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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

BIOLOGICAL RESPONSE MODIFIERS ADVISORY COMMITTEE

MEETING #37

Thursday, March 18, 2004

8:30 a.m.

Hilton Hotel
Silver Spring, Maryland

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GUEST HEALTH CANADA REPRESENTATIVE

Norman Viner, M.D.

GUEST SPEAKERS

Stephen Epstein, M.D.
Silviu Itescu, M.D.
Robert J. Lederman, M.D.
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Emerson C. Perin, M.D., F.A.C.C.
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C O N T E N T S

C O N T E N T S (Continued)

P R O C E E D I N G S

Call to Order

DR. RAO: Good morning. Welcome to the 37th meeting of the Biological Response Modifiers Advisory Committee.

Today's topic, as you all know, is related to use of cells in cardiovascular disorders, and we have a pretty full schedule for the next couple of days, but before we can start the meeting, we have to have a few sort of committee stuff that needs to be gotten through, so I will turn the mike over to Gail, so that she can make the mandatory announcements.

Conflict of Interest Statement

MS. DAPOLITO: Good morning.

The following announcement addresses conflict of interest issues associated with this meeting of the Biological Response Modifiers Advisory Committee on March 18 and 19, 2004.

Pursuant to the authority granted under the Committee Charter, the Associate Commissioner for External Relations, FDA, appointed Drs. Jeffrey Borer and Susanna Cunningham as temporary voting members.

In addition, the Director of FDA's Center

for Biologics Evaluation and Research, appointed Drs. Jeremy Ruskin, Michael Schneider, and Michael Simons as temporary voting members.

Based on the agenda, it was determined that there are no specific products considered for approval at this meeting. The committee participants were screened for their financial interests. To determine if any conflicts of interest existed, the agency reviewed the agenda and all relevant financial interests reported by the meeting participants.

The Food and Drug Administration prepared general matters waivers for participants who required a waiver under 18 U.S.C. 208. Because general topics impact on many entities, it is not prudent to recite all potential conflicts of interest as they apply to each member.

FDA acknowledges that there may be potential conflicts of interest, but because of the general nature of the discussions before the committee, these potential conflicts are mitigated.

We note for the record that Dr. John Neylan is participating in this meeting as a non-voting industry representative acting on behalf of regulated industry. Dr. Neylan's appointment is

not subject to 18 U.S.C. 208. He is employed by Wyeth Research and thus has a financial interest in his employer.

With regards to FDA's invited guest speakers and guests, the agency determined that their services are essential. The following disclosures will assist the public in objectively evaluating presentations and/or comments made by the participants.

Dr. Stephen Epstein is the Executive Director, Cardiovascular Research Institute, Washington Hospital Center. He receives research support, is a consultant to and has financial interests with, firms that could be affected by the committee discussions.

Dr. Philippe Menasché is employed at the George Pompidou Hospital in Paris, France. He has an association with a firm that could be affected by the committee discussions.

Dr. Emerson Perin is employed by the Texas Heart Institute. He receives consultant fees from, and is a scientific advisor to, firms that could be affected by the committee discussions.

Dr. Doris Taylor is employed by the University of Minnesota, Center for Cardiovascular

Repair. She receives consultant fees from a firm that could be affected by the committee discussions.

Dr. Norman Viner is employed by the Biologics and Radiopharmaceuticals Evaluation Centre, Biologics and Genetic Therapies Directorate, Health Canada, in Ottawa, Canada.

FDA participants are aware of the need to exclude themselves from the discussions involving specific products or firms for which they have not been screened for conflicts of interest. Their exclusion will be noted for the public record.

With respect to all other meeting participants, we ask in the interest of fairness that you state your name, affiliation, and address any current or financial involvement with any firm whose product you wish to comment upon.

Waivers are available by written request under the Freedom of Information Act.

Thank you, Dr. Rao.

DR. RAO: Now you know why I always have Gail read that statement.

Before we start any committee work, I would like to welcome two new members to the committee, Dr. Murray and Dr. James Mulé. We

generally introduce everyone on the committee first, and we generally go in alphabetical order, but this time I will try and start with the new members, so that they can tell us a little bit about themselves before we have the others introduce themselves.

Introduction of Committee

DR. MULE: I am Dr. Jim Mulé. I am currently the Associate Center Director for the H. Lee Moffitt Cancer Center in Tampa. I oversee all translational research at the Center including all cell-based therapies for the treatment of cancer as it applies to the clinical treatment of patients with advance tumors.

Prior to being in Tampa since September of last year, I was at the University of Michigan Cancer Center for 10 years, and prior to that, the NCI for another 10 years, and I am delighted to be here.

DR. MURRAY: Good morning. I am Tom Murray. I am President of the Hastings Center, which is celebrating its 35th years as the world's first research institute devoted to ethics in medicine and the life sciences.

I spent 15 years as professor at medical

schools including 12 at Case Western Reserve University School of Medicine. My interests are fairly broad. I write a lot about ethics and ethics in the life science and science policy.

Thank you. I am delighted also to be here.

DR. RAO: If we can go down the table, Dr. Tsiatis.

DR. TSIATIS: Hi. I am Butch Tsiatis. I am from the Department of Statistics at North Carolina State University.

DR. BORER: My name is Jeff Borer. I am a cardiologist. I work at Weill Medical College of Cornell University in New York City. I run a division and an institute at Cornell and, relevant to this meeting, I am the Chairman of the Cardiorenal Drugs Advisory Committee of the FDA.

DR. CUNNINGHAM: Good morning. My name is Susanna Cunningham. I am a professor in the School of Nursing at the University of Washington in Seattle, and I am the consumer representative for the Cardiovascular Renal Advisory Committee.

DR. SCHNEIDER: I am Michael Schneider. I co-direct the Center for Cardiovascular Development at Baylor College of Medicine, and our interests

are in the molecular genetics of cardiac muscle formation, cardiac growth, cardiac cell apoptosis and its relation to heart failure, and, relevant to this meeting, cardiac progenitor cells of different kinds.

DR. SIMONS: Hi. I am Michael Simons. I am Chief of Cardiology at Dartmouth Medical School. I work in the area of vascular biology, gene and cell therapy.

DR. RUSKIN: Good morning. I am Jeremy Ruskin. I am a cardiologist and electrophysiologist, and I direct the Cardiac Arrhythmia Service at Massachusetts General Hospital.

DR. NEYLAN: Good morning. I am John Neylan. I am a nephrologist and an organ transplanter by training. Currently, I am Vice President of Clinical Research and Development at Wyeth, and I serve as a industry representative to the committee.

DR. KURTZBERG: Hi. I am Joanne Kurtzberg. I am a pediatric oncologist. I direct the Pediatric Bone Marrow and Stem Cell Transplant Program at Duke University and the Carolinas Cord Blood Bank at Duke.

DR. ALLAN: Hi. I am Jon Allan. I am a virologist at the Southwest Foundation for Biomedical Research. My area is nonhuman primate models for AIDS pathogenesis.

DR. CANNON: Good morning. I am Richard Cannon. I am at the National Heart, Lung, and Blood Institute. I am Clinical Director of NHLBI, and I am representing NHLBI at this meeting.

DR. ROSE: Good morning. I am Stephen Rose. I am Deputy Director for the Recombinant DNA Program in the Office of Biotechnology Activities in the NIH.

DR. JENSEN: Good morning. My name is Nick Jensen. I am a reviewer in the Center for Devices and Radiological Health. I am a veterinarian and an engineer.

DR. McFARLAND: Good morning. I am Richard McFarland. I am a reviewer in the Pharm/Tox Branch in the Center for Biologics in the Office of Cellular, Tissue and Gene Therapies.

DR. RIEVES: Good morning. My name is Dwaine Rieves. I am a medical officer in FDA's Center for Biologics Evaluation and Research.

DR. GOODMAN: Good morning. I am Jesse Goodman. I am the Center Director of the Center

for Biologics. I would just like to join in welcoming especially the new members. My background is as an infectious disease physician in academic medicine for many years.

DR. NOGUCHI: I am Phil Noguchi, Acting Director of the Office of Cellular, Tissue and Gene Therapies in CBER.

DR. RAO: Thank you, everyone.

We are very fortunate in having some really leaders in the field come and present some of the data which will be the basis of where we can address some of the questions that have been raised by the FDA.

I am going to ask them to just briefly introduce themselves, as well.

DR. EPSTEIN: I am Steve Epstein, a cardiologist. I am head of the Cardiovascular Research Institute at the Washington Hospital Center. We are involved in vascular biology, gene, and cell therapy.

DR. MENASCHE: I am Philippe Menasché. I am cardiac surgeon at the Hospital European George Pompidou in Paris, France.

DR. PERIN: Good morning. I am Emerson Perin. I am an interventional cardiologist and

Director of Interventional Cardiology at Texas Heart Institute in Houston.

DR. TAYLOR: Hi. I am Doris Taylor. I am a scientist. I just moved from Duke University to the University of Minnesota to head the Center for Cardiovascular Repair.

DR. ITESCU: Hi. I am Silviu Itescu. I am Director of Transplantation Immunology at Columbia Presbyterian, New York.

DR. RAO: I would also like to welcome Dr. Viner who is from Health Canada. Health Canada has been following a lot of what the FDA has been doing and it is nice to have them there.

I would like to invite Dr. Goodman to make a statement.

FDA Opening Remarks

Presentation of Certificate of Appreciation to Retiring Member

DR. GOODMAN: My main purpose is to thank Joanne Kurtzberg for I guess about four years of service to the BRMAC. We really appreciate that tremendously. She has also interacted with CBER before that.

One of the reasons I really wanted to come by this morning. Joanne is rotating off this

committee. I know from interactions both within this committee and outside, and from all the leadership and staff within CBER, just what a tremendous advisor and asset Joanne has been for FDA and for your various fields here.

Of course, she has mostly contributed very extensively in her areas of hematopoietic stem cells, et cetera, but she has also been a very important thinker and discussant and contributor on the whole range of other cellular therapies and even gene therapy.

