NATIONAL INSTITUTES OF HEALTH

THIRD GENE TRANSFER CLINICAL RESEARCH SAFETY SYMPOSIUM

SAFETY CONSIDERATIONS IN CARDIOVASCULAR GENE TRANSFER CLINICAL RESEARCH

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LANA SKIRBOLL, Ph.D.

DR. SKIRBOLL: Good morning to the brave souls who came out in this lovely Washington weather. We love ice storms here. If you are from the real north, you get snow storms; on the west coast you get fog; when you come to Washington, you get ice storms. The only thing that is good about it is the trees look pretty.

(Slide.)

Well, welcome to the third National Gene Transfer Clinical Research Safety Symposium.

The idea of convening periodic safety symposium originated with a meeting that was held on the NIH campus a year ago this very month. One of the recommendations that emerged from that meeting, the 1999 Adenoviral Vector Safety and Toxicity Conference, which we sponsored jointly with the FDA, was that comprehensive reviews of aggregate gene transfer clinical trial data should be convened on a periodic basis.

Those recommendations were then embraced at the very highest levels of the department in March of this year as part of an over arching departmental effort to enhance the protection of participants in clinical research studies.

Secretary Shalala launched this very program of national safety

symposia for gene transfer clinical research.

Our goal is simple. It is to provide public fora for the review by scientific experts of emerging issues in medical, scientific and safety aspects of clinical gene transfer research. And we hope that by fostering such discussion and information exchange, we will enhance the understanding of the safety and toxicity of gene transfer; that we will identify critical gaps in current knowledge; that we will maximize patient safety; enhance the informed consent process; and optimize the development of gene transfer clinical trials.

The first of these safety symposium was held on March 8th, in this year, on the topic of a new vector system, the internally depleted helper dependent adenoviral vector. Symposium participants at that meeting identified a need for improved quantitative and qualitative assays for measuring the vector and they suggested the need for further preclinical studies and clinical trials to help confirm the vector safety profile.

The FDA took the lead in the second national safety symposium in November on a very important topic, long-term follow-up in gene transfer clinical trials. That conference and this topic raised a number of critical policy issues that were not more in-depth exploration and we anticipate sponsoring a policy conference forum on this topic in the near future.

We chose for the third symposium the topic of cardiovascular gene transfer safety issues, a growing area of interest in gene transfer research and clearly one in which there are some important safety issues emerging. We are heartened by the interest and willingness of clinical investigators that came from

across the country and many institutions today to participate in this meeting and help exchange information that will help advance our collective mission of optimizing the development of clinical research and the safety of human research participants in gene transfer trials.

So with that, I will -- I am Lana Skirboll, Director of Science Policy at the NIH. I introduce myself because I do not think to a group looking at cardiovascular research, anybody, the next speaker needs an introduction more than to say Dr. Claude Lenfant, the Director of the National Health, Lung and Blood Institute.

Claude?

WELCOME FROM THE NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

CLAUDE LENFANT, M.D.

DR. LENFANT: Well, thank you very much. I am very pleased to be here and add my welcome to that which was just extended to you but I am also very pleased to see that one of these symposia is, indeed, focusing on cardiovascular disease.

I want to, if I have to, remind you that the very first gene transfer was actually done in our institute in, I think it was, 1988 or 1989. I cannot remember the date exactly.

And at that time a great deal of excitement emerged certainly in the institute but in the country as well.

And there was so much excitement that lots of people perceived

gene transfer as becoming some sort of a universal panacea that would treat a great number of conditions, if not all actually.

I think it is absolutely marvelous that we saw such enthusiasm and excitement but then already at that time some of us said, yes, we should have as much enthusiasm and excitement as we must have caution with regard to gene transfer.

Well, the excitement has continued and grown, and the knowledge has increased a great deal but perhaps for some reasons, which I would not -- are not within my purview to discuss, cautions may have been a little bit set aside and for that reason gene transfer may have received a bad name, and that is most unfortunate because the potential of that therapeutic approach remains and must be pursued very actively.

We at the NIH and in our institute can decide to support research which is necessary to first the development of this technology but safety is a joint responsibility. It is a joint responsibility from us but also from those who are doing the research.

And it is for that reason that we in the institute very much welcome a symposium such as this one because it will give us the -- all the opportunity to share our views and to explore how we must best assure the safety.

Let's keep just one thing in mind.

New therapies are developed for only one purpose, which is the benefit of the patient. And if you keep that in mind, it is indeed very clear that at the same time we must be sure that the therapy is safe and is not doing more harm

than what is really intended.

So I again want to welcome and assure to you all who are interested in gene therapy research in cardiovascular diseases and other areas of interest to our institute that we are here to help you and to support you but the thing that we cannot do for you is to assure the safety of what you are doing, and that is what the symposium is about for you to talk about and make sure that everybody knows what is the best way to do what we want to do.

So thank you very much and have a great time here.

(Applause.)

DR. PATTERSON: It is my pleasure to introduce the next speaker, Dr. Kathryn Zoon, Director of the Center of Biologics at FDA.

WELCOME FROM THE FOOD AND DRUG ADMINISTRATION, CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

KATHRYN ZOON, Ph.D.

DR. ZOON: Good morning and thank you all for coming. This is a very special opportunity that FDA has to co-host these safety symposium regarding gene transfer studies and, as Dr. Skirboll has already introduced, this is one in a series of steps that the NIH and FDA are proceeding with, with respect to gene therapy safety, but as well as looking at how we proceed in these cases when there are implications to clinical trial safety issues overall.

We are happy to be here. Many of my FDA colleagues are participating in this meeting to provide some of the advice and expertise that we have in this area and we are also here to listen. To listen and to work with you as

a team to do the very, very best to further these new therapies while keeping in mind and paying close attention to human subject protection, and I think that is the fundamental step that we need to keep in mind that we keep these conferences open that there can be public participation and interest in these issues and try to make sure that we do the very best as public health service agencies and you, as investigators, in moving the science forward safely because at the end of the day this is very important to getting new medicines out to the public. Medicines that will improve their lives and their quality of life, as well as have public confidence that we are doing the best job in making sure that we can provide them a sense of the safety of these products.

As with any product, there is no such thing as zero risk. We all know that. When we have medicines we look at a risk/benefit ratio. What is the opportunity to give potential good and clinical benefit versus the risks that come, some inherent with the product themselves or other possible interactions that lead to side effects?

So we need to be constantly vigilant at doing the analysis of risk assessment and an iterative process especially during the clinical development of these drugs.

In moving forward in this particular area of cardiovascular disease there is a need for new products. This is an opportunity to explore the applicability of gene transfer products to this field and I think it holds great promise. However, we need to pay attention to the details. We need to pay attention to the vectors, the properties of the vectors, the safety of the vectors.

We need to pay attention to how we are designing our clinical trials, how we are analyzing our clinical trials, and how we are monitoring our patients.

So I welcome all of you today. I wish you an excellent meeting. We at FDA are happy to cosponsor and participate in this meeting and we look forward to the fruits of your discussion so thank you very much.

(Applause.)

WHY A SYMPOSIUM ON CARDIOVASCULAR GENE TRANSFER RESEARCH?

AMY PATTERSON, M.D.

DR. PATTERSON: Good morning and welcome.

(Slide.)

I thought it would be helpful to just spend a few minutes giving you a bit of a background about the context and rationale for having a safety symposium on cardiovascular gene transfer research and also to describe the organization and process and purpose of today's meeting, and then set forth the goals that along with your expertise and input we hope to achieve today.

(Slide.)

I think it is safe to say that probably everyone in this room has at least a friend or a family member or maybe themselves affected by cardiovascular disease. The high prevalence of coronary artery disease as well as diseases of the peripheral vasculature demand new strategies for both the prevention and treatment of cardiovascular disease. This is part of the reason for the increasing interest in the use of gene transfer to treat cardiovascular disease.

(Slide.)

This slide shows the relative distribution of cardiovascular indications. About nine percent of all transfer trials ever submitted to NIH address cardiovascular disease.

(Slide.)

But if we look at those trials over time we see that there has been a somewhat dramatic increase over the past three years. This year alone 17 percent of the trials address cardiovascular indications.

(Slide.)

There are varied approaches that these trials use, including different clinical indications from unstable angina to problems with restenosis. There are different vectors that are being used and I will talk about those briefly in a moment, as well as different transgenes.

There are also different routes of product administration as well as different contexts for clinical administration.

(Slide.)

If you look at the cardiovascular disease indications you will see that a little over half or 53 percent of current trials address diseases of the coronary arteries and a little less than half or 47 address diseases of the peripheral vasculature.

(Slide.)

When we look at the vector systems that are currently in use, although others are under preclinical studies, we see that a little over half of the

trials employ adenoviral vectors or 53 percent and 39 percent of trials utilize plasmid DNA and eight percent use DNA liposome complexes.

(Slide.)

When you look at the various transgenes used we see that there are approximately four major categories. Transgenes that encode angiogenic growth factors, transgenes that encode transcriptional regulators of angiogenic factors, genes that encode inducers of vasoprotection by nitric oxide, and genes that encode the angiogenic endocrine receptor ligand.

(Slide.)

When we look at the relative distribution of transgene usage we see that the predominant categories shown here in varying shades of blue are the angiogenic growth factors with VEGF occupying the predominant majority of 64 percent.

(Slide.)

It is important as this critical field moves forward that it move forward with the best science, the best medicine and the best ethics, and there are currently a number of challenges that we need to face in terms of optimal product development, and those include issues revolving around the selection and design of vectors, the selection and design of transgenes, and this morning's session will focus on optimizing product development and the discussions about transgenes you will see will focus on the angiogenic growth factors predominantly because they are the major class that is currently in usage, although we will touch on other types of transgenes.

(Slide.)

In the afternoon we are going to explore strategies to optimize clinical trial design and we are going to talk about the selection and monitoring of patients, the short-term and long-term follow-up of these patients and finally the selection and design of study controls. A particularly vexing issue for the field, and it is not alone in this regard, is how to differentiate toxicities and serious adverse events that are due to the underlying disease as opposed to the use of a gene transfer product. We are going to again explore how to potentially utilize study controls to make that differentiation.

(Slide.)

I would like to emphasize that the purpose of today's symposium is the collegial exchange of information, strategies and perspectives garnered from basic preclinical and clinical experience. By discussing the specific questions that have been posed, and these are included in your meeting materials, these questions were collaboratively developed by NIH, FDA and members of the RAC, and these questions will serve as a guide to our panel discussions.

Each of our speakers has been asked in the course of their presentation to address those questions that their particular clinical experience or basic research has relevance to. By virtue of working through these questions today in a collaborative fashion we hope to develop a set of principles and recommendations to help advance the field.

(Slide.)

I would just like to underscore as we go through our agenda today, I think, we have some common goals to use the knowledge that we have gained so far to inform current and future research in cardiovascular gene therapy and also to make sure that we optimize patient safety so we have a paradigm where every single patient counts.

At this point it is my great pleasure to say that the meeting is -- we are through with welcomes and we will begin the meeting, and I would like to introduce Dr. Peter Libby, who has had a long standing interest in this field.

A PLACE FOR GENE THERAPY IN CARDIOVASCULAR THERAPEUTICS

PETER LIBBY, M.D.

DR. LIBBY: I hope now that we have a president that we will have a budget and the NIH will be able to get a VGA switcher.

DR. PATTERSON: While we are waiting for the audio visual, some people have mentioned that they were not sure where the questions were and they are in your briefing packet, and they are entitled, "Safety Considerations," and it probably is not clear until page two that they are actually questions but they are in your briefing material.

DR. LIBBY: Thank you very much and thank you very much for the opportunity of addressing this august group on this very important problem.

(Slide.)

My charge today is to try and situate gene therapy in the constellation, in the firmament of cardiovascular therapeutics where it will

inevitably seek its rightful role.

(Slide.)

Now we live in exciting times in cardiovascular therapeutics because we have had a panoply now of clinical trials that have shown that addressing low density lipoprotein, LDL, that we can make substantial end roads into cardiovascular mortality. I am not going to go through all of these acronyms, these are statin mega trials all showing significant reductions in coronary events and when sufficiently powered decreases in mortality.

This has been the holy grail of much atherosclerosis research and led some of our esteemed colleagues to declare that heart attack would be gone with the century. Unfortunately, when we go to the coronary care units they are not empty and I think that we need to face the other side of this coin, and that is the majority of deaths that are not prevented. Here in some of the statin mega trials when you just plot the data backwards you see that we have a long road ahead of us in trying to push back the residual burden of morbidity and mortality due to coronary heart disease.

(Slide.)

And it is in this context that we must think beyond LDL and cardiovascular gene therapy as we have just heard and as you will hear throughout this conference is one of the bright lights on the horizon of being able to make incremental end roads into this devastating problem and one that is becoming of increasing importance worldwide.

(Slide.)

It may seem strange that I, who am not known as a practitioner of cardiovascular gene therapy, was asked to speak here but I was able to participate as the vascular biologist on a team that was one of the first to report the feasibility of vascular gene transfer. Here is Jim Wilson when he was working with Richard Mulligan. Two of my post-docs and myself were able to show the proof of principle that one could actually do cardiovascular gene transfer here with the characteristic B gal stain showing gene transfer with a retroviral vector.

(Slide.)

And that really was published in 1988 together with the Nabel's pioneering work, and that set the stage for what has been a really ferment of activity in trying to apply this in cardiovascular therapeutics. And as we heard, the majority of the trials that are ongoing are targeting angiogenesis, myocardial angiogenesis.

(Slide.)

Heart attack shown here by this dusky region in the left ventricle of this heart is still with us and is still a very pressing problem.

(Slide.)

You see here in this Spaetholtz preparation, which is a cleared heart with the vessels injected, where there has been a myocardial infarction we have a paucity of these vessels which supply the blood to the myocardium, and it is clearly a worthwhile goal to try to stimulate new arterial formations so that one may re-perfuse these territories so that one may come to the succor of ischemic myocardium.

(Slide.)

But life is complicated. Biology is complicated. The disease is complicated. And as we sit here and think about safety of gene transfer, we have to think that neo vascularization in the ischemic heart occurs not only in the myocardium but also in the blood vessels. This is the classic work of VanBuchs and Cliff Barger. They have cannulated the coronary artery here and they are injecting a latex resin. And in the later phases the microvasculature of the plaque itself here fills up and you are able to appreciate this rich plexus of micro-vessels, which is part and parcel of the atherosclerotic process, often missed by classical histologic stains.

(Slide.)

But when one performs specific stains such as von Willebrand factor to mark endothelial cells, here shown at higher power, we see the profiles of these vessels make the atheroma look like swiss cheese. This is an example from a study which I was able to do with some of my colleagues where we proposed that a mechanism of neo angiogenesis involved over expression of matrix metallo-proteinases to allow these angiogenic sprouts to drill their way through the dense matrix, extracellular matrix of the advanced atheromatous plaque.

(Slide.)

In work that we did with Eddie Brogi and Jeff Winkles a number of years ago, we were able to localize endogenous angiogenic growth factors such as acidic viral blast growth factor within the plaque associated with inflammatory

cells and the presence of these plaque micro-vessels.

(Slide.)

What I really do for a living is to study the mechanisms of atheroma de-stabilization. I spend a lot of time thinking about fracture of the fibrous cap and about superficial erosion but also intra-plaque hemorrhage from these micro-vessels is an additional potential mechanism of lesion complication.

(Slide.)

Here are some observations that were made by Dr. Brogi where she localized one of these regions of plexi of micro-vessels in the heart of a human atheromatous plaque and she saw extravasated von Willebrand factor, indicating that maybe these neo vessels in the plaque, like those in the diabetic retina, were particularly fragile and prone to microaneurysm or disruption. And here we have evidence for bleeding within the plaque.

(Slide.)

Here is a Prussian blue stain showing deposition of iron rich hemosiderin just in this very area where there has been a probably clinically silent disruption of those micro-vessels.

(Slide.)

So intra-plaque hemorrhage due to these micro-vessels with thrombosis in situ might actually promote plaque complication by thrombin generation, which can incite activation of many kinds of cells, release of fibrogenic mediators from platelets at sites of thrombosis in situ and also promote oxidative stress through fentin chemistry because of that iron deposition

extracellularly.

(Slide.)

I think many of you are aware that one of our faculty members at the Brigham, Karen Moulton, working together with Judah Folkman, has been able to show micro-vascularization in the experimental plaques in the APO lipoprotein E deficient animal.

(Slide.)

And has been able to actually then inhibiting angiogenesis can reduce intimal re-vascularization and plaque growth in this experimental model of plaque vascularization.

(Slide.)

So I just think when we are considering safety, when we are talking about the abnormal heart with arteries that have preexisting disease that if there is a spill over from the myocardium to the blood vessel that we must consider the plaque neo vascularization might increase and promote plaque complication by hemorrhage and thrombosis in situ. So I think we just have to be aware that life is complex, disease is complex, and take that into account when designing and analyzing our trials.

(Slide.)

Now another clinical application of gene transfer in the cardiovascular arena that has been quite popular is restenosis, and I would like to continue to weave this theme of complexity and change.

(Slide.)

It is amazing when you do this to an artery, put a balloon in it, blow it up and crack it wide open that you do not always get this growth of neo intimal tissue shown here leading to renewed symptoms and causing the need for another intervention.

(Slide.)

Histologic studies have shown that the cells in the neo intima following injury are primarily smooth muscle cells stained here with an actin stain. You will note, however, that the cells are actually imbedded in a rather loose appearing extracellular matrix.

(Slide.)

