it was an irreversible effect on binding, that the kinetic

considerations really fell by the wayside, if you will, that really what is the critical determinant in that clinical situation was, in fact, that irreversible binding, and so it's pharmacodynamic rather than pharmacokinetic which is the critical determining factor. Is that correct?

DR. FRANK: Yes, sir, that is correct, and I would emphasize further that even if there is not an irreversible binding of an enzyme, it still can easily be imagined that accumulation in the brain or the half-life in the brain might be quite different than it is in the periphery.

So, this just emphasizes the importance of looking at the kinetics at the site of action whether it's the brain or the kidney or the prostate or muscle.

DR. DOULL: Does anybody in the audience have a burning question? We are running a little late, but since we won't have Dr. Frank available--I think in that case, then, we will go ahead and take our break.

Why don't we come back at 20 of.

[Break.]

DR. DOULL: Before we start, there was one question which Dr. Frank wanted to--it had to do with your question, Jack.

DR. REYNOLDS: The question I asked was who were those persons or organizations, professional, academic, or otherwise, that we could partner with in terms of PET

methodologies.

DR. FRANK: In my answer to that question, I mentioned a number of groups including academia. I failed to specifically mention the National Institutes of Health, and I think they have a very great potential to contribute to this process, and I congratulate the subcommittee on their intent to add an NIH member to this subcommittee.

Thank you for the opportunity to clarify that.

DR. DOULL: You may have noticed if you looked at the Backgrounder, that the Backgrounder does biomarkers in somewhat a different order. We have changed the order of the program somewhat to accommodate our speakers.

We will move now into the noninvasive imaging section of the program, and we will start that off with Dr. David Lester. He is a team leader in Neuropharmacology Research for CDER, Division of Applied Pharmacology Research.

Dr. Lester.

Magnetic Resonance Imaging

David Lester, Ph.D.

DR. LESTER: Thanks very much.

[Slide.]

I am going to be giving you an introduction to an application of magnetic resonance imaging which I consider has tremendous potential for the drug development process.

I will be followed by Dr. Allan Johnson, who is going to be presenting some spectacular examples which I think will convince you really of its potential.

MRI, its unquestionable impact on the clinical sciences, is well known to you all. It is interesting, in a recent issue of Biophotonics, I saw that over the next five years, they expect that diagnostic imaging instrumentation, the sales of it will double to a value of about $16 billion, of which the largest increase is going to be in MRI.

[Slide.]

Why MRI, why has MRI been so powerful? As I mentioned, its unquestionable impact in clinical science is well known, but in terms of some of its characteristics, have really reached these results, is the noninvasive nature of it, the fact that we can image soft tissue.

The data that we obtain is intrinsically three-dimensional. We can look at both intrinsic and extrinsic activities. In intrinsic, we can look at the proton in NMR in particular, extrinsic, gadolinium is a very good example. It is used for blood flow measurements and vascular responses.

Pulse sequences, these are the different scanning protocols that can be used, and there is a tremendous variety of them, which I am not a physicist, and I wouldn't dare to go into, but you will hear terms like T1, T2, echo,

FSE. All of these things refer to the different pulse sequences, and that really is sort of what gives MRI so much power.

The clinical applications are obvious, I don't even need to go into that.

The ability for quantitation is a new direction that is going on now, and it is beginning to develop and radiologists are beginning to recognize that they can analyze their data and do quantitative analysis, not only those beautiful images that they have been looking at for many, many years.

Lastly, it use as a tool for diagnosis and drug development. It has primarily been used for efficacy, and as Dr. Frank and Dr. Collins talked about earlier, it is the applications in imaging have generally been in efficacy. Toxicity or safety has been neglected, and it has even called, to some degree, a dirty word.

[Slide.]

I would like to turn your attention to an application or an extension of MRI, called magnetic resonance imaging microscopy, and I will often refer to this as MRM. Magnetic resonance imaging microscopy was first applied about 16, 17 years ago, and I would say in the last 10 years, we have seen a spectacular increase in its application and in its potential, and a lot of this is

actually due to Dr. Johnson's lab and his center for in vivo microscopy.

[Slide.]

What I would like to focus on today, one of the applications with MRM is its use in pathology, and the reason why I would like to bring this to your attention is due to a number of concerns that I have been posed with by the reviewers in terms of pathology analysis when they receive INDs and NDAs, and we believe that MRM can answer a lot of these questions.

