

DEPARTMENT OF HEALTH AND HUMAN SERVICES
UNITED STATES FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

Cardiovascular and Renal Drugs
Advisory Committee Meeting

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8:00 a.m.

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Silver Spring, MD

PAPER MILL REPORTING
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P A R T I C I P A N T S

William R. Hiatt, M.D., Chair
Elaine Ferguson, B.S. Pharm., R.Ph.

Cardiovascular and Renal Drugs Advisory Committee
Members (Voting)

Steven D. Findlay, MPH (Consumer Representative)
John M. Flack, M.D., MPH
Frederick J. Kaskel, M.D., Ph.D.
A. Michael Lincoff, M.D., FACC
James D. Neaton, Ph.D.
Emil P. Paganini, M.D., FACP, FRCP
Lynne L. Warner Stevenson, M.D.
John R. Teerlink, M.D.

Temporary Voting Members

Ruth Day, Ph.D.
Mark A. Fogel, M.D., FACC, FAHA, FAAP
Tal Geva, M.D.
Sean Hennessy, Pharm.D., Ph.D.
Eric S. Holmboe, M.D.
Ruth G. Ramsey, M.D.
James L. Tatum, M.D.
Paul H. Zanetti, M.D. (Patient Representative)

Industry Representative (Non-Voting)

Jonathan C. Fox, M.D, Ph.D., FACC

Guest Speakers

Robert Hamlin, DVM, Ph.D.
Sanjiv Kaul, M.D.

FDA (Non-Voting)

Karen Weiss, M.D.
Dwayne Rieves, M.D.
Ira Krefting, M.D.

P R O C E E D I N G S

Call to Order

DR. HIATT: We are going to start the meeting. I appreciate everyone being here. My name is William Hiatt. I am from the University of Colorado, Denver, School of Medicine, and I will be chairing today's session.

I would like to begin with the introduction of the committee. Dr. Weiss, could you start over here and we could just go around the room or continue on to the right there.

Introduction of Committee

DR. WEISS: Good morning. I am Dr. Karen Weiss. I am the Deputy Director of the Office of Oncology Drug Products at the FDA.

DR. RIEVES: I am Dwaine Rieves, Division Director of Imaging and Hematology at the FDA.

DR. KREFTING: I am Ira Krefting, a medical officer in the Division of Medical Imaging and Hematology Products.

DR. PAGANINI: Emil Paganini, private nephrologist out of Cleveland, Ohio.

DR. FLACK: John Flack, Chairman, Department of

Medicine, Wayne State University.

DR. KASKEL: Rick Kaskel, pediatric nephrologist at Albert Einstein College of Medicine.

DR. LINCOFF: Mike Lincoff, an interventional cardiologist and Director of the Center for Clinical Research at The Cleveland Clinic.

DR. NEATON: Jim Neaton, biostatistician, from the University of Minnesota.

DR. STEVENSON: Lynne Warner Stevenson, Brigham and Women's Hospital, Boston. I am a heart failure transplant cardiologist.

DR. ZANETTI: Paul Zanetti, Patient Representative.

DR. FINDLAY: Steven Findlay from Consumers Union. I am the Consumer Representative on the panel.

MS. FERGUSON: Elaine Ferguson, Designated Federal Official.

DR. TEERLINK: John Teerlink, University of California, San Francisco, Director of Echocardiography at San Francisco VA Medical Center, and Director of Health Failure Clinic.

DR. DAY: Ruth Day, Director, Medical Cognition

Laboratory, Duke University.

DR. HENNESSY: Good morning. My name is Sean Hennessy. I do pharmacoepidemiology research at the University of Pennsylvania.

DR. GEVA: I am Tal Geva, Director of Cardiac Imaging at Children's Hospital, Boston.

DR. HOLMBOE: I am Eric Holmboe, Senior Vice President for Quality Research and Academic Affairs at the American Board of Internal Medicine.

DR. FOGEL: I am Mark Fogel, Director of Cardiac MR, pediatric cardiologist at Children's Hospital, Philadelphia.

DR. RAMSEY: Ruth Ramsey. I am a neuroradiologist, Medical Director, Premier Health Imaging, and I am also a clinical professor of neuroradiology at the University of Illinois in Chicago.

DR. TATUM: I am Jim Tatum, Associate Director, Division of Cancer Treatment and Diagnosis, and I am also Molecular Imaging Branch Chief at NIH.

DR. FOX: Jonathan Fox, Industry Representative.

DR. HIATT: Thanks, all.

Next will be the Conflict of Interest Statement.

Elaine Ferguson.

Conflict of Interest Statement

MS. FERGUSON: The Food and Drug Administration is convening today's meeting of the Cardiovascular and Renal Drugs Advisory Committee under the authority of the Federal Advisory Committee Act of 1972.

With the exception of the Industry Representative, all members and temporary voting members of the Committee are special Government employees, SGEs or regular Federal employees from other agencies and are subject to Federal conflict of interest laws and regulations.

The following information on the status of this Committee's compliance with Federal ethics and conflict of interest laws covered by, but not limited to, those found at 18 U.S.C. 208 and 712 of the Federal Food, Drug, and Cosmetic Act is being provided to participants in today's meeting and to the public.

FDA has determined that members and temporary voting members of this committee are in compliance with Federal ethics and conflict of interest laws under 18 U.S.C. 208, Congress has authorized FDA to grant waivers to special Government employees and regular Federal employees who have

potential conflict of interest when it is determined that the Agency's need for a particular individual's service outweighs his or her potential financial conflict of interest.

Under 712 of the FD&C Act, Congress has authorized FDA to grant waivers to special Government employees and regular Federal employees with potential financial conflicts when necessary to afford the committee essential expertise.

Related to the discussions of today's meeting, members and temporary voting members of this committee have been screened for potential financial conflicts of interest of their own as well as those imputed to them, including those of their spouses or minor children and, for the purposes of 18 U.S.C. 208, their employers. These interests may include investments, consulting, expert witness testimony; contract/grants/CRADAs; teaching/speaking/writing; patents and royalties; and primary employment.

Today's agenda involves discussions of safety considerations in the development of ultrasound contrast agents, based upon the experience of the New Drug Application 21-064, perflutren lipid microsphere injectable suspension, Bristol-Myers Squibb Medical Imaging, now

Lantheus Medical Imaging, Inc., NDA 20-899, perflutren protein type A microspheres injectable suspension, GE Healthcare and the Investigational New Drug Application for sulphur hexafluoride microbubble injection, Bracco Diagnostics.

Perflutren lipid microsphere injectable suspension and perflutren protein type A microspheres injectable suspension are indicated for use in patients with suboptimal echocardiograms to opacify the left ventricular chamber and to improve the delineation of the left ventricular endocardial borders. This is a particular matters meeting during which specific matters will be discussed.

Based on the agenda for today's meeting and all financial interests reported by the committee members and temporary voting members, no conflict of interest waivers have been issued in connection with this meeting.

With regard to the FDA's guest speakers, the Agency has determined that the following information to be provided by these speakers is essential. The following interests are being made public to allow the audience to objectively evaluate any presentation and/or comments made by the speakers.

Dr. Sanjiv Kaul has acknowledged that he is currently a blind reader for a Phase III study for one of the competing firms and that he has served as one in the past for another competing firm.

With respect to FDA's invited industry representative, we would like to disclose that Jonathan Fox, M.D., is participating in this meeting as a non-voting industry representative, acting on behalf of all regulated industry. Dr. Fox's role at this meeting is to represent industry in general and not any particular company. Dr. Fox is an employee of AstraZeneca.

We would also like to remind members and temporary voting members that if the discussions involve any other products or firms not already on the agenda for which an FDA participant has a personal or imputed financial interest, the participants need to exclude themselves from such involvement, and their exclusion will be noted for the record.

FDA encourages all other participants to advise the Committee of any financial relationships that they may have with any firms at issue.

If you can give me one more minute, I just wanted to mention that we would like to introduce a member of the FDA press, Karen Riley, if you would please stand up. Thank you.

DR. HIATT: Thank you very much. I think we will then move into the content of the meeting. Dr. Rieves is going to give us opening remarks.

FDA Opening Remarks

DR. RIEVES: Good morning.

[Slide.]

My name is Dwaine Rieves and on behalf of our Division of Medical Imaging and Hematology Products, I welcome you to our discussion of safety considerations in the development of ultrasound contrast agents.

Before we delve into our specific presentations today, I want to highlight a few items to set the stage for today's discussion.

[Slide.]

I am going to touch on three areas. Specifically, I am going to introduce the products, then, provide some background, and finally, I will summarize our goals for today's meeting.

[Slide.]

Ultrasound contrast agents are unique among our drug products in that they are sometimes described as bubbles or, more specifically, microbubbles, since they contain a core gas surrounded by a molecular shell.

The products which are generally less than the size of red blood cell are administered intravenously, and following administration, the larger bubbles embolize to the lungs while the smaller bubbles traverse the pulmonary capillaries and enter the left heart.

As they travel through the left heart, the bubbles provide echogenic contrast during echocardiography and assist in visualization of the left ventricular chamber and the endocardium.

[Slide.]

Two ultrasound contrast agents are approved by the Food and Drug Administration for use among patients with suboptimal echocardiograms in order to improve visualization of the left ventricle and the endocardial border.

However, multiple other used for these types of products have been proposed and potential approval goals include the use of the products in visualization of the

peripheral vessels, such as for the detection of stenoses within carotid arteries, visualization of altered vascularity in organs as may occur, for example, in liver lesion, as well as use of the products to assess myocardial perfusion in multiple other potential uses.

[Slide.]

Three ultrasound contrast agents are currently marketed in the United States or Europe. Optison and Definity are the brand names for the products marketed in the United States and both contain the same core gas perflutren. However, Definity and Optison differ in the molecular shell. The outer shell is human albumin for Optison and certain lipids for Definity.

The third product, SonoVue, is marketed outside the United States and consists of sulfur hexafluoride surrounded by lipid shell.

All three manufacturers are here today to summarize their experiences and potential uses of these products.

[Slide.]

As background for our discussions, I will summarize some of the safety information we reviewed last

year and the actions that followed from this review.