Well, many have thought about how to apply gene therapy to this restenosis problem following vascular intervention but it requires that we know what gene to transfer and the biology of this disease has proven more elusive than might have seemed at first.

(Slide.)

We focused for decades on smooth muscle proliferation but as I have also shown you extracellular matrix accumulation accounts for the bulk of the volume of these restenotic lesions and a lot of this, particularly in this new disease which I will come to in a moment, in-stent stenosis, is due to water accumulation because of proteoglycans highly negatively charged, which combined water.

(Slide.)

So is smooth muscle proliferation a sensible target for inhibition

post-injury? Does smooth muscle cell division occur rapidly enough? Work from Ed O'Brien and from Steve Schwartz suggests that it is rather indolent. And, if successful, could inhibition produce unwanted complications?

(Slide.)

Aneurysm formation or actually plaque destabilization by impairing the ability of the smooth muscle cells to repair and maintain the critical extracellular matrix skeleton of the plaque that is all that stands between many of our patients and unstable coronary events.

(Slide.)

And the pathogenesis of loss of lumen post injury, post coronary intervention, is complex. It is not only intimal thickening. As a matter of fact, some believe intimal thickening does not play a major role at all. Other mechanisms such as elastic recoil, vasoconstriction, failure of compensatory enlargement or adventitial cicatrization or a negative remodeling may contribute to the loss of lumen following injury.

(Slide.)

Kakuta and Lafont, experimentally, and Mintz and many others, have presented evidence that constrictive remodeling, not intimal thickening, contributes substantially to restenosis following balloon angioplasty in human subjects with preexisting atheroma.

(Slide.)

So in this part of medicine, as in many others, you have to keep your eyes on a moving target because the field has moved quite a bit since the

days of balloon angioplasty and we have now entered an area where stents have proliferated.

(Slide.)

Now stenting is an extremely appropriate and life-saving therapy. This is an angiogram of a patient of mine who is hanging on by a saphenous vein graft to a totally occluded native circulation and whose life was saved by insertion of the stent.

(Slide.)

But it can be taken to extreme. Here is a post-mortem angiogram of a patient who ended up with nine sequential stents in their left anterior descending coronary artery, sort of armor plating of the vessel and that is why this led to an autopsy.

(Slide.)

So the widespread use of stents have changed the biology that we have to consider when we are thinking about targets for gene transfer. It is rendered restenosis obsolete and created a new disease, in-stent stenosis, and that has led to new therapies.

(Slide.)

Brachytherapy where one can give beta radiation, delivering through fancy catheters.

(Slide.)

And that can in one of the recent studies decrease the late lost of lumen here in the beta irradiated segments of the vessel.

(Slide.)

But here again we are chasing our tail. The use of brachytherapy to prevent in-stent stenosis has spawned yet another new disease known as the candy wrapper lesion where this is the part that has been treated by the radiation but at the margins we often get a stenosis and this is turning out to be a rate limiting problem, and the excitement now in interventional cardiology as of the recent American Heart meeting is coated stents with say rapamycin as a way to avoid this particular new disease complicating one of our interventions.

(Slide.)

So life is complicated and we have to think of the changing targets when the technologies advance and the disease changes before our very eyes.

(Slide.)

Now another question which has already been alluded to is the safety of the vectors. Are our current vectors advanced enough for routine clinical evaluation? Now you will hear later from Dr. Dichek, with whom I was fortunate to be able to collaborate, taking our long-standing interest in vascular inflammation and coupling it with his expertise in vascular gene transfer, and he will tell you at greater length that we were able to show that these early first generation adenoviral vectors, technology has progressed considerably since we did these experiments in the mid '90s but that there was evidence for inflammation shown by expression of these markers of inflammation and accumulations of T lymphocytes not only at ten days following intervention but thirty days as well. So there is prolonged inflammation with these early first

generation and now perhaps obsolete, as you will hear, kind of vectors.

(Slide.)

Indeed, we were able to document intimal hyperplasia, which is of course the target for much arterial gene transfer. So the question is, is the intervention at least with these early first generation viral vectors going to actually complicate the problem rather than solve it?

(Slide.)

And like any therapeutic agent, I think that we must consider very importantly some of the same things that we would consider with drugs, the efficiency of transfer, the control of the dose and the time, the control of delivery, and take into account the inflammatory effects of some of the current vectors.

(Slide.)

I just want to give a little bit of balance and say that ultimately gene therapy for many acquired cardiovascular diseases will be a halfway technology, as defined by Lewis Thomas, awaiting fundamental understanding of the disease process for prevention. So for those of us in the room who already have atherosclerosis it is too late to think about prevention perhaps as a fundamental approach but we must still continue to invest in the basic science of the pathogenesis of the disease rather than sticking our finger in the dike with a halfway technology.

(Slide.)

Now in the 1990s, four years ago, I showed this slide when I was presenting some of the work that we did in collaboration with David Dichek. I

urged short-term caution because of raised public expectations and raised expectations of funding agencies, regulatory agencies and investors.

But I was and I continue to be a long-term optimist about vascular gene therapy taking its role in the firmament of cardiovascular therapeutics because these technical problems are suprabral in principle. And if we do our homework in the biology and we do our homework in the vectorology, and that is what we are here assembled to address, that we will be able to benefit our patients.

(Slide.)

Unfortunately, there has been, as Dr. Lenfant told us very eloquently, some notoriety to this gene therapy enterprise. This is from this week in the Boston Globe. You will be interested to learn that all of the editorials were about the Supreme Court on Monday but here since biomedicine is an indoor sport in Boston this was on the op-ed in the editorial pages of the Globe.

(Slide.)

So gene therapy, like all other advances in medicine, follows a cycle. This is the curve of enthusiasm which burgeons in the initial introduction days and then inevitably reality sets in, there are some reverses, and perhaps we are in the nadir. But then I am confident and I believe many of you here share my confidence that we will find an even keel and that we will settle out to a plateau of application of gene therapy in cardiovascular therapeutics that will leave us at a higher baseline than we started.

(Slide.)

Advances in medicine, ladies and gentlemen, requires several kinds of people and that is why I commend the agencies that are sponsoring this meeting for having gathered and assembled this group of people. You need the Columbus's who have the courage to sail forth into the unknown and you need to also pay some attention to the Cassandra's who point to the complexities.

That is why I think that we are on target to find the place of gene therapy in cardiovascular therapeutics.

Thank you.

(Applause.)

DR. FRIEDMANN: Good morning as well from me. My name is Ted Friedmann and I have had the pleasure of helping with some of the aspects of the organization of this meeting on behalf of RAC.

Let me just go ahead straight away since we have a very crowded morning and introduce the next speaker, Dr. Robert Simari from the Molecular Medicine Program at the Mayo Clinic, and he is going to continue the discussion of general principles of cardiovascular gene therapy again with particular emphasis on the questions that are central to the program as in the brochures that we all have.

CURRENT APPROACHES TO CARDIOVASCULAR GENE TRANSFER

ROBERT SIMARI, M.D.

DR. SIMARI: Thank you very much. I appreciate the opportunity to share my perspective on sort of historical and current aspects of cardiovascular

gene therapy. It has been a very exciting field to play a role in and I would like to share it from a historical perspective, and by doing so will share some of the work in brief of people in the audience but I would first like to apologize if misrepresented and I look forward to the discussion section.

(Slide.)

In 1995 the Orkin-Motulsky report concluded that somatic gene therapy is a logical and natural progression in the application of fundamental biomedical science to medicine and offers extraordinary potential in the long-term for the management and correction of human disease. And as cardiovascular scientists and as early adapters in technology, the cardiovascular field has generated a lot of interest in this area over the last 15 years.

(Slide.)

Orkin and Motulsky also concluded that significant problems remain in all basic aspects of gene therapy and as cardiovascular scientists this is our challenge.

(Slide.)

I would like to walk through the field of cardiovascular gene transfer in the following areas:

As Dr. Libby pointed out, the great interest in vasculo-proliferative diseases, gene transfer, and the subsequent development of the area of induction of angiogenesis. There is a burgeoning area of gene transfer from myocardial dysfunction or electrical instability but because of the time constraints I will not go into these today.

(Slide.)

It is my feeling that in the gene transfer field we have two circles that we have to deal with. We have to deal with the preclinical circle and the clinical circle. The preclinical studies, many of which have been pursued by people in this audience, generally have taken the form of looking for transgene expression, whether it be in normal animals or in animals with disease, and then looking at transgene function in those same animal models.

Subsequently, with biological function then one can undertake studies looking for "therapy" in these preclinical models. When this has been demonstrated, one can then branch out into the clinical arena. Again I would propose that the same circle applies to the clinical arena. One needs to demonstrate function -- excuse me, expression, followed by function before one can really put together studies that would identify whether there would be therapeutic benefits from such a procedure.

(Slide.)

In terms of cardiovascular gene transfer, the vector systems which we have at our disposal and which Dr. Dichek and others will speak more on this morning, have basically broken into categories of either viral vectors or nonviral vectors. And the limitations in applicability of these vectors in the cardiovascular system are such that in the vasculature, which is a relatively quiescent tissue, and only in certain instances such as balloon injury or stenting is there much proliferation.

A vector such as adenoviral vector, which does not require

proliferative cells for transduction has really risen to the forefront over the last decade in terms of gene transfer to the vasculature. Of course, the efficiency with that vector is relatively high. The stability is relatively brief and we will get into much of the discussion today about the toxicity of that vector.

Early studies done with plasmid or DNA liposomes which demonstrated the proof of principle have shown to be relatively limited in their scope to large vessels but recently there has been some data with new liposomes that that may actually be changing.

To the cardiac myocytes, some of the same principles apply.

To skeletal muscle, there seems to be an advantage or at least a potential advantage to plasma delivery with relatively low efficiency.

But for a potent transgene such as VEGF or other growth factors, this may be sufficient to get a biological effect.

This dependence upon adenoviral vectors in cardiovascular gene therapy I am sure will be a discussion and a theme that will follow throughout the day.

(Slide.)

Human vasculo-proliferative disease, as Dr. Libby mentioned, are a wide range of diseases. On the lower range, atherosclerosis, which has a very low baseline proliferative rate, to, at the higher range, arterial venous fistula stenosis and graft stenosis, which have a relatively higher proliferative potential.

Many of us in the field chose a simple model for vascular proliferative diseases, that is the renarrowing following balloon injury or

restenosis following angioplasty, as both an animal model and an animal model of clinical disease to study.

As Dr. Libby mentioned, this is not a straightforward simple disease but this provides a model for some of the aspects of clinical restenosis.

(Slid.e)

Now work from Betsy Nabel's lab early on, and many others, showed that, indeed, in this simple animal model that proliferation is an important factor and the proliferation associated with balloon injury or vascular injury plays out over a relatively finite period of time. This is in the ileo femoral pig model following balloon injury. One can see Brd C labeling that increases over approximately the first week following injury.

So the goals with gene transfer originally were to inhibit this wave of proliferation that might have downstream effects not only on the number of cells but on the matrix on which they produce.

(Slide.)

Shown clinically, as Dr. Libby mentioned, the error of stents has really changed interventional cardiology such that in most laboratories about 80 percent of the patients are receiving intra-coronary stents rather than plain old balloon angioplasty. This is an intravascular ultrasound study that shows that proliferative index -- proliferation is very important in the stent system, such that late lumen loss, that being the in-growth of the vessel following stenting, is directly related to neo intimal hyperplasia following stent placement. That is because the vessel -- the constriction that is seen following balloon angioplasty in

the remodeling is restrained by the stent placement such that in the clinical -- current clinical scenario neo intimal hyperplasia is correlated with this late lumen loss.

So in spite of the complexity of the disease, at least in stented patients neo intimal hyperplasia is an important factor, albeit a factor in the disease.

(Slide.)

The original gene transfer studies into the vasculature were using relatively crude delivery devices. Shown here is the original device used by Betsy Nabel, the double balloon catheter, with an installation port between these two balloons to isolate a segment of the vessel shown here in the porcine iliac artery, when these balloons are inflated, we can isolate a portion of the vessel for installation of a gene transfer vector.

Using that system in the late '80s, early '90s, they were able to demonstrate the use of transgene betagalactosidase both with retroviral vectors and with DNA liposomes that one could transduce the endothelial cells within these vessels showing proof of principle of gene transfer into large vessel.

(Slide.)

Subsequent studies that we performed when I was at the University of Michigan with Betsy, in this rabbit atherosclerotic model, show that this is a hyperlipidemic rabbit artery in which the animals have been denuded, the endothelium has been denuded and the animal fed lipid for three weeks in the neo intimal forms here that are somewhat reminiscent and have some features of

human atherosclerosis.

We came back and injured these vessels with balloon catheters similar to angioplasty disrupting the intima and delivered, using a similar catheter, a first generation adenovirus expressing human placental alkaline phosphatase, sacrificed the animals two days later and saw transgene expression within the intima portions of this vessel, as well as some of the intermedial portions, suggesting that again in this more clinically relevant model that we could get transgene expression in large vessels.

(Slide.)

We also showed with immunostaining that -- double staining that we could transfect smooth muscle cells, which make up a predominance of the vessels within the intima as well as macrophages within this macrophage rich area. Therefore, we demonstrated transgene expression in the major cellular players within atherosclerotic plaque.

(Slide.)

Now a number of studies have progressed since that time in terms of vascular gene transfer to show a therapeutic effect in animal models. These have ranged from the antiproliferative effects using either cytotoxic approaches or cytostatic approaches, antithrombotic effects using both native proteins such as thrombomodulin and TFPI, but also exogenous proteins such as the work from Dr. Dichek using Hirudin.

Other approaches have been rather pleiotropic such as using proteins such as ENOS and INOS as well as some of the naturetic peptides that

may have multiple effects, including antiproliferative effects and antithrombotic effects. As a model of how these studies have progressed, I will show our work using the HSV-tk system to demonstrate some of the principles of therapeutic effect in an animal.

(Slide.)

So we took advantage of the tk gancyclovir approach to maximize our transgene effect with the limitation of transgene delivery such that the tk gancyclovir approach allows the bystander effect, that is cells which are not directly transfected may be killed through their presumed diffusion of phosphorylated gancyclovir, that being the toxic compound generated from thymidine kinase expression and exposure to gancyclovir, which can then diffuse into neighboring cells and kill neighboring cells. Thus amplifying a relative -- or decreased gene transfer expression due to catheter limitations.

Using this approach in the rabbit model of atherosclerosis we were able to show decrease intimal proliferation and decreased medial proliferation in those animals that were treated with the prodrug gancyclovir in the adenoviral gene transfer as those that did not receive the gancyclovir, suggesting that not only could we get gene in but we had an effect on proliferation.

(Slide.)

Three weeks later when we looked at all the control groups, including those that were not infected and received gancyclovir, those that received a null adenovirus and received gancyclovir or those that received the transgene without gancyclovir, we showed a decrease in intimal area compared to

all the control groups, suggesting that with this genetic approach we could inhibit proliferation and inhibit intimal formation.

(Slide.)

These studies have been replicated in a number of different laboratories, actually both in Europe and in the United States, and you will notice that the effect has been generally about a 30 to 40 percent decrease in intimal formation following balloon injury.

And it should be noted that, out of interest, these studies really were studies of the mid 1990s and really have not been moved forward to a clinical level because of the reasons which I will discuss.

(Slide.)

In other studies, just to keep things in perspective, rather than killing proliferating cells, one can take a cytostatic approach and over express inhibitors of the cell cycle such as the cyclin dependent kinase inhibitors such as p21, p27 or p16 and get similar effects.

(Slide.)

When we over express p21 in a porcine model of balloon injury we saw a similar effect, about a 30 percent decrease in intimal formation compared to control vectors or noninfected arterial segments, suggesting that there are a multitude of ways in which to inhibit intimal formation in animal models, both of normal animals and of atherosclerotic animals.

(Slide.)

However, these studies lack some of the complexity, as Dr. Libby

mentioned, of human disease. So to approach that we asked the question of whether we could infect or transduce human arteries to a similar degree as we could do animal arteries. This is a study that Mark Rechter and I performed at the University of Michigan taking human coronary arteries and human carotid plaque in organ culture, using the same adenovirus expressing placental alkaline phosphatase, and showed similar degrees of transgene expression shown here on the lumenal portions of human coronary arteries.

Of interest, this was a human carotid plaque at the site of plaque rupture and there was transgene expression at the site of plaque rupture, suggesting that this injury to the atherosclerotic plaque was advantageous in terms of gene transfer.

So we extended the studies at least ex vivo into humans.

(Slide.)

And the following study by Seppo Yla-Herttuala in Kuopio, Finland, really went the next step, and that is could we use first generation adenoviral vectors to transduce human atherosclerotic arteries.

In a study that was very unique, Dr. Yla-Herttuala put an adenoviral vector expressing beta galactosidase delivered through an intraarterial catheter into the limbs of patients which were doomed for amputation. So these were patients with ischemic limbs that were scheduled for amputation. He delivered, using a catheter, a beta galactosidase adenovirus into the atherosclerotic limbs and the lessons he learned here were actually quite interesting.

(Slide.)

This is a cross section of a portion of an artery that was delivered with the highest level of highest titer of adenovirus and one could see transgene expression, Seppo quantified, in up to five percent of the cells. But, interestingly enough, the transgene was in the more relative portion of the vessel rather than the more disease portion of the vessel, suggesting a potential very important feature of gene transfer into a disease that is patchy such that there may be more gene expression in the normal vessel rather than in the disease part of the vessel.

(Slide.)