Please join me in thanking Joanne for her service over these years. Also, we like to say, particularly CBER, that we are a family and that nobody ever leaves it, and that we, just like a family, we will keep asking for favors in the future and probably causing grief in return.

Thanks so much, Joanne. We have a plaque for her, of course.

[Applause.]

DR. GOODMAN: I guess I will just turn it over to Phil to just give a brief introduction for the meeting, but just to say that, as I mentioned a little while back about the islet cell therapies, we, at FDA, are extremely excited about cellular

therapies and their potential, and I think nowhere is some of that potential clearer, but also perhaps more difficult to evaluate and help move forward than in the area of cardiovascular disease whether it is for ischemic disease or heart muscle disease or trauma, et cetera, some of the uses where there have been some very promising reports.

So, we think this is a very timely meeting. It is very important to get input about how to go forward with efficient development of those products, how to address some of the clinical and safety issues, and how to hopefully make this field positioned to realize its successes in the most efficient manner and also help FDA get that right to the extent that we all can based on incomplete information.

Again, we really look forward to this. I apologize, my usual schedule means I will be in and out, but I really appreciate it.

Phil.

DR. NOGUCHI: Thank you, Jesse, and, of course, Dr. Kurtzberg, our sincere thanks for the many years of service. Jesse is absolutely right, don't be surprised if the next meeting, you get a funny call early in the morning.

This is one of our, in a way, continuing series of dealing with things that seem really wonderful and amazing when they come up, where there is a lot of hope and there is perhaps a little bit of hype, but what we have always found over the years, and here I would like to just acknowledge Dr. Rose in the Office of Biotechnology Activities and the Recombinant DNA Committee, what we have learned from them is that one of the best ways that we have of really dealing with things controversial and where there is both hope and there is some trepidation about whether or not this is actually going to work or not, is to bring everyone together, put them in the same room.

Our continuing--and this really goes back at least 25 years through the RAC and many years for the BRMAC--is that when you get reasonable people together who may have differing opinions about things, but are presented the facts and the realities, as well as the unknowns, we all basically pretty much come out with the same conclusion, and then we can make significant progress in making these therapies not just experimental, but a reality.

With that, what I would really like to

do, because we have such a full schedule, is now turn it over to Dr. Rieves for the introduction.

DR. RAO: As Dr. Rieves comes up to the mike, I just want to remind people of a few simple rules. Remember that when you want to ask a question, make sure that you are recognized. Use the button. You will see that the light comes on. When you are done, just hit the button again to switch it off, because otherwise, there is sort of a feedback loop and noise. Make sure you identify yourself when you ask questions.

Cellular Therapies for Cardiac Disease

FDA Introduction and Perspectives

DR. RIEVES: Good morning. My name is Dwaine Rieves. I am a medical officer within FDA's Center for Biologics Evaluation and Research. This morning I am going to present a brief overview of FDA's perspective on cellular products used in the treatment of cardiac diseases.

As will be covered in a subsequent presentation, certain cellular products, when either perfused into the heart or directly injected into heart muscle, are thought to be capable of regenerating heart tissue and/or augmenting heart function.

Consequently, these products may have special utility in the treatment of heart failure and certain other cardiac diseases. Today and tomorrow, we will discuss issues in the early clinical development of these products.

[Slide.]

This talk is divided into three major sections. First, I will cite the purpose in convening this advisory committee. Secondly, I will provide a regulatory background on FDA's understanding and activities within the realm of clinical development of these products. Finally, I will introduce the major questions we have proposed for discussion.

[Slide.]

Unlike many advisory committees where the topics center around assessment of data associated with a specific product or data related to a specific regulatory concern, our purpose in convening this committee is not to obtain definitive regulatory advice, instead, FDA has convened this committee to listen to, and learn from, the voiced thoughts and perspectives with the understanding that this information will enhance our ability to promote the safe clinical

development of these products.

As you are aware, the clinical development of cellular products is in its infancy and many questions surround the very early stages of product development. Consequently, our purpose today and tomorrow is to stimulate a solid scientific discussion of the major facets associated with the very early clinical development of these products.

As noted here, we will focus upon three major areas: manufacturing aspects of the cellular product, preclinical testing of the products, and finally, items related to the early clinical studies.

[Slide.]

What are the cellular products we will be discussing? These products may be broadly grouped into two categories.

Firstly, those manufactured without ex-vivo culture methodology, that is, the cells are harvested from humans, processed, and then delivered to a recipient without maintaining the cells in culture for a period of time.

In general, these cells consist of bone marrow mononuclear cells and certain peripheral blood mononuclear cells, hematopoietic progenitor

cells that are variously referred to as stem cells, cells thought to be capable of assuming phenotypic characteristics of non-hematopoietic cells.

The second category consists of cells that, following harvesting, are maintained in ex vivo culture for a period of time before final processing and administration.

In general, these cells consist of those derived from skeletal muscle tissue, cells frequently referred to as myoblasts, and certain bone marrow stromal cells, cells also referred to as mesenchymal cells. Whether these cultured cells should be regarded as forms of stem cells is more questionable than that for the hematopoietic progenitor cells.

Lastly, as the slide notes, most of the cellular products we will be discussing today and tomorrow are of autologous origin.

[Slide.]

The many questions surrounding the scientific basis for cellular product development illustrate the very nascent nature of the field. As we are probably all aware, there is almost no precedent for the clinical development of products intended to regenerate and/or augment disease

tissue.

The scientific data surrounding this field are relatively new, such that the data are limited in depth and the extent of replication. Hence we come to the table of clinical development with many hypothetical considerations and some, but relatively limited background supportive data.

[Slide.]

Given these limitations, our discussions today and tomorrow assume a scientific focus in which certain insights and perspectives are presented, and you, the committee members, will be asked to share your thoughts. Three points are cited here.

First, we acknowledge that these thoughts are all tentative and susceptible to revision based on accumulating data.

Secondly, we are not requesting any definitive assessment of data, and we note that the data presented here today are within the public arena, and have not undergone FDA vetting.

Finally, I reiterate an earlier comment, that no specific cellular product discussed here is under review with respect to regulatory decisionmaking.

[Slide.]

This slide illustrates the interconnectedness of clinical research and regulatory paradigms. The connecting link between the two fields is the science. Clinical research generates the scientific background for clinical development of cellular products and the scientific background forms the major basis for our regulatory paradigms.

[Slide.]

FDA is charged with many responsibilities, but as cited here, two are especially relevant to this discussion. Specifically, FDA's mission is to promote and protect the public health by optimizing pre-market product development and ensuring sufficient post-marketing product monitoring.

The key word in these two statements is "product." A notation that whereas we frequently hear the terms transplant, graft, and procedure, we need to think in terms of a cellular product, a product that is manufactured, labeled, and potentially marketed.

[Slide.]

A little over 10 years ago, FDA clarified the regulatory basis for oversight of clinical

development programs for cellular products. In general, this regulatory framework is the same as that for the drugs and biologic products we commonly recognize as marketed products.

Hence, the commonly cited biologic product, drug, and device regulations applied to the clinical development of these cellular products, and the clinical studies must be conducted under the purview of submission of a investigational new drug application.

The last bullet on this slide reminds us that clinical development programs may be divided into early and late stages, with the late stages focused upon the ascertainment of data definitive to safety and efficacy, and the early stage, what we are talking about today and tomorrow, focused upon the ascertainment of exploratory safety and bioactivity data.

That is, we hope to examine the nature and extent of background data necessary to introduce the cellular products into small, sample size, Phase I clinical studies.

[Slide.]

As previously noted, the keystone consideration in early clinical development is

safety. Specifically, we need to ensure that the tripod of product development is solid. That tripod consists of manufacturing control and testing information, sufficient preclinical testing information, especially information that may inform the design of a clinical study, and finally, the clinical study itself.

The next few slides will cite each of these three components.

[Slide.]

Cellular products must be manufactured in some manner, that is, the cells must be harvested and processed prior to administration to a recipient. Manufacturing aspects may be divided among four major areas, three being shown on this slide.

The top bullet notes that documents should describe the cell source and reagents used in the manufacturing process, such as growth factors, sera, salt solutions and additives. We need to be confident that all the reagents used in the manufacturing are of clinical or pharmaceutical grade, or that if they are not pharmaceutical grade, they are sufficient for human use.

One may envision many potential concerns

with these materials, such as the use of sera that may contain infections agents, or the use of only partially purified reagents that contain harmful excipients.

Secondly, documents should describe the procedures used in manufacturing, specifically describing how cells are aseptically harvested, isolated, and potentially selected.

For example, a distinct population of cells may be selected based upon the presence of certain cell surface markers, such as the CD34 antigen with the selection process involving incubation with an antibody to CD34.

As we know, many investigational antibodies have been developed to target cell surface antigens, and we need to be confident that these selection techniques are performed in a reproducible and safe manner.

Additionally, documents should describe the storage and tracking of the cellular products, this being of special concern because certain cellular products may be patient-specific products.

For example, measures must be in place to ensure that for autologous products, the cellular product is returned to the correct donor. Of

course, the cellular product needs to be labeled as one for investigational use only.

The bullet at the bottom of this slide emphasizes the importance of testing the cellular product, an especially important concern since cellular products cannot be sterilized in the same manner as one might sterilize a drug product or a device. Notable aspects of testing include tests for sterility, endotoxin, viability, enumeration, or cell counting.

[Slide.]

The fourth component of manufacturing information is product characterization as highlighted here. When one speaks of product characterization, we are generally talking about cellular phenotype and/or functional characterization and the characteristics of the product's final formulation.

For example, a product containing solely CD34 positive cells in saline with no preservatives or media. Product characterization is especially important from a clinical perspective, because failure to consistently manufacture a product makes the clinical data virtually uninterpretable.

As noted here, the major aspects of

product characterization consist of a description of identity, purity, and potency of the final cellular product.