During this review process and over the several months after the labeling change, we dealt with additional information and concerns that prompted development of a risk management plan for these agents, as well as a second label change which was completed only a few weeks ago.

In October of last year, following the first label change, we issued a statement that described the most notable findings from postmarketing reports of serious events.

At that time, the FDA had received approximately 190 reports of patients with serious non-fatal events that included a variety of manifestations as listed here, such as abrupt onset of hypotension, dyspnea, loss of consciousness or seizures, as well as arrhythmias and cardiac arrest.

Some reports described urticaria or other types of rash, which were suggestive of a hypersensitivity reaction. There were reports also included 11 deaths with 4 deaths occurring within 30 minutes of administration of product.

Overall, the deaths and serious events occurred predominantly among patients in one of three categories, specifically, patients with underlying chronic conditions

with multiple comorbidities, patients undergoing stress tests or patients with acute unstable conditions, such as acute myocardial infarction or other critical illness.

[Slide.]

Given the occurrence of the serious events among patients with multiple comorbidities or unstable conditions, it is important to note the premarket testing of the products among these types of patients.

This slide highlights some of our background considerations to assessing these postmarketing reports. With respect to Optison, which was approved in 1997, three items are notable.

First, until November of last year, Optison had not been marketed for nearly two years, hence, the most recent postmarketing reports related mainly to Definity.

Secondly, the premarket safety database for Optison was relatively small by contemporary standards with approximately 300 patients described in the label. However, the postmarketing experience, the exposure was much larger as illustrated by usage data obtained from the Premier Hospital Healthcare Alliance database which cited at least 200,000 patients exposed and. as we will hear later,

apparently as many as a million or even many more than that have been exposed to Optison, as well as to Definity.

Definity, which was approved in 2001, had a premarket safety database of approximately 1,700 patients. However, patients with unstable conditions were systematically excluded from the major clinical studies.

The Premier database also indicated a sizable postmarket exposure with at least 260,000 hospitalized patients exposed. Of some note was that Definity was approved with a postmarketing commitment to perform a surveillance study that examined the safety of the product as it was actually used in clinical practice.

At the time of last year's safety review, this study had not been initiated.

[Slide.]

The SonoVue experience also provides useful background. SonoVue is an agent that was approved in the European Union in 2001. The approved indications related to three areas--echocardiography, macrovascular doppler or peripheral artery visualization, and microvascular doppler or imaging of vascularity in the liver or breast.

Following marketing of SonoVue, three fatal

adverse events prompted a product relabeling to contraindicate its echocardiographic use among patients with acute MI or certain types of angina, acute or class 3 or 4 cardiac failure, severe cardiac rhythm disturbance, severe pulmonary hypertension in patients with the acute respiratory distress syndrome.

As we will hear subsequently today, the manufacturer SonoVue has performed studies in pigs to show that the marketed contrast agents cause transient, but notable systemic arterial hypotension and pulmonary artery hypertension at doses similar to those administered to humans.

Some investigators have questioned the relevance of these data obtained from pigs based upon a unique sensitivity of pigs to these types of products. But the following slide illustrates the potential usefulness of porcine data.

[Slide.]

This slide highlights the recent publication that implicated a contaminant within marketed heparin as responsible for a number of serious cardiopulmonary reactions. The article emphasized how in vitro and in vivo

studies in pigs as compared to other species were uniquely helpful to assessing the hemodynamic consequences of the products contaminant.

[Slide.]

Last year, several changes to the Definity and Optison labels were made as outlined here. These included the addition of a boxed warning for the occurrence of serious cardiopulmonary reactions, addition of new contraindications for use of the products among certain unstable or high risk patients, and directions for the monitoring of all patients during and for 30 minutes after product administration to include EKG and oxygen saturation.

[Slide.]

Several actions followed the relabeling as shown on this slide. As indicated in the top bullet, many physicians expressed concerns about the contraindications and the monitoring plans in particular, and noted that the diagnostic information obtained from these agents may prove lifesaving in some situations even among patients with unstable cardiopulmonary conditions.

Secondly, publications were supplied that described no pulmonary artery pressure changes in patients

following administration of some agents although the 750 publications were very limited in the description of the data.

Consequently, the manufacturers and the FDA developed a risk assessment and management plan for the products and a few weeks ago updated the labels.

[Slide.]

The recent label changes and components of the risk assessment and management plan are shown here. These label changes altered but maintained the boxed warning, reverted to the small number of contraindications present in the original product labels, and revised the monitoring plan to focus upon high risk patients.

The risk assessment and management plan consisted of educational efforts plus two new clinical studies for each product, which were approved under the recently enacted FDA law regarding required postmarketing studies.

One study is a prospective examination of pulmonary hemodynamics in patients receiving the products, and the other study uses an observational design to analyze outcomes among critically ill patients.

[Slide.]

Three major goals for today's discussion are highlighted here. The first goal is to heighten the visibility of these very unique products by means of sharing the experiences mainly from the sponsor summaries and identification of lessons learned from these experiences.

Secondly, we hope to stimulate discussion in a manner that will educate us all and help identify those essential considerations for future preclinical and clinical development of the products.

Lastly, we bring this topic to this committee in anticipation of future diagnostic imaging drug discussions which, as highlighted at the bottom of the slide, involve very unique understandings of diagnostic efficacy--that is, efficacy demonstration that often importantly differs from the type of efficacy shown in studies of therapeutic products and of course the very unique study design considerations both for efficacy and safety for diagnostic products.

[Slide.]

Two caveats. I emphasize that we are not focusing today upon any specific contrast agent, and we are not seeking any recommendations for specific regulatory actions.

However, we are focusing upon shared experiences from the companies in order to obtain relatively independent perspectives from our committee members.

The agenda is on the next slide.

[Slide.]

We have two guest speakers this morning. We are very privileged to have Dr. Sanjiv Kaul, who will discuss clinical aspects of the agent, as well as Dr. Robert Hamlin, an expert in drug studies among animals who will discuss certain animal testing considerations for drugs in general.

Subsequently, the three manufacturers will discuss their products, followed by lunch and open public hearing, and our brief introduction to the question.

We look forward to the presentation and discussions, and I will return the podium to our chairman.

DR. HIATT: Thank you very much.

Let's go ahead and move on to the next speaker.

Guest Clinical Speaker

Overview of Echo Contrast Agents

DR. KAUL: Good morning.

[Slide.]

My name is Sanjiv Kaul. I am from Oregon Health

and Science University, and I appreciate being invited to speak to you today.

I am only going to speak on cardiological application since my expertise is limited to cardiology, and I cannot speak on the other radiological applications.

[Slide.]

I have been in this field for 25 years when we started using contrast agents that we manufactured ourselves. A lot of these agents we made by hand and then Steve Feinstein developed the technique of sonication and we started sonicating and it was years later that companies got involved for this application, so I have quite a retrospective on this field.

I will try to give you some idea about not only the current applications but a lot of off-label uses which, to people like myself, are a much larger part of contrast than the clinical applications that have been approved.

[Slide.]

Here is a bunch of contrast agents, and Dwaine did mention Definity, SonoVue, and Optison. But we have had other agents in this sphere. Sonazoid is induced in Japan, Levovist has been used in Europe and Japan, and Cardiosphere

and Imagify are two agents that are under consideration for myocardial perfusion.

[Slide.]

Before I start, I will give you a little update on something we did at the American Society of Echocardiography. Dr. Bill Zoghbi, the president of the society will speak to you this afternoon, but after the first relabel of contrast agents, American Society of Echo decided to look at some safety data.

We collected data from around 80,000 patients, from 13 cardiac ultrasound laboratories that do a lot of contrast, 5 percent were trans thoracic and 28 percent were stress echos, which is not an approved indication. I will show you some of those data. More than 10,000 injections given to critically ill patients in ICUs. Severe reaction probably related to ultrasound contrast agent developed in 8 patients out of 80,000.

Of these, 4 were consistent with anaphylactoid reactions, all were outpatients, no events in 8 patients, no events in inpatients, and no deaths. So, I myself have probably used 40,000 injections in patients over the years. I have to give a caveat, though, that I almost always use it

as a continuous infusion of diluted agents, because that is the way we look at myocardial perfusion, and not as a bolus.

And this may be something that could be discussed later in the day when we look at safety.

[Slide.]

So, on the left, now I don't know how well we can see this with the lights, but this is the application for contrast. On the left is a patient with atypical chest pain, comes to the echo lab with an abnormal EKG, and you can see there that is your typical type of echo without contrast. And we gave contrast. And we made a diagnosis of apical hypertrophy.

We are making these diagnoses much more now in all types of myopathies which we were not able to make consistently because of echo quality previously.

[Slide.]

On the left is another echo. You can see that maybe there is a wall motion abnormality in the apical region on the left, on top. But, when you give contrast, it becomes very clear that the patient has an aneurysm and a pseudo-aneurysm, which of course makes it surgical candidate, an emergent surgical candidate, something we

would not have normally picked up on this patient.

[Slide.]

Here is another patient and this is liminal like this. Here is a patient we can see bad LV function, but that's about it. And then when we give contrast, we know this is non-impacted left ventricle, which we are finding to be much more common in dilated cardiomyopathy than before the use of contrast.

So, our incidence of non-compacted LV is going up steadily in our laboratories. In fact, anybody with myopathies, Becker's dystrophy, anything like that that is sent to us, we always give contrast now because we pick up these cardiac manifestations of myopathies which we were not able to pick up before.

[Slide.]

We are picking up thrombus. A lot of times there is something always there in the noisy area of the apex. Those who do echo will know when you give contrast, you can make a very good diagnosis. It has become the gold standard in our practice now for thrombus and when there is a thrombus, of course, your treatment is different. You give anticoagulation.

[Slide.]

Of more real instance, here is a patient who came to the emergency department with chest pain, known to have myeloma before, and there is this mass there, and it looks like a thrombus, but when you do contrast, you see that the mass has perfusion.

There is perfusion here in the mass and this turned out to be hemangioma of the coronary artery, a rather rare tumor, and something again we would not have been able to diagnose.

[Slide.]