Now Seppo has gone on to do several studies in the human vasculature with gene transfer and he showed data at the American Heart Association meeting on safety in this regard using either a plasmid liposome construct, an adenovirus or control, in the plasmid liposome or adenovirus -- first generation adenovirus expressed VEGF. He used intra-arterial delivery following percutaneous angioplasty of either the coronaries or the peripheral vessels and Seppo documented a significant adverse event of zero percent in the plasmid but seven percent in the adenoviral group.

One of the patients had a severe reaction with fever and joint pain, and one of the patients in follow-up developed chronic myeloid metaplasia, which Seppo put as possible interactions with the adenoviral vector. He also found fever in 35 percent of the patients that received plasmid and 48 percent of the patients that received adenovirus compared to three percent in the control group.

Elevated CRP T reactive protein was a feature in a majority of patients, whether they received plasmid or adenoviral vector, and in a portion of the control presumably from the vascular injury. And he found increased adenoviral titers in 35 percent of the adenoviral patients that received adenovirus.

(Slide.)

So -- and he has yet to show any functional effects of VEGF in these patients.

So I would suggest that as far as targeting the large vasculature, we have shown expression in vessels, we have shown function in preclinical models, and we have shown therapy in preclinical animal models. Seppo's work has taken that to the next step and has shown expression in humans.

I believe we are at a block here and I believe that this block prior to function is a very important one and I think it is based upon the following four factors: I think it is based upon the fact that we have relied on preclinical models on adenoviral vectors and their attendant problems.

We have a lack of optimized clinical delivery devices for the vasculature given the branching nature.

We are in a setting of changing clinical imperatives. The idea that balloon angioplasty and restenosis was a major problem, as discussed by Dr. Libby, has been changed by stenting. It has been changed by radiation and it has been now changed perhaps by coated stents.

So I think there is changing clinical need for this in the vasculature.

(Slide.)

I also think that clinical trial scenarios have been very difficult to sort out and the need for correlative science such as the transgene expression and function in the vasculature is one that we should talk about in the discussion session because that is something that has provided great challenges to the field.

(Slide.)

As the field of delivery to the large vasculature progressed through the 1990s it was really leap frogged on the work of Dr. Isner and others who came up with the idea that we might be able to use gene transfer for therapeutic angiogenesis, that is the growth of new vessels in ischemic tissue.

(Slide.)

And this slide from Dr. Isner's work that was actually protein delivery really highlighted the prospects for this approach and that is that this was a -- using the rabbit ischemic hind limb model, delivery of intra-muscular or in this case it was intravenous VEGF protein, one could develop a collateral network and increase blood flow into the ischemic hind limb, as shown in this slide versus a control experiment, showing minimal collateral formation. This being the treated group.

And really it was this concept of therapeutic angiogenesis and the potential for gene transfer that really blossomed and really surpassed the field of delivery to large vessels in the mid 1990s.

(Slide.)

The angiogenic approaches, which we will discuss in detail this

morning, have really been of several types. One is the delivery of these vectors have either been intramuscular, either into ischemic skeletal myocytes or into cardiac myocytes in the case of the heart. But there has also been a study, which we will hear about later today, of intra-coronary delivery, that is just dripping the viral vectors into the ischemic bed.

The vectors have ranged from taking advantage of the potency of the transgene effect to either use plasmid alone or adenoviruses or in preclinical studies now, adeno associated viruses, for this approach. The growth factors, as were mentioned earlier, have included the VEGF family, the FGF family and transcription factor such as HIF-1 alpha and VP16 combinations.

(Slide.)

Now Jeff's work has really been extraordinary in that he has been able to deliver plasmid DNA into ischemic limbs or limbs destined for amputation or those with nonhealing ulcers and get clinical results in these Phase I studies as well as an improvement in the blood pressure in the distal limb as measured by the ankle brachial index. He has also showed evidence of serum expression of VEGF systemically as shown in this publication from 1998.

(Slide.)

When one looks at the group one can see following baseline injections and injections in four weeks into the musculature with these plasmids expressing VEGF that one can get a bump in circulating VEGF levels, suggesting transgene expression in these Phase I studies.

(Slide.)

Other studies by Dr. Crystal and others using another isoform of VEGF, VEGF-121, this in the ischemic amaroid constrictor model in the pig, one can show that if they delivered an adenovirus expressing VEGF-121 they could get recanalization of the occluded circumflex artery as compared to the control animals which receive the null vectors.

(Slide.)

This blood -- this recanalization was associated with a decrease in the ischemia as noted on the radionucleid studies. In the Ad null group there was this -- this is -- the differences between these rest and stress curves is a representation of the ischemia within the myocardium, and one can see that following amaroid constriction there is the ischemia and following transgene delivery that remains with a null virus.

(Slide.)

With the 121 adenovirus one can see resolution of that stress ischemia, suggesting a functional effect in this animal model, and we will hear more about these studies later today.

(Slide.)

In a really landmark study by Kirk Hammond, who is in the audience, Frank Giordano delivered an adenovirus expressing FGF-5 but rather than using intra-muscular delivery, these investigators used intra-coronary delivery and not intra-arterial delivery that I mentioned before, targeting the wall but merely a dripping of the vector into the distal bed and showed transgene expression using the Lac-Z virus and then showed their therapeutic gene or FGF-

5 in this case, their expression within the myocardium.

(Slide.)

This has led to a trial, a Phase I/II trial, that is randomized using intra-coronary delivery of a similar growth factor family member, FGF-4, which we will hear more about today.

(Slide.)

These FGF-5 studies in the animals were associated with capillary development, both in the nonischemic region and the ischemic region, associated with a transgene delivery.

(Slide.)

So I would suggest that it is my impression that the angiogenesis field, which started a little bit later than those of us looking at vasculo-proliferative disease has really leap frogged and made major advancements, that is expression and function in preclinical models generated -- early models of preclinical therapy and on to clinical studies which have demonstrated expression.

I await information regarding functional and therapeutic effects from further studies in this area and I look forward to discussion in this later today.

(Slide.)

I would like to present one abstract that came from Stefan Yanson's group, which speaks to perhaps some of the safety concerns of gene delivery to the heart. Stefan's group preimmunized pigs with a null adenoviral

vector at five times 10^9 pfu using a retrograde delivery system via the coronary sinus or used control animals. They generated neutralizing antibodies in all the immunized pigs to the titers of greater than one to 2,000. In those pigs they came back and used a similar method using coronary sinus delivery of an adenoviral vector expressing a transgene.

And they showed that preimmunization decreased subsequent transgene expression three-fold in the group that received the preimmunization. In these animals when one looked histologically at the hearts at the area of delivery, one saw histologic Grade 3 to 4 rejection in the immunized pigs as compared to lack of rejection in the nonimmunized pigs.

Stefan went on to caution the audience at the Heart Association that he measured neutralizing antibody titers in patients undergoing coronary bypass surgery and saw that about 50 percent of the patients had similar titers to those receiving the preimmunization in these studies, suggesting caution regarding transgene -- adenoviral delivery to humans with neutralizing antibodies. Now whether or not this relationship holds in patients is obviously yet to be seen.

(Slide.)

So I would summarize with some thoughts in a slide very similar to Dr. Libby's in that the field has undergone many changes. It was founded with innovation and it led to unbridled enthusiasm, and I would agree with Dr. Libby that we are in an era of harsh realities as the biology has met the patient, and since our major concern is the patient, this is, I believe, currently where we live.

I think there are a number of thoughtful adaptations that are ongoing that will get us out of some of these conundrums.

(Slide.)

And I would suggest that the thoughtful adaptations, as I perceive them, are to improve the vectors either to get -- to have improved nonviral vectors as the studies from Heike van der Leyen and Victor Zao using novel liposome transgene delivery into the vasculature that looked very promising, using adeno associated virus in the vasculature, which we will hear about today, or helper dependent adenovirus. The use of lentiviral vectors has really not been fully explored in the vasculature and may have some advantages.

The use of stem cells or endothelial progenitor cells as systemic targeting for the vasculature has great promise, and I believe it is the systemic delivery and targeting of the vasculature that may ultimately play a role.

Targeting of the vasculature, either using transcriptional targeting, which might limit transgene expression to target cells and avoid transgene expression in antigen presenting cells perhaps may play a role.

Viral display as was discussed at the RAC meeting by Steve Russell yesterday may be able to target the vasculature.

I think there are improvements made in the catheters. I think recent work by Maria Palaisis and colleagues at Boston Scientific showing that they could develop catheters that are more compatible with adenovirus may play a role in the improvement of the field.

There are obviously new potent transgenes that will be developed

that might have affects.

I think the opportunity to describe unique clinical scenarios is really a very important one. That is how we can generate clinical trials where we could identify and describe transgene expression and transgene toxicity is a very important one that has challenged the field.

Seppo Yla-Herttuala did a very important study in patients undergoing amputation. I think there are similar clinical scenarios that should be discussed regarding transgene expression. I think transgene monitoring in a noninvasive method either using imaging techniques, which are available, say for the tk gancyclovir approach or using some systemic monitoring such as Steve Russell is doing at Mayo with clipped transgenes to be able to use systemic monitoring of transgene expression may be very important.

I think what we are doing here with education, both at the professional level and in the public, will really be -- will really improve the lot with which we are working in the cardiovascular gene transfer field.

I just want to end with one slide to give perspective.

(Slide.)

This was taken from Science. It was the ups and downs of therapeutic antibodies. And I think we are reliving this with the ups and downs of cardiovascular gene transfer. Clearly to a cardiologist who uses a therapeutic antibody in terms of Ad sixam and Ad reapro, in many of the patients that we deal with currently we can see that there was therapeutic benefits from this field in spite of the ups and downs over the years.

The only thing I would bring to your attention is actually the X axis here, which is not weeks, months, years, but it is decades. And, in fact, one can say that this played out over 25 years. So it is my opinion that we are going to see similar phases of cardiovascular gene therapy but we should keep in mind that this axis is decades.

Thank you very much.

(Applause.)

SAFETY CONSIDERATIONS IN PRODUCT DEVELOPMENT

DR. FRIEDMANN: Okay. Well, thank you very much, both Dr. Libby and Dr. Simari.

Let me just back up a little bit and give you one minute's worth of rationale for this meeting and tell you why we are here -- why you are here.

It occurred to a number of members of RAC in reviewing protocols that were coming in front of us that it was getting increasingly difficult to know what questions we should be asking and we have all become aware, as beautifully reviewed now by the previous speakers, of how complex cardiovascular disease is and ont only how complex it is but how much more complex it is when it moved just from peripheral vascular system to the heart. And it really began to pose major questions to us as members of RAC in trying to review protocols what questions we should be asking and what clues we should be looking for in evaluating protocols.

And so I thought -- a number of us thought that it would be very useful and instructive to the RAC to hear from experts in the field, both people

who are devoting their work to gene transfer studies per se but also people who are distant and at arm's length from the field of gene therapy but who know the issues of the vascular system, both peripheral and cardiac, issues of the electrical systems in the heart, how they are affected by manipulations that are being proposed, conductivity, contractility of the heart, and all those things began to make us convinced that we ought to hear from all these people, both experts in the gene therapy aspects of it and the hands off experts in the physiology and function of the heart.

So that is really the purpose of this meeting, to look at principles knowing and as has been reviewed already beautifully how the ups and downs of the field are typical of many areas of rapidly moving science, difficult science, and there is no question in any of our minds that we will get there sooner or later but it will continue to be a difficult road with a trajectory obviously in the right direction.

So what we would like to do now is move to a series of talks on the general issue of vector choice. How does one choose how to move genes into the vascular system? With particular emphasis again on -- and we have instructed and asked the speakers to pay particular attention to the issues as laid out in the discussion questions, and we will have the same issues in the panel discussion as well -- to pay particular attention on what features of cardiovascular disease affect the selection of the vector that is going to be used to introduce transgenes, what particular features of the pathogenesis of cardiovascular disease should affect the selection of the vector; how -- again what features of disease

and pathogenesis affect how the vectors are introduced into the cardiovascular system; and what critical questions need to be posed and answered with respect to the safety of all this.

So those four questions we hope that each of the speakers will touch on and that the panel will continue to pick up after the three speakers.

So we will start off with David Dichek from the Gladstone Institute at the University of California, San Francisco, who is going to discuss the mechanisms of selection of vector for delivery to the cardiovascular system.

David?

OVERVIEW OF VECTOR SELECTION

DAVID DICHEK, M.D.

DR. DICHEK: Thank you very much, Ted, and it is certainly nice to be here today.

(Slide.)

I am at Lassiter Institute, UCSF. Next month I will be moving to the University of Washington in Seattle.

(Slide.)

So I thought I would start by reviewing what is the optimal vector for cardiovascular gene transfer. This is what many of us are working towards. It should be efficient in terms of number of cells transduced per vector particle infused. It should be able to be modified to target specific cell types. It should have a large cloning capacity to include regulatory elements and large transgenes. There should be no proinflammatory effects. It should be capable of achieving

targeted integration into the genome for many applications. There should be no preexisting immunity, a problem alluded to by the previous speaker. And it should itself be nonimmunogenic so that it can be administered. That is certainly a tall order.

(Slide.)

So I know the title of my talk is "overview" but I thought that I would really overview what I know best so I will stick with our experience with cardiovascular gene transfer vectors. We have been working in the area since 1988.

We initially started with a retrovirus. We did in vitro experiments showing that it worked quite well, although it did require a selective step in those days. In vivo it was very inefficient and we, therefore, switched to adenoviral vectors and have been working with them since '92.

They were attractive to us because of the tremendously high efficiency with no selection in vitro and even high efficiency in vivo. However, we have been dealing with numerous problems that I will discuss today. I know that Barry Byrne will be talking about AAV so I will not touch on that at all but I think some of the things -- a lot of the things I will say will be generally applicable to other vectors, though really restricted to our experience with adenovirus directly.

(Slide.)

Peter Libby discussed this study that we did a number of years ago asking the question what is the baseline effect of adenoviral vector infusion in an

artery. So that would be the baseline from which one would work in introducing a transgene. There is -- at the same time we were doing these studies -- tremendous interest in viral pathogenesis of atherosclerosis and since there are many open reading frames in first generation vectors we wanted to see whether there were any effects.

Infusing into the rabbit femoral artery, Kurt Neuman did a study. Here is an in vivo view. And then harvesting the artery.

(Slide.)

And as Peter Libby mentioned, we did find inflammation both at 10 and 28 days associated with virus infusion.

(Slide.)

And as he mentioned as well, we found intimal hyperplasia also. This is a vehicle infusion. The media is there. There is a large neo intima in the Ad Lac Z infused artery and that was present in the adeno-infused artery as well and not a specific effect of the Lac Z transgene.

(Slide.)

Quantitatively at ten days after infusion of vector looking at the Lac Z vector versus the control vector, this was a vector originally supplied by GTI, at ten days we had a borderline significant increase in intimal medial ratio. However, that was highly significant at thirty days and was present with both Lac Z and control vector. This is obviously not something you want in interventions where you are trying to decrease intimal hyperplasia.

(Slide.)

So we then tried to make the system more complex and more realistic in terms of human applications. Adenovirus infusion in normal rabbit arteries causes inflammation and neo intimal formation. What is the effect of adenovirus infusion in a setting conducive to the development of atherosclerosis so a setting of hyperlipidemia. Maybe it makes no additional difference if the vessels are already becoming diseased due to hypercholesterolemia.

(Slide.)

So we set up an experiment where we fed rabbits with either a standard or atherogenic diet for four weeks and then infused either vehicle or a null first generation vector and harvested the arteries up to four weeks later, looked with morphometry and immunostaining. So it is really a two by two study, regular diet, atherogenic diet, vehicle or Ad null, four groups.

(Slide.)

And what we found is that at least in terms of the lesion size that the effects of adenovirus and hypercholesterolemia on lesion size were additive so infusing vehicle in rabbits on a standard diet, if you have a good surgeon who handles the arteries carefully, you do not get any neo intima. You do the local infusion and nothing happens.

On the other hand, if the rabbit is hypercholesterolemic, infusion of vehicle alone is sufficient to cause a lesion to form, that along with hypercholesterolemia.

Adenovirus infusion with a regular diet causes this more highly cellular lesion, the easily recognizable foam cell, foamy lesion here, very highly

cellular lesion here with smooth muscle cells and inflammatory cells as well and the two of them together creates a larger lesion.

(Slide.)

Quantitatively, no virus, no cholesterol. This is the IM ratio, cholesterol, no virus. Difficult to detect a difference with this measurement technique but with others it is there. Virus and no cholesterol, a significant increase, and the two of them even greater. Measuring macrophages by RAM-11 immunohistochemistry, the synergy here is quite remarkable. There are no macrophages except in the setting of cholesterol feeding. With adenovirus there is a moderate number of macrophages but with cholesterol and virus is really quite increased macrophage density.

(Slide.)

So, you know, we were concerned because we had not seen this in rats, that maybe the rabbit was a particularly proinflammatory host, and maybe we ought to look at nonhuman primates, and we were fortunate to have collaboration with Randy Geary at Bowman Gray, who had some cynamalogous monkeys that had been on a cholesterol diet for a year and were coming up for harvest for other purposes, and he offered to infuse Ad Lac Z or vehicle into their brachial arteries ten days before they were due to be harvested. Their cholesterols were around 440. And look with histology and immunostaining to see whether there was a difference caused by vector infusion.

What we found was that even in that short period of time it did promote lesion progression, vehicle infusion, Ad Lac Z. You can see there is a

bit more of a neo intima here and that it is highly enriched in macrophages. So similar to the rabbit studies where the combination of adenovirus and hypercholesterolemia seems to particularly accelerate macrophage accumulation.