[Slide.]

Pre-clinical testing is the second major component of product development, and the major aspects of this testing are cited here. The top bullet notes that consistent with the science, the extent and depth of preclinical testing necessary to support a clinical study is an evolving paradigm and is a major topic for discussion at this meeting. However, we generally take the stance that this preclinical testing paradigm should be consistent with that used for other biological products.

The last bullet notes another important aspect of preclinical testing, the testing of the product administration procedure.

This is especially important because many cellular products involve injection directly into heart muscle either through the epicardial surface or the endocardial surface. These techniques represent inherent safety concerns that may be best evaluated in animals prior to their use in humans.

As noted, all available catheters, whether

marketed or not, are regarded as investigational with respect to administration of cellular products.

[Slide.]

This slide highlights three aspects of preclinical testing that will be the focus of the preclinical questions tomorrow.

Firstly, the choice of the relevant species is central to designing preclinical studies with the major choices being between large animals, such as pigs, versus small animals, such as mice, as well as the choice between immunocompetent animals where, for autologous products, the cellular products would be the animal cells, not human cells, or immunocompromised animals, where the actual human cellular product may be tested.

Secondly, designing preclinical studies raise questions of the choice of model, that is, a disease model, such as ischemic heart disease induced in the pig versus a healthy animal.

Lastly, preclinical concerns relate to testing of the administration procedure itself, such items as the impact of the catheter materials upon cells, the potential for occlusion of catheters by the cellular product, and the safety

concerns associated with manipulation of the catheters in the heart.

[Slide.]

The third component of the clinical development program for cellular products is the clinical study. There are many aspects of clinical study design that could be discussed, but at this meeting, we are focusing upon two, the first shown here, that is, adverse event detection.

This slide highlights two aspects of clinical study design that are frequently engineered to optimize adverse event detection, the evaluation plan with attention to the duration of clinical follow-up, the frequency of evaluations, and the extent or nature of these evaluations.

Secondly, the clinical study safety monitoring plan may be optimized through the use of close scrutiny of each study subject based upon the sequential, not simultaneous, enrollment and treatment of the subjects, as well as the prespecifications of the types and numbers of adverse events that should prompt interruption of the study, that is, the study stopping rules.

Tomorrow, the committee will be asked to discuss potential adverse events in these early

clinical studies, both the nature of the events and ways to optimize the safety of the studies.

[Slide.]

This slide illustrates an additional clinical study design item that we will bring to the committee, that is, a discussion of the analysis of adverse events.

Exploratory clinical studies are, by their nature, small sample size studies in which it is often difficult or impossible to distinguish treatment-related events from adverse events that might occur in the natural history of the disease, potential study design mechanisms that might help, but certainly not resolve this issue are cited in the bullets, design features that incorporate randomization of subjects among groups, such that comparisons may be made, the use of controls, especially placebo controls, to make comparisons, the use of masking or blinding to help lessen the bias associated with concomitant therapies or clinical care.

Tomorrow, the committee will be asked to discuss mechanisms that might aid in adverse event attribution.

[Slide.]

In this presentation, we have covered three major topics. Firstly, we have noted that the focus of this meeting is upon a discussion of the scientific aspects of early cellular product development.

Secondly, we have noted the regulatory precedent for the cellular products.

Finally, we come to the questions.

[Slide.]

This slide highlights the four major areas of tomorrow's questions. Specifically, questions related to manufacturing, we will request a discussion of the extent of safety testing and characterization that should be performed prior to the release of a cellular product for administration to humans.

The second and third discussion areas are especially critical and may consume the bulk of our time, that is, the extent and nature of preclinical testing necessary to support the introduction of a cellular product into humans, testing that involves questions related to the product itself, as well as the delivery mechanism, the catheter.

Finally, we will pose clinical questions centered around adverse event detection and

analysis with a discussion of the pros and cons associated with the use of controls in these studies.

[Slide.]

Our agenda is summarized on this slide. As you can see, today, we have a series of invited presentations by FDA staff and leading investigators in the field, as well as the opportunity for public presentations.

Tomorrow, we will have another opportunity for public presentations followed by a discussion of the questions.

[Slide.]

In closing, listed here are some documents that are especially pertinent to our discussions. All these documents are available at www.fda.gov under the CBER sites, specifically the guidance section.

The first document is entitled "Draft Guidance for CMC Reviewers: Human Somatic Cell Therapy Investigational New Drug Applications." This document describes the types of information FDA reviewers will examine following the submission of an IND. Consequently, it provides a very clear description of the types of manufacturing

information that needs to be submitted with an IND application.

The second document is from the International Conference on Harmonization of Regulatory Practices, and it is entitled " Preclinical Safety Evaluation of Biotechnology-derived Pharmaceutics," the S6 document.

This document is cited because it contains a paradigm that one may apply to cellular products.

Finally, the last bullet cites one of the most useful guidances to sponsors and investigators, the ICH Guideline on Good Clinical Practice.

This guideline provides detailed information on how to design and conduct a clinical study, information presented in a simple to read, yet relatively comprehensive format.

This concludes my presentation and I thank you for your attention.

[Applause.]

DR. RAO: Before we continue with the rest of the presentations, I would like to just welcome Dr. Harlan and ask him to introduce himself.

DR. HARLAN: I apologize for being late,

but I am David Harlan, NIDDK. I study transplantation of islets and immunotherapies.

DR. RAO: Our first speaker will be Dr. Perin, whom you already were introduced to.

Guest Presentations

Overview Cardiomyopathy and Ischemic Heart Disease

DR. PERIN: I want to thank you for the invitation to be here to present to you today, especially Dr. Grant, who has helped me put this together in a way.

So, what I want to do here this morning, the task that has been laid before me is that of in a way setting the stage or giving you a general idea of the kinds of patients that we are treating.

Obviously, this is fundamental if we are thinking about doing clinical trials. It is very important to understand the nature of the disease in which these kind of therapies will frequently be applied.

What I plan to do is talk about the following topics. First, we will start from the beginning, define what heart failure is, look at the scope of heart failure, talk a little bit about the pathophysiology, look at some prognostic markers, talk about the treatment to some extent

and that is important in terms of monitoring, and then really work our way towards end stage heart failure because that is where I think the focus of most of the future clinical trials will likely be initially, and finally, talk about adverse events, which I think is a major concern, and the monitoring of there adverse events.

Now, I know many of you are not cardiologists, so hopefully, I can go from a level where we are not getting too complicated, but not too simple.

Starting with the definition of what heart failure is. Firstly, heart failure is a clinical syndrome very simply defined by certain symptoms and certain signs that come together. These symptoms are fatigue, shortness of breath, and congestion, and these are translated on a physical exam by being able to hear a third heart sound, the patient manifesting peripheral edema, and jugular venous distention.

If we start looking at this problem and have a broad overview of this, first, I want to show you a graph from the HOPE trial. This is a trial that was conducted in thousands of patients, as you can see here, over 9,000 patients. It was a

study primarily of ramipril and vitamin E in patients with hypertension over a long period of time, involved a five-year follow-up.

But it is just very interesting, as we start out looking at heart failure, to look at this patient population, and here we have over 500 days, so here is about a year out, and if we look at this population, who is not primarily designated as particularly sick or harboring heart failure, that identified the patients that did have heart failure and we look at their survival, you will see the mortality.

It separates from the beginning, and when we get out to about a year, you have got a 10 percent mortality in the group that has heart failure compared to less than 4 percent mortality in the general population. So, you can see that the problem that we are dealing with seems to be very serious.

If we go here and let's just look at the placebo arms of some very large heart failure trials, these are trials pretty much aimed at evaluating different forms of therapy now in heart failure patients, and looking at different severity of heart failure patients, for example, in the

V-HeFT trial, inclusion criteria might be an ejection fraction less than 40 percent.

If we look at PRAISE, which evaluated amlodipine in more severe heart failure, an ejection fraction was less than 30 percent, comparing this with Class III and Class IV patients, very sick patients.

So, you can see here if we look at just the placebo arms of all these trials, a very striking mortality as we go along. If we look at 1 year here, this will vary from 10 percent down to around 30 percent.

If we go out to 2 years in the very sick patients, we see that half of the patients are dead. So, heart failure, depending on the presentation, carries a very ominous prognosis.

It is a very broad problem, 5 million Americans are living with heart failure now, 550,000 new cases are diagnosed each year.

From 1979 to 2000, heart failure deaths increased by 148 percent. Now, what is interesting, over this period of time, we have actually gotten a lot better at treating heart failure, and we do treat it. I will get into this a little later, and I will show you the modern treat

of heart failure and how much better we are doing, but at the same time that we are treating heart failure better, we are also treating the patients that have coronary disease, which is a very dominant problem in this country and around the world, we are treating those patients better, too, so what happens is we are getting more patients with heart disease that normally would have died earlier, to live longer, and as we are able to bypass and stent and do all these revascularization procedures and come up with better treatments, we are getting people that go further down the road, that otherwise would have succumbed a long time ago.

So, despite our improvements in treatment of coronary disease, we are dealing with an increasing amount of heart failure deaths.

In individuals diagnosed with heart failure, cardiac death occurs at 6 to 9 times the rate in the general population. If you are more than 40 years old, you have a 1 in 5 chance of developing heart failure, and 22 percent of men and 46 percent of women that have heart attacks will be disabled within 6 years with heart failure.

So, as you can imagine, the high

prevalence and multiple complications have an implication in terms of health costs. If we look at the costs, and these numbers vary, and it depends on what you are looking at and what year you are looking at, but this is a very significant financial burden on the country, over 5 percent of the total health care costs.

You can see that most of the cost involved is really involved in inpatient care, and as I will show you hopefully, that really translates to the sickest portions of these patients, that as you get sicker with heart failure, you start coming into the hospital more, and that is what runs up the cost of treating these patients. It is interesting that transplant is just a little sliver out of the pie here.