Moving on, here is a patient, and this is what we do. We have done about 2,500 patients like this in a study. Here is a patient who comes with chest pain, 50-some-year-old male, some risk factors.

Emergency Department. We do echo in everybody in the Emergency Department with chest pain without ST elevation. And here is an echo and that is what it looks like. I mean you have to be a genius to be able to pick up anything there.

We didn't pick up anything. And then you do contrast and there is this discrete but definite wall motion

abnormality on the lateral wall which we would never have picked up on routine echocardiography.

[Slide.]

The patient, despite the normal EKG, goes to the cath lab, has a totally occluded intermediate coronary artery. You can see right here, which we opened, and it makes a completely different treatment for a patient who otherwise would have waited for repeated EKGs, enzyme determination and, by the time we would have picked it up, would have been hours later, and would have a persistent wall motion abnormality for life and reduction in LV function, which we prevented.

[Slide.]

Here is another patient who comes in with a stroke, and when she wakes up, complains of chest pain. And that is the EKG. A lot of people would have called this an inferior MI and taken this patient to the cath lab. But, as I told you, we do echo in these patients, and so this patient has an apical wall motion abnormality, large apical wall motion abnormality. But, when we do perfusion, this is blowing the bubbles and then watching them come back into the myocardium.

[Slide.]

Myocardium perfusion is totally normal, so this is takotsubo syndrome or apical ballooning. Again, we are picking up a lot of this in atypical chest pain in our emergency department.

[Slide.]

So, these are just manifestations of some off-label uses. And our interest has been in the Emergency Department about 20 million ED admissions for chest pain annually in the U.S.A. EKG is diagnostic in 30 to 40 of all undergoing AMI, 30 to 40 percent. Cardiac enzymes take hours to become positive and 5 million people are admitted to hospital for observation or admission and we as a nation spend \$15 billion a year on this.

[Slide.]

So, we did a study, as I told you, about 2,500 patients, and I am going to share the results of the first 1,000. This is a patient with a wall motion abnormality despite a normal EKG, so these are non-diagnostic EKGs.

You can see the wall motion abnormality and you do perfusion. These are still frames with high MI imaging, but the apex and the anterior wall shows no perfusion. This

patient is taken to the cath lab. His proximal LAD is open. Myocardium now shows very nice perfusion as you can see on the left and one month later wall motion is completely normal.

So, this is a real saver as far as I am concerned, early detection, early diagnosis, early intervention, and the LV function is totally normal in the patient with an acute myocardial infarction that was electrically silent.

[Slide.]

I show you another example. This is a patient with contrast in the LV, showing a big apical defect, wall motion abnormality, but when you do contrast, the apex is completely perfused.

[Slide.]

So, here is a patient who has a wall motion abnormality, but totally perfused apex. Angiogram showed spontaneous recanalization of the artery, which we see 1 in every 6 patients now giving them heparin and aspirin in the ED. By the time we take them to the cath lab the artery is open.

[Slide.]

So, this is the stem myocardium, which just based

on regional function assessment could not have been determined without myocardial perfusion.

[Slide.]

In the first 1,000 patients, we had 90 acute myocardial infarctions and a total of 166 heart events. We took these people with heart events out for analysis and followed the others for another two years to look for events, and we got 13 percent event rate in those patients.

And so these are people with non-diagnostic EKGs who come to the Emergency Department with a 30 percent two-year event rate, and a lot of those are acute MI cardiac deaths.

[Slide.]

So, if we look at the first 48-hour event rates, you can see that hypotension and diabetes, those kinds of risk factors are not important. An abnormal EKG, that is, any EKG abnormalities, and these people didn't have ST elevation, any nonspecific abnormality gives you about twofold chance of having an event.

An abnormal function with contract in the LV cavity will give you 5 times more chance of having an event in 48 hours, while abnormal perfusion and abnormal function will give you a 14-fold higher incidence of having an event,

so it is extremely powerful. And, if you think about it, it makes sense because acute myocardial infarction is affecting the myocardium and the microcirculation, and if you can detect that, you are better off.

[Slide.]

This is the two-year follow-up. If we had normal perfusion and function on contrast. We had a less than 5 percent event rate. If both were abnormal, we had about a 50 percent event rate, and if the perfusion was normal, but function was abnormal, as we saw in patients with reperfused myocardium, stem myocardium, dilated cardiomyopathy, non-ischemic cardiomyopathy, et cetera, there, the event rate is somewhere in the middle.

[Slide.]

We did a cost analysis. If a patient comes through the ED, and goes to the usual care, we saw what happened to these people in terms of tests being ordered on them, you know, maybe a stress test after leaving the ED or whatever, or a catheterization, and we just formulated another approach where if we had used MCE data, which way would these patients have gone and how much would we have saved, and we saved about \$900 per ED admission patient,

which is a substantial saving despite the cost of the ultrasound contrast agent.

[Slide.]

Now, there is another offline application, which is very common in most of our laboratories, including mine. We do about, in my lab, it's a modest size lab, we do about 50 to 60 echos a day, not like Mayo Clinic that does 500.

We do 50 to 60 echos a day and about 10 of those are stress echos. And, in my lab, nobody has a stress echo without contrast because, as you know, once you are having stress, the echo quality is very difficult to interpret. And, without contrast in the cavity, even the best of us are at a loss, and it is a lot of waste of resources not to make a diagnosis if you can give contrast.

[Slide.]

This is a normal, in a three-chamber view, showing your normal echo, stress echo. And here is an example of an abnormal stress echo with multi-vessel coronary artery disease, which I can tell you, having done a lot of these, is difficult to do without contrast.

So, unless a patient objects to contrast, at least in my lab, every stress echo gets a contrast, which is an

off-label use.

[Slide.]

I will move on to the detection of coronary disease with myocardial perfusion. As you well know, your resting flow is normal until you have about a 90 percent stenosis and so making the diagnosis of coronary artery disease for most of us clinicians requires increasing blood flow. Because it has been shown that when you increase blood flow, you can separate 50 percent or less stenosis from 50 percent or more stenosis, and this is the entire basis of stress testing which a lot of us do in our clinical practice.

The idea is that, when you have increased hyperemic flow, areas subtended by 50 percent or more stenosis will not exhibit as much of a hyperemic response as areas with less than 50 percent, and that's how you make your diagnosis whether it's nuclear or echo or whatever.

[Slide.]

So we developed a method of assessing myocardial blood flow or myocardial perfusion. Now we use it in all organs accessible to ultrasound. What we do is we give these bubbles as an infusion, as a diluted infusion, and in

about two or three minutes we reach saturation; that is, the blood pool becomes saturated, the rate of bubble introduction and elimination becomes equal, so we have a steady-state condition.

If we do--if you look at the right panel, if you look at any blood pool, whether it's the myocardium or any other organ, you will find that if you take an image at that point in time, that represents the number of bubbles in that tissue, which is the blood pool of the tissue since these are pure intravascular tracers, so it's the blood volume of the organ.

What we then do is destroy the bubbles in the ultrasound beam by increasing the power that we transmit through the transducer. These bubbles oscillate in an ultrasound field, that is how we see them, because if they didn't oscillate, we wouldn't be able to see them.

The oscillation produces harmonics and we then tune our echo systems to pick up these harmonics. So we suppress the fundamental frequency at which we interrogate these bubbles and then instead pick them up at the harmonic.

The same procedure of making these bubbles oscillate, can be used to destroy them, and when we destroy

them, there is nothing in the beam. The beam is about 5-millimeters thick and we watch the bubbles reappear into the beam.

At rest, the bubble velocity or red cell velocity in the myocardium is 1 millimeter per second. So it takes about 4 to 5 seconds to replenish the beam, and the rate of replenishment is velocity, which we can quantify. So velocity multiplied by volume is flow, so this is a very nice way of measuring flow to tissue whichever tissue can be accessed by ultrasound.

[Slide.]

We started doing studies long ago. This is a study we did in patients with normal LV function and coronary disease because those are the patients where you want to make a diagnosis of coronary disease. If somebody has already had a prior infarction, you know that they have coronary disease.

So we took these patients in a multi-center study, three institutions, and here is a patient with a normal nuclear scan.

[Slide.]

But where we destroyed the bubbles there. You can

see that there is destruction--one, two, three, four is filled. The entire myocardium in that three-chamber view is homogeneously filled at rest.

Now, when we repeat this at stress, you will watch the bubbles being blown. It will be a flash and then you will see things reappear, so let me just show that to you.

Flash 1, 2, 3, 4. You can see the anterior wall is filled and the posterior wall fills much, much slower. You can see it is still dark. So, we can see that the posterior wall has a stenosis. This was normal, by the way, as I showed you. Of course, the patient has a very proximal circumflex artery stenosis.

In this study, we did quantitative coronary angiography as you can see on the X axis, and compared MCE with SPECT. And you can see at about 20 to 40 percent stenosis. That is probably false positive, 40 to 60 percent we started seeing that MCE was superior to SPECT at moderate levels of stenosis.

Only at severe levels of stenosis were these equal, and our reading is that, because we destroyed the bubbles and watched them reappear, the slower velocity we can measure and see that gives us added sensitivity that is

not available for SPECT since SPECT is simply a blood volume imaging technique, and there is no velocity information.

I will come back to some of this later on if I have time because the gold standard, as Dr. Rieves indicated earlier--one of the things we have to discuss today is how do we go forward, what are the benchmarks, what are the gold standards, and coronary angiography is very questionable as a gold standard, and I just put it in front of the committee later on.

[Slide.]

So, the question then becomes I just showed you an example where we use a vasodilator for stress. Can we use dobutamine? The question always arises why would we use perfusion when we can use function, like stress echo.

This is ischemic cascade. Blood flow mismatch always precedes systolic dysfunction, so if we had a technique that was a little more sensitive, we may pick up coronary disease better.

[Slide.]

Here is an example. With Definity, we can see a normal resting image on the left at 20 mcg/kg/minute there is a perfusion defect despite normal function, and at 40 mcg

there is again a perfusion defect despite normal function. This is because of the ischemic cascade.

[Slide.]