As a neurotoxicologist, one of the major issues we hear from reviewers is that when they receive data for neuropathology, it is usually one to five sections of the brain, and a lot of the work I am talking about is all preclinical, needless to say, and those one to five sections will be coronal sections. They will be cut in this direction and this plane.

That is the second issue here, they are only obtained in one plane. Obviously, when you cut a sample for pathology, you have basically destroyed its intrinsic integrity. So, these is one to five sections.

There is a concern there in that maybe they are not getting a section or they have not looked at a section which is showing where a potential lesion or a potential adverse effect is occurring.

Secondly, the section of the plane, can you visualize a lesion in that particular plane or is it better visualized in another plane?

The third thing is which stain. There is a plethora of stains out there. Generally, H & E, the Nissl stain or the standard stains. Are those stains capable of detecting the potential lesion?

I mentioned previously the destruction of the intrinsic structure.

Then, another issue is how do you extrapolate that from the animal to the human. Also, there is the fixed tissue biopsy where you are taking the issue out, you are doing ex vivo analysis for a lot of the pathology. You are not going to do the whole animal.

So, these are major concerns that the reviewers have brought to our attention, and I think based on that, we began to search for potential technologies that could help us in providing answers and satisfying these issues.

[Slide.]

MRM as a tool for pathology. Until today, it has been used analyzing water and the distribution that changes the property of water. Water, as you know, is the most abundant biological molecule in any organism or organ system, so it is actually a very good marker to use.

It is also going to be a very sensitive marker.

Now one of the problem with MRI is its intrinsic lack of sensitivity. You need millimolar concentrations to detect things. If you are looking at water, you have those sorts of concentrations, so you can get around that issue.

Imaging microscopy is also nondestructive. You don't have to slice and section the tissue of interest.

Multiplanar, because it is intrinsically three-dimensional like MRI, you can look in any plane, and I will show you some examples, and Dr. Johnson will go into that further.

The images that you obtain are intrinsically digital, which is wonderful for image analysis, very, very powerful, and it allows ultimately automated and quantitation of the system.

You can do it ex vivo, you can do it in vivo, and again you will see examples of that.

It has the potential of detecting adverse effects. It has the potential of monitoring toxicology. One of the things about MRI is it monitors changes in structure, and structure is usually considered to be the best indicator of some toxic type response or the strongest indicator I should say.

[Slide.]

I would like to just provide you an example of a study that we did together with Dr. Johnson, and I think

this is an excellent example of how MRM is very, very powerful.

It is a study on the effect of an excitotoxin, an established excitotoxin on rats. This excitotoxin is very, very problematic as it is with a number of other neurotoxins. This excitotoxin can induce convulsions, and it can induce lesions of various forms.

The appearance of a lesion or convulsion varies. You can use the same dose in two animals and see no convulsions in one, convulsions in the other, and the same thing in terms of the lesion.

So, basically, you really don't know, you have got no way of predicting unless you go ahead and do a complete analysis of the brain as to whether there is a lesion occurring, whether there is structural damage.

The other issue is that this toxin, because of the convulsions, you get breaks in the blood-brain barrier, you see lesions in a number of different regions of the brain, so to section the brain and to go ahead and do the pathology is really a very daunting task.

[Slide.]

This is just a demonstration of the scanner that was used especially to some degree--and Al will correct me if I am wrong afterwards--it is a modification of a standard NMR spectroscopy system.

What we are analyzing here, rat brain hemispheres, this is just a picture of it, a standard 35-millimeter picture, and this is the MRM scan, and you can see the detail is really extraordinary.

The resolution here is around 45 microns. Standard MRI, clinical MRI I should say, is around 1 millimeter to maybe half a millimeter resolution. This is 10-fold greater and potentially, with some of the data you will see it goes even beyond that.

[Slide.]

If you look at standard MRI from a human, you can see the detail, and we look at the MRM of the rat brain, what you can see already at this level, at the 45-micron, you can see more detail in terms of the structural integrity in the animal, but one thing that is very, very important that we point out, and that is very useful, is that the image that we obtain here looks very similar to what a pathologist is used to looking at in terms of a stained section, and I will give you some examples a little bit later on.

[Slide.]

I have mentioned a couple of times that this data, 3-dimensional, well, these are the sorts of things we can do. There is the image. We can cut and present the tissue in any way we desire.

[Slide.]

The issue of one to five sections here with the one dataset, we can generate what we call the slices or virtual sections anywhere we want, at any region within the brain, and we get quite high structural resolution.