So, let's look at the causes of heart failure, and I am not going to get into all the little minor details, but let's look at the major causes of what brings on heart failure.

Seventy-five percent of people that go on to develop heart failure had hypertension previously. Valvular heart disease is a big contributor and also heart failure engenders valvular heart disease, mitral regurgitation

further contributes to the problem.

Coronary artery disease, you are all familiar with this, the number one problem in this country, and this is really what we are going to focus majorly on in terms of causing heart failure and the specific kind of heart failure that this engenders.

In cardiomyopathy, there is many different kinds of things that get a heart to perform poorly, all the way from an idiopathic cardiomyopathy to such things as iron overload, et cetera, which are not as common.

Now, what I want to talk about here is really systolic heart failure. There is something called diastolic heart failure, and that really has a lot to do with compliance problems of the ventricle, and in these patients, we are going to see a normal ejection fraction.

So, this is really a different animal and it is really not what we are focusing on, so what I am going to be talking about today is systolic heart failure, and as I will show you, with the hallmark being a low left ventricular ejection fraction.

This is just to give you a practical

example. This is an angiogram from one of the patients that we treated with stem cell therapy in Brazil, who all had an ejection fraction that averaged about 20 percent. This patient has an ejection fraction of 10 percent.

You can see the coronaries are calcified. This is a catheter in the left ventricle. This heart is supposed to be pumping this contrast we just put into the aorta. As you can see, it is not doing that very well at all. Only 10 percent of what is in here gets out with each beat.

So, you can tell this is a dilated big heart that just doesn't contract well. That is the picture of severe heart failure right there, and this is what I want to talk about.

Now, when we talk about heart failure, I think everybody is aware of the classification. There is Class I, II, III, IV, which are commonly used, but it is important to acknowledge this. Class I involves no limitation of physical activity, Class II slight limitations, Class III marked limitations, you can't walk up a flight of stairs without getting short of breath, and Class IV, you have symptoms at rest.

If we look at this, if we put Class III

and Class IV together, you see the division is about a third for each of these pieces of the pie here.

Now, if somebody comes in with Class IV heart failure, they are very short of breath at rest, you can give them some diuretics and they will feel better. They are not Class IV anymore, they are Class III.

So, it is interesting, there has been a want in development of a little different way of looking at heart failure, and a staging or classification put out by joint AHA and ACC shows four different stages, and really looks at heart failure more like a disease like cancer.

So, where we can identify patients that are at high risk of developing it, we can screen patients, and then we can start treating patients before they really manifest symptoms of the disease.

Again, this is a progressive disease and we are going to end up with people that are refractory even to all kinds of treatment. I am going to go over this a little bit more in detail a little later.

So, in defining what heart failure is, I

hope I have given you a general idea of the scope of the problem, just talk a little bit about what causes it because it is important to understand that to be able to know how we treat it and how we monitor these patients.

Usually, we are talking about ischemic heart disease and we are dealing with a myocardial insult, which is usually a heart attack, so that heart attack causes damage to the heart muscle, and that is going to result in dysfunction of that heart muscle.

Well, the body is going to try to compensate this dysfunction and especially in two major ways. One is neurohumoral activation, so we will talk a little bit about this in more detail, but essentially, these compensatory mechanisms are going to make the heart change its shape and its size. It is something we call remodeling. It involves hypertrophy of the myocytes and then it involves fibrosis and dilatation.

So, these mechanisms that the body helps, to try to help to reverse what is going on, actually wind up causing toxicity, hemodynamic alterations that all lead to remodeling, and remodeling really is the hallmark.

You saw that big heart. Well, remodeling is how you get from a normal small heart, which you have, to a big boggy heart that doesn't contract. That is the problem of heart failure.

This was very simply put by Doug Mann in a nice editorial a few years ago. Basically, here is the heart over time, as we have an index event, and basically, remodeling occurs, the heart gets bigger, the ejection fraction goes down as time goes by and symptoms occur as time progresses, as well.

So, I have told you we have a myocardial insult. This leads to LV dysfunction and remodeling, and this really instigates a neurohumoral response. In return, this is going to have an impact on remodeling again.

So, what are these neurohumoral things that happen? Well, first of all, most importantly, is the renin- angiotensin-aldosterone system, and there are several points in which the body upregulates the system and ultimately, it acts on the AT-1 receptor, which will cause vasoconstriction, proteinuria, again LV remodeling.

As you can identify, here are several sites in which medications, the mainstay of some of

the therapy for heart failure works, namely ACE inhibitors that work at this point, ARBs that work at this point, beta blockers have a role in inhibiting renin, as well. So, some of the mainstay of therapy is actually directed at one of these mechanisms of compensation.

On the other side, we have sympathetic activation. We have increased sympathetic activity that again leads to myocardial toxicity and arrhythmias, and then on the other side, with the sympathetic outflow, we get vasoconstriction. This impacts negatively on the kidney, sodium retention, more vasoconstriction, and progression of the disease.

Just to get a slightly little bit more complicated, just to mention that it is really not all that simple, there are other things involved, and we have cytokines, TNF-alpha, IL-6, inflammation that actually progresses with the progression of heart failure.

Endothelin is a potent vasoconstrictor. All these things lead to apoptosis and unfavorable effects upon the myocyte, but then lead to LV remodeling, which I have told you is one of the mainstays of reasons for heart failure.

Now, natruretic peptides are important, as well. It's another compensatory mechanism that the body has. I am sure you are familiar with these BNP, it's a B-type natruretic protein that actually comes from the ventricle, the A types comes from the atrium. We will just focus on the B type.

What this does, basically, in response to elevated pressure inside the heart, we secrete BNP. This suppresses the renin-angiotensin-aldosterone system and suppresses endothelin. It helps with peripheral vascular resistances, decreases vasodilatation, and it increases natruresis.

So, if we go on to understand now that there is an interplay between LV dysfunction and remodeling, and that basically, this will lead to low ejection fraction, and that is what we see in the patients.

On the other hand, as a result of this, we will start getting a constellation of symptoms, and it is the combination of having a low ejection fraction and symptoms that defines heart failure.

Let's look a little bit at the prognostic markers. I just talked a little bit about BNP. Well, it is very interesting. If we divide BNP in

quartiles here, depending on the amount of BNP that you have circulating, your survival will go down. It is a prognostic marker, as well as a treatment. Norepinephrine, the same way. So, these are markers of prognosis.

It is very interesting. These are levels of BNP, and if you can decrease them, decrease to a less degree, or here, we have an increase. So, depending on which direction your BNP goes, your survival varies as well, and that is an important concept.

Let's look at another different kind of marker. Exercise capacity, peak oxygen consumption. In the transplant world, this is very important. Here you see the number 14, so a peak oxygen consumption greater than 14 or less than 14 has very different prognostic indicators and in many centers, this serves as a marker threshold for one of the criteria for entering the patient into a transplant program.

You can see here a difference in mortality from 53 percent mortality over two years in patients that have an NVO2 of less than 14, to that of 11 with greater than 14, so this is another important number in patients with heart failure.

Then, if we look overall and look at symptoms and hospitalizations, here is a New York Heart Class I to IV, and this is fairly intuitive, but as we get more symptomatic, we have an impact on survival, and as we are getting more symptomatic, we have an increase in rehospitalization.

What about ejection fraction? I just talked about ejection fraction, and you can see here, similarly to NVO2, ejection fraction can divide prognostically how patients will do. Here we see more than 20 percent, less than 20 percent. Here you see a two-year survival, 54 percent, so half the people dying that have an ejection fraction less than 20 percent. At one year, that is a little over 20 percent.

The same thing, this is a large randomized clinical trial, ejection fraction less than 40 percent. Over time, people die more frequently.

Now, let's add a little arrhythmia to this. Looking at different levels, the first two are greater than 30 percent ejection fraction, here less than 30 percent, so that stratifies that out, but then if we just add the amount of extra ventricular beats to this, and if we have less than

10 per hour, more than 10 per hour, and then with a poorly contractile ventricle, your survival goes down as we add extra ventricular beats.

One attempt that has been made to sort of graph this problem, because now I have shown you many different prognostic markers and different things we can use to classify these patients to decide what to do and how to follow them.

One of them is a heart failure survival score. There is an invasive model, there is a non-invasive model. So, things like cause of heart failure, resting heart rate, EF, mean blood pressure, if there is a conduction delay electrically in the heart, oxygen consumption, and serum sodium can enter into a risk classification.

Here, you just basically have a graph that shows according to low, medium, and high, your survival will vary according to the risk.

In our little schema here, that leads symptoms and low ejection fraction to heart failure, what are really the things, though, that are driving mortality? They are going to be pump failure, on the one hand, and arrhythmia, on the other, because sudden death, as I talked to you about before, is a very prominent problem in people

that have heart failure.

So, it is the combination of these three things that will pretty much drive patients to a lethal exit.

Let's talk a little bit about treatment now. What are the goals of treatment of heart failure? You want to delay the progression or reverse remodeling, which you can do in some patients, and delay the progression and reverse myocardial dysfunction.

You want to reduce mortality, relieve the symptoms, improve functional capacity, and reduce disability, also decrease the intensity of medical care and hopefully reduce economic cost.

I have shown you we go from initial injury, initial infarct, we suffer remodeling, we get a remodeled heart that now has a low ejection fraction, and over this course of time, we have a worsening of symptoms, so how are we going to impact this in terms of treatment?

Well, the two mainstays are neurohumoral blockade, we have kind of gone over some of the things that we can do, and we will look at those, and the other is revascularization. So, many times with the use of medication or with the use of

revascularization, we can reverse some of this remodeling in some patients, and in some patients we don't.

One thing that is very important in terms of being able to recover patients that have remodeled hearts, and that are in this road of heart failure, is identification of viable myocardium.

Myocardial viability has clearly been shown to influence the prognosis of people that are undergoing revascularization procedures, so if you have a viable myocardium, you are going to do better. You have a chance of improving more than someone who doesn't.