Here is an example. This is from Tom Porter's lab. Tom does a lot of these. He has got enormous experience with dobutamine. We tend to do dipyridamole or adenosine in our lab, but he does a lot. You can see it's a normal stress on top with perfusion defect during stress, normal wall motion, and you see normal on the left bottom with perfusion defect in the inferior wall.

So, this is multi-vessel disease in a patient that has no wall motion abnormalities.

[Slide.]

What he showed was that even at peak dobutamine dose, the sensitivity is better for perfusion than function and, at intermediate dose, at 20 mcg alone, you can get a very high sensitivity because you can start seeing blood flow mismatches already, low doses of dobutamine act like a vasodilator without increasing myolopsin[?] consumption, so you get blood flow mismatches and a high sensitivity even at small doses of dobutamine.

[Slide.]

He was able to show that multi-vessel disease detection is also much better using this technique than a regular stress echo.

[Slide.]

Then, he did a follow-up in about 800 patients over four years, and again he showed if your wall motion and perfusion were both negative in this combined analysis, you had a very low event rate. If both were positive, you had a very high event rate. If wall motion was negative, but perfusion was positive, you had an intermediate event rate.

Again, it is adding information to what we already know.

[Slide.]

I just want to quickly then look at gold standards because we have been looking at coronary angiography as a gold standard. This is an intravascular ultrasound. You can see here this is a coronary angiogram and different portions are taken.

This is left main that looks totally normal, left main has a huge atheroma, and the wall is way out here. It has been remodeled, so that the cavity on the angiogram looks okay, but the actual lesion is enormous. You can go

back, come to the middle of the LAD. You can again see there is a lot of atherosclerosis and again further down.

[Slide.]

Basically, what happens is the vessel remodels. The lumen looks lovely, but you really have a lot of disease. When you have disease like this, it becomes hard to use coronary angiogram as a gold standard when you look at angiographic stenosis versus intravascular ultrasound defined stenosis.

I mean if you have data like this, it is very hard to see how we can continue to use coronary angiography as our gold standard. Nobody--even an optimist couldn't fit a line to these data.

[Slide.]

The other issue, of course, is vasodilatation. We know that when you increase flow through your distal coronary microvasculature, your flow increases through the large artery, and the large artery dilates, flow-mediated vasodilatation.

[Slide.]

We know this is necessary for normal flow but if we have atherosclerosis. Even if we have mild

atherosclerosis, just very little, this flow-mediated vasodilatation will not occur, so we will have a normal flow reserve.

[Slide.]

This is a study we did in the cath lab. All these patients have chest pain, even those with so-called normal coronary angiograms could at the most have a flow reserve of 2.5. Nobody went up to a flow reserve of 5, because these are all diseased people, they have diabetes, hypertension, they have all of the risk factors that decrease flow reserve.

With contrast echo, we are looking at physiology, which is abnormal flow reserve. That is what we are looking at, and then we tried to compare it to anatomy that is static and inaccurate. I will show the last two slides.

[Slide.]

When you exercise you release catecholamines and your coronary arteries dilate. A normal coronary artery dilates with catecholamines. It has been shown repeatedly.

[Slide.]

However, if you have atherosclerosis, your coronary artery will vasoconstrict. At rest, you can

measure that stenosis, and it may be 40 percent. But, at stress, it may have become 70 percent and cause ischemia. So, when you compare a 40 percent coronary angiogram stenosis to something happening at stress, they don't jibe.

I will end here. Thank you very much for your attention.

DR. HIATT: Thank you very much. I will note that in order to keep on time, there is time later in the day for questions to the presenters. So I hope you will be available for that because I think there will be some questions from the committee.

In the interest of staying on schedule, let's go on to the next presentation, Dr. Hamlin.

Guest Preclinical Speaker

DR. HAMLIN: I also thank you for the invitation.

[Slide.]

This slide goes without saying, the inability to predict toxicity with 100 percent sensitivity and specificity may be tragic, expensive, and embarrassing, and I would like to discuss this in the context of animal models.

[Slide.]

If one Googles perflutren, you see that serious cardiopulmonary reactions occur seldom in normal subjects, but in subjects with specific diseases, and we will expand on this quite importantly.

[Slide.]

If you examine a phase encoded enema image, you can really be impressed by the spectacular structure called the heart. What you might not know is that the normal heart for a normal human beats about 2.4 billion times in a lifetime and, if you are lucky, it can beat 3.5 billion times.

However, when you look at the animal surrogates for man, we find that a cat heart beats 1.2 billion times in its lifetime, and all of the other animals from shrew to elephant beat about 600 million times, which should tell us that there are significant differences among the animal species and these differences may relate to differences in usefulness for predicting toxicity.

[Slide.]

Well, it ends up that predicting toxicity is a daunting problem for a number of reasons. It is easy to identify toxicity to strychnine because you give the drug

and instantly there are tragic signs.

However, because of the relative rare occurrence of most toxicities, this constitutes a portion of the dauntiness of the task.

Again, if we study toxicity acutely, it is very easy, but we must remember that much toxicity is insidious in onset. For example, fen-phen takes years to be manifested, doxorubicin may take decades to be manifested, so it makes it very difficult for us in safety pharmacology or toxicology to identify toxicity.

Furthermore, there are many potential targets and variations of those targets, in due no small part to polymorphisms, we understand that there is hysteresis in responses that, if we achieve a blood concentration after administering the drug, there may be no toxicity. Then, as that drug concentration decreases, toxicity may be manifested for a number of reasons.

It is very important that we understand that there are differing responses dependent upon the states of health or specific diseases. We understand that diabetes, heart failure, and obesity constitute substrates for sensitizing for making propitious the presence of toxicity.

We understand that there are logistic problems that interfere with our identifying toxicity, that costs regulations, animal rights activists were not clever enough, we don't communicate well enough, so there are a number of reasons that make this task so difficult.

[Slide.]

But what I would like to talk about today is using--part of the problem is that we use potentially inappropriate surrogates. For example, we know not to use rats to identify terfenidine toxicity, dogs for procainamide toxicity, rabbits for atropine toxicity. We should not use pigs for exercise stress.

Furthermore, even if we picked the correct surrogate, we might not study the animal under appropriate conditions. We understand that restraint with anesthesia can obfuscate the response to drugs.

I will show you how important temperature is, how instrumentation may interfere with the ability to identify drugs or drug toxicity, or, in fact, produce that toxicity, issues of husbandry excipients, many other factors that pharmacologists understand are important.

[Slide.]

Well, we have to understand that toxicity can be described as something that produces a state different from health. Everybody understands what health is. A healthy person is one who feels good and lives to 120 years, because that is what some people do.

We also understand what disease is. You feel bad and you don't live a full life. However, very few of us are in a state of health and not terribly many of us are in a state of disease. SBut a whole bunch of us, and most importantly our animal surrogates, are in a state that I call "absence of health."

Now, what do I mean by this? It ends up that if your blood glucose differs postprandial, the magnitude with which it differs from 102.5 determines whether or not you have dental caries.

So, we might say that a blood glucose of 110 is normal but, in point of fact, it puts us at a state of absence of health because we have dental caries, and that clearly is not healthy.

It used to be taught that a blood pressure of 125 was fine. Now we know that that is not fine, it is not a state of health, it's an absence of health. We can do the

same thing for many, many parameters which we previously thought were within acceptable limits, that now we know it puts us in a state of absence of health.

It is important to understand that when we attack our surrogates, we have to identify what is the physiological or possibly pathological state. Okay.

[Slide.]

But all is not lost. It sounds like a daunting task. But we can identify a best surrogate quite easily. I say that with tongue in cheek. It can be identified best by regulatory agencies who, by experience, have identified this surrogate as predicting with highest sensitivity and specificity the likelihood of a toxic reaction in man.

That must be interpreted in the context of what Bernard Lown said some 30 years ago, you can know all there is to know about mechanisms of disease and all there is to know about mechanisms by which a therapeutic agent works, but the only way to know if it does work is to give it in a clinically relevant manner to a target species with a specific disease for which it is indicated.

The best surrogate may be the best surrogate only for a specific test article for a specific disease and in a

specific patient. Therefore, the following comments I made are doomed at the outset.

[Slide.]

So how do drugs and diseases affect biological systems?

I am interested in the cardiovascular system and therefore I identified with asterisks, just some of the potential targets that drugs or disease might affect, and it is clear that no safety evaluation is complete unless one identifies the potential toxic effect on all properties of the cardiovascular system, which, if affected, may translate to morbidity and mortality.

Here, you see a whole plethora of potential targets that must be explored.

[Slide.]

Now, a few questions. Do these individuals look alike to you? Is there any reason to believe that they act alike or that their physiological or pathophysiological processes are alike? The answer is clearly no.

[Slide.]

However, if you are lucky, and if you are skillful, you study a human population heterogeneous enough

to extrapolate results to the general population, so that we may identify potential toxicities.

[Slide.]

Question 2. Do these individuals look alike? Is there any reason to believe that they act alike or that their physiological or pathophysiological processes are alike, or that findings on one may be extrapolated to another?

[Slide.]

Again, if you are lucky enough, you pick animal surrogates that possess physiology close enough to human physiology that the results matter.

[Slide.]

So, let's look at the universe of potential surrogates, each of which has extreme value because of its similarity and because of dissimilarity to man.

At the upper left, you see a bar-headed goose flying over Mount Everest over 30,000 feet where the partial pressure of oxygen is less than 5, the temperature is 40 to 50 degrees below zero Fahrenheit.

This animal is suited to do something that man is not suited to do, and so would it be reasonable to study

drug toxicity or potential toxicity in a bar-headed goose?

I think not.

Here, you see a Weddell seal who routinely dives one mile below the surface and stays down for about an hour.

It wouldn't suit studying sudden infant death syndrome in an animal like this, that shares certain characteristics of sudden infant death because its physiology is so terribly different.

Would we study the effect of a test article on exercise capacity in a pronghorn antelope that consumes 240 cc's of oxygen per kilogram body weight per minute, which is orders of magnitude greater than most subjects can consume? It wouldn't be a good model.

Would we study reproductive physiology in a *Schistosoma haematobium*, whose male lives in permanent population with a female? Although this is an enviable state, it clearly is a state in which it would not allow you to extrapolate data on reproduction from Schistosomes to man.