[Slide.]

We can look at any planes we want. This again is the same hemisphere. We can via what we call the virtual sectioning, we can look at the coronal, the sagittal, and the horizontal simply by orienting the dataset that we obtain.

[Slide.]

One of the important things to demonstrate is that what we see in MRM and what we consider a lesion is very often a change in contrast or difference in contrast in the region of the brain. At this stage, we have to then go back and validate and do standard histology or the conventional histology to really demonstrate that that change in contrast represents a true lesion, and one of the beauties of the MRM, it is nondestructive, this is fixed tissue that we look at in this case here.

We have taken the same brains that we scanned, and then we have looked at a region where we see a lesion, for instance, here. In the amygdala, we see a darkening here. Then, we can go ahead and cut sections in that region, and

we can stain them and determine the mechanism and the nature of the toxicity.

What I would like to point out, there are three different stains here. This is the standard Nissl stain. You don't really see that much, this is the control here that is treated. You can see there is a lightening there.

We look at IgG, which is an indication of blood-brain barrier, we get very, very strong staining here, and then in GFAP, you see a darkening in that region there which would indicate this is an area of toxicity, there is glial activation, but you have to examine it quite carefully, whereas, here, with the MRM, it is a very noticeable effect.

[Slide.]

Just another example I want to present, this is a very large lesion we found in the cortex from a different animal. We can see the staining here. Again, this is the control. You can see the staining using Nissl. It is not too strong. There are some breaks in the blood-brain barrier. Myelin staining is quite weak, but what I would point is that myelin, if you look at it, looks quite similar to the MRM stain.

Then, we have here, there is also a change in the cell layer, and we look at early immediate gene response, and we can pick up a change using early immediate gene response, and the reason why I have shown these two slides

is what you have seen is a variety of different stains.

These scans were done about two, two and a half years ago. In order to obtain these scans, each sample was scanned for a period of eight hours, which is a long time, but the information that was obtained, we could identify up to six or seven different lesions with these single scans.

[Slide.]

While people sort of balk at eight hours, if you look at it in comparison to the standard histology, it took seven different stains to identify all of the lesions that we could characterize with a single MRM scan.

Those stains, it took a period, as you can see, 162 hours total in terms of time versus 11 hours for the preparation, the staining, and the analysis, so it is well over 10 to 15 times less or faster in obtaining all of the relevant data.

What I should also point out is that the stains we used here were basically chosen, and the regions of the brain we looked at, though, directed by the MRM, it is the MRM that told us this is where the lesions appear to be, and then went in with the conventional histology.

A lot of the stains that I have talked about, you would not routinely use in a standard screen, so a number of the lesions that we detected with the MRM would not have been detected using standard pathological stains.

[Slide.]

What I would like to just finish up with is how I could see this committee developing, what sort of a plan it could propose in terms of development and application of this technique, and I think there is three directions.

One is the ex vivo analysis that should continue and be expanded. The second one is development of in vivo approaches for monitoring acute and chronic responses. The third is the development of pulse sequences.

I mentioned earlier about pulse sequences. Dr. Johnson likes to refer to them as stains. We talk about histological stains. It is the same sort of phenomenon there.

[Slide.]

In terms of the ex vivo, the steps that could be followed there, first, would be obtaining a number of well-characterized samples where there is known adverse reactions of a variety of different organ systems, and certainly we could include the National Toxicology Program, NCTR, and industry.

The second one is acquisition of data at a number of different sites with the same data, and these samples could be transported from one site to another to establish standardized acquisition practices.

The third is the digital analyses. These also

could be done at distinct sites.

The fourth step is the subsequent pathological analysis of identified lesions to verify and validate these lesions, what they are and what the changes in contrast actually mean, and then the evaluation determining how the MRM analysis compared to the conventional pathology.

[Slide.]

The second program, the in vivo program, we first need to identify specific animal models for acute and chronic drug studies, then, whole animal scans that should be done optimizing temporal and spatial resolution because both of these factors provide very relevant information and important information in terms of the action and the toxicity of the reagents.

Conventional pathology then again on organs where there has been identified lesions, should be analyzed, and then again comparing the MRM data with the conventional pathology.

[Slide.]

In terms of the stains, the development of specific pulse sequences for identification of specific pathologies, and this work is going on, and then cataloging various lesions and their related pulse sequences, and basically, this is an informatics issue.

[Slide.]