Just to shift gears for just a second here, these are electromechanical maps. These are representations of the left ventricle. This is from a patient in our Brazil stem cell study.

This is an electrical map, this is a mechanical map. Let's just look at the electrical map because I just talked to you about viability. Very simply, if your cells are alive, they have an electrical signal that is high. If you have a big scar with no cells, you have no electricity, you have a low electrical signal.

We put it on a little color scale. Red is dead or red is very little voltage. Purple is high. Here, you see on this electromechanical map, an area of myocardial viability. Again, just as it is important to understand viability when you are vascularizing patients that have heart failure, that have coronary disease, it is also going to be important, in my view, to understand myocardial viability when we are applying some of these therapies, and I think there will be differences in bone marrow therapies and myoblast therapy, but that is something to keep in mind.

I just wanted to show you an example of the very common things that we deal with, so this is not some esoteric difficult patient to find. We come across people like this all the time in the hospital every day.

This is a patient who was 41 years old, he had bypass, he stopped up all his vein grafts and his memory artery, and he had ejection fraction of 20 percent, very similar to the one that I showed you, and Class IV congestive heart failure.

This gentleman was really delightful. He was actually a pilot for a major airline, and because of his bypass, he had to be put off the

flying, and he was actually in charge of all the simulators, and he was the guy that graded all the pilots when they had to come in and do the simulation testing.

Basically, here, we have a 41-year-old guy, very active man who has gone bypass, he has lost his graft, he obviously has very aggressive disease, and why I hear the talk about why some people have more aggressive coronary disease than others.

You see this is his right coronary, it is completely blocked up, X's mean that you can't see anything on angiography, so this kind of fills from the other side by collaterals, see these little twigs down here.

Then, the circumflex is completely occluded. This is a floating marginal branch. This is supposed to be connected, but this is totally occluded, as well. The only artery he has got left is the one down the front of his heart, but this is very much infarcted, and has a very significant blockage here, as well as the takeoff of this.

So, this patient, there is really nothing to do, and we are faced with this a lot every day.

This patient, as I have shown you these curves of mortality, this patient at our hospital wound up going for an LVAD type procedure and died, and that is what we see again and again, so this is a very serious problem.

So, looking of an overview of treatment of heart failure, let's see, we have medical-based therapy, on one hand, we have device-based therapy, on the other.

On the medical side, we need neurohumoral blockade, we can have a hemodynamic approach and also antiarrhythmic approach, so we are going to use these drugs, ACE inhibitors, aldosterones, diuretics, beta blockers, and then antiarrhythmics, such as amiodarone, and then we are going to use more potent i.v. inotropes that improve hemodynamics, and asaratide [ph], which is basically similar to BNP, it is like giving the patient BNP.

On the other hand, we are going to have a device-based approach using resynchronization therapy. It really hasn't shown a benefit in survival, but in combined endpoints. We are going to put defibrillators into people, and I will show you how that has improved survival.

Then, we will have ventricular assist devices, and when all this fails, we have an option of heart transplant, that is very little available actually, and as you saw, it is a very little sliver of what we are able to do.

But as you cumulatively add these therapies, you are able to impact on survival and make patients live longer. Here, you see sort of adding digoxin and diuretic, adding an ACE inhibitor, and then adding a beta blocker, we get progressive improvement. So, this is pretty well established in terms of medical therapy.

When we look at defibrillators, here is a curve. This is from the MADA-2. This is primary prevention, defibrillator in patients, previous MI, LVF less than 30 percent, a very significant survival difference in the patients that get a defibrillator, so treating the arrhythmias is also important.

Back to our schema of the different classification of stages of heart failure. You see that we can gradually, we start with ACE inhibitors and gradually add different medications, but everybody kind of goes up these stairs and ends up here at the top, and that is why we have increasing

mortality from heart failure, because we are getting people to get to this point where before they really didn't reach that stage.

Then, we get to a stage of basically refractory symptoms, so they have been bypassed, they have had stents, everything has been done for them, and they have that bad heart, it doesn't pump well, they have a lot of symptoms, they can't breathe very well. Many of them have angina. I want to want to give you a little bit of my own perspective on that.

If we look at current trends, this was published last week in JACC, very interesting. Heart failure treatment--this is the survival curves--heart failure treatment in 1994 to 1997. Here is a survival curve. We have improved the treatment of heart failure.

1999 to 2001, gee, we are doing a lot better, and this is comparable actually to transplant from 1993 to 2000, and it really raises the question if transplant, with the modern management in medical management of heart failure, how important is it and what the role of transplant really is.

Really, there is a gap between a very

invasive transplant or LVAD and the medical therapy, there really is, and we are here to talk about stem cell therapy. There is a gap of something that could be done that is not quite as invasive and traumatic as an LVAD or transplant, and that can improve the patient significantly since we are doing so well with medical therapy.

I want to talk to you a little bit about my perspective on end-stage ischemic heart disease. Basically, as I have told you, we have improved the medical management, so we have longer survival, we have improved the vascularization treatments of coronary disease, we have improved the survival following a heart attack, and that is why we have more patients, and now we are using widely defibrillators, and that is why people are living longer.

So, this is sort of my understanding of this end-stage patient. You progress with coronary disease until you get to the Stage III and Stage IV, Class III/Class IV heart failure.

If we look at these patients, sometimes there will be a little surprise, because some patients really just have shortness of breath, so this is a variable. This may occupy the whole

square or angina may occupy the whole square.

So, some patients predominantly have heart failure, and these patients that predominantly have heart failure probably weren't very good at forming collaterals when they had heart attacks and developed a lot of scar tissue, and have a very low ejection fraction. These are the sickest patients and the patients that are going to have a very high mortality.

On the other hand, but also in the Class III or Class IV, and sometimes we pool these people together in trials and that is why I am making this distinction, some people have angina more than they have heart failure. These probably have a much better collateral formation when they had these events, so their ejection fraction is a little more preserved.

I have had many patients that have lived on one artery. Their whole heart is beating okay. That one artery feeds everything by collaterals, but they are in really bad shape. I mean it's an illusion that they are doing okay, but they do have a preserved ejection fraction, and their manifestation is a lot of chest pain.

So, symptoms can vary from one side to the

other and some patients have a balance here, and I think we need to keep this in mind when we are designing these trials.

So, there is a predominant angina, and this is the kind of patient that got, let's say, these TMR type procedures. That is the kind of population you are dealing with. The predominant aspect is disabling angina, preserved EF, 100- to 200,000 new cases per year, and constitute about 5 percent of the patients undergoing angiography at tertiary referral centers. This has been studied in this particular case at the Cleveland Clinic.

One year mortality is still very high. Then, that other group, predominantly heart failure symptoms, very low EF, myocardial ischemia, though, is still present, but with more scar. No option really for any kind of revascularization. One year mortality, 20 to 50 percent. I have shown you one curve where it is up to 80 percent, I mean it can be really bad.

Here, we have ICD therapy trials. If we look at secondary prevention trials, very sick patients in this study, treated with amiodarone, you see here one year mortality 44 percent. I mean heart failure can be worse than cancer.

Here is the REMATCH trial. This is an LVAD. This is the impact of LVAD, and there is an impact of survival, but again you are dealing, in this case, with Class IV patients that are unresponsive to medical therapy, so these very sick patients, but again an invasive, costly, not widely available kind of therapy, but it does have an impact on failure.

I want to finish now talking a little bit then, hopefully, I have given you an overview of the problems with heart failure, and how are we going to look at adverse events.

Well, what are the things that are going to drive the adverse events here, are going to be arrhythmia, ejection fraction, and symptoms, and I think if we focus here, we can pretty much decide what we need to look at in these patients over time as we use new therapy towards these patients.

Let's look at low ejection fraction, how are we going to monitor that? Well, we need to look at cardiac function, cardiac size, and the perfusion status of the ventricle. We can do that very simply, if you take a simplistic approach, with echocardiography.

I empirically have placed this here based

on my own limited experience here, but I read in the document that you wanted some more practical advice, so I will give you my own sort of practical feel for what I would do.

If we did echocardiogram on these patients, we could do it monthly for the first three months and then at six months follow-up. We can do SPECT, we know that we don't need it too early, and that is a very simple way of doing it, three to six months. Clinical visits, which will be very frequent, and I will talk about that, and BNP can be done for that, as well.

Now, we can get fancy and use alternative imaging strategies, we can use MRI, electromechanical mapping, PET, depending on the institution, and depending on what we are really looking for and want to find.

Cardiac arrhythmias, it is important to monitor cardiac rhythm. Holter monitoring is very simple, probably should be done after the procedure, one, three, six months later. Q-T interval when the patient comes in for his clinic visit is a strong predictor of survival, just a plain-old, good-old 12-lead EKG, and that should always be looked at.

In the patients I guess that are getting myoblast therapy, there may be a little bit more concern about this, and this is really not my area of expertise, but these patients, many of them already entering with an AICD, that have sort of a built-in little computer that is already monitoring their rhythm as it is. If they don't, you might want to consider event monitoring.

For symptoms, well, clinical visits biweekly for 8 weeks, monthly up to 6 months. We are going to look at heart class, we are going to look at EKG, CBC, CRP, look for inflammation. Exercise capacity, ramp treadmills, as you know, if you put a patient that has end-stage heart failure on a graded treadmill test, every time the treadmill bumps up and goes a little faster, he just may not be able to exercise at that point.

So, the advantage of a ramp treadmill protocol is that you have a gradual continuous increase, so these people that really can't do very much at all, they will be able to tolerate the exercise and probably get further than they could in any other kind of exercise test.

There is a very simple way of evaluating an exercise test, a 6-minute walk test. You just

define a distance, walk the patient walk for 6 minutes, see how fast he can go. You can do that at a clinic visit, and it is very simple to do. So, you can do something like this at one, three, and six months.

Rehospitalization. We look at the rehospitalization rates. It is important to look at the use of i.v. medications that are used to control symptoms, because this is, as you saw, the biggest part of the pie in terms of costs, and is a real problem in the end-stage patients.