Suppose you are interested in studying the effect of a test article on the interdependence between the left and right ventricle. Here, you see a typical heart from a

sea cow. Notice that the right ventricle and left ventricle don't touch each other, so you can't possibly study the interaction. It would be the wrong model to pick.

Suppose you were interested in studying diastolic heart failure responsible for morbidity and mortality in 40 to 60 percent of the humans with heart failure, would you study a spider? Clearly not. Why? Because a spider has a heart that pumps in systole and it also has muscles that are responsible for diastolic filling. It wouldn't be a good model.

But let's go from the ridiculous to something a little more appropriate. Let's take a look at two categories of animals, animals that are characterized by this heart, which is the heart from a primate or a carnivore, or animals from this heart, which are all the other animals in the kingdom.

If you will notice the distribution of these specialized conductile fibers in the hearts of man and dog.

They differ dramatically from the hearts of all other animals, such that if you have interruption of the conduction system in animals, in man or dog, you see that you have tardy activation of this region of heart.

But it doesn't happen in the hearts of most animals in the kingdom. Why? Because they have complete collateral channels or great collateral channels, and wide distribution of these specialized conductile tissue. You are stacking the chips against yourself trying to anticipate a toxic manifestation studying a pig if the toxic manifestation is mediated by conduction through the heart.

Coronary artery disease is enormously important obviously, so here we see the coronary arteries of most animals. And here are the coronary arteries of a guinea pig. You ligate a coronary artery, a major coronary artery of most animals and you have a myocardial infarction suffered by oxygen deprivation.

You ligate a coronary artery or two major coronary arteries of guinea pig and nothing happens. Why? Because the guinea pig is endowed with spectacular collateral channels. Please give me a heart that is part guinea pig.

[Slide.]

So, you see it is enormously important to identify the correct animal surrogate.

[Slide.]

Question. Is it reasonable to believe that

information gleaned from a healthy mouse raised in a foreign environment is extrapolatable to a sick person?

Corollary question to the clinicians among us.

How many of you would conduct clinical trials on persons all at 20 degrees Fahrenheit? Not many of us live our lives at 20 degrees Fahrenheit.

[Slide.]

Another question and we will get back to that very shortly. Why do you dose between 8:00 a.m. and 10:00 a.m. for a drug to be taken at 10:00 p.m.? Diazepam taken in the morning, it's okay to dose in the morning.

Zolpidem, the soporific, is given at night to help us sleep. And so because we understand the differences in physiology, in particular use dependence and inverse use dependence of toxicity of some drugs, we should study drugs that are taken at night, at night, and drugs that are taken in the daytime, in the daytime, and why do we study them at 8:00 to 10:00? Because that's when we come to work and people don't like to dose at night.

Why would you study an IKR blocker in a rat that doesn't have an IKR channel, or an IKS blocker in a rabbit that doesn't have a vigorous IKS channel, or atropine in a

rabbit that has an atropinase that prevents any effect of atropine? You are stacking the chips against finding the truth as it is extrapolatable to humans.

But now let's get back to the issue of temperature as a classic example. What can you trust from a study conducted on a freezing mouse? And I cite data from Steve Swoap and others at Williams College.

Could you please look at these graphs which depict the heart rate, the oxygen consumption, the mean arterial pressure, and the activity level of a mouse.

Here we see the mouse at thermoneutrality, which is 80 degrees Fahrenheit. Notice that the heart rate is 300, the oxygen consumption is less than 0.75 ml/kg/minute, the mean arterial pressure is about 75, and the mouse is relatively inactive.

Why? Because he is happy. He doesn't have to eat an awful lot because his temperature is just right. However, we don't study mice at 80 degrees Fahrenheit. Why? Because the USDA recommendation is you study them at 70 to 72 degrees Fahrenheit. I guess that's because we are comfortable at that temperature. That happens to be our thermoneutrality.

So, let's see what happens if we were to study the rat at temperatures that are comfortable for us. The heart rate is doubled. It is 600, and so many of us honestly believe that a mouse has a heart rate of 600. Sure, in a terribly foreign environment, it has a heart rate of 600, but that is not where it normally wants to live.

How about his blood pressure? How about his oxygen consumption? Way high. How about his mean arterial pressure? It's between 100 and 103. Look at how terribly active the mouse is when he is at a temperature that is foreign to him.

So, why is it that we think that we could extrapolate information from a freezing rat to a human living their existence at thermoneutrality? It is not reasonable.

Now, it doesn't matter what I think. It doesn't matter whether I think or you think it's reasonable or not. What matters is the truth. And that is why we have to look to regulatory agencies and PhRMA and universities to find out whether information is, in fact, extrapolatable.

[Slide.]

Let me give you another specific example. We are,

of course, very interested in clotting profiles. And take a look at the clotting profiles of a whole bunch of species - man, chimpanzee, monkey, pig, dog, cat, and rat.

If we look at the bleeding times, clotting times, and preferment times in minutes for these animals, you can see that they are all pretty much alike and we are interested in how these animals compare to man. When a point is missing, it means that the data is not available.

So, it doesn't really matter I guess so long as you don't pick a cat or rat. It really doesn't matter whether you study bleeding times or clotting times or preferment times in any of these species.

However, let's now take a look at 13 very important parameters of clotting listed 1 through 13. The values for humans are shown here and we would think that if we studied the effect of a test article searching toxicity and clotting mechanisms, we would search an animal that shares as many parameters with man as is possible.

Well, let's take a look at it. Of the 13 parameters, the chimpanzee, if there is an empty space means it's good, the chimpanzee shares many parameters except this one is three times greater than man.

Monkey looks very good, but look, three and four times as great as man, so you would make an error, but take a look at the animal that is used most commonly or very commonly in studying clotting profiles, drug effects, the pig. Of the 13 parameters, 9 of them are very different from present in the man, so the question is why would we expect to uncover toxicity in clotting mechanisms using pigs. The answer is probably not well.

[Slide.]

Okay. Coming down the home stretch. We can rename some of what we showed in the first slide. The best surrogate can be identified best by regulatory agencies who, by experience, have identified that surrogate as predicting with higher sensitivity and specificity the likelihood of a toxic reaction in man.

In the context of Lown's statement, rephrased now using toxicity instead of therapy, you can know all there is to know about toxicity and all there is to know about the pharmacology of a drug, but the only way to know if it is toxic is to give it in a clinically relevant manner to a target species with the specific disease for which it is indicated.

The best surrogate may be the best only for a specific test article for a specific species and in a specific patient. Therefore, as admitted, this presentation was doomed at the outset.

[Slide.]

The final question. What is the best model to predict the toxic potential for a test article? To answer, you must know three things; what the potential targets of the toxicity are, how do those targets vary among surrogates or recipients, and the precise conditions of use of the test article.

[Slide.]

One thing is true, however. Preclinical trials are important to protect subjects of clinical trials because exposures can be used, higher exposures can be used because they provide clinical investigators with hints as to what targets should be explored in humans and because they frequently do expose toxicities.

Our job, however, is to identify the appropriate surrogate for man, and that is a daunting task indeed.

Thanks very much for your attention.

DR. HIATT: Thank you very much.

I think we will move now to sponsor series of presentations, from Bracco Diagnostics. Dr. Denaro will introduce this.

Sponsor Presentations

Bracco Diagnostics

Introduction

DR. DENARO: Mr. Chairman, Committee members, and ladies and gentlemen, good morning.

[Slide.]

My name is Maurizio Denaro on behalf of Bracco. I would like to thank FDA for the invitation to participate this session even if our product is not yet approved for commercialization in the United States.

We are particularly pleased because we think this meeting comes to the right time when we believe contrast ultrasound is establishing as a novel imaging modality which has proved to be very useful in clinical setting, certainly in the clinical setting for which indication has been approved and also in clinical setting for which data are emerging where contrast in ultrasound appear to be extremely valuable.

Where Bracco can help in the discussion is because

SonoVue, as has already been said, has a broader indication in the country where it is approved, so we have indication which is not just cardiac bolus, other indication, and you will see that in these indications, the product is proved clinically extremely valuable.

[Slide.]

Just a short introduction of what is SonoVue. SonoVue, of course, is microbubble which contain inert gas, sulfur hexofluoride. The bubble diameter is between 1.5 and 2.5 micrometer. The human dose is between 2 and 2.4 mL when it is dissolved in saline.

Very important I think is to remember that when we give a human dose in total we give a volume of gas of 16-20 microliters, so very tiny amount of gas.

[Slide.]

These are the countries where product is marketed, mostly European Union and other non-European countries, in Asia, China, and South Korea are the two major countries.

[Slide.]

As I was telling you, we have three major indications. We have the echocardiography indication similar to Definity and Optison, but we also have in Europe

the indication for stress echo. But then we have the indication for microvascular enhancement, particularly for liver and breast lesion, which allow us to do lesion characterization, which we have exploited in the clinical setting.

Then, macrovascular enhancement allows the detection or exclusion of abnormalities in cerebral arteries, extra-cranial carotid, or peripheral arteries.

[Slide.]

I think there are a couple of important elements which I would like to start with my introduction, which are the unique characteristics of contrast ultrasound. The fact is that these are blood pool agents, so what they give you is real-time information of the vasculature.

In doing that, we have improved diagnostic information in the macrocirculation. But, when we go to microcirculation which are vessels diameter below 200 micrometer, what we can do and what we do now, we visualize and characterize organ perfusion as has already been said.

Now, if you can visualize and characterize organ perfusion, in reality what you do, you do tissue characterization.

Here, let me say something which I think should be discussed during the day, because when we started our development and also the initial marketing, microcirculation was detected with doppler.

Nowadays we detect microcirculation with the dedicated specific contrast software, so we have much better image about microcirculation and we can do much better analysis of perfusion.

Now, whenever you talk about contrast, you always have to remember that there is a strong correlation between technology and the contrast. Evolution of technology is extremely important and is much faster than the evolution of contrast.

[Slide.]