The impact on the drug development process. What MRM can potentially provide is a rapid, sensitive, predictive initial screen for toxicity. It can provide a more standardized series of populations, so we scan the animals before and after. It provides a much better comparison and much more standardization of populations.

We can identify potential biological endpoints that can then be transferred to the clinic using standard MRI.

We get complete datasets, not just specific sections. We can reduce the number of animals used because these can be done ultimately in vivo, so you can follow the progress of the effect in the same animal, and we can also speed up the analytical process by introducing automated techniques for quantitation, such as being introduced like order segmentation in the MRI.

I will leave it at that. Thank you.

DR. DOULL: Thank you, Dr. Lester.

We will go ahead then and hear from Dr. Johnson from Duke University. He is the Center Director there for in vivo microscopy.

Magnetic Resonance Imaging

Allan Johnson, Ph.D.

DR. JOHNSON: Thank you. I am reminded of George Goebel's comment some years ago. He said, "The world is

formal, and I am a pair of brown shoes." I have a Macintosh, so if you will bear with me for a moment while it boots and finds its sync, and all that sort of stuff.

DR. REYNOLDS: Mr. Chairman, maybe while his computer is booting up, I could ask a question of our last speaker.

DR. DOULL: Sure.

DR. REYNOLDS: Are there examples or models that you could help us focus on, that would help us build a bridge from the preclinical to the clinical area? If you could just maybe mention a couple of those, or things that we could do, and do you see the application of this in the preclinical area really being directly relevant to those areas in clinical practice, or are we just in the process of building endpoints or surrogates that can be subsequently measured in humans?

DR. LESTER: There are a number of examples where studies have been done on both animals and in the clinic using MRI, not using MRI microscopy, and some of them, they corroborate the work that has been done in animals, and some of them don't. So, yes, that has been done.

DR. JOHNSON: Several of my first slides, David, from Marcus Rudin might address your question.

[Slide.]

I will crank up now since we have all the computer

technology in hand. I would like to thank the committee for giving me the opportunity to speak today.

[Slide.]

I am going to talk about 3D MRI microscopy and a little broader topic, magnetic resonance in general. I am going to try to cover three different areas.

I am going to talk first about MRI in drug discovery. I am going to try to distinguish MRI from MRM, and then I am going to talk about MRM in drug discovery.

[Slide.]

I am going to take almost the reverse order that David took in his presentation. I am going to start at this end of the spectrum and go to this end of the spectrum, and I am going to pause a moment and define resolution in a slightly different fashion than David did.

MRI and MRM, as PET and CT, are also all volumetric imaging techniques. We usually excite a slice. There is a slice of some finite thickness, and that slice contributes to your resolution, as well. So, I like to define the resolution in terms of voxel volume. For each picture element, each pixel, you have a volume of tissue that is being mapped to the image, and it is that voxel volume that is important in defining our resolution here.

So, for example, in clinical MRI, we will excite a slice that is usually 10 millimeter thick in a whole body

study, and the spatial resolution will be 1 by 1 millimeter in plane, that is, each voxel is 10 cubic millimeters, and I will show you some images that are down towards this range in MR microscopy where we have 10 micron by 10 micron by 10 micron resolution.

So, I think keep the idea of differentiating the resolution volumetrically is important.

I am going to go from this end of the spectrum down towards this end of the spectrum. I am going to start with a couple of studies that have been done with MRI using small animal models, work that has been done by my friend and colleague Marcus Rudin at Novartis.

[Slide.]

Marcus has been in the game actually almost as long as I have, and I think Marcus started in 1987, I started in '82. Marcus has done a beautiful job of demonstrating the utility with the n's of animals that are necessary.

We differentiate ourselves in the sense that I can take all day. I am an academic, I don't have to really work, so I can spend the whole day scanning an animal, but Marcus works in the real world where he has to get results out, and Marcus has been doing work with these number of compounds in stroke, this work published in NMR and Biomedicine, and has done a wonderful job of quantitating

the volume of stroke in one of these animal models, segmenting the volume, and then come out with very specific quantitative information, comparing infarct size with and without drug treatment. It is very quantitative and it has been very helpful in their drug discovery process.

[Slide.]

He has applied it in many, many different models. I have just picked a couple of his, this again from some cardiovascular work in which they were looking at some normal--these are rats now--about 250-gram rates, and they are looking at cardiac images in the normal, with the pressure overload model, and then with the volume overload model. You can see quite strikingly the difference in the volumes of the chambers, and they are able to quantitate both the chamber volume as well as the mass of the heart.