Quality of life, it is important to assess quality of life, for example, SF36, Minnesota Questionnaire.

Just some suggestions. I want to wrap this up and saying I hope I have given you a general idea and scope of this problem. We deal with a very, very serious problem, which is heart failure, specifically, that which is ischemic heart failure and specifically, end-stage ischemic heart failure.

I hope I have given you a flavor of this and set the stage for the discussions.

Thank you very much.

[Applause.]

DR. RAO: Thank you, Dr. Perin.

There is time for questions, and we can open it up to the committee.

Q&A

DR. SCHNEIDER: Emerson, one of the things that you did very nicely was lay out the clinical spectrum for people who may not be familiar with it in this context.

I wanted to follow up on that point because work presented at international meetings recently by the Frankfurt group of Andreas Sire and Stephanie Dimler suggests that bone marrow derived cells and circulating progenitor cells from patients with established heart failure may be deficient relative to the performance of bone marrow derived and circulating progenitor cells from patients with an acute infarct.

So, while it is not quite an apples and oranges comparison to envision cardiac cell grafting immediately post infarction or in the first week post infarction in patients without severe ventricular dysfunction versus patients, let's say, two to four months out with mild or no ventricular dysfunction versus the end-stage heart failure patients who have been a focus in your talk

this morning, it does seem to me that that clinical heterogeneity introduces a couple of problems.

I am curious to know how you have worked those through in your own work. One of them is because what we are discussing today and tomorrow, is autologous cell therapy, I believe that there is a serious issue of patient-to-patient cell heterogeneity which has been relatively little discussed in the field except in these still unpublished or perhaps one paper has come out in a secondary journal from Stephanie and Andreas about the defects.

So, one question is what kinds of standards should a proposed production center be required to meet in terms of their ability to generate cells that perform in accordance with some standard when there is patient-to-patient variation of this kind.

Secondly, if you are envisioning putting cells of different kinds into a so severely an ischemic background as the 41-year-old former pilot that you mentioned, doesn't it become important to clearly distinguish, as the prefatory remarks did, between mechanisms of action for proposed donor cells that are aimed at regeneration specifically

versus benefits that are achieved through entire different mechanisms, such as angiogenesis?

If you put new cells into an ischemic background, they will surely die, and if the goal is to achieve angiogenesis in a background where the native coronary circulation has failed and the graft has failed, then, it seems to me we need a clearer resolution of the problem of which cells do which things well, and really fine-tune much better than the field has to date, you know, which are the cells that we want where the spectrum is normal vasculature, insufficient muscle cells versus the hypothetical ischemic patient that you described where revascularization is the major goal.

DR. PERIN: Well, that's fantastic.

[Laughter.]

DR. PERIN: I think the basic answer to your question is I don't know, but, you know, these are all very good points, starting with the cell type, we really don't know.

Actually, we have submitted a manuscript in which we have had the pathology of one or our patients in our study in Brazil who received autologous bone marrow, died 11 months later, and I really can't preempt I guess our publication, but I

think we will be seeing some evidence of myogenesis and angiogenesis from autologous bone marrow cells, but we really don't know what we are getting when we are putting, let's say, autologous bone marrow, and even in that patient that has, let's say he has predominantly ischemia, if we want to revascularize, can we get a predominantly angiogenic effect, so we really don't know, and we need to define that.

Mononuclear fraction of the bone marrow is a very simple approach, the one that we have taken, and it seems to initially, and we haven't really done efficacy studies and we are continuing on, but there is a suggestion that it does, so I think that we need to take every step that we take should be put one foot in front of the other, and if the mononuclear cell fraction works, I think we can go from there and keep investigating that.

Now, the average age in our trial was about 58, and you mentioned the problem--

DR. RAO: Can I interrupt? These are really important questions, but they discuss data which was not presented in the talk right now. I would like to at least focus the questions initially on the issues that relate to the

presentation right now.

We should really come back to these questions tomorrow when we discuss exactly these sorts of issues.

Do you think that that would be okay with you, Dr. Schneider?

DR. SCHNEIDER: We will certainly return to them tomorrow, but I was discussing issues that were raised in this talk, which was clinical heterogeneity.

DR. RAO: Let's then focus, not on the cells per se, and the choice of cells, because none of the presentation was related to the production facility or how the cells would be, or the quality would be, or how you would choose the mechanism, but maybe how do you choose patients for a trial or is there some reasonable way of selecting patients, that there would be consensus on.

DR. PERIN: Okay. So, we will get back to your first question and really, that is something that actually, we are working on trying to understand, is there a thumbprint or is there a profile in the study by Dimler and their colleagues looking at the characteristics of cells in certain patients, and obviously, they may not be the same

in a diabetic, in a severe heart failure, we don't know, so there is another important we don't know.

Age obviously is a very important thing, so harvesting cells from a 75-year-old may be very different than doing that in a 55-year-old, so these are all questions that need to be answered.

DR. RAO: Dr. Mulé.

DR. MULE: Given the slides you showed of the steps toward progression of heart failure, and given the current interventions along that pathway, from your perspective, where would you see cell-based therapy intervention falling into that step toward complete heart failure?

DR. PERIN: Right now, at close to the last few steps, I think ethically, we are propelled to really study the problem in the patients that really don't have a proven conventional option for treatment. In brief, I would say in the patients who can't be revascularized, because really medical therapy, we are going to apply to everyone, so then we are left with revascularization.

Well, can we revascularize? Well, we do, and we do it again and again, and there is a point where you are out of revascularization options, and I think that is one place we are initially now,

then, you could think about applying this kind of treatment.

DR. HARLAN: Building upon what Dr. Rieves mentioned when he gave his introductory comments, I want to just congratulate you on, it seems like our task is to weigh the risk-benefit, and you have outlined very clearly the risk, and I accept that it is severe, and I also want to congratulate you on mentioning the JACC paper that was just published, that showed how dangerous it is to look at historical controls, because we are making such rapid progress.

My question is along those lines, not in this field, I just read in the journal, the Washington Post, about the great advance that has been made in super-high statin therapies, and I wonder if you could comment on that study, that these super-physiologic statin doses seem to have a major impact on mortality.

DR. PERIN: I really don't have an expertise in a lot of things, and that is not one of them, so it is really hard for me to comment on that. I know that it looks like giving people HDL in the future may be a very exciting thing, and we may be able to finally find our liquid plumber kind

of solution for people.

Then, again, statins are just--more and more if you study statins, you have probably come to the conclusion it should be in the water pretty soon, I mean the patient benefit is on every single aspect of cardiovascular disease.

DR. RAO: Dr. Kurtzberg.

DR. KURTZBERG: You mentioned some practice-based methods to evaluate outcomes and function in these patients, but I think the challenge is to determine what the cells are doing, you know, are they differentiating into other kinds of cells, are they mediating inflammation, are they mediating angiogenesis, and I don't see how you can sort that out by clinical-based study.

Do you know of other technologies that are on the horizon that may help with that, that are non-invasive, or would you consider serial biopsies in patients like this to answer those questions?

DR. PERIN: That is a good question. I don't know that serial biopsies would be a very efficient way of evaluating that. You would have to have a very precise way of being able to identify where you put the cells and be able to go exactly to that same spot.

We do have that technology. Dr. Lederman is going to follow me eventually here. The MRI field, I think is very promising in that regard in terms of labeling and following cells.

Now, I really don't know that even labeling a cell, even if it died, if the label stays there, you still see the label, so I think that we have to even go a step further and be able to prove the functionality of the cell that is alive and was implanted.

That can be done on an experimental basis, so we figure ways out to do that, but this is a very intriguing problem and a very difficult problem to evaluate. I think you have put your finger on something that is going to be hard to know.

DR. DINSMORE: Jonathan Dinsmore from GenVec.

I just had a question on your angina heart failure continuum. I was confused because most heart failure patients present without angina, with symptoms of fatigue, so what percentage of heart failure patients actually experience angina?

DR. PERIN: If we are talking about ischemic heart failure, we are not talking about

other kinds of heart failure, actually, idiopathic heart failure, you kind of get the same remodeling and everything except you didn't have that infarct in the beginning, but you go through the same sort of pathophysiologic processes.

So, we are talking about ischemic heart failure. People that have ischemic heart failure have coronary disease. Coronary disease is narrowing of your coronary arteries.

Depending on what your response is, you will or will not have angina, but angina is one of the manifestations of coronary disease, and it is really not a good thing to base a lot on, because the expression of angina is very variable.

It depends on your pain threshold. I mean if you are a diabetic, you may not have as much pain. It is a subjective thing subject to interpretation by the actual patient, so it is something that is very difficult to evaluate, and that is why I put the continuum, because it is all there and you really shouldn't take a patient population based on angina or based on shortness of breath.

I think you have got to bring both of these things together to understand they are sort

of in the spectrum of a similar underlying pathophysiologic process.

DR. SIMONS: I would like to come back to the issues of the differences among the patients having these kind of therapies. We have learned from a number of trials of growth factor therapies that there is a very large difference in how the patients respond.

This issue that there are different subgroups that we are not defining is fairly critical to the field. You mentioned one or two biomarkers, but there seemed to be a general association of markers as opposed to really identifying which patients respond in which manner.

What would you suggest as a way of trying to sort of stratify these patient groups? Not suggest ejection fraction, that is probably in a way sort of crude measure, but in terms of biological responses.

DR. PERIN: If we look at the trials of devices, I think that probably a common way to look at these patients is exercise capacity.

I think that probably is one of the unifying parameters that we cannot only use at entry, but also you are able to follow as a patient

goes along, and if he has a response to therapy, he will have a positive response in terms of what he is able to do in terms of function.

That has a very practical translation into quality of life and people feeling better. I would say in a broad sense, that exercise capacity, peak oxygen consumption might be something that I might consider an important thing to follow in these patients, and not just ejection fraction, which is dependent on a lot of things, how much loading the ventricle has that day, the amount of mitral regurgitation, et cetera, so there is a lot of things that will make that extremely variable.