In preparing this presentation, we have asked external people to help us and two of them will make the major presentation; Dr. Senior, which is Director of Cardiac Research of the Northwick Park Hospital in Middlesex in UK. He is an expert echocardiographer, has experience with SonoVue, but also experience with Definity or Lumenity as it is called in Europe, and Optison; and Dr. Patricia Williams, Chief Operating Officer of Summit Drug Development Service.

We elected to start the presentation with the clinical more than the nonclinical, because we think we have learned a lot while we are in the clinical environment, and we are trying to take and implement the lessons learned from the clinical in the way we develop new agent for contrast and ultrasound.

[Slide.]

Now, these are the key messages which will be delivered. We think that SonoVue has a full established safety record. Whenever we talk about the clinical indication we think that there is a clear high positive risk-benefit profile of the product.

We have among the rare serious adverse events, which is the rate, the most frequent are allergic-like reactions. And, as I said, we think that today we have nonclinical tools which have been identified, which may help to provide new agents to reduce potential risks based on the experience which we have had in the clinical environment.

I would like to ask Dr. Senior to come and present the clinical and the safety profile of the product.

Clinical Experience and Safety Considerations

DR. SENIOR: Thank you very much, Maurizio. Thank

you, ladies and gentlemen.

[Slide.]

I will divide my presentation into three broad groups, my clinical experience in using this agent in assessment of LV structure and function of both regional and global, which is approved indication, and to assess and to also talk something on the microcirculation which is not approved use, then, go on to the side effect profile, and risk-benefit ratio of this product.

[Slide.]

Now, the commonest indication, or rather the commonest reason for referral in echocardiography, is assessment of LV structure and function, and particularly left ventricular ejection fraction.

It is not surprising why this is so because, if you look at this data here, these are patients with heart failure, and this is looking at LV ejection fraction and mortality. You can see that with decreasing ejection fraction, there is increasing mortality, and this ejection fraction is one of the very strong independent predictor of outcome.

So, not only if the ejection fraction is reduced,

the mortality is raised but, with decreasing ejection fraction, there is increasing mortality. Therefore, it is not surprising that there are criteria for actually implanting a biventricular pacemaker or implantable cardiac defibrillator in patients to assess ejection fraction before proceeding on to expensive therapy or preventing patients from having those therapy unnecessarily.

[Slide.]

However, although assessment of LV structure and function is the commonest indication for echocardiography, we get images like this, and 20 percent of our patients--I mean throughout in all scenario, you know--we get images like this where it is impossible to know what the LV function is, let alone to describe what the LV structure is.

This is a 55-year-old male who had a previous pacemaker implanted who presented with shortness of breath and we were asked to assess the cause of shortness of breath and therefore the LV function and structure.

[Slide.]

However, when we injected SonoVue, you can clearly see now, you can see the LV cavity very clearly, the borders very clearly, and we know that the ejection fraction is

completely normal. You don't have to actually sit down and calculate. You can see very clearly that the heart is contracting everywhere.

Also, what you see here, the black areas are the myocardium, and what you see here right at the top, the myocardium is seen here but it is very thick at the top. And this is an example of apical hypertrophic cardiomyopathy. So we have identified the patient who has a normal epidural[?] ejection fraction, but he has a substrate for heart failure, and that is apical hypertrophic cardiomyopathy. So, with this just single injection at the bedside, in real-time, we have made a diagnosis.

Now, if you hadn't had the contrast, this patient would have had undergone a transesophageal echocardiography, which is invasive, semi-invasive, which requires sedation, which requires local anesthesia. And it's a fairly prolonged procedure with lots of other ancillary management, and, if not, this patient would need to have another examination like MRI, which is actually regarded as the gold standard for assessing LD function. But it is not available in all hospitals. It is expensive, and the patient has to be sent away. And it is not real-time, we cannot do it at

the bedside.

[Slide.]

But this is a comparison of contrast echocardiography against MRI, which is considered as the standard technique for assessing ejection fraction. And this is looking at the differences in the values obtained in more than 100 patients with SonoVue in comparison with MRI.

You can see this is divided into those with a poor baseline image quality like you saw before, and also those who had good image quality.

But, if you look at this group first, you will see that the difference of ejection fraction is 6 percent, which is pretty high. It is not really acceptable to be that different from a standard, but as soon as we inject contrast we can see the difference goes down significantly to only 1.9.

[Slide.]

Not as true with good image quality. But you can see the difference with baseline echo is better than without, you know, good baseline echo. However, it still further improves when you give contrast.

This is important because if the criteria is that

the ejection fraction has to be 35 percent or 40 percent or 30 percent, We have to actually show it accurately that it is so and certainly, under that circumstance, we would be using contrast echo even with good baseline image quality to assess the ejection fraction under those scenarios.

[Slide.]

Now, these are various techniques that has been tested in a multi-center trial where images were off-line, and these techniques are used in cardiology to assess LV function.

Now, for a technique to be good, it should be accurate, but also reproducible. And the reason why it should be reproducible is that today we know that when we give herceptin therapy or cardiotoxic therapy like doxorubicin, we need to follow this patient up longitudinally to see any change in LV function, and therefore, that technique not only has to be accurate, but reproducible.

So, this is testing the reproducibility on the vertical axis, the mean percentage of error meaning the higher the value, the worse the reproducibility, and this is looking at echocardiography, this is looking at

cineventriculography. This is looking at MRI and this column is contrast echo.

If you look at it, yes, echo wasn't that good in terms of reproducibility. But, with cineangiography, it improves, and with MRI it is much better. However, if you look at contrast echo, it is even superior to MRI, which is the standard technique to assessing LV function in terms of reproducibility.

So, there is no question today--actually, in Britain, one of the requirements for following these patients up in terms of, you know, patients receiving cytotoxic drug, it is a requirement that we use contrast echo to assess LV ejection fraction to follow these patients up.

[Slide.]

Now, I move on to another patient who is a relatively young lady, although diabetic, who presents with chest pain, shoulder pain, chest pain, who delivered about two months ago and was getting discomfort during pushing the pram.

Now, the major question here, of course, is whether this patient has got coronary artery disease. Now,

the ECG wasn't very helpful because it's normal, and in UK and in many other countries, I think they do a lot of exercise ECG to assess myocardial ischemia, which is one of the other tests to assess myocardial ischemia, but that turned out also to be non-diagnostic. So this patient was advised to have a stress echocardiography.

[Slide.]

Now, again, we are faced with a problem of this image. Now, as Dr. Kaul has said previously that to actually do a stress echo, we need pristine images because we need to look at every part of the left ventricle to decide whether that wall motion is normal or abnormal, and there is no way we can actually do this with this image quality.

Now, what is alternative? The alternative is we can send the patient for myocardial scintigraphy, but in this particular patient, that is not a good alternative, because this patient is feeding her child, radionuclide agents are not indicated in these patients.

We could have proceeded to coronary orthography, but this patient has intermediate likelihood of having coronary artery disease, and you don't want to subject this

patient to an invasive procedure which has got a high mortality and complication rate, so what we did is we injected contrast.

You can see immediately after injecting contrast, -this is the rest images. We can identify the fact that this patient not only has normal LV function--I mean this patient has normal ejection fraction, but right at the top you can see there is a wall motion abnormality at the apex, and we know that this patient is likely to have coronary artery disease.

But she still further went on to have an exercise echo because we want to know the extent of ischemia that the patient has, and you can see, on exercise echo, the left ventricle dilated. And there is a large area of wall motion abnormality right at the septum, and this was predictive of a proximal LAD disease.

[Slide.]

You can see here she then underwent angiography, and there is a proximal LAD lesion which was opened up, ballooned and stented.

[Slide.]

Now, she came back again three months later with

chest pain. We immediately proceeded for stress echocardiography. And this time the stress echocardiography with contrast was completely normal and this patient did not undergo any further testing in terms of diagnosis of coronary artery disease because we know this patient doesn't have any narrowing of the artery.

So, this test illustrates that, on the one hand, we appropriately directed the patient to a more invasive test, on the other hand, we prevented the patient from getting on to coronary orthography, which is an invasive test.

[Slide.]

Now, this is actually a trial done at Northwick Park in our hospital--because we are faced with this problem like Dr. Kaul has said, patients presenting with chest pain.

And we do cardiac enzymes first and, if they are negative and if there were high-risk factors, we still don't know whether they have got coronary artery disease or not.

So, the commonest test again used was exercise ECG and we said, okay, let's compare it with stress echo and echocardiography and see which is the better technique to identify patients at high risk.

[Slide.]

We can see this is randomized trial. By the way, about 200 patients in each group, and you can see the pre-test likelihood of having coronary artery disease if 69 percent in each group, meaning there are a lot of patients on whom we don't know whether they have got coronary artery disease and they need further testing.

Now, after the testing was done with exercise ECG, we are still left with 39 percent of the patients on whom we don't know what is going on and needed further testing. But when we look at stress echocardiography with contrast, we were left with only 3 percent of the patients.

While we could discharge the majority of the patients, saying that they are at low risk compared to only 33 percent of the patients in the exercise ECG arm, now, yes, it is well to discharge them, but what happened to those patients?

[Slide.]

Well, you can see now that when those patients who were discharged have excellent outcome, there was no difference between the stress echo and exercise ECG group, but bearing in mind that stress echo managed to discharge

many more patients than did the exercise ECG. Therefore, it was potentially cost effective to have this test performed in these patients for proper restratification.

[Slide.]

Now, this data looks at assessment of regional wall motion abnormality again in a multi-center trial compared to gold standard like MRI and also cineventriculography. This is looking at the accuracy of contrast echo versus echo for accurately identifying wall motion abnormality.

You can see that with contrast echocardiography, the accuracy is about 88 percent higher than, of course, unenhanced echocardiography, but also higher than cardiac MRI.

Using contrast echo, one can effectively, quickly diagnose with a fair degree of accuracy and reproducibility at the bedside and also assess patients for coronary artery disease by accurately identifying wall motion abnormality at the bedside.

[Slide.]

Now, stress echocardiography is not without complications. This is looking at large registry, 85,000

patients, and this is with dobutamine, which is one of the commonest used test in stress echo, and you can see the death rate is about 1 in 7,000, acute myocardial infarction is about 1 in 3,000.

[Slide.]