(Slide.)

So the model of what is going on here is illustrated here and I am sure many people are familiar with the difficulties of adenovirus and certainly I would not credit myself with having formulated this model that is based on the work particularly of Jim Wilson and many others in the area. And that is that adenoviral vectors transduce cells, they can be vascular cells, pulmonary cells, liver cells but cells in any event.

Viral peptides are made from the opening reading frames. The transgene product is there also and can potentially be a neo antigen. These antigens are presented by MHC 1. In the meantime exposure of professional antigen presenting cells to those antigens causes them to present those antigens to the cells of the immune system, both T cells and B cells. Those cells home to the site of transgene expression and can extinguish transgene expression by killing off the transduced cells and causing inflammation at the same time.

So according to this model, local inflammation and loss of transgene expression are linked, and this model suggests many ways of intervening, eliminating the neo antigen by using species homologous proteins, gutting the vector viral proteins or decreasing expression by other means, whole cell immunosuppressive therapy that eliminates cytotoxic T cells, or more focused interventions that interfere with costimulatory signals or antigen

presentation, sequestration of MHC 1, for example, and that really brings us into the area of second generation and third generation vectors, which we have tried extensively in this rabbit model.

(Slide.)

Many of you are familiar with the second generation vectors, including temperature sensitive E2a mutation. We also used an E2 null virus provided by IntraGene of Holland with similar results to the E2a temperature sensitive vector. And, also, a vector expressing gp19k that might sequester MHC 1 or have other immunosuppressive effects, and compared these vectors to the first generation vector all expressing beta gal in our rabbit model.

(Slide.)

What we found was that neither of these first generation vectors prolonged transgene expression. So at three days this equivalent expression of the first and second generation vectors log scale -- two log drop by 14 days of both first and second generation vectors. So we asked ourselves the question can anything prolong transgene expression in this system and so we used high dose cyclophosphamide, sublethal doses for these rabbits and found that that did prolong transgene expression, although there was instead a one log drop instead of a two log drop. However, with this intervention we really completely ablated the immune response of the virus as is shown here.

(Slide.)

So we also asked does this second generation vector have decreased inflammation potentially because of less antigen synthesis or less

antigen presentation. So measuring a CD5, a T cell marker in rabbits, VCAM and ICAM expression inflammatory markers in the artery wall, we found that cyclophosphamide really completely eliminated VCAM expression and nearly completely eliminated T cells. One means there is a one T cell per section on the average so on the average there were no T cells per section or less than one.

No significant change in the second generation vector so similar inflammation with the second generation vector so you can suppress inflammation with whole cell immunosuppression but the second generation vectors were not effective here.

(Slide.)

The cyclophosphamide was effective in completely eliminating the antibody response to adenovirus. There is no antibodies -- no detectable antibodies in rabbits that have not seen adenovirus. Those that are infused with first generation virus and treated with cyclophosphamide similarly have no detectable antibodies. If you leave out the cyclophosphamide there is high antibody titers. And in the second generation vectors there is no difference also.

So it is interesting that completely eliminating the immune response in this case really -- it had some impact on prolongation of transgene expression but did not preserve it so it in many ways dissociates the immune response from persistence of transgene expression.

(Slide.)

More recently inspired by some very exciting reports from Marshall Horwitz on the function of the full E3 region, we made a vector that

expresses the full E3, which has not only gp19k but proteins that modulate TNF locally and compared vector with and without E3. E3 is so big that you can only get luciferase in there and not beta gal so we had to switch to that as a marker gene.

(Slide.)

We found similar to the other -- to the other second generation factors that there was no difference in expression at 14 days. Quite variable but the medians are really identical. So it did not prolong transgene expression.

(Slide.)

However, and this is, you know, after so many negative studies, this was really quite exciting, there was a very significant decrease in inflammation caused by this vector delivered at identical dose and with matched transgene expression as well. So looking at the E3 plus vector, CD5 T cells significantly decreased, VCAM really brought down to nearly undetectable levels at the median here. ICAM decreased substantially and the neo intima also was cut substantially. All these are highly significant p values.

This is very encouraging but again it dissociates inflammation from loss of transgene expression so the model needs to be rethought. These are really two separate processes and probably will need different solutions.

(Slide.)

These are representative arteries. You an see neo intimas here in the first generation vectors and really essentially none here and no staining in the E3 plus so we seem to be able to solve inflammation with E3 with

cyclophosphamide or by lowering the vector dose, although you do that at the expense of transgene expression but persistence remains a real challenge.

(Slide.)

So I will now go through some of the questions that were posed by the organizers in the context of choosing vectors. What are the important features to consider in selecting gene delivery systems for use in cardiovascular gene transfer research? Well, efficiency certainly. Duration of expression has been a hard nut to crack with adenovirus in the vasculature.

Potential for inclusion of regulatory elements. That -- in initial studies people have not really worried about that in vascular gene transfer but I think it is going to become more important later on.

Inflammation. There are ways around it but they are not perfect solutions.

Insertional mutagenesis, also something to be taken into account. It is certainly not a problem with adenovirus.

And preexisting immunity. The study that Dr. Simari cited. I believe actually it was a three log decrease in transgene expression and we had found a similar result that we reported in 1997 in vascular gene transfer in rats by preimmunizing them with adenovirus. We really rendered them untransducible by adenovirus.

(Slide.)

What aspects of the pathogenesis of cardiovascular disease should be considered in the selection and design of gene delivery systems? Well, for

vascular gene transfer we really need to keep in mind the inflammatory etiology of atherosclerosis and when we see data showing from Seppo in Finland that CRP is up in 70 percent of patients and we see reactive protein, and we also know that that is a marker for bad prognosis of atherosclerosis, that is cause for concern. So this is a specific disease aspect that needs to be taken into account.

Increasing inflammation in a vascular lesion and promoting macrophage accumulation is exactly the opposite of what many vascular therapies intend so that needs to be taken into account.

Potential for low efficiency in areas of advanced vascular disease presented by Dr. Simari in the last talk. There are necrotic acellular areas in vascular tissue and it is unrealistic to think that one is going to get high gene expression when there are not any living cells in particular parts of the artery wall.

Need for exposure of gene transfer vectors to the circulation. This problem of preexisting immunity will be particularly serious when vectors are infused in the bloodstream. If they are infused into the pleural space or into the middle of a necrotic tumor you can imagine that the immune system may have less access. However, with vascular gene transfer, by definition, the vector has to be in the vasculature. That is where the antibodies are. That is where the T cells are and the problems are going to result from that.

(Slide.)

For cardiac, I think we need to be aware of the potential for introduction of electrical heterogeneity, which could be the substrate for

arrhythmias. That is not a concern in other tissues or it may be a theoretical concern but it is not a concern in terms of provoking something lethal.

Another issue in cardiac therapeutics is the dissociation of symptom reduction and survival benefits that is familiar to many cardiologists. There are heart failure trials where drugs have actually made people feel better, although they died sooner, and many of our therapies for atherosclerosis for ischemic heart disease make people feel better but they do not make them live any longer. So we need to be aware of what it is we are actually accomplishing with these therapies. It is tempting to think we are going to get a survival benefit, which is what everybody wants, but many times it does not go with symptom reduction.

Both coronary artery and cardiac, we need to be aware of the high lethality associated with cardiac dysfunction. You can -- with an occluded artery or an arrhythmia that is a lethal event and perhaps some of the problems ought to be worked out in the peripheral circulation where you can come into the hospital with a white leg and walk out but you cannot come in with a white heart.

(Slide.)

What are the important factors to consider in selecting the route of administration? Is targeting possible, that is with peripheral delivery and a targetable vector, or is direct local delivery required? So an issue here is, for example, crossing the endothelium in a large artery with a peripheral injection. I do not think that can be done so if you want to deliver into the smooth muscle cells, a targetable vector is not going to get you there because it is just going to

stay in the blood and not cross the endothelium.

What controls distribution of the vector from the administration site? So once the vector is injected where does it go? Is it all absorbed to the cells? Is it cleared? Which direction is it cleared in? Is it cleared essentially through the lymphatics? Does it reenter the circulation? We need to know this. If it is infused into the epicardium, where does it go? If it is infused into the pericardium, if it is infused in the endocardium, does it distribute? We need to know what controls vector distribution in order to determine the route of administration. What controls the distribution of the gene product? Once the gene product is made and if it is secreted, is it cleared centrally? Does it accumulate locally? What does it bind to? That is going to determine our choice of routes of administration? Can we deliver into the coronary arteries? Do we need to inject directly into the heart? These are important issues.

(Slide.)

What are the critical questions to be resolved to optimize the safety of gene delivery systems for use in cardiovascular clinical trials?

It is pretty simple. There are only three of them.

What is the effect of the vector on local cell and organ biology? And our studies show that the effect of adenovirus on the vasculature is proinflammatory at least at the doses we used.

What is the effect of the transgene on local cell and organ biology? It starts to become very complex when you choose a transgene. A lot of the transgenes that are involved in the coagulation system, for example, have

separate roles in developmental biology, in smooth muscle cell migration, in arterial remodeling. When they have been knocked out they have caused lethality. What is going to happen when we over expression uro-kinase or TPA or tissue factor pathway inhibitor? Will it just affect coagulation or will it affect other cellular processes?

Is there systemic delivery of a vector or transgene product and, if so, what are the effects? Will VEGF cause neo vascularization in the retinas or tumor progression? That is the sort of question.

(Slide.)

So once again the issues are efficiency, duration, potential for inclusion of regulatory elements. It is going to be a problem in AAV because it has such a small cloning capacity although there now potential ways around that. Inflammation, insertional mutagenesis for integrating vectors, and preexisting immunity, which at least for adenoviruses is quite prevalent.

So thank you very much.

(Applause.)

DR. FRIEDMANN: We have left a few moments after each talk for questions from the audience. I might just -- if I could just start by asking David -- first of all, I think one of the really terribly important lessons that he just left us with is that one really ought to begin to think seriously about understanding vectors as agents, drug delivery agents, and one really ought to understand them in the same way that one understands small drugs. Where do they go?

I mean, it is an exercise in pharmacology and pharmacodynamics and pharmacokinetics. One has to understand these vectors. Where do they go? How do they get there and how do they recognize their sites?

But let me ask a quick question about the gutted vectors. Is there any experience yet in the gutted adenovectors and do they offer any additional promise that you are aware of?

DR. DICHEK: We have some data, although it is really too preliminary to present. I am not aware of other groups' results with gutted viruses. I believe that -- it is -- there is a controversy in the field in that I am sure many of you are familiar with Inder Verma's data where he suggests with the UV treated psoralen, UV treated virus, where he produced data suggesting that it was the protein inoculum that was sufficient to cause inflammation and the inflammatory response, and that would suggest that gutted viruses will not avoid these problems.

But then there are data from other groups that suggest that they will and we trust our work in the vasculature. It is at an early stage but I anticipate in the next several months we will have some more definitive answers about how well they work. We do not know yet.

DR. FRIEDMANN: Are there any other questions from the audience? If so, I would just ask for you to use the microphones set up in the auditorium. Questions or comments for Dr. Dichek?

Okay. Thank you very much.

Let's move on then to Dr. Barry Byrne, who is Associate Professor

of Pediatrics at the University of Florida, Gainesville, and a member of the Gene Therapy Division there.

Dr. Byrne is going to tell us about AAV vectors.

AAV VECTOR BIOLOGY AND APPROACHES TO CARDIOVASCULAR DISEASE

BARRY BYRNE, M.D., Ph.D.

DR. BYRNE: Well, thanks very much and I am glad to be here to share our experiences with this vector system with the group.

(Slide.)

AAV is relatively new on the front of gene therapy tools in the cardiovascular system. As has been mentioned previously, most of the experience has been with adenoviral vectors.

(Slide.)

This is an electron micrograph of AAV, which, shown here next to the larger adenovirus, is an unusual virus and I will just put this in context of the work that has been done in the cardiovascular area and particularly when we are talking about transduction of myocardial cells.

(Slide.)

This concept really goes back, as has been said, not to -- in the not too distant past, only ten years, and AAV kind of spans that arena first with the isolation of the virus in a cloned form in 1982 and this work historically has been conducted at the University of Florida. Nick Muzyczka and Jude Somulsky published this paper in PNAS. But even as recently as six years ago, Nick wrote

an editorial in the JCI that said, "Will AAV vectors work?"

(Slide.)

I will give you an overview of the properties of this virus because it is -- has some features which may make it very amenable to use in the cardiovascular system. First of all, it is based on a nonpathogenic virus. When one looks at the role of the virus in treating a disease, we may want to take advantage of the fact that the vectors follow a similar pattern and lifestyle and make use of latency and persistence of the vectors if there is a chronic disease that is being approached in the gene transfer study.

The viruses -- the vectors both transduce dividing and nondividing cells and much like a helper dependent Ad although the AAV vectors contain no viral genes.

(Slide.)

And what has been determined thus far in some elegant studies really that were conducted first in the adenovirus field by Jim Wilson and then his group also described the inability, relative inability of AAV to transduce antigen presenting cells, there is minimal -- both humoral and cellular immune response to these vectors and the particles are very stable.

I think one of the things that has been difficult in this field is that it has been very difficult to produce or manufacture these vectors and this has led to the use of viral stocks which may have limited potency or decreased physical stability. And certainly the doses have not been available to study in great detail.

(Slide.)

If you contrast them directly, I think it has been mentioned one of the major limitations of this vector system is that the native virus is only 4.7 kilobases and I am going to emphasize some of the aspects of the vector biology because I think that helps us understand how they may be used in this arena and to determine what the right tool is for the job that is the clinical problem.

Certainly adenovirus and the helper dependent Ads have a much greater capacity. The particle is very small, although unlike many other viruses, has an extreme physical stability and can tolerate heating and even organic solvents.

The virus by nature is part of the dependent virus group of parvoviruses and is defective for replication on its own. So this would enhance the safety profile because even a wild type AAV vector virus, which is present, requires adenovirus to replicate and hence the name.

This was first isolated from Ad infected children from the nasal and GI tracts. This has had been observed to have little clinical effect on its own other than the adenovirus syndrome and, in fact, it mildly attenuates adenovirus infection. And in some tissues there has been an observation that latency of the virus actually is inversely correlated with malignancy.

In contrast, adenovirus is known to cause inflammatory illnesses in its wild type form. Now vectors that are attenuated for these properties have helped them be deployed in the many disease applications that have been described.

(Slide.)

This can be shown -- the latency issue can be shown kind of schematically here, is that the wild type virus can either enter cells and stay integrated within a site specific in chromosome 19. Vectors do not share that property directly but they do -- they are integrating vectors. And a latently infected cell can be rescued when it is infected with adenovirus or herpes virus leading to a productive infection and then two types of particles are released from that process. And this is also a schema which needs to be followed in the production of vectors and these tools -- new tools have helped accomplish that.

(Slide.)

The replication and integration of the virus is rather complicated but I just use this slide to remind us that the single stranded genome, which is within the capsid is not immediately expressed on entry into the cell and that ultimately through head-tail concaterimization (?) of the vectors there may be episomal persistence of these genomes and ultimately through a unique rolling circle pathway these can lead to head-tail integrations in the genome.

So, in fact, one virus may be multimerized at a given site and this may influence the ability of integrated viral genomes to persistently express the transgene.

(Slide.)

So this is the wild type virus in which there are two elements of 145 base pairs known as the inverted terminal repeats. They are the only cis elements required for viral replication and packaging as well as integration. One can remove all of the material between the terminal repeats and place a control

element and a gene and then utilizing a complementing DNA, which expresses those wild type genes, one can then encapsidate this vector plasmid into an intact vector.

(Slide.)

So how might these be applied in cardiac gene therapy? I think these are some principles that are worth covering because it gets back to the point of the pathology being treated. If one has a disease target, it is important that the molecular pathology be understood. Otherwise we would not be taking a gene therapy approach. Whether one is replacing a gene and augmenting gene expression versus gene correction has an effect on the potential success of the approach.

Secreted products versus cell specific correct, that certainly influences the ability to achieve a success with this approach and then having a predictive animal model would help us evaluate this in a preclinical setting.

(Slide.)

So just to give you a few bits of data about how AAV works in myocardium, certainly there has been considerable experience since the mid '90s by our group and others with the use in other striative muscle, in the skeletal muscle, and this is some of our data in heart muscle where we are actually looking at a transplant setting where virus infused into the coronary circulation or at high dose and recirculated into the myocardium can give very extensive transduction. So the virus actually can be quite efficient at transducing myocardium and similar pictures have been obtained by direct interstitial delivery

of vector in skeletal muscle as well.

(Slide.)

In the vasculature we think that there also is potential for this vector in imaging microvasculature transduced with a GFP virus. We can see on end here, on edge, transduction of endothelial cells and lower down in the image one can see the smooth muscle cells also transduced by AAV GFP.

(Slide.)

So the utility of them seems to be there but the challenges are understanding how to apply them because the -- as I said, the vector biology is quite different than adenovirus. Because the genome is single stranded, the onset of gene expression is not immediate and, in fact, in skeletal muscle the peak of gene expression is at about six weeks post-delivery. In myocardium it seems to be very similar. This may be influenced by dose but that brings me to this slide.

The dose quantification is an important measure that we have addressed in the field because there are many ways of preparing this virus because of its unique biology and so I thought I would take a minute to emphasize how one analyzes the dose because this is after all what controls how we enter with this into the clinic.