[Slide.]

So that is MRI. I am going to shift and move up that spectrum to MRM, and we were discussing during the coffee break what my role is. I am the mouthpiece of the group. This is really the group of people that do the work. I have the privilege of working with some wonderful people for the last, oh, almost 18 years doing MR microscopy on that volumetric scale.

[Slide.]

This is the visible man, which you probably have

seen on the web, on video, et cetera, and to scale down here is the visible mouse. You see him sort of tucked into his hand there. This is the visible mouse here, blown up a little bit, and next to the visible mouse is the visible mouse embryo.

We have done MR microscopy on these specimens at the same relative organ definition as we do in the man, and if you consider 100-kilogram man and a 1-gram mouse, there you have it, quite a bit of difference in spatial resolution required.

[Slide.]

Getting the resolution increase in vivo and in vitro is not just a simple trick. You don't just add one gizmo to make your MR system work a little bit higher resolution. It has been the focus of our laboratory for the last 15 years or so, and that focus has been on integration.

I am a physicist and engineer by trade, but within our group we have some wonderful folks doing biology and physiology. We have some very clever engineering people, some wonderful computer science people, and we are beginning to attract some chemists to the operation.

[Slide.]

This shows one of our systems. We have three magnets, a 2 tesla, 30-centimeter bore magnet, a 7.1 tesla, 15-centimeter bore magnet, and a 9.4 tesla, 89 millimeter

bore magnet.

The typical clinical system is a 1.5 tesla magnet. I have the dubious distinction of being the Director of the Physics Section within Duke Medical Center, so that half of my life is spent seeing what technology is relevant for our Radiology Department for the day and looking at the translation of that technology into our environment.

By bouncing between the basic and the clinical facilities, we are able to balance those technologies, so we can sort of keep on the edge of both. In 1982, I started working with friends at General Electric in putting the first high field system anyplace in a clinical environment. That was Duke in 1983.

At that time, I was working with large animals. It was Rags, the wonder dog, and several of his colleagues. Rags became a permanent member of my household. We would travel up to Milwaukee and we would scan me. We could get my head in the bore of the magnet, but at that time we could only make coils that were about 30 centimeters in diameter, and this may come as a surprise to you, but this is not 30 centimeters here, it's a little bit more.

So, we would take Rags, and I would sneak Rags into the back door of the Midway Motor Hotel at night, just sneak him up to the room, and then take him down to scan him at General Electric. About the fourth or fifth time you

have been trying to smuggle a dog in the door at 11 o'clock in January in Milwaukee, or you are trying to get him through O'Hare, you say, boy, if I had something I could put in my pocket, it would be a lot easier getting through the air scanner.

So, we started thinking about what was necessary to do very high resolution on the mice, on rats, and any other small animal models we could get our hands on.

This shows the magnet here and a lot of the physiologic support gear that I will talk about in a little more detail here.

[Slide.]

One of the key elements of this has been the work of my colleague, Larry Hedlund. Larry is a physiologist. About the second or third time we went to Milwaukee to look at this, Larry came along with just tons and tons of stuff. He had physiology stuff and ventilators and stuff. I said this is physics, man, we don't want that stuff along, but Larry persisted.

It is his persistence that has enabled much of what we do, because once you put the animal in the bore of the magnet, he is gone, you don't see him. You have no physiologic metrics. You don't know whether the animal is alive or dead.

Larry has developed this very elegant system where

we are able to measure EKG, airway pressure, airway pressure here, trigger the magnet, temperature in the bore of the magnet, and heart rate, virtually all of the physiologic metrics, so that we can keep an animal stable and physiologic in a magnet for up to 24 hours.

[Slide.]

This is a guinea pig and with the triggering that Larry is able to accomplish, we can do this in vivo cardiac microscopy at about 100, 150 micron resolution, and you will notice that you can see mitral valve there. The field of view here is about a centimeter.

[Slide.]

Cardiac imaging has one of our focuses. About three or four years ago, we struck up a relationship with some really bright people at Princeton to start looking at lungs.

Actually, Larry and I have been interested in looking at the lung since 1993. This is a proton image of a 150-gram rat, and there is very little signal in the lung because there is very little water in the lung, but these very clever people at Princeton, Will Happer and Gordon Cates, figured out how to make helium gas magnetically active, and this is the very first hyperpolarized helium gas.

Essentially, we shine laser light on a mixture of