Now, this is the data with SonoVue in 5,000 patients who underwent dobutamine stress. You can see that there were no deaths in this study, no acute myocardial infarction. There were allergic reactions, but they were non-life threatening, and the other side effects that occurred are similar to what you see with dobutamine anyway, so there was no difference whether you give contrast or not in terms of complications.

[Slide.]

Now, the other advantage of using contrast in our clinical practice is simultaneously as you see wall motion, you can see microcirculation, and this is a patient who came with heart failure.

You can see there is a huge amount of wall motion abnormality, but also you can see microcirculation. You can see a contrast within the myocardium here, but no contrast in the apex at all. So, what does this information mean?

Now, it potentially means if there is contrast in the myocardium, meaning there are capillaries there, the myocytes are alive, and this part of the heart should do good, while this part of the heart is unlikely to recover, so it has both prognostic and even therapeutic implications because many of these patients will undergo revascularization if there is a lot of tissue viability as suggested by microcirculation compared to if it hadn't.

[Slide.]

These are examples in two patients with LV dysfunction. And you can see in one patient, there is a very good microcirculation meaning good perfusion and, in other patients there is no perfusion.

Look at the recovery after that, and this is the outcome. Those with good perfusion had a smaller ventricle contracting well and those with reduced perfusion had a dilated left ventricle and was not contracting well.

In other words, a good perfusion translates into better outcome compared to a reduced perfusion.

[Slide.]

We actually looked at the data in terms of cardiac mortality. You can see in this group of patients those with

good perfusion had a much better outcome compared to reduced perfusion, and this information is on top of wall motion and other clinical criteria, so it is an independent predictor of outcome.

This, you can do it simultaneously with contrast when we are looking at wall motion and then you look at the microcirculation, too.

[Slide.]

Now, we move on to extra cardiac application for assessing microcirculation. Now, this is ultrasound of the liver and what you see is echo-free space here. The question is--ultrasound is the first port of investigation anyway for liver--the question is whether it is benign or malignant.

Now, until recently, these patients would have gone on to have a CT ultrasound to decide whether this is malignant or benign with CT, and they use contrast, too, in CT. They inject contrast and they look at the phase.

The first phase suggests malignancy, in other words, there is vascularity, and then late phase suggests washout, and if there is a washout in that area with homogeneous opacification of the rest of the liver, that is

suggestive of malignancy.

[Slide.]

Now, this is with contrast and looking at exactly the same thing, this is the first phase. You can see the tumor here and then there is late phase you see it is gradually disappearing, and you can see it matches exactly what the CT showed, a hot spot, hot spot, and then gradually washout, washout.

It gives you the result what you can get with CT, but at the bedside, in real-time, not sending the patient away, with a technique which uses radiation and on top of it, the contrast, too.

[Slide.]

This is a direct comparison in large studies using this technique to identify focal liver lesions. You can see, of course, contrast, ultrasound is better than unenhanced ultrasound in terms of accuracy for characterizing the lesions, but if you look at the comparison with CT, there was no difference between the two.

[Slide.]

Based on these results, the European and the American group had recommended that contrast ultrasound

should be an alternative technique to assess focal liver lesions in patients.

[Slide.]

Now, another use of contrast is in stroke patients. We know that today, the treatment of acute stroke is thrombolytic therapy. It is also known that thrombolysis is not without complication.

You know, the patient may have bleeding, in fact, further stroke with thrombolysis, so it is important to triage patients, those who actually will benefit from thrombolysis versus in those who it would not.

Now, this is an example of a patient where you see it's a transcranial doppler examination. You don't see very much here, but as soon as you inject contrast, you can identify the circle of Willis in the brain, and you can see that the blood flow is stopped suddenly in the middle cerebral artery, and that is a thrombus. This is a corresponding image here.

[Slide.]

Now, this patient underwent thrombolysis and with thrombolysis you can also follow whether the thrombolysis is successful or not. And you can see here this is time zero.

This is one hour after thrombolysis you begin to see contrast in the vessel. In three hours, you see the contrast within the vessel.

So, with this technique, not only you can triage patients, but also you can actually identify in which patient the thrombolysis was successful.

Now, coming to the safety profile.

[Slide.]

These are the Bracco-sponsored clinical trials. There were 4,700 patients. In these, 0.02 percent had drug-related serious adverse events.

[Slide.]

This is looking at 6-year data. This is postmarketing surveillance analysis again looking at serious adverse events. You can see that even in this large group of patients. The serious adverse events remain below 0.02 percent and it doesn't change over the period of time despite increased use of contrast as people began using contrast even more.

[Slide.]

This is looking at deaths during this postmarketing surveillance data. You can see there were 5

deaths and that is equivalent to 1 in 145,000 patients, and 1 was clearly unrelated because it occurred 9 hours after SonoVue injection and a few minutes after morphine administration.

One seemed to be related to SonoVue because there was evidence of anaphylactic reaction to that, but the 3 cardiac deaths were in patients who had severe underlying cardiac disease whose spontaneous mortality itself was very, very high.

[Slide.]

Now, looking at the serious adverse events, it is found that 76 percent of the serious adverse events are related to allergic-like reaction, and these are characterized by the fact that they are independent of dose, it occurs within 1 to 2 minutes after injection, and the first symptom is hypotension and syncopal attacks, but these resolve with adrenaline and cortisone.

[Slide.]

Now, amongst all those serious adverse events, there were 30 patients who demonstrated cardiac manifestation--that is, 1 in 25,000--and these are the various cardiac manifestations at the bottom.

I am not going into each of them, and the bars indicate actual number of patients who actually manifested these symptoms. The red bar that you see here is association with allergic-like symptoms, so you can see the majority of these cardiac manifestations are associated with allergic-like symptoms as I have described before.

[Slide.]

Now, this is really summarizing the risk, you know, the classification of risk based on incidence of adverse events. This is a classification by the International Collaborative Study. You can see that SonoVue falls in the low risk category similar to MRI agent and certainly the iodine contrast agents in the medium range group.

[Slide.]

Putting everything into perspective in terms of mortality in cardiac procedures, the competing cardiac procedures that we may have to use if one technique is not helpful, you can see here with contrast echo, the mortality is 1 in 145,000 with SonoVue.

With myocardial scintigraphy, it is 1 in 10,000. That includes radiation hazards, et cetera. With exercise

ECG it is 1 in 2,500; with coronary arteriography it is 1 in 1,000.

[Slide.]

In conclusion, ladies and gentlemen, contrast echocardiography or, sorry, contrast enhanced ultrasonography has significant clinical impact. It certainly is a low risk procedure, and it has an excellent risk-benefit profile.

Thanks.

Preclinical Data and Implications for

Future Development

DR. WILLIAMS: Good morning.

I would like to share with you all some highlights of the nonclinical program with SonoVue.

[Slide.]

Starting out with the first slide, we will first discuss the studies that were conducted to support not only the clinical development but the marketing approval of SonoVue in Europe. Those were the pharmacology studies, toxicology studies, and studies that were conducted with ultrasound exposure in addition to SonoVue.

Secondly, we would like to talk about some

retrospective studies that were conducted after observations of the anaphylactoid reactions that Dr. Senior has indicated occurred in postmarketing and studies that we have done to look at potential mechanisms of these reactions both in vitro and in vivo.

[Slide.]

Moving to the pharmacology studies, as is typically done with these agents, imaging studies were done in large animals, pigs and dogs, to define the optimal imaging dose.

Importantly to these discussions, a very extensive series of safety pharmacology studies were done, over a dozen studies were done at significant multiples of the human imaging dose based on a body surface area, shown here, in mouse, rat, rabbit, dog, and monkey.

These studies were conducted to assess cardiovascular, respiratory, CNS, gastrointestinal, and renal function in animals. Importantly, these studies were done in conscious animals as well as anesthetized animals.

[Slide.]

The key findings of all these studies are shown here and really the only significant findings in these

studies were seen in dogs, and in conscious animals. No cardiovascular effects were noted at doses up to 0.3 mL/kg, which is 10 times the exact clinical dose; at 1 mL/kg, there was transient hypotension in 2 out of 7 dogs.

In anesthetized dogs, and to Dr. Hamlin's point, this was done in a pulmonary hypertensive model. There were only transient and minimal increases in pulmonary arterial pressure seen at 1 mL/kg, and effects were not seen at lower doses.

Importantly, of all those other studies that were done in primates and other animals, as well, there were no significant cardiovascular, CNS, respiratory, or other functional changes in these animals.

[Slide.]

Moving to the toxicology studies, these were done by intravenous bolus administration in animals. The clinical formulation being used, and the maximum doses were 27 to 54 times the human dose, again on a body surface area conversion basis.

These studies consisted of single dose studies, 4-week repeat dose studies in both rats and monkeys, genetic toxicology, reproductive toxicology, and other special

studies such as local tolerance and blood compatibility testing in human blood.

[Slide.]

The results of the toxicology program were unremarkable. In fact, there was no mortality in any species at any dose with SonoVue. There were no significant findings in the repeat dose studies in both rats and monkeys. The repeat dose no effect level in monkeys was the high dose of 5 mL/kg.

In rats, there were lesions in the cecum that were noted and this is considered a rodent-specific effect that is observed with other ultrasound agents. Otherwise, the no effect level was the top dose in the rat study.

Importantly, in these studies, there were no signs of immunological reactions in either species upon histopathological examination of thymus, spleen, and lymph nodes. Also, very importantly, there were no lung lesions or emboli noted in either species.

If you go back, please, there was another special study that was conducted. Since many patients may have cardiac shunts that will bypass the pulmonary filtration system, a special study was conducted by direct injection of

SonoVue into the carotid artery in rats specifically to look at the potential for brain emboli and toxicity.

In that study, there were no effects noted with intracardiac, carotid artery injection of SonoVue.

[Slide.]

Lastly, studies were done with SonoVue with concurrent ultrasound exposure. Again, up to 1.9 mechanical index, as shown in the instrumentation, and 1.2 in the dogs, and in rats there was no histological lesions in organs with SonoVue treatment in the presence of ultrasound.

In dogs, as well, this was particularly a study that was designed to look at electrocardiographic potential effects, and in this study, there was no effect on ECG parameters, in particular, QT interval, or on the heart histopathology in dogs up to this high dose of 1 mL/kg.