DR. RAO: As an extension of that, it's a very general question. Is there any problem with many of these studies which are in high-risk patients enrolling people for the placebo arm of the trial? Not in cell therapy, but maybe when you do devices or you do assists, has this been historically a problem for the cardiovascular field?

DR. PERIN: Well, it has been done as you can see, so I have showed you a bunch of studies where it has been done, and it can be done.

Personally, the way I like to see it is I

want to offer patients that get in the placebo arm some kind of a treatment, so in our future upcoming study, what I am going to do is I will tell a patient you are going to get randomized to maybe not getting treatment, but if you don't get that treatment at an X period of time, six months, you will cross over to get the treatment.

I think that is a humane way of doing it, in which these patients are very ill and desperate to get something to help, so again, if you can cross over, sometimes these placebo patients at some point after you have achieved your assessment, then that makes it a more palatable or fair way to do things maybe.

DR. RAO: Dr. Cunningham.

DR. CUNNINGHAM: I just wonder, in your data, if you see any difference by either socioeconomic status or by gender, or by any way of culture, dividing populations, whether it would be race or ethnicity or any other factor like that?

DR. PERIN: You mean in our own--

DR. CUNNINGHAM: Yes, reading the JACC data, was there anything by gender, for instance, or by subpopulation?

DR. PERIN: Females, there are some

differences in the female population in which there are some differences. There is the catch-up phenomenon in the end, but socioeconomic differences, I am not aware that it would have an impact on that, as well, but maybe gender differences, yes.

DR. RAO: One question to sort of follow on Dr. Simons' question, in at least the way I understood it, it is really kind of difficult to stratify patients or to extrapolate from one class of patients to the other. Historically, that has always been a problem.

Again, it's a general feeling when one conducts studies in the cardiovascular field, is there some consensus that everybody says that, well, if you measure by ejection fraction, and we take patients, which is what it seemed like a lot of studies have done, that that is a reasonable criteria that you can extrapolate from one classification of that kind to the next, or one cannot? Just as a general statement.

DR. PERIN: It has been done, and it is a general way of separating--there is definitely a correlation with your ejection fraction and your survival, so it is probably not the most refined

way of dividing patients, and it depends where you make the cutoff, so if you make a fairly high cutoff, let's say, patients that had ejection fraction less than 40 percent, then, you are including most of the population of patients that have heart failure, so it's a general way to divide things.

If you start decreasing that number of that cutoff, then, you are really selecting out more I think subpopulations we were talking about, maybe some different kind of subpopulations of patients with heart failure.

DR. RAO: Dr. Borer.

DR. BORER: Dr. Rao, a few minutes ago you made a point, and I would like to restate it in another way, because what Dr. Perin did, as I see it, is very well present an overview as an outline, was a scaffold upon which we can conduct subsequent more specific discussions.

I think that right now we are getting into a series of questions that are way beyond the data that exist, and you couldn't expect Dr. Perin to respond to them in a meaningful way because the data don't exist.

In specific response to your question,

which was a very fundamental one, I think we are at a point now with this form of therapy where if we could define any group in which we saw a response which seemed credible, which was statistically valid, we would then have a series of hypotheses that would have been generated that would allow one to move further, but I think that is the level we are at.

The idea of defining a general population in which to test therapy the way we do with drugs, we are not there yet, so I think the specific questions have to come a little later in this forum.

DR. RAO: I just wanted to get it clear to people that that was the case, but your point is very well taken.

Dr. Neylan.

DR. NEYLAN: Thank you.

That was a very nice clinical overview, and I wanted to ask you from your perspective as a clinician, there are obviously many parameters whose relief or improvement would be significant in terms of the lives of individual patients, and many of these could be utilized as endpoints for proof of concept.

But ultimately, what do you believe is the most relevant clinical endpoint for defining registration criteria for this form of therapy, is it patient mortality or something else?

DR. PERIN: I don't know if we are going to be impacting patient mortality. That is a very difficult question. I would go back and what I had said earlier, and use an endpoint, I would use something like the LV02 as an endpoint.

I think that is a little bit more palpable, and obviously, looking at mortality, this is such an initial incipient field in which we have barely treated any patient, so to think about looking at mortality, which involves a much larger number of patients, I think that is probably getting ahead of ourselves a little bit.

We need to first verify if this is efficacious and if there is some objective improvement in these patients, and one of those objective ways of doing that would be something like exercise capacity, like I mentioned.

DR. RAO: Dr. Ruskin.

DR. RUSKIN: Just two quick comments on Dr. Perin's very nice presentation.

One is that we have learned from drug and

device trials that both ejection fraction and heart failure classification are critically important predictors, but that they are not necessarily fully interactive, that is, they are independent, so using both, I think in any classification with regard to these kinds of interventions would be critical because the outcomes are very, very different in Class III and IV even with the same EF.

The other relates to a question that Dr. Rao raised about recruitment and controls. I think that given the excitement in this area, but the unknown issues that have already been raised, doing trials that have adequate controls perhaps is more important here than anywhere else one can imagine given the severity of the illness that we are dealing with and the kinds of outcomes that Dr. Perin has described.

As someone who recruits for device trials, though, I can tell you that it is not easy, and randomizing patients to acceptable controls in this kind of illness is going to be a huge challenge, but I think it is important for this group to emphasize that there is no place where this could be more important, otherwise, we will never get an

answer, and I think that mortality ultimately will have to be a critical part of any trial that is done.

DR. RAO: Go ahead, Dr. Borer.

DR. BORER: I agree completely with Jeremy that controls are essential in this kind of research and really in any clinical research, but I think again to put this whole area in context, and in response to Dr. Neylan's point and question, we are at the point now of looking at physiological variables and what we would call in drug development "surrogates," to see whether cardiac performance, cardiac perfusion, this, that, and the other thing, is affected in one way or another, so that one could extrapolate to the point where it would be legitimate to define hypotheses about clinical outcome.

We are not there yet, and the clinical outcome, just to put it in context from the drug world, is perfectly legitimate in the view of most people who deal with this area and these agents to think of a therapy as being approvable if it makes people feel better, but doesn't make them live longer.

If it makes people feel better, even if it

makes them live a little bit shorter, as long as you know how much shorter that is, and if it makes people live longer while not making them feel too much worse.

I don't think we are at a point yet again to define what the outcomes variables should be. I think we are at the point of defining physiological and pathophysiological surrogates, and that is what is being done in the studies to date, and then we can decide what the outcomes are, clinically important for registration.

DR. RAO: I guess that leads us to the fact that many of these things should be discussed tomorrow, just like you pointed out.

If there are no critical questions remaining, I will thank Dr. Perin.

[Applause.]

DR. RAO: We are going to take a short break.

[Break.]

DR. RAO: We are really extremely fortunate in having Dr. Menasché here to present his findings, and I look forward to a really interesting talk.

Clinical Experience of Autologous

Myoblast Transplantation

DR. MENASCHE: Good morning. First of all, I would like really to thank you for the privilege of this invitation and this unique opportunity of sharing some data on the clinical myoblast transplantation.

What I would like to do in this talk is first to briefly touch on the preclinical data which have paved the way for these early clinical trials, and then, as requested by Dr. Grant, to focus on the various aspects of the clinical experience which has accumulated so far before drawing some perspectives which may have clinical relevance in the near future.

Now, I think just to make things clear, that the basic assumption is fairly straightforward, and the objective of this therapy is really to try to repopulate areas of dead myocardium with new contractile cells with the hope that these areas can regain some function, and given the close relationship between function and survival, which has been already mentioned, the ultimate hope is obviously that it can have a significant impact on clinical outcomes.

The reason why we initially started with

the skeletal myoblasts are actually listed here. These cells are not really stem cells, they are better termed precursor cells for muscular fibers in that they are very committed to their skeletal muscle phenotype as you will see.

The first advantage of the myoblasts is that they can be very easily retrieved from the patient himself, thus overcoming any problem associated with rejection and immunosuppressive therapies.

These cells feature a very great expansion potential which is important given the relationship which exists between the number of cells which are injected and the ultimate functional outcome.

As I have just said, they are pretty well committed to their myogenic lineage, and the risk of tumor development is virtually negligible. Finally, they are pretty resistant to ischemia, and although unfortunately, many of them die shortly after the injections, fortunately, some of them will survive and may positively affect function.

So, this is type of animal model which has been used initially in rodents. You see here the heart and the needle injecting the cells. I just would like to mention that it took us seven years,

seven years of preclinical work before I did operate on the first patient June 15, 2000.

During the seven years, we moved from the rodent models to the large animal models, which I think is absolutely necessary before arriving to clinical trials.

Just to summarize the bulk of this data, we can say, number one, that when you inject skeletal myoblasts into an infarcted area, they retain the possibility of differentiating into typical myotubes. Here is a typical myotube, elongated structure, and this is a sheep heart and this is a human heart.

This is an autopsy specimen. One patient of our Phase I trial died 18 months after his surgery from stroke, and we had permission for the autopsy. You will appreciate the striking similarity of these two slides. Here you find in this human heart, a typical myotube embedded in scar tissue.

At closer magnification, you can appreciate the typical cross-striations, and I think two observations are important to be made at this point. Number one, these cells really remain committed to their skeletal muscle phenotype. In

other words, there is virtually no evidence that they can ever turn to cardiomyocytes. They will not become cardiac cells.

Number two, they remain electrically insulated from the surrounding myocardium, which obviously raises major mechanistic questions regarding the underlying mechanisms by which they can improve function, but the fact is that there is no real evidence that they develop connections with the neighboring cardiomyocytes.

Nevertheless, when you subject them to strong depolarizing currents, they show excitable properties, and you see here, this is a fluorescent myotube which has been grafted in a myocardial scar. This is an in vivo study and definitely they can respond to currents by generating action potentials followed by contractions.