One can either assess the physical titer and, in fact, probably best that all these things be done to obtain a complete profile of the virus. Physical titer is assessed by an analysis of the number of genomes that are DNAse resistant. This can be quantified by a quantitative competitive PCR, a slot blot assay or most recently we have utilized TaqMAN analysis very effectively for

this titer.

A functional titer, identity test, as sometimes known in the FDA, would look at the particular transgene being expressed and we have, in fact, chosen GFP expression to be a gold standard, which I will discuss in the next slide as having a bench mark to measure against.

Infectious titer is a very important parameter which in the older literature had not been thoroughly addressed because it is a difficult study to do but one of the ways to assess the infectious nature of the virus if there is not a markable transgene included is to use a replication center assay which emphasizes that the virus infected into cells where rep is expressed will replicate. And those replicating genomes can be scored on a per cell basis.

Certainly the other concern with analyzing infection is the content of replication competent AAV since the wild type virus can exist latent in stocks of cell lines or other reagents it is important to quantify this in the final outcome of the vectors.

(Slide.)

So the quantification of the total particle number and the infectious particle number lead to very important assessment of what the particle to infectivity ratio is, this is essentially an assessment of potency of the virus and again there is tremendous variability on that parameter, depending on how the virus is prepared.

So we also have assessed the purity of these stocks by SDS-PAGE. This had been difficult in the past because one would not sacrifice a whole

preparation of material for this analysis and now the quantity of material produced is sufficient to analyze this and we like to look for the physical characteristics of the capsid because empty and full capsids may behave differently in vivo.

And a lot of these evaluations have led the field to meet as a group over the past year-and-a-half and propose to the NGVL that a reference standard be created and so our group at Florida received funding from NCRR and the NGVL to produce a reference standard which will be accompanied by a set of protocols that establish these parameters and sent out to the ATCC for any laboratory to order and evaluate their own studies.

(Slide.)

So to get back to the clinical application, the studies that had been proposed, again another collaboration with the FDA has been to examine at least in the skeletal muscle platform, and this is very applicable to cardiac muscle as well, what are the vector specific toxicities and whether these can be generalized into platform studies where the transgene is not so important but the vector in the context of a muscle cell is important and so the standard approach has been to evaluate biodistribution, and we have done this now for several different vectors being delivered to muscle to build a drug master file which could be used by multiple groups heading in the same direction but with a different transgene.

It has been important to evaluate the dose related and direct vector toxicity, and this has been found to be minimal with these vectors, interestingly enough, because the capsid structure is quite simple and there are no viral genes.

So we have not observed any -- at the maximum dose we could even deliver -- any dose related vector toxicity.

And the harder questions to assess are going to be the risk of insertional mutagenesis because this is an integrating virus. In some cells there is mostly episomal persistence but in striated muscle the virus inserts into the genome and as a vector this happens in a random fashion. We will need to approach this with a large study looking at the potential for carcinogenesis and tumorigenesis.

The germ line question is a little easier to answer. This has been addressed by the study that Kathy High is doing at the University of Pennsylvania where AAV Factor IX is being expressed from a depot of skeletal muscle, and this has been reasonably reassuring that there is not transmission to the germ line with this vector.

(Slide.)

And the greatest amount of data thus far in patients treated has been gathered by Terry Flawed in our group and his colleagues at Targeted Genetics and Stanford University, and this is the data from ten patients that were treated with AAV in the maxillary sinus in a dose escalation study. And, in fact, they really found no lymphocytes accumulating in the site of administration, very low levels of IL-8 across all the subjects. And interestingly even though 80 percent of the population is seropositive for AAV and all of these patients had an antibody, a low antibody titer to AAV, there was not any increase, substantial increase, except in this one patient.

So it appears from some other studies that humoral immunity to the virus for serotype II can be neutralizing but this can be overcome with dose so a little different again than the adenovectors because those humoral antibodies tend to be completely limiting of repeat administration.

(Slide.)

So as was mentioned before, these are lengthy processes, and I just thought I would close by seeing how one might develop an AAV based product for cardiovascular gene transfer. As I mentioned, a predictive animal model is important in any specific disease one is addressing.

We focused a lot in this space on this problem because when one enters into preclinical toxicology studies, it is important to have identified the method by which you are going to produce vectors in sufficient quantity to treat patients. So this is a rather lengthy process and we have spent really the last two years addressing this issue.

And then one can proceed with those toxicology studies as we are trying to do in a collaborative fashion to make this data available to the community for others that are going to pursue this approach of treatment of both cardiac and other muscular diseases, and then enter into one study.

So I will stop at that point and take questions if there are any.

(Applause.)

DR. FRIEDMANN: Questions or comments for Dr. Byrne?

DR. RUSSELL: Steve Russell, Mayo Clinic.

I was just interested to know which vascular structures AAV can

actually transduce? I mean, it is one of the concerns about the virus is that there are certain human cell types that it is very efficient on, for example skeletal muscle and others that there is a very low efficiency, for example, early progenitors in the bone marrow.

And you have showed us data here of transduction of cardiac myocytes but I am just wondering if you have any data on intima, media, adventitia, endothelial cells?

DR. BYRNE: Sure. I did not go into great detail on the attachment and entry of the virus but there are two principle viral receptors. One is heparin sulfate proteoglycan, which seems to be present in reasonable abundance on most cells, although its presence on bone marrow progenitors may be one of the variable factors from donor to donor that has been observed in that field and then there are two co-receptors, FGF and the alpha-V beta 5 integrin.

These seem to be present in pretty high abundance in the vasculature and, in fact, the second slide I showed was microvasculature that had been transduced with the vector and demonstrating both smooth muscle and endothelial transduction. And in conducting arteries we see in uninjured or injured vessels, we see a lot of transduction of the adventitia, and this may be due to the small size of the particle and its ability to get out of the vessel but I think most of the tissues that we have examined are transducible by this serotype.

Now I will also mention that there are several other serotypes of AAV which may have a preference for other compartments of the vasculature and that is being evaluated now by many groups.

DR. ENGLER: Engler, San Diego.

Are there important differences in efficiency, insertion, transcription and persistence between dividing and nondividing cells?

DR. BYRNE: Yes. I think that has at least, in one example, that we have in our lab evaluated myoblasts that are either dividing or exiting the cell cycle in differentiation and it appears that the attachment and entry is the same but the transcriptional activity seems to be different in the terminally differentiated cells such that we saw much greater expression of a marker transgene like EPO.

And the virus does seem to have a favoritism towards terminally differentiated cells. It works extremely well in the brain, the retina and in striated muscle and the transduction of hepatocytes also seems to be fairly efficient.

DR. AGUILAR-CORDOVA: Estuardo Aguilar from Harvard.

If there are such differences in tissue target specificity between the various serotypes, would the standard that you have produced and the platform studies that you have proposed, would those be limited only to that serotype or would you think that then each one of those things needs to be done for each serotype?

DR. BYRNE: Right. I think each one would need to be done through each capsid code. Now there are different ways in which these serotypes can be produced.

One can mix and match the terminal repeats from the given

genome with a different serotype so there are potentially many different versions of these viruses that could be created.

I think with what little we know now about these other serotypes it will be a while before there is enough call for those as a reference standard. Right now we have only seen three clinical studies of AAV Type 2 and I think there will be more with Type 2 because there is experience with that but as we move towards using the other serotypes that can be certainly addressed.

DR. AGUILAR-CORDOVA: You mentioned also the presence and the potential variability of ratios between empty particles and capsids containing DNA. Two questions related to that. Is that a fairly standard ratio and, secondly, the ones that do contain DNA, is there good information as to what percentage of those actually contain the viral DNA or the vector DNA?

DR. BYRNE: As -- by viral DNA you mean DNA from rcAAV or other DNA?

DR. AGUILAR-CORDOVA: DNA from what you expect the DNA to be versus just stuffer DNA?

DR. BYRNE: I do not think anyone has examined the ratio of other DNAs that might be incorporated into vector. Certainly when we look for the genomes either by infectious center assay or by slot blotting, we only find the DNA that was contained between the TRs because that is the substrate for packaging.

The ratio of empty to fully capsids has been something that we have started to examine now. There are probably over 300 consecutive

preparations. We think that that ratio is about one in five and so we will continue to kind of look at that.

DR. FRIEDMANN: That is a very important issue that has arisen here that we have lost -- that we had not had sight of until in the last few -- couple of years, that is that what we call a virus preparation is, in fact, a mix. It is quite a soup of different kinds of particles, again different from other kinds of pharmacological agents.

What we are delivering really is a mixture of many different kinds of particles, some infectious, some not infectious, a lot of debris and a lot of other material.

Let me just ask one quick question. Are we to kiss good-bye forever the idea of specificity of integration of AAV vectors?

DR. BYRNE: No, not necessarily. In order to accomplish that -- take advantage of that aspect of life cycle it is necessary to apply some amount of the rep protein in trans with the virus. That could theoretically be done as an adjunctive protein delivery. There have been some recent reports of how it can be supplied through a crelox recombination system.

DR. FRIEDMANN: In the absence of further engineering.

DR. BYRNE: If the fully rep deleted vectors appear to integrate randomly.

DR. FRIEDMANN: Okay. Any further questions?

All right. Let's move on then to the last of this series of talks. Dr.

Alexander Kuta, who is Vice-President for Regulatory Affairs at Genzyme is going to come back to the adeno system.

Dr. Kuta?

Can we have one of the audio-visual people for some help, please?

We are a few minutes late and this might take a couple of minutes to set up so why don't we, in fact, have our break now. It is scheduled for now. And reconvene, let's say, at twenty-five after 10:00. It gives us twelve-and-a-half minutes.

(Whereupon, at 10:17 a.m., a break was taken.)

DR. FRIEDMANN: Okay. Let's proceed then.

Dr. Alexander Kuta, again from Genzyme Corporation, Vice-President for Regulatory Affairs there, and I think he is going to summarize again some of the issues related to the adenovirus system.

Dr. Kuta?

USE OF ADENOVIRAL VECTORS

IN CARDIOVASCULAR DISEASES

ALEXANDER KUTA, Ph.D.

DR. KUTA: Thank you.

(Slide.)

Actually I would like to thank OBA for asking me to speak today.

What I would like to do is show you some of the topics I am going to speak about today.

(Slide.)

Product development; the adenoviral vector platform; use of adenoviral vectors in cardiovascular gene transfer; and then also address assessment of risk versus benefit in product development.

On the real sort of themes that I want to hit on today is development. We presented a lot of these data in detail at the September RAC meeting so I am not going to go into a lot of data review, although I will talk about some of it and how it influenced our choice of adenoviral vectors for cardiovascular gene therapy.

But my goal is really to run you through our thought process as to how we chose this and I promise not to give you too much sort of old time regulatory stuff in this talk as well.

The development effort for the use of adenoviral vectors for our cardiovascular disease trial and for our peripheral vascular disease trial really began in May of 1998 in efforts to get us into clinical studies and then designing those studies. The proof of concept development actually began in about 1996 so about two years before that. The peripheral vascular disease trial began in October of '99 and the cardiovascular coronary artery disease trial began in November of this year.

(Slide.)

So what I would like to do a little bit briefly is go through some of the general outlines of drug development. So looking at issues like your CMC. And again these things are outlined in detail in the CFR. One of the reasons I bring this up is because we are talking a lot about research, we are talking a lot

about clinical research, and at the end of the day what we are really talking about, I think, is drug development, okay, and how to go about thinking about that because that then leads you to some of the questions and how to think about the questions and perhaps answer those questions and make a choice on what questions are relevant for getting into the clinic versus those that can be addressed while you are in the clinic.

(Slide.)

So obviously this process is familiar to everyone in this room. Okay. Looking at characterization of your vector, looking at developing a manufacturing process that is capable of delivering a consistent product that you have already identified and characterized, defining attributes of product quality so that you know that you are delivering a consistent product as you move through clinical development.

As far as executing the clinical studies, obviously in early studies you are looking at safety evaluations and so when the discussion comes up about risk/benefit sometimes the issue that is raised is, well, there may be potentially be no benefit at this point because it is a safety study, although those are the studies that really formulate the foundation for then moving on into studies that are well-designed to clearly demonstrate efficacy.

So really the goal is not generating enough data to get into the clinic but rather --

(Slide.)

How did that happen -- not generating enough data to get into the

clinic but what are you going to be able to get out of the clinic?

So gene transfer development, the development as I mentioned earlier is basically drug development. So while vector classes are going to have their individual issues associated with them, regardless of what they are, okay, some of the fundamentals apply to all of those.

A year ago there was the Ad safety and toxicity working group that was asked to comment on issues associated with adenoviral gene therapy. And to briefly summarize this, and this is in the minutes of that meeting, but to briefly summarize this, they talked about characterization, they talked about defining preclinical models of safety and efficacy, defining attributes of product quality to ensure safety and consistency. Again those things that go back to the previous slide talking about development and talking about working to understand what your product is, what your therapeutic is.

I think what is unique about gene therapy at this point is that there are many individual investigators running trials and that also brings a lot of excitement into the field and I think adds for certainly a different perspective than perhaps in the development of other therapeutics.

What I would say is that all these recommendations again that came out of this Ad working group really are applicable to all viral -- all vector classes and nongene therapy therapeutics.

(Slide.)

So looking at the decision making process, obviously it is data that drives the decision process. We have talked a lot about that this morning. The

previous speakers went into some extensive data. And I think the important point is how do you use those data to assess risk versus benefit. As Dr. Zoon alluded to, there is no risk free therapy.

And again the development process does not stop once you have gotten into the clinic. Again the goal is how do you maximize the information you are going to get out of the clinic. As far as beliefs go, all the opinions are great, the discussion is great, but they still have to be backed by data, and I know that everybody here knows that. I am not telling you anything new and so this is where I do not want to get too preachy.

But the literature and all the data that exists as different pieces, that is really your guide. Okay. Because at the end of the day, the data that support you moving safely into clinical studies that are of value is really the data that are generated on your product.

(Slide.)

So then the question comes up how much is enough? Okay. And I guess part of the answer to that is what is meaningful? What do you need to know? Because there is always going to be another question and again preclinical data really indicate what may be. There is nothing definitive about preclinical data per se. They are predictive. You still end up needing to explore all these things in the clinic.

So you need to determine what is nice to know versus what do you need to know and work to establish a meaningful preclinical safety and efficacy profile.

(Slide.)

So when these issues come up there is always the question of balancing. How much do I need to know and, well, I do not have the resources to answer all those questions. Okay. Last month at the Biological Response Modifiers Committee meeting the discussion came up about sequencing of vectors. And one of the comments was, well, there is no way that a lot of investigators or small companies could ever afford to do that.

Regardless of what the issue is, really what that means is that your planning of your preclinical studies and planning of how you are going to generate the data and the questions you are going to ask become very critical at that point because obviously limited data or limited resources, excuse me, do not -- are not an excuse for a lack of data.

So again it is balancing the level of detail versus your ultimate goal which is really providing a useful therapy. It is not just getting to the clinic.

And I believe I have already addressed risk versus benefit, I think, enough.

(Slide.)

So to maybe reiterate some of the issues that Dr. Dichek raised earlier today, what is the ideal vector for gene therapy. Obviously it would not have the associated toxicities. It would be nonimmunogenic so you could give it on a repeat dose basis. There would be efficient gene transfer. Your duration of expression would be -- you would have an extended duration of expression if that, in fact, is a desirable attribute for your vector in the therapy you want -- in

the disease you want to have an impact on.

It would have high levels of expression and ideally you would be able to regulate those levels of expression. Again, readministration would be possible. Readministration would be possible. Targeting, and again the ability to manufacture this at a scale and at a purity that allows you to go into the clinic efficiently.

(Slide.)

So perfection is a goal but it is not necessarily a reality because at the end of the day your vectors are just tools and I think it is pretty safe to say that there are no perfect vectors suitable for all applications.

So -- but having said that it may not be critical to have a perfect vector to have a significant impact on the practice of medicine.

So again the level of risk needs to be appropriate for the disease you are trying to impact, which also means that you have to have a clear understanding of what the characteristics of your vector are and when they are useful and when they would not be useful.

(Slide.)

So when is an adenoviral vector -- when is it suitable to use? When it is the best tool for the job. So you have to consider -- for example, we heard about how adenoviruses were not suitable for intra-arterial injection this morning. That may -- that may be the case. That does not mean that they would not necessarily be useful in other applications.

(Slide.)

So when would you use adenoviral vectors? Well, certainly where local delivery is efficient and where you can maximize safety.

When the risk/benefit ratio is appropriate, understanding again that none of this is risk-free.

Where there is a medical need and the severity of the disease warrants its use and current treatments have questionable efficacy or there may not be current treatments.

Where your preclinical studies, safety and efficacy, allow you to predict what your dose and what your dose schedule would be and what toxicities to look for.

(Slide.)

So short-term expression is -- excuse me. Short-term expression -- when short-term expression is adequate and desirable, adenovirus is a suitable candidate. Adenovirus would not be suitable for chronic treatments, for example, in genetic diseases.

So what would suitable targets be? Well, that would -- we would propose be cancer gene therapy but also cardiovascular gene therapy.

(Slide.)

So current adenovirus vectors are really tools. So what are their positive attributes? We know about their genetics. The genetics are well-defined. They are efficient in delivering genes to a target tissue. They have a large carrying capacity. They can be produced at large scale to high purity and they can be well-characterized.