In this particular study, 1 animal did have hypotension that was consistent which was seen in the other conscious dog studies.

[Slide.]

The conclusions of the nonclinical program that supported the clinical development and marketing approval, SonoVue was well tolerated in all standard and safety

pharmacology studies when administered either alone or with ultrasound.

The cardiovascular effects that were observed were transient and observed at very high doses in dogs, and in general, the nonclinical studies corroborated the overall safety profile that was subsequently seen in humans that Dr. Senior has summarized.

[Slide.]

Now, on to the postmarketing effects and the anaphylactoid reactions. As discussed, very low incidences of allergic-like reactions were observed in humans in postmarketing surveillance.

Bracco has conducted additional in vitro as well as animal studies to investigate the potential mechanisms of this rare event.

[Slide.]

As you can imagine, having to investigate a rare event when you have such a clean safety profile represents a unique problem, but Bracco has maintained that the hypothesis is as presented by many in the literature, that these particle compounds, whether they may be micells or liposomes or ultracontrast agents, that the toxicity that

one sees with these allergic reactions is due to the particulate nature, and it is likely due to complement activation.

Again, the dilemma of trying to study something that is so rare, so Bracco has looked at cardiopulmonary studies in pigs. We have gone to very high doses in rats, as well, and also resorted, if you will, to in vitro studies to look at complement activation in pig and human, as well as in vitro basophil activation.

[Slide.]

We are starting with the pig again, which is an animal model that has its pros and cons. As we have alluded to, the pig is a good model in terms of echocardiography and was used to select the imaging doses and is commonly used in cardiology. However this species is known to have severe reactions to the injection of particulates, which is well documented in the literature.

Pigs also have very high concentrations of pulmonary intravascular macrophages, PIMs, relative to other species including humans, which under normal conditions you would not have PIMs in the pulmonary system, and this may be an important risk factor to discuss later.

Imaging studies in pigs then are commonly done with pretreatment with anti-inflammatory agents to control this particulate reaction.

So, while it is useful for imaging, the pig has not been considered as an animal model for safety pharmacology studies due to its overreaction to injected particles.

However, and alluding to Dr. Hamlin's comments, based on the human and rare reactions, Bracco has pursued studies in naive pigs to gain insights into the mechanisms of these anaphylactoid reactions, again a very sensitive species.

[Slide.]

Shown here is in anesthetized pigs, the effects of SonoVue and two marketed ultrasound contrast agents, and as you can see, there is a rapid and pronounced hypotension that is observed, accompanied by an increase in pulmonary arterial pressure, and pretty much the patterns that you see with these three agents are more superimposable, so there is a very consistent response.

Again, these are at human doses with these ultracontrast agents.

[Slide.]

The next slide is really to demonstrate that as with all--as individuals, whether they were pigs or people or rats, there is individual variation.

These are just individual responses with the hypotension with SonoVue. But, as you can see, with the exception of maybe one animal here, this is a very consistent reaction which is pretty much unlike what you see in humans. But, nonetheless, there is a consistent hypotension in this animal model.

[Slide.]

In further trying to understand the mechanisms related to this hypotension, and again you can see the increase in pulmonary arterial pressure shown here, and then there is a concomitant increase in thromboxane release vasoactive mediator--and, as you can see, it is very closely correlated with the increase in pulmonary arterial pressure.

[Slide.]

The key findings of the mechanistic studies in the pig model are many. SonoVue and other marketed ultrasound contrast agents were tested again in naive pigs, unpremedicated, and the doses ranged from 1 to 4 of the

human imaging dose.

There were decreases in arterial pressure, increases in pulmonary pressure, increases in heart rate, increases in airway resistance, and decreases in lung compliance.

The effects were dose and injection rate dependent and they were associated with increases in plasma thromboxane levels. There were no detectable increases in complement mediators C3a and C5a in vivo. But again Bracco is concerned that the release of these mediators may be so rapid that they would be very difficult to detect in vivo.

The effects were blocked by aspirin pretreatment and the effects again were similar to those reported for other injected particulates, liposomes, micellar lipids, et cetera.

[Slide.]

Continuing on, there was a marked individual variation, but again there was a very consistent response at the imaging dose of SonoVue and other UCAs.

Importantly, and again for discussion purposes, the pig shows a sensitivity not seen in humans. The symptoms, however, in cardiovascular effects resembled the

anaphylactoid reactions in human.

The release of vasoactive mediators is considered a key event in pigs. The relevance again to humans is unknown because of the differences in the anatomy in the lung but, nonetheless, cause us to look at this model seriously in terms of whether it is telling us something about the human response at very, very low frequency.

[Slide.]

In moving to the rat, again, we hadn't used this as a cardiovascular model. It was, can we push the dose in rats and elicit this effect, and, indeed, transient hypotension was observed in rats at very, very high doses, greater than or equal to 5 mL/kg.

There were increases in thromboxane similar to the pigs. However, the hypotension was not blocked by aspirin pretreatment contrary to the pigs. The hypotension was blocked by platelet aggregating factor antagonists pretreatment, and this was contrary to pigs, data not shown, but the PAF antagonist did not block the hypotension in the pigs.

The hypotension was blocked by complement depletion, with cobra venom factor. Our conclusion really

is the rat and pig mechanisms may differ in mediators or target cells involved. Maybe the PIMs in the pigs and maybe the Kupffer cells in rats is an example, but there may in addition be an underlying commonality in the release of complement.

[Slide.]

Just shown here is the dose-response to rats at very high doses going from 3 to 10 mL/kg of SonoVue, a very dose-related, hypotension and reversible importantly in all these studies, reversible hypotension.

[Slide.]

Going on to the in vitro studies, SonoVue and other UCAs were tested at very high doses and they showed similar findings after in vitro incubation. There was dose-dependent increase in C3a and C5a in pig plasma and a dose-dependent increase in mediators in human serum, as well.

There were really no marked differences between pigs and humans in vitro. So again a cautionary note in terms of this information may be qualitatively good information but doesn't explain the quantitative differences in the sensitivity between the species.

There were no effects on human basophil activation

in these studies.

[Slide.]

In summary, looking at these retrospective studies. we conclude that the symptoms observed in pigs are similar to human anaphylactoid reactions, in particular the cardiopulmonary changes.

The incidence of these anaphylactoid reactions in pigs is far greater than in humans, again great sensitivity maybe due to high density of PIMs relative to other species.

The rats show hypotension at very high doses, and the complement activation could be one of the mechanisms involved in the reactivity in all species, rats, pigs and humans.

[Slide.]

Just to kind of close with lessons learned in the overall safety evaluation, SonoVue again was very well tolerated in nonclinical studies and was corroborated by the result in clinical trials.

The lack of cardiovascular effects of SonoVue in safety pharmacology studies at doses relevant to humans correlates with the lack of anaphylactoid reactions in clinical trials.

However, another way of looking at that is that the studies did not predict the very low incidence of anaphylactoid reactions in postmarketing. We can't do toxicology studies with thousands of animals, can we? So, something to think about.

The anaphylactoid reactions seen in humans were similar to those seen in naive pigs, and this has been shown with various classes of particulates in the literature. The reactions of pigs is attributed to a high density of PIMs, which normally does not exist in humans, but may exist under pathological conditions.

Again, the relevance of the findings in pigs is unknown, but there are some similarities that we think are important in terms of the development of these agents moving forward.

[Slide.]

In continuing our lessons learned, in vitro complement activation may be an early triggering event in the reactions observed in humans and in animals and represents a potential screening tool.

Bracco is incorporating both in vitro and in vivo testing in the selection of next generation products.

The result in in vitro and in vivo models may be useful qualitatively in looking at potency of compounds, but certainly not quantitatively for risk assessment as we have all seen with the numbers in terms of sensitivity.

The rare anaphylactoid reactions may be thus reduced through these screening efforts.

Thank you very much.

Closing Remarks

DR. DENARO: Thank you, Dr. Williams.

[Slide.]

Just short conclusion. We think that the data demonstrate the excellent record of safety of SonoVue. Particularly, we think that the risk-benefit profile of the product in the clinical indication is clearly demonstrated.

As I told you, I think some of the data demonstrate that contrast ultrasound is a new emerging modality and I will give you some more information about that. This is important because there are clinical data emerging, suggesting that this modality has a large potential for new clinical use.

[Slide.]

These are areas where we, as Bracco, or

independent clinical people, have demonstrated the use of contrast in ultrasound with SonoVue. Clearly, myocardial perfusion, which was discussed already by some of the previous speakers for which we are going to have Phase III clinical trial for the indication, but also very interesting the ability to characterize the plaque by visualization and characterization of vasa vasorum plaque.

In oncology, in Europe, local percutaneous ablation of tumor are conducted under real-time examination with contrast in ultrasound, very interesting data for which we are going to have clinical development is the fact that you can monitor response of chemotherapy in tumors as much as you can increase the accuracy of biopsy for detecting prostate cancer.

One of the things which I wanted to briefly mention is that there are now very interesting data concerning the use of contrast in ultrasound in the rejection or toxicity of the drug for drug transplantation.

[Slide.]

If I go to the next, this is what you see. When you inject SonoVue, this is a normal kidney, you see the perfusion of the entire organ, you see and you can discuss

the dynamic of the perfusion.

This is an early rejection. Now, you see how different is the perfusion, how the perfusion is different from the normal. The entire organ is perfused, but the way it is perfused, the time of perfusion is totally different, and this is a kidney which will be rejected, total different perfusion, only perfusion in the central part of the kidney, no perfusion at all in the cortex.

Now, this is a patient where you will not use CT, because you cannot use hydrogen[?] compounds in these patients. Nowadays you start to have problem using MRI. The only thing you are left with is biopsy.

Now, this is a method which allows you to monitor constantly the patient because you can assess perfusion, so I think this modality has a tremendous potential, and I think we need to discuss how are we to facilitate the introduction of the modality also in the United States.

Thank you.

DR. HIATT: Thank you.

We will take a short break and commence at 10:15.

[Break.]

DR. HIATT: The next series of presentations will