This translates into an improvement in function, both regional function here in the sheep model, and global function, the LV ejection fraction. This improvement, as you can see, seems to be sustained over time until one year in our rat studies, and basically, these kinds of observations have been made by several other investigators already past 10 years.

So, there is a fairly good consistency showing that these myoblasts can, to some extent, improve function at least in animal models, and obviously, the gap with the humans is a wide one.

So, if we now move to the clinical experience, so far there are 44 patients who have been included in early Phase I trials, and 34 patients currently included in our ongoing randomized, multi-centered Phase II study .

This list is by far not exhaustive. I have not tabulated anecdotal case or me-too cases. I have just kept those studies which have been published in peer-reviewed journals.

Basically, the inclusion criteria have been fairly straightforward across all these studies. Patients with low ejection fractions, usually below 35 percent, patients with a history of myocardial infarct, and obviously, patients requiring concomitant coronary bypass surgery since for ethical reasons, it is difficult to open the chest just for injecting a product we don't really know whether it is effective or not.

If we try to summarize the main results, we can say, number one, that multiple epicardial injections look to be safe. I have never seen any

bleeding from the needle holes, and overall, this experience has been shared by the other surgeons who have practiced the operation.

Number two, it is possible--and we will come back on that--that the procedure increases the risk of arrhythmia postoperatively, at least in the early post-op period.

Number three, I will be extremely careful and cautious about that, there are some data suggesting that maybe function can improve, but it is clear that until we have the results of the ongoing randomized, placebo-controlled study, we cannot make any meaningful conclusion.

This is the list of the studies and of the patients. I have just added the last one a few days ago. Professor Siminiak presented at the American College of Cardiology another series of 10 patients who got the cells through a percutaneous catheter using the coronary sinus route. I will come back on that catheter in a few minutes, but I will rather concentrate on the surgical implantations listed here.

Dr. Smits also injected cells through a catheter using the interventricular approach similar to the one alluded to by Dr. Perin.

This goes back to the inclusion criteria which have previously been mentioned. I think it is important to look at all words, because as you will see, differences in definition may really be confounders in the interpretation of the results.

It is important to look at akinetic areas that is really dead myocardium, not simply ipokinetic or dyskinetic, really akinetic myocardium, which are not amenable to revascularization and obviously, it is also important that the bypass surgery be done in other areas.

For example, you will see that in one study, the area which was transplanted with cells was also revascularized, so when the authors conclude that cell therapy improves function, it is clearly meaningless since the same area has got simultaneous revascularization.

For those of you who are not familiar with the procedure, I just would like briefly to show you this three-step operation. It starts with a muscular biopsy. We take it at the thigh. It is a very simple procedure under local anesthesia.

We remove a chunk of muscle, which is then cut into small pieces, put in this sheeping medium

and sent to the cell culture lab where a multiple tri-cell factory is being designed to allow for large-scale cell production.

Then, there are regular morphological controls. Obviously, the key point is to inject the cells before they reach confluence. What you would like to do is that confluence occurs in vivo following the engraftment, not before, so it is important to check the morphological state of the cells on a regular basis.

This is how human myoblasts look like during the cell culture process, and this is how the cells look like when they are back in the operating room.

Then, with the curved needle, we inject the cells all across the infarcted area including the borders. It's a time-consuming, I would say 10, 12, 15 minute procedure, rather tedious and boring procedure, by the way, where you have to mentally construct the grids and then go with the needle from side to side, so we are working on the multiple shot device, but it is more tricky than we initially thought.

So, right now we have the requirement for these multiple injections all across. This is

another view of the injections.

So, if we start by feasibility, I think it is quite well established that this technique is perfectly feasible. In other words, it does demonstrate that provided you have the appropriate techniques, you can take a small piece of muscle which contains, say, 3- 4 million skeletal myoblasts initially and expand it over two to three weeks until approximately 1 billion cells.

These are the results of our cultures during the Phase I trial, during which the target numbers which have been prespecified have consistently been obtained and even overshoot it.

You will note that you can get up to 90 percent of skeletal myoblasts in that--and this is an important point--you really end up with a pretty well defined cell therapy product. You really know what you are injecting.

Importantly, what we have seen is that heart failure does not prevent skeletal myoblasts to differentiate into myotubes, and this was a question because when we did preclinical rounds, I got pieces of tissue from orthopedic colleagues, but often these patient were young, and the question was are the myoblasts from this Class

III/IV heart failure patients going to differentiate normally, and the answer is yes, so far we have had no failure.

The only thing is that it may take a little bit more time for some patients until we get the target number of cells, but at the end of the day, it has always been possible to achieve the prespecified target number of cells in myoblasts.

What about safety now? These are the different adverse events we were concerned with by the time we started the trial, and fortunately, I must say that none of them has occurred except--and we are going to discuss that--possibly the arrhythmias, but it is important to emphasize that, for example, there was never any particular bleeding from these multiple puncture sites.

There was no unusual complication in the postoperative course of these patients, and when the cells were injected in newt immunocompromised mice, there was never any evidence for tumor formation.

Obviously, before we started the study, we had to go through a lot of regulatory constraints, indeed, what I did is to discuss with the French FDA and ask them what was approved or not, and the

game was not so easy because as previously mentioned, there was no precedent.

So, they told us, well, this is what you are allowed to do. This is the kind of culture medium, ancillary product additives which are permitted for human use, so we immediately from the onset designed our cell culture in accordance to the prespecified instructions, and obviously, it was timesaving because when we came back with the process, there was nothing else than to accept it.

Well, what about the V-tachs? In the initial series we had 4 patients with sustained episodes of ventricular tachycardia.

All of them occurred during the early post-op period, the early three first week, postoperative weeks, and there was virtually no recurrence later on because these patients had a defibrillator put on and only one of them experienced firing of the defibrillator one year later, so it really appears to be a relatively early post-op event.

Now, there are different mechanisms which could account for these arrhythmias, in particular, the differences in electrical membrane properties between the grafted cells and the neighboring

cardiomyocytes. Obviously, other mechanisms can also be considered, but we really favor the first one because we did an EP study in which we looked at the different membrane properties of the cells.

Here, you see a typical action potential of a muscular fiber and here of a cardiomyocyte. Now, if you graft skeletal myoblasts back into a muscle, these cells retain a typical skeletal muscle phenotype, and this is also true for myotubes which grow in culture.

The question is how does it look like when you graft the skeletal myoblasts into the heart. Well, definitely it remains very similar to what it was initially and different from the action potential of the cardiomyocyte.

If you expressed it graphically, you would see that the action potential duration is quite different between the cardiomyocyte and the myotube, and this heterogeneity might account for some of these arrhythmias.

Now, having said that, the picture is probably more complex and the reason, as you know, and it has been mentioned by Dr. Perin in his talk, is that heart failure by itself predisposes patients to arrhythmias.

So, I think that as long as we don't have the results of the randomized trial in which all patients have been instrumented with a defibrillator, it will be difficult to conclusively establish a causal relationship between grafting of cells and the occurrence of arrhythmia.

I can also tell you that we currently have randomized 34 patients in the Phase II trial and the incidence of arrhythmia has been strikingly low, much lower than in the initial study we had done, so things are probably less clear than they were initially, and once again we have to wait for the results of the randomized trial before we can definitely say yes, there is no relationship between myoblast transplantation and arrhythmia.

Anyway, these patients or most of them would require at one point a defibrillator, so it was not a big issue for us to implant those defibrillators in all the Phase II patients.

Now, what about efficacy? Now, we have to be extremely careful in the interpretation of the results which are presented because of the multiplicity of the confounding factors.

The culture conditions, for example, the Spanish group has used a culture medium which

contains the patient's own serum, and the conclusion is we had no arrhythmia, so if you use the patient's own serum instead of fetal calf serum, you prevent arrhythmia.

I think it is really a simplistic conclusion based on 12 patients, but it can introduce an additional bias. There is currently no evidence that fetal calf serum is really responsible for the arrhythmias.

Dosing has been extremely different and variable from one study to the other, as well as the kinetics of the grafted area.

Once again, any kinetic area is different from a dyskinetic area, which features a paradoxical motion, and, for example, in the U.S. trial, some patients were included who had hypokinesia, which we know can improve just because of the revascularization even if revascularization is not targeted at this particular area.

The same for bypasses. In the Spanish study, for example, the cell grafted areas were also bypassed, which makes the interpretation of results impossible.

Type of surgery has also been different. In the U.S. study, for example, some patients had

additional reconstructions of the left ventricle in addition to the bypass surgery, which make things still more complicated.

Finally, the method of outcome assessment, in some studies, the assessment has been centralized at one side, in others, each center has made its own assessment, which obviously makes big differences.

This is just to illustrate the variability in the number of cells which have been injected. I don't have the figures for the initial surgical study from Professor Siminiak, but as you can see, there is a wide variability.

The U.S. study of Dr. Dib was, as you know, was a dose escalating study accounting for this variability in the numbers. Dosing is probably important. This is one study among others showing that there seems to be a tight relationship between the number of injected cells and the functional outcomes.

This is the reason why, in our early Phase I trial, we have targeted a high number of cells, 800 million. In the Phase II, we have two arms with two different doses of cells, but the number probably makes a big difference given the high rate

of early cell death.

The characteristics of the grafted segments, as I previously mentioned, have also been different from one study to the other, as well as the method for assessing viability, usually, dobutamine echocardiography, occasionally MRI or PET scan.

Same variability in the characteristics of injections, but you see that you can go up to almost 60 injections without any concern related to bleeding, and obviously, the number of injections depends on the extent of the area of infarction.

It is also important to look at the cell concentration. We extensively studied that before I started doing patients. You have to find a tradeoff because if you use a large needle, then, you can have large holes and some bleeding problems.

If you use a too small needle, you will eliminate the bleeding problems, but the cells may be packed and damaged through their passage, so we ended with a 27-gauge needle which gave an acceptable rate of cell viability.

The concentration of cells is important, and probably still more important when you are