And we do have and can generate additional data on understanding the preclinical toxicity in a preclinical therapeutic window. And there is a significant human experience with these vectors. Negative attributes obviously are immunogenecity, the acute toxicity related to that, and duration of expression, although that may be application dependent. Short duration of expression may not be a bad thing if, in fact, that is appropriate for the disease that you are trying to impact.

(Slide.)

So things we need to improve: The toxicity, the immunogenecity and perhaps the duration of expression.

(Slide.)

Now what we are exploring in peripheral vascular disease and coronary artery disease is Ad 2/HIF-1 alpha VP16, which I will just end up calling HIF-1 alpha from now on. But the vector is a recombinant -- it is a recombinant replication deficient adenoviral vector that is based on the Ad2 serotype. It is a second generation vector that is E1 and E4 deleted. It contains the open reading frame 6 and protein 9. It is controlled by the CMV promoter, the human CMV promoter, and the simian virus 40 poly-A signal, and is produced in 293 cells.

(Slide.)

Now the transgene, HIF-1 alpha, induces a pleiotropic angiogenic effect and it potentiates adaptation to hypoxia by regulating gene expression in response to changes in oxygen tension. So you are up regulating things like

INOS, VEGF, VEGF receptors, glycolytic enzymes. I think really it sort of uses the natural response to hypoxia to stimulate angiogenesis.

It is hybridized with VP16 of herpes simplex virus which results in sustained activation so that it is expressed in normoxic tissue.

(Slide.)

So some of the characteristics of other vectors that guided us to choose adeno: We looked at plasmid and in our hands it had a lower efficiency. Anywhere from 10 to perhaps 100-fold less than adenovirus. That is not to say it may not be efficient enough to get the appropriate response but we felt in order to maximize our chances in seeing that response we would pursue adeno.

AAV we felt had inappropriate expression kinetics. It is initially delayed and then stabilizes.

Retrovirus we had -- there is no evidence of efficient gene transfer. The cells are not mitotically active.

And again adenovirus affords efficient delivery, appropriate kinetics, ability -- there is the ability to determine a safety and therapeutic window, and there is a broad clinical experience.

(Slide.)

What I would like to do is just run through some of our preclinical efficacy studies.

Covering both the studies that supported the peripheral vascular disease protocol as well as the studies that supported the coronary artery disease protocol because they are really interdependent when you are looking at the

effects of the transgene.

We looked at the rabbit hind limb ischemic model and induction of angiogenesis and also looked at determination of an optimal volume that we were able to inject into the muscle.

We also looked at the Yorkshire pig amaroid pig model and performed a pilot study to look at transgene expression as well as the pilot study to look at what lesions to identify in further studies. And then we performed a bioactivity study following myocardial injection and also looked at safety parameters.

(Slide.)

What I am going to do is give you a brief overview of that pig study.

An amaroid constrictor was placed on the left circumflex artery and then after three weeks when the amaroid constrictor was allowed to swell and actually constrict the artery the animals were treated with ten injections of 100 microliters each of the vector. So the dose was spread out through a wider area of the heart.

And then the animals were sacrificed after four weeks and analyzed.

(Slide.)

Now these are measures of efficacy of angiogenesis ranging from what are generally considered the least effective, the coronary angiography, to probably the most sensitive or what is often referred to as the gold standard, the

labeled microspheres.

(Slide.)

And here you can see that the vector at 10^8, 10^9 and 10^{10} gave you a significant angiogenic response and gave you maximal blood flow in the ischemic zone when compared to vehicle control, the plasmid that expressed HIF-1 alpha VP16 or to the empty Ad vector.

(Slide.)

The other thing we looked at was the ability of the material of the vector to potentiate tumor cell growth because by stimulating angiogenesis you may stimulate tumor cell growth in well vascularized tumors.

So we used the human neuroblastoma cell model in nude rats, injected vector in control intra-myocardially as well as intra-muscularly. And we used VEGF as a positive control. I think the thing to bear in mind here is that the system was designed so that VEGF would be the positive control. And tumor volume was then monitored over a three-week period and we found that Ad2 VP16 -- Ad2 HIF-1 alpha VP16 -- did not potentiate tumor cell growth.

(Slide.)

Just to run through some of the other studies that we performed. Actually -- okay.

Biodistribution studies. There was a 28 day study of myocardial transgene expression following an intra-myocardial infection. This is for the coronary artery disease indication.

By day 28 the vector transgene was expressed at approximately a

log less than it was at peak. There was a 60 day study of myocardial expression and systemic distribution following intra-myocardial injection. Most of the vector DNA was deposited at the transgene -- transgene expression was at the injection site.

At 60 days there was a lower level of DNA in transgene expression at the injection site and systemic distribution was observed with transient low level vector expression only in the liver and the lung and there were no drug -- sorry about that. There were no HIF-1 alpha related toxicities detected.

(Slide.)

There was also a 120 day study of the myocardial transgene expression following the intra-myocardial injection. Again vector DNA levels decreased over time.

This is really kind of irritating.

(Slide.)

Decreased over time. There was no vector related toxicity by clinical pathology and then the systemic distribution of transgene expression following an intra-arterial administration, sort of a worse case if you will, in which case vector DNA was detected in almost all organs sampled on day two with a low level of expression only in the spleen and the liver. By day 14 expression was only in the spleen and no expression detected on day 28.

(Slide.)

There was a 60 day study performed. This is now for the peripheral vascular disease study. It was a 60 day study of skeletal muscle

transgene expression following an IM injection and most of the vector was deposited at the transgene site.

(Slide.)

I have to apologize for the technical difficulties here.

At the transgene site.

Let's see.

(Slide.)

Yes, it is, indeed. Okay.

So 60 days following the intra-muscular injection the systemic distribution was limited to the spleen and transgene expression was not detected in the spleen beyond day 30 so the expression was transient.

(Slide.)

So, in summary, again the intra-arterial study. This study was used to support both studies so again I have already discussed this.

So, in summary, the development of gene transfer based therapeutics really is drug development and I think it needs to be considered that. There needs to be a balance between the level of detail and the ultimate goal of providing a useful therapy.

There is always going to be another question and so it is going to be critical to determine what is nice to know versus what you need to know to give you enough data and the appropriate data to advance into the clinic and do that carefully.

The perfect vector does not really exist and it is not necessary

probably to have -- a perfect vector is not necessarily to necessarily have a meaningful impact on the practice of medicine.

Adenovirus based vectors are useful when the local toxicity is acceptable, when local delivery and short-term expression are desirable, and in which case I think the example for today was coronary artery disease.

(Slide.)

What I would like to do is thank the people on the team who helped me and endured my sort of tedious questions and provided me with information.

Thank you.

(Applause.)

QUESTIONS FROM THE AUDIENCE

DR. FRIEDMANN: Let me invite the audience to ask any questions or comments to the talk by Dr. Kuta. Any issues that people want to raise?

One of the issues that came up during the break, of course, is that there are many vector systems that have not been included and are lurking behind the scenes. Maybe to appear very soon, the lentivirus system is obviously going to be interesting and useful. Other systems, even papova viruses are making their reemergence and synthetic complexes will also be used in the cardiovascular system.

So this discussion of vectors is not in any way meant to be exclusive. We have tried to illustrate issues that are related to the selection of

vectors which are relevant not only to the systems we have discussed this morning but others.

So any questions or comments?

DR. MARKERT: Louise Markert at Duke.

Do any of you have data in an animal model about effects of systemic growth factors on eye vasculature, and I am looking forward to the comments by the investigator here from the Eye Institute, if there are systemic growth factors? And is there any animal model that would be similar to diabetic retinopathy? And then, of course, I will be very interested later today if there is any data from autopsies in patients.

DR. CSAKY: My name is Karl Csaky. I am from the Eye Institute. So the question, I guess, relates to potential for systemic growth factor administration to affect the eye vasculature and we will get into this, I think, more in the discussion, panel discussion, but just briefly I think one of the problems is going to be that there are very poor models of eye neo vascularization in animals.

For diabetes, for instance, there really is no diabetes induced retinal neo vascularization model in animals. Most investigators use an oxygen deprived neonatal mouse model as a surrogate but there are very poor models.

In terms of other complications there is subretinal neo vascularization which can occur in patients in age related macular degeneration and those patients obviously also are affected by cardiovascular disease and may be candidates.

We have published recently a paper where we used an adenovirus vector system with VEGF 165 and was able to induce subretinal neo vascularization with fairly low doses.

So I think there are some systems available to screen systemic applications.

DR. ISNER: Was that by local administration of the VEGF?

DR. CSAKY: Yes, absolutely. That was a local administration at the site where we think the VEGF is being produced in the disease state.

DR. O'REILLY: Can I make a comment?

The data actually may be out there, if you just think about it, that if the theory is correct that giving an exogenous angiogenesis stimulator is going to worsen angiogenesis in the eye then a tumor that is producing excess angiogenic factor that is getting in the circulation should do it as well. I do not know if any studies have been done but it would be relatively straight forward to look at levels of vascular endothelial growth factor or FGF in the urine and see if they are high. And then in patients that have preexisting neo vascularization of the eye, does it get worse. I do not know if anyone has done that study but I do not think -- or if there is any anecdotal data to support it but that would seem to be -- to me to be the most straight forward way to address that question or at least a straight forward way to address the question.

DR. FRIEDMANN: Yes?

DR. RUSSELL: Yes, this is a question for Dr. Kuta on the gene therapeutic as a drug. It seems that the vector is not really the drug. It is a

prodrug and it is being converted in the body and inside the cells of the real drug, which is the therapeutic protein. So when you administer this gene therapy agent it is the number of cells transduced and their distribution is fairly erratic and differs between different patients.

And you do not really have a direct handle usually on how much protein is being produced by how many cells and for how long.

So I am just wondering how in the development process can you deal with this issue of how you will actually provide this drug to the patient at the end of the day as a specific dose of agent that will somehow reliably produce a therapeutic effect.

DR. KUTA: I think your preclinical studies are key in developing preclinical efficacy models so that you can try and understand what your -- what the efficiency of your delivery is and what the rate of gene expression is and are you getting the response that you predict.

I think in the case of our studies here we are looking at very local effects and local effects are really what are desirable in this case.

DR. RUSSELL: Right. But, I mean, do you not think there is a need for some mechanism built into the vectors to allow you to actually know after you have administered the patient the drug how much drug they actually got, i.e. how many cells, what the distribution is? It is a transgene monitoring question, I guess.

I mean, do you think that is a necessary component of the final product or do you think you can make the leap from these preclinical studies and

clinical studies to say, okay, if you get this dose it is going to work?

DR. KUTA: I do not know how you would necessarily do that in the patient unless you were able to look at systemic levels of whatever the gene product was. So I think in some cases your preclinical studies are the best thing that you had to try and do that.

DR. RUSSELL: But are you indicating that you would like to know the concentration of the protein expressed from that particular gene and you are saying in the same way that we would think of pharmacology today that you would want to have some kind of levels to be a criteria in terms of its efficacy? I mean, you are saying you do not want to measure the protein or do you think that that is a feasible way to go?

DR. KUTA: I do not know if it is always feasible. When you are talking about a small molecule it is everywhere. When you are talking about delivering a gene you are talking about, you know, trying to target a particular tissue. If you do that and you are looking at a local response and you want a local effect, I think that is difficult to do.

Now if you are looking for an overall effect and you are looking at the transduced tissue as a depot, well, then, yes, that is a different issue. That is a different application. So if you are looking, for example, for -- looking at gene therapy for -- as an enzyme replacement therapy or a hormone therapy then those are issues that are clearly important.

DR. RUSSELL: Yes, I mean, I agree it is easy where you have some transgene product that is secreted into the circulation and you can measure

it but in many of the transgene products the cell is associated or retained within tissues. So what I am driving at is there is clearly a need to know how many cells are actually producing the transgene product. What is their distribution and what is the appropriate timing of the repeat dose and was is the first dose sufficient?

And it seems to me there is a real lack of technologies available at the moment for monitoring both the distribution of transduced cells by some kind of direct imaging and, secondly, the total quantity of protein being produced by those cells collectively.

DR. KUTA: But is it the number of cells or is it the level of the protein that you are really interested in?

DR. RUSSELL: Ideally you want both.

DR. KUTA: Right. But what you probably are more able to get is the level of protein and you want to -- you understand those effects or the effects of that protein. So it may not tell you exactly how many cells but there should be something in your system that you can measure that would give you an idea of what those levels are.

DR. RUSSELL: I think, probably. Yes.

DR. FRIEDMANN: I would like to just comment on that point. I think this relates to the issue in general of pharmacokinetics and pharmacodynamics. I think again depending on what the goal of your treatment is, if the goal of your treatment in angiogenesis is to improve myocardial blood flow and ultimately myocardial function, if you can demonstrate that you have achieved your pharmacodynamic effect and observed duration of action via

pharmacodynamics, you may not necessarily need the pharmacokinetics in detail to answer your question.

For example, I believe with the antihypertensive drugs, blood levels do not correlate at all with efficacy of those drugs so that again pharmacodynamics and pharmacokinetics -- that you may not need to assess the kinetics to show you have efficacy.

DR. ROBERTS: Yes, one last question here.

DR. BREAKEFIELD: Yes. As a member of the RAC I guess --

DR. ROBERTS: Can you please identify yourself?

DR. BREAKEFIELD: Xandra Breakefield.

As a member of the RAC I was just trying to hope from this audience -- I mean, from this panel, and maybe we cannot do it all now, is just to get some advice particularly related to use of adenovirus vectors for cardiovascular uses. I think those plots that I saw kind of, of enthusiasm versus, you know, almost despair over the use of gene therapy for cardiovascular research almost is basically a parallel of adenovirus. I think that initially there was great confidence and now we have realized there are all kinds of complications.

I guess the question -- and we have protocols that are sent to us now that basically were proved in the mid '90s but now we have a very different view. Now we agree that they are inflammatory and we agree that they are cytotoxic.

So what kind of guidelines when we see a protocol should we use

in terms of things like dose? We know that animals do not -- you know, are not infectable with adenoviruses the same way that humans are. So to what extent can we look at these preclinical studies in other species and say that, gee, it is not toxic or whatever?

And, you know, I was very impressed with the E3, putting it back in now to decrease inflammation. Is that an issue that we should look at? So how are we going to cull out now from these adenovirus cardiovascular protocols the ones that people would consider to be -- and also, I guess, how sick does the person have to be? And it is a lot of questions here but any insights would be appreciated.

DR. ROBERTS: Well, I think that although we were to go on to the panel, I think that is a very crucial question because one of the things -- although we have not listed it specifically this morning -- is what animal model is appropriate. We have heard from the rat, the rabbit and the pig, and I think that in one of the earlier presentations that the amount of information induced by the vector in terms of atherosclerosis was the same or certainly qualitatively the same in the rat as it was in the rabbit.

But I would like to ask some of our -- or any of our speakers this morning what sort of lessons or anything do we know in response to part of your question is one of you certainly pointed out that it is important that we had a predictive model. I think everybody would agree with that.

And have we learned any lessons in selecting which animal model is to give us more appropriate information because I think clearly one of the

things that is bothering all of us is the inflammatory response and granted there are other problems but the inflammatory response. And knowing that the immune system, although it is pretty similarly evolved across mammals that clearly there are significant differences, and so I think that that is one of the, I think, fundamentally unanswered issues here because most of your data in the experimental is going to come from animals that we do not know, I think, enough about that.

And so who would like to take on that one? Go ahead.

DR. NEMEROW: I think the issue of biodistribution really speaks to that. It is not just where the transgene is being expressed but where is the virus going. And that is a very complex question because the issue of receptor distribution is not always predictable from a mouse model to rabbit to human or nonhuman primates.

So, for instance, we learned in the liver that in the mouse, CAR, the primary receptor for adenovirus, is expressed at a reasonably high level and adenovirus delivery into the liver in the mouse is fairly predictable. But in humans that might not -- does not appear to be the case. There is lower levels of expression in the liver and so the virus does not end up in the cell type to which you are expecting it to go. So that is a major issue.

In the mouse, the integrin, which is a coreceptor for adenovirus, we do not have good reagents to measure alpha V integrin expression in the mouse so using the mouse as a model for viral tropism is fraught with those kinds of issues.

So there -- the integrin, the primary receptor distribution is important to know in terms of which animal model you are going to look at for virus tropism in biodistribution.

DR. ROBERTS: Yes, did you get the answer you want?

DR. BREAKEFIELD: No. Well, I think for now I will just -- I think we will probably move on and then I will just probably raise more questions.

PANEL A DISCUSSION OF SYMPOSIUM

DR. ROBERTS: Okay. Thank you very much. I think we now will go on to the panel this morning, Panel A.

I first would like to make a couple of comments before we introduce the panelists. It is my understanding that what we want to do is we have got -- although they look like multiple questions, I am going to look at them as two basic questions because there is the significant overlap in them. And what we want to do is take those two questions, and we are looking for input we will get from the panelists and we also want input from the audience, and we recognize that there are some people in the audience from RAC and there are people from the audience who are using this, there are people from the audience who are involved with doing the trials and so forth, as well as more fundamental development. And we certainly would like to get the input from everyone.

I am hoping, and I understand that it is correct, we are not here to get a consensus. We are here to try and collect the different views and get information on it. We recognize, as you heard from the previous speaker, there is

no perfect animal model. There is no perfect vector and there is no perfect transgene at the moment perhaps. We do not necessarily have to wait until we get the perfect for either of those to get some success as we have already had.

I guess what we want to do is try to enhance that success and look at its safety given the knowledge that we know today and what can be obtained. And it is on that basis that we are trying to record the various responses.

So what I am going to turn to first is have our distinguished panelists introduce themselves and then we will start with the first question and maybe we can start.

DR. SPRINGER: Yes. I am Matt Springer from Stanford University.

DR. SKARLATOS: Sonia Skarlatos from National, Heart, Lung and Blood Institute.

DR. SIMARI: Rob Simari from the Mayo Clinic.

DR. SERABIAN: Hi. I am Mercedes Serabian. I am an expert toxicologist with the Center for Biologics.

DR. O'REILLY: Michael O'Reilly, University of Texas, M.D. Anderson Cancer Center.

DR. NEMEROW: Glen Nemerow, Scripps Research Institute, La Jolla, California.

DR. MARBAN: Eduardo Marban, Johns Hopkins.

DR. KUTA: Alex Kuta, Genzyme.

DR. ISNER: Jeff Isner, Tufts and St. Elizabeth's Medical Center.

DR. DICHEK: David Dichek from Gladstone Institute, UCSF.

DR. CSAKY: Karl Csaky from the National Eye Institute.

DR. BYRNE: Barry Byrne from the University of Florida, Gene Therapy Center.

DR. BAUER: Steve Bauer from the Division of Cell and Gene Therapies, FDA.

DR. ROBERTS: I am Bob Roberts from Baylor College of Medicine.

DR. PATTERSON: Amy Patterson, Office of Biotechnology Activities, NIH.

DR. FRIEDMANN: Ted Friedmann. I am a member of the RAC and the University of California, San Diego.

DR. ROBERTS: All right. Thank you.

We are now going to take our first overall major question and that has to do with the -- both selection and design of delivery systems and so I am going to turn to our panelists first and get started on that. Certainly although we had listed four subquestions underneath that, I think that they overlap and I am sure if I do not get to move along and get all the answers that Dr. Patterson will make sure that I do, and will jump in from there or so will Dr. Friedmann.

The -- I am going to turn to the first panelist to try and talk about what they have learned or how they want to put this together with respect to selecting a particular delivery system.

Sonia, do you want to start?

DR. SKARLATOS: Sure. What I would like to know and find out is how significant is the expression of the gene as far as long-term expression for cardiovascular disease? It seems like when you look at vascular proliferative disease that it is not necessarily that you need a long-term gene expression delivery system.

So I was just wondering if anyone wanted to address that issue.

DR. O'REILLY: I could address it. I think there are actually -- that it would be not indicated to have long-term expression of the angiogenesis stimulators in this disease. I think you need to go in and target the area of disease and allow for revascularization and then perhaps turn it off. One idea would be to have regulatable promoters that if you needed to have it back on again, you could.

I think Dr. Libby showed that very clearly when he showed that the intimal plaques actually are dependent upon neo vascularization.

And Karen Moulton, a cardiologist who was working in Dr. Folkman's lab while I was there, also in Dr. Libby's department, showed that very elegantly where she actually found that she could prevent the process of plaque formation in an APO-E mouse model by giving angiogenesis inhibitors, endostatin or TNP470.

So I think that having prolonged delivery of the stimulators of angiogenesis may, in fact, have an initial benefit followed by a detriment, particularly if it was widespread, if you were giving it not targeted to the area of disease but perhaps to a larger area of the vascular system. Again a lot of that is

theoretical but there is data to support it with the work I just quoted from the Folkman and Libby group.

DR. ISNER: Yes. I think that is one of the things that has made cardiovascular disease imminently suitable for early trials of gene therapy because it is not at all dependent on long-term gene expression. If you look at the issues that have been discussed today, angiogenesis, restenosis, both of those appear to be goals that can be achieved with short duration of expression.

I think the third issue that I think maybe Rob Simari had on one of his slides this morning, congestive heart failure, that may be a different story, and depending on what your strategy is there, that may be more in line with some of the more classic notions of vectors required for gene transfer.

But I think, you know, I would be surprised if -- there is a slide that I think Amy Patterson showed indicating that half of the cardiovascular trials that are currently being conducted are being conducted with nonviral vectors. I would be surprised if there are many other areas of gene therapy right now where the use of nonviral systems has achieved that proportion of use and I think it speaks to the fact that even with something as relatively low -- with a low efficiency, short-term duration of expression, those -- that is the kind of perhaps -- those are the characteristics that are required right now of current vectors for treating some of these cardiovascular diseases and may allow us to have a better chance at improving the safety profiles of this technology than is the case with some other areas of gene therapy.

DR. ROBERTS: So is it fair to say then that at the moment in

terms of cardiovascular disease that one target we can look at primarily is to try and have the short-term treatment because many of the diseases that we know would be applicable to that and perhaps in the time being we should more concentrate on that sense. That is the big hurdle but trying to do it on a chronic basis at the moment seems to be further in the development than it is in the application.

DR. SPRINGER: I do not want to get ahead of my own talk later on today but I am going to show -- you certainly do not want to have too long expression of something like VEGF. You can stimulate autofeedback loops as a matter of fact and things can get out of control. So I think you definitely want to have a mechanism of either turning off the gene expression or having a transient system.

DR. SIMARI: It should be kept in mind, though, that there are reasonable goals in the longer term, including vasoreactivity for pulmonary hypertension, for systemic hypertension, and for antithrombotic strategies that might require long-term expression but at different issues and different toxicities.

DR. ROBERTS: Sure. No, I realize. And if you look at diabetes as one of our risk factors, I suspect if we were going to treat that it would be a long-term story, too, and certainly other aspects.

I think at the moment, certainly we have got lots of problems to deal with and we have not made a true major big impact in our therapies and clearly the people who are looking at this and where we are going, I would say at the present time, a good target is that cardiovascular disease is very applicable

because there are some short-term applications. And I think that we will be looking more at that as we talk about this problem this morning.

And in terms of -- I guess one of the questions that is answered then is to say that for those things, the appropriate -- whether it is a transgene or a vehicle, we want to do something that will last and give us some effect in the short-term and at the moment long-term is, indeed, longer away.

In line with that, the diseases that we are looking at for short-term such as whether it is to prevent a plaque from rupturing or whether it is to grow blood vessels into that infarcted tissue, or whether it is to try to prevent restenosis in whatever format you want to do that, all of those, I think, are looking at we are selecting a certain disease.

Is there anything in common with those pathogenesis that have in common with a particular vector?

My first answer would be yes and no, I suppose.

I mean, Jeff, what do you see in common with what you do with preventing both growing blood vessels as opposed to preventing restenosis?

DR. ISNER: Well, I think the most obvious thing, Bob, is the requirement for the level of gene expression and the duration of gene expression. And the fact that each of those appears to be something that can serve as a target for short-term, low-level gene expression.

I think that, you know, in our specific case there is a further commonality and that is the fact that rather than use the antiproliferative strategies that Bob Simari nicely outlined, we have attempted to use the same

mitogen to promote endothelial cell proliferation for the growth of new blood vessels in the case of angiogenesis strategies and then use that same ligand to promote reendothelialization of a site of arterial injury. That would have the effect of prohibiting redevelopment of plaque or restenosis.

I mean, that happens to be, I think, you know, specific to that certain transgene but I think those would be the similarities that I could identify.

DR. DICHEK: I am a bit less convinced than perhaps some of the others that short-term interventions are ultimately going to be effective in these diseases. In terms of restenosis, not involving stents for the purposes of this statement, but that develops over several months. Whereas the animal models -- in humans. Whereas, the animal models it is pretty much maximal in 14 days.

So I think that what we have seen is people taking advantage of the vectors that are available that work and hoping that short-term intervention is going to be definitive but I do not think we know the answer to that. And if you have a vector that prevents intimal growth in a rat or pig ballooned artery in two weeks, to think that that is going to prevent the process of restenosis that develops over three to six months, particularly if that is due to vascular remodeling, a biological process that is completely not understood in humans, it is a leap of faith. It might work but I do not think there is any guarantee.

I also do not think there is a whole lot of hard data to support that an initial burst of angiogenesis is going to provide long-term perfusion and mature vessels that are going to persist. That is really uncertain from my reading of the literature.

So I think people have appropriately made efforts to move these therapies into the -- or towards the clinic but it is an untested hypothesis in my view as to whether very short-term interventions are going to provide long-term success here.

DR. BYRNE: I would say David and I were looking at each other a little earlier because I think we agree on this issue and then it begets a new question. How does one then if you achieve the perfect vector system, how does one control transgene expression?

And I think we will hear later about the deleterious effects of over expression of some of these transgenes but these issues help us design the experiments appropriately for the disease. And I think these -- aside from the issues related to cardiomyopathy, particularly ones that are in genetic origin, many of the patients with vascular insufficiency have a whole panoply of problems related to chronic vascular insufficiency.

And I -- if there is data about the long-term benefit of a single angiogenic event, that would be great but I think we are going to be looking at other therapies for these patients down the road.

DR. ROBERTS: Anyone else?

DR. NEMEROW: So one of the issues related to driving longer term expression, today we heard a lot about using the CMV immediate early promoter to drive transgene expression, and that promoter is broadly specific and also susceptible to shut off by other host cell factors. So I wonder whether there has been experience with using tissue specific promoters that might give us

longer term expression and a greater safety in terms of having the transgene express where you want it?

DR. BYRNE: Actually, David, you have done some of those studies and Nabel have -- Betsy Nabel has some very excellent work both in smooth muscle and in cardiac specific promoters. Eduardo has experience with those as well. I think those help the situation tremendously because they do provide stable expression. They also prevent expression in antigen presenting cells and if there are immune responses to transgene, those would be limited with those vectors. So certainly an appropriate.

One can even combine those promoters with switches and I think that is probably part of the ideal system, is to have a tissue specific switchable promoter.

DR. ROBERTS: Yes, go ahead.

DR. SIMARI: I would suggest that one other -- in regards to the original question, which is what binds these diseases and these potential treatments together, in that every issue that we have talked about today, both the diseases and the vectors, involve inflammation. And I think that atherosclerosis is an inflammatory disease and we have to keep in mind, both when we look at what the vector's effect on the disease is but also how we look at toxicity in regards to inflammatory disease.

So I think inflammation is a key as we start to understand the viral -- possible viral etiologies of atherosclerosis and the viral possible treatments, that that is something we definitely need to keep in mind as we look at toxicities.

DR. ROBERTS: I agree. I think that all of us -- if we have not been emphasized this morning -- when you see how well that vector could generate the atherosclerosis and assuming that the similar might happen in humans, I think that is a very sad reminder that the vector will have to be truly evaluated in great detail from that point of view.

I suppose it is fair to say that both -- the rabbit we know is very prone to atherosclerosis. It is one of the favorite animals for it but nevertheless you saw in the rat as well and I think most people feel that that is going to be -- and I guess also bringing us back to the pathogenesis of the disease.

Although we have not got any data that infections can induce atherosclerosis in humans, I think that is definitive, but we know there is more and more concern that part of that atherosclerosis may be immune response or immune reaction to some form of injury.

And so putting all of that together, I think for atherosclerosis clearly the pathogenesis is important to us in selecting whatever vector or gene you want and you would have to keep that one in mind, and I suspect it would be the same for many others.

Go ahead.

DR. ENGLER: Okay. English from San Diego.

I just wanted to follow-up on the first question of vector selection and David Dichek's remark. I think that when we are dealing with a problem of organ remodeling such as angiogenesis for myocardial ischemia, such as preventing restenosis, perhaps such as regrowing endothelial cells after

angioplasty, it would seem ideal that transient expression would be desirable from the remarks that were made.

And clearly adenovirus has some of those characteristics in terms of transient expression but I would emphasize what you said, David, that no animal model is going to completely predict what is going to happen when we get into patients. And if we wait around for perfection in the animal model that will be the enemy of success.

What we have to do is get the best available animal model we have, test it the most we can and then what this human research is about is it is a human experiment. It is experimental medicine and what we have to do then is apply what we learned in the animal model skillfully in the clinic, carefully and with protection of human subjects, and so forth but we have to move into the clinic and try it because, as you point out, we do not have perfect animal models that are going to predict what is going to happen.

So my view is for organ remodeling. Transient expression would seem to be the best type of vector approach to take and people are clearly moving that into the clinic.

DR. ROBERTS: I think I will encourage some comments from the audience or people from RAC who are in the audience or otherwise, if you would like to make some comments or ask some questions.

DR. O'REILLY: You give me a microphone and you cannot seem to shut me up from the panel here but in any event I just wanted to make a comment that I think with the earlier discussion about not only looking at the

different vectors but also comparing the different growth factors. My personal opinion is that vascular endothelial growth factor may be -- at least with looking at all the available data -- may be the best candidate to focus on at this point. Not to belittle the work of others.

The reason for that is that if you look at the role of vascular endothelial growth factors, I see it being less of an angiogenesis stimulator, although it certainly has that function, and more of a regulator of vascular integrity.

So I think that there is the potential with the anti-VEGF agents and also perhaps with the HIF-1 alpha in that they can up regulate some of the VEGF in terms of not only restoring vascularization but perhaps having better vascular integrity of the vessels that have formed certainly as compared to basic fibroblast growth factor.

Again there is no data in the gene therapy area yet but in terms of looking at VEGF as being critical in vascular integrity, there is now a lot of data that supports that contention, and so I guess the point is, as I said, initially is to not only look at the vector but also look at the different angiogenic factors.

And it may very well be that once the fact -- there may be different factors that are for different conditions so whatever animal models are found to be the best, that should be studied as well.

DR. ROBERTS: Okay. Go ahead.

DR. FRIEDMANN: I just wanted to make the point that we are obviously going to spend a lot of time on transgene selection later in the

afternoon. It is obviously a very major question. Not only which vector you use but what you are going to deliver. So we are going to spend most of the early part of the afternoon on exactly that issue.

DR. DICHEK: I would just like -- I would like to respond to Dr. Breakefield's question earlier about animal models.

This is an evolving science. What is the best animal model and what is going to be predictive and we do not what is going to be predictive until we have human data and we can go back to the animals and see which one was right.

But for us making the animals hypercholesterolemic revealed things that we would not have predicted and so I would think that that needs to be taken into account.

It is -- there are -- certainly some of my colleagues in atherosclerosis research say, "No hypercholesterolemia/no atherosclerosis."

So if you want to model that you probably need hypercholesterolemia and the vessel behaves differently in the setting of hypercholesterolemia.

The same is true of preexisting immunity. We certainly find very different results in animals that have been preimmunized to adenovirus as compared to animals that have never seen it.

I do not know how predictive that is about -- for humans. We may be inducing a state of hyperimmunity by giving these large intravenous doses and maybe overstating the case. I do not know but it is certainly true that one gets a

different result in a preimmunized animal both in the vasculature and the data presented at the AHA by Stefan Jatsons in the myocardium.

So those have been useful for us. Whether ultimately they will be useful and the only way to model or to have predictive data in animals, time will tell.

DR. ROBERTS: Yes?

DR. SERABIAN: Can I just make a quick comment?

DR. ROBERTS: Go ahead. Please do.

DR. SERABIAN: And as Dr. Kuta was saying, I think it is important that it be the total database and maybe not just one model, not just one study, but the whole database in vitro and in vivo, both for efficacy as well as safety.

DR. ROBERTS: I agree.

DR. GREGORY: Rich Gregory, Genzyme.

I would sort of like to comment on the last two comments, as well as Dr. Breakefield's comments.

And part of my concern about trying to come up with a universal statement about vectors is that there is a lot of contradictory data in the field and I respect the opinions of all the members of the committee here this morning and the data that has been presented has really been very nice but I would have to say there are probably a lot of people in the audience when certain things were said, said, "I have seen exactly the opposite." An example would be the study that was just cited in terms of the pigs at the AHA where the preimmunized animals had a

different response than animals that had not been preimmunized.

We have actually seen something different in our models, which is that animals that have been preimmunized have the same response as animals that are naive but in both cases it is inflammatory so I am not saying the vector is not inflammatory. I am saying the inflammatory response is equivalent in both cases and that is something that we have worked into our preclinical models and we understand.

But, you know, this point about different data from different groups, I think makes it difficult for us to, you know, make universal broad statements about vector platforms. I think that one thing -- is I would have to agree with Alex. No surprise. -- is that it is specific to the drug, which is the combination of the vector and the transgene and that we have to understand each individual vector fully through a complete set of GLP studies and not just going out into the literature and trying to assume that what had been seen with another vector is the same virus.

DR. ROBERTS: All right. I am going to ask a question a somewhat different way and looking at it from the NIH point of view and looking at it from if you sit on the advisory council.

If you had say $200 million dollars to spend per year for the next ten years, what would be the things you would like to concentrate on in terms of, you know, looking at factors that would enhance or otherwise alter in selecting the route of administration. Keeping in mind, I think all of us are aware today that local delivery is systemic as far as the immune system is concerned.

I think that is one thing that is clear from all of this data, whether you inject it into some little tiny area exactly where you want that most likely the macrophages will come along one way or another, the immune system will take it and make it a systemic response in some way, shape or form.

But what sort of factors, what sort of things would be exciting if you have enough money to do it to be looking at the route of administration or improving it or completely changing the picture and to go around sniffing it rather than doing what we do?

So, Jeff, why don't I ask you to start addressing that?

DR. ISNER: Well, that is a lot of money.

DR. ROBERTS: Well, I tried to make it attractive for you.

DR. ISNER: I guess to be honest with you, I -- you know, I think there are a number of very clever strategies that could be investigated. Just ask -- you know, going around the people on this panel, the people in the room.

I guess, however, you know, I think that following up on some of the questions that were just raised, these animal models only take you so far and I think that, you know, the disease is not the same in the animals as it is in the human. The background of the human is not the same as the animals. It is much more heterogeneous and the delivery problems are much more complicated in general in humans than they are in animals.

And so, I guess, you know, one of the issues that, you know, probably needs to be considered is if you have some animal data that suggests that a vector or a transgene or a delivery approach is reasonably safe to use, that

no red flags have come up, can you then proceed to the human trial in a responsible and careful systematic way to try and address your question.

And, frankly, I think that some of that money -- one of the issues that is going to come up is an appropriate use of that money to, you know, take care of some of the monitoring that is now being proposed.

I mean, the safety profile, you know, is in these initial clinical trials in patients has not been terribly bad. And I think one of the issues is if you are going to require some of these investigator-initiated studies, which are often the incubator in academic centers for what ultimately become larger commercially sponsored trials, if you are going to encourage those kinds of trials in the future, and then you add the need for monitoring of these safety issues by a CRO, is that something that is going to be affordable by, you know, academic institutions or whatever?

And, you know, I suppose one of the questions -- one of the requests that would come up for that kind of support would be if it is going to be mandatory to utilize that kind of expensive high powered outside monitoring. Is that an appropriate use of those kinds of funds because then you have the opportunity to answer some of these questions in the real -- in the field rather than in another animal model?

DR. ROBERTS: So your story is that we should be more intense and take the methods we have got and be very intense about characterizing them and looking at the safety profile and monitoring that. And certainly I think that everybody would agree that there is going to be a benefit to that.

I guess the other part to think about, though, as you look ahead as we go into this -- I mean, if you ask any person in the pharmaceutical world, you know, what they want, they are easy. They want something that is small that goes into all cells, take it by mouth and it has got no toxicity.

Now gene therapy is a different ball game and probably always will be but we have to look ahead to when you get this little insert to tell you how to use this therapy. And not that we have to get there today but certainly the fundamental part in terms of looking at routes of administration is you have to take that into account.

And at the moment we go to the target organ first and foremost because you want to get a high dose of your gene there. The side effect that we are most worried about, the inflammatory one, though, does not much matter which organ we go to. And perhaps it does not matter a whole lot how you get there because those cells are going to seek it out and give you that response to some extent.

Or is that true? I think that is only partly true? Go ahead.

DR. BYRNE: I think I have a comment on that. I think it actually is critically relevant to the toxicities of what the dose is and the route of administration really dictates the dose. Obviously if one is targeting a cardiomyocyte, direct intra-myocardial injection is the most effective way of achieving the local concentration that would result in transduction, there may be ways to do that through the vasculature. But one has to then contend with the fact that coronary blood flow is substantial in the working heart.

And, unlike other organs where it may be possible to obstruct arterial blood flow, that is usually not favorable for any length of time in myocardium.

So I think that the route of administration is first important to establish what is the target cell and there are many implications being discussed so I will not suggest that one way is better than the other. If endothelial cells are the target then the intravascular approach seems sensible.

But one thing that influences this, too, that has not been brought up is what is the formulation of the material. If one wants to improve dwell time, sustained release preparations may be appropriate. It may even be appropriate in some scenarios that the circulation be supported for the patient while coronary -- intra-coronary delivery is given.

So these are, I guess, some of the questions that I would have.

DR. ROBERTS: All right.

Eduardo, you have been very quiet. Do you have any comments to make about this?

DR. MARBAN: Well, I think that unintended consequences is a big issue that needs to be contended with, with all of these trials. As smart as we are, we are never going to be able to figure out or anticipate what the problems will be and so I think a corollary of that is that there is some thought that should be put into rescue strategies.

It is not obvious to me what the rescue strategy would be if angiogenesis produces retinopathy or if angiogenesis produces distal tumor or

aggravates existing atherosclerosis by paracrine effects on neo vascularization in the adventitia of otherwise indolent atherosclerosis.

Or if the sprouting of vessels keeps alive marginal tracks of electrically conductive tissue that would create an arrythmogenic substrate what is the rescue strategy? I do not think we would ever launch a pharmacological trial without a rescue strategy. It is usually obvious with pharmacology. You stop taking the pill. But there is a certain hubris in gene therapy because we launch into these things without thinking about what we are going to do if it goes wrong.

DR. SPRINGER: I would like to comment on that. I guess in addition to thinking of gene therapy as a drug, you could also think of it as a transplant even if you are just putting in virus or DNA. So I think you have to look at it in those terms. What is the rescue strategy for a transplanted kidney in an experimental situation? Some of these things are just permanent and so you have to go into it with as much caution as you can.

DR. ROBERTS: So if I were to summarize briefly, and I will come back, that we all agree we have got to go to the target organ, whether you get there through a catheter or through some other mechanism that it is important to get high density gene to the target.

And what I am hearing is that it is very important and more important perhaps at this stage that we concentrate on monitoring both expected and unexpected events in a very intense way and that you feel that at least half of that $200 million should spent every year doing that rather than trying to come up

with some other fancy methods. Because the organ to some extent dictates the route you are going to use to get there and everybody seems to agree, and I think they should, that we want to get a high concentration of that gene at the target organ.

And that the monitoring and trying to look at -- because of the diffuseness of the immune system and otherwise, those effects are far more important and at the moment they seem to be the thing we should go for.

Go ahead.

DR. RUSSELL: Can you hear me?

DR. ROBERTS: We would like to have it on because it is being recorded.

DR. RUSSELL: We have heard a lot about the different vectors that are being used and the pros and cons of the vectors but whenever you do cardiovascular gene therapy there is a device used to deliver the vector and that is an integral part of the therapy. Depending on the precise target, it is a different type of device and we have not really heard much about devices today and the potential risks associated with the different devices and the potential for future developments there.

So I wonder if the panel could address that question?

DR. ROBERTS: Who wants to put their favorite gene in the stent and whatever?

DR. ISNER: I think that is a great question and particularly, you know, for this subspecialty because many of us have spent a lot of our careers

using devices and that is really perhaps one of the things that cardiovascular specialists have to actually contribute to this field. And it ought to be possible and I think it has already been shown to be possible to enhance gene expression using physical means that perhaps may not carry some of the same kinds of safety baggage that we have heard described this morning.

There have already been probably two dozen papers that have been published in the literature using catheter electroporation systems, catheter ultrasound systems, novel types of just conventional catheters that appear to at least in experimental animals yield multifold biologically significant enhancement of gene expression.

And I think that is -- particularly when we look at, you know, trying to reduce the amount of gene that we have to deliver, whether it is naked DNA or viral vector. There is probably still a lot of unexplored room there to improve the safety benefit profile -- the risk factor safety profile here by taking advantage of some of these technical delivery device related improvements.

DR. ROBERTS: Go ahead.

DR. SIMARI: One of the issues is there are only a -- less than a handful of approved catheters for local vascular delivery. So if one is going to carry a clinical -- a trial forward, one has to either coopt one of those devices or bring a new device in parallel, which adds a complexity.

I think the thing that has been brought up by Dr. Roberts, and we would all agree that local is systemic and if you can get systemic expression -- systemic delivery from injecting into a tumor, you are certainly going to do that

from delivering into an atherosclerotic plaque using a catheter.

So I think like vectors there are no perfect catheters. There are applicable systems that have shown effects and the question is how can we optimize those and that is another collaboration with industry because that is not going to come from the academic realm.

DR. ROBERTS: Yes?

DR. FRIEDMANN: I think this question of devices is very useful but it seems to me again it goes back to the issue of drug delivery. Delivery of a pharmacological agent. And I would like to come back to something that Steve Russell said earlier about the vectors per se not being the drug but rather the transgene product being the drug.

I think I disagree with that and what I would like to think is that -- maybe I misunderstood but it seems to me that what we need more than anything else is not the identification of a perfect vector because we will never have that. That will always be defined by the specifics of the disease and the delivery and the physiology.

But what we need at the moment is a -- is sort of a shift in strategy and a shift in thinking from phenology to pharmacology. We need to understand what we are doing better at the level of what we are delivering. Whether you call it a drug or whether you call a transgene a drug, it does not matter. We need to understand better what happens when we deliver something and we need to understand that at the same level as we have to understand a new antibiotic or a new cancer chemotherapeutic agent.

We need to understand what the material is. We have not been very careful about characterizing our materials. We know that they are very heterogeneous and very messy. They are a cell soup in essence partially purified but that is all they are.

And what I would -- and the -- what would help me as a RAC member is some instruction and some guidance on how to make that shift from phenomenology to sort of pharmacological rigor. What should we demand of characterization of the material that we get as a drug? What should we demand in terms of knowledge about interaction of this material with serum proteins, with the first cellular barrier that they see, about the pharmacogenomic issues involved in variation in the way drug -- these drugs will act?

I think just that sort of dissatisfaction now of phenomenology to characterization is what I would hope that we would strive for in the field in general but what I suspect the RAC is going to more and more want to hear and certainly that is part of the impetus for this meeting, is to help us understand what we should be demanding of these materials as drugs or as transplant or as xenotransplants.

DR. SPRINGER: I would just like to comment about that and about the question that you were commenting about. I think it really is -- there are two questions. You put a vector into the body, what happens to the vector, where does it go. Does it go to the germ line? Does it go to its target?

It then makes the gene product. Now how does that gene product get from where it is made to where it needs to go?

So I think you need to ask those questions about both sets, two processes.

DR. ROBERTS: Yes. You have been waiting patiently.

DR. ANDO: Dale Ando. I am on the RAC and also at Cell Genesis.

In having reviewed a lot of these protocols in other areas like cancer, so one specific question is that we will be confronted with a variety of adenoviruses, first generation, E4 deleted and gutless, and I was struck by the acceleration of atherosclerosis associated with elevated cholesterol that Dr. Dichek showed.

So one issue here would be what is the mechanism and what would be the best way to sort that out? Would that be preclinical in a particular model or should that be basically dependent on clinical research? And if it is in clinical research then what kind of endpoints would you use? So that is sort of a kind of group question that I would like to get some kind of input.

DR. ROBERTS: Well, that seemed to go to to the heart of the matter.

Go ahead, Dave.

DR. DICHEK: I will go after that one first.

You know, I can give a pretty standard answer. The -- it is going to be risk/benefit in advancing it to clinical trials. If the potential risk is a coronary occlusion and death and the potential benefit is, you know, to cut the nitroglycerin pills in half then that is probably not that great.

But on the other hand if the animal data look really safe and you have never seen a plaque rupture and you have never seen worsening atherosclerosis and you are able to use a lower dose, then it might be reasonable to go ahead in the peripheral circulation.

I think it was in Molecular Therapy this month where someone was quoting a -- that perhaps a standard should be applied. Would you enroll one of your family members in this trial? And I think that, you know, you can apply that and say, well, is it safe enough to go ahead in humans.

I would prefer to work out a lot of this in animal models and I think it is able to be worked out. I mean, I am not resigned from the gene transfer area because of these difficulties. I think we will figure them out and when we do we will have vectors that we do not need to worry about these issues with.

Whether it is possible to go ahead in humans with an adenovirus and deliver into the vessel wall and not risk catastrophe, I think, you know, it is with lower doses in the peripheral arteries there are treatments even if you do start to get an occlusion.

But I would be cautious about enrolling one of my family members in a trial where they were getting adenovirus in their coronary arteries right now.

DR. SERABIAN: Can I just add to that real quick? With respect to risk/benefit, I think one thing that is important is you may see something in the animal studies and it is crucial to attempt to follow it and monitor clinically. It may be something that may be an invasive. You only found out through an invasive procedure in animals that -- you know, how do you follow it clinically

then. That is an issue. That is a problem.

Maybe there are certain biomarkers that can be developed preclinical that somehow can provide some type of correlation clinically. Those are other ways to go.

DR. ROBERTS: Yes?

DR. _____: I would just like to continue with what Richard Gregory said that there are lots of contradictory data floating around and instead of considering each of these data I would like to spend the $200 million, what you just offered previously, and I would suggest, for example, two ways that we could do. One is the incredible lack of overall understanding of each of the vectors. That is we have no database where published data could be put in and analyzed. This would take substantial effort and would spend some of your money I am pretty sure.

The other way I could spend some money is we are establishing right now standards for adenoviruses and AAV is doing the same thing. Now these standards are essentially will be paper standards and based on donation. Now there is a major problem with this. I am sure that academics and companies actually would be very willing to donate reagents which could be standardized and standardized by companies themselves already.

However, this is substantial expense.

So if you give me some of this money I would be very willing to produce some of those reagents and deposit it. It was done for HIV research by NIH, which is extremely successful in establishing reagents, gathering and

distribution.

DR. ROBERTS: So one of the things you feel that is missing at the moment that could help to both understand more about safety or lack of as well as in terms of success is to be able to get the information in a better format and get it all together. I suspect that you have a good point there. Certainly coming from where I do in molecular genetics that if we did not have gene bank we certainly would be much farther behind today in terms of what we do and how we find genes. I would think that because of the heterogeneity of the models and the transgenes and the targets and the disease and the substrate on which they work as well as the vehicles that I suspect you are right that it might be better to sift through and get some general principles. I think that you -- we will let NIH know that and you and everyone else can apply in the same way to get some of those millions.

DR. _____: Thank you.

DR. LAO: Gene Lao from GTI.

I actually did -- I had the exact same point that Amy wanted to make regarding standardization. I think that is really important because of the -- at least from my understanding with respect to this adenoviral vector being a soup, a complex soup. I think there is a tremendous variation between preparations.

In addition, the titering, the biological titering versus the molecular titering is also a key issue. It is done very differently at different labs and you can have a ratio of one to three all the way to one to forty, and I think that is a

really important point in terms of the standardization process.

The second question I had is more regarding an issue that has just barely been touched on really and that is that I think there is a lot of interest in terms of vascular genesis, adult vascular genesis, and these endothelial cell precursors. I think that there is a tremendous potential in this area and it really drives a whole different set of questions which I have not heard at all addressed by the panel, which I think that potentially will need to be addressed, if not now, in the near future.

DR. ROBERTS: Thank you. Well, I agree. I am sure that uniformity obviously had a big factor in deciding our last president from the Supreme Court's point of view and I think it will probably play a major role, and I respect it. That is a good comment.

Go ahead.

DR. AGUILAR-CORDOVA: Estuardo Aguilar. I am also a member of the RAC as well as at Harvard.

One of the things that, you know, I have heard throughout the morning, of course, is all different vectors, different applications and such. But one of the things that is really intriguing to me at least is whether -- what are the major obstacles, what is our biggest fear in as far as safety? Is it the inflammation as we have heard multiple presenters talk? Or is it the duration of expression, either that it is too long or too short?

And perhaps we could get a feel from some of the members of the panel as to what you think really is the major risk factor and major obstacle there.

Whether it is, as I said, too long of a duration of the transgene expression, too short of a duration, or is it the inflammatory response? Which of these may be the most critical factors that we must be concerned about?

DR. ROBERTS: Which one? Go ahead.

DR. SPRINGER: Actually rather than answering the question, I will add to the list. It is not just how long or how short but also how high or how low, which gets back to comments that several people have made about long-term or short-term duration. It is more complex than that. You have got long-term low level and you have got short-term high level. This all has to be worked out.

DR. ROBERTS: But you would agree in terms of looking at the possible neoplasia or inflammatory response -- well, let's say neoplasia -- that longer term, either with low level or high level, is more likely to be more risky than short-term.

DR. SPRINGER: I think that depends. Something like VEGF, as I think Jeff will agree, if you have short-term high levels of VEGF as was done in some clinical trials just with protein bolus, you can get transient deleterious effects such as hypotension. So you might argue that in that case you would not want even a short-term high level delivery.

There are other cases where a low level long-term might prove to build up problems along the way so I do not think we can say --

DR. ROBERTS: So basically -- I mean, it depends on the threshold in the eye when you give it in the heart and so from that point of view you are saying that if you had low levels you may never reach the threshold for

neoplasia in the eye and, therefore, do the job you want to do in the heart.

So it is complex from both points of view.

DR. SPRINGER: Very complex, yes.

DR. KUTA: I do not know if you can come up with one answer for all these. I mean, realistically it is going to depend on your application. It is going to depend on your vectors. It is going to depend on whether you want a longer duration of expression or a shorter duration. Okay. You are going to have to develop preclinical models and, yes, there are cases, like Dr. Dichek said, where you are going to go from those preclinical models to the clinic and back to the laboratory and back to preclinical models.

That is a very realistic path and I think you will find that that is what you are going to end up doing.

Regarding the inflammation, there will be situations where that inflammation is acceptable. There will be situations where it is not.

DR. ROBERTS: Get your answer?

DR. AGUILAR-CORDOVA: I realize the complexity of it all and the levels of expression and such, and one would assume that you will try to minimize that complexity in your preclinical models, as Alex just mentioned, but given all those parameters and that you have chosen your vector and you have chosen your best guess as to route of administration.

I just wanted to know if there was a sense from the panel, especially the clinical components of the panel, as to what would be the major risk for those applications that we are talking about here that you would be

concerned about for your patients?

DR. BYRNE: I have a comment because I think this gets to Eduardo's point. If one has a method of dealing with a complication and it can be mitigated by that approach, it is you can build that into your safety profile.

One thing that is very difficult to mitigate against in heart are acute toxicities because immediate dysfunction is hard to manage in patients. Not impossible. But certainly that is the acute vector related toxicities which are directly related to dose are, I think, an issue that are very adequately dealt with in the FDA and the RAC but that would at least be my first priority and it is probably why it is so effectively considered.

DR. ROBERTS: Yes. This lady has been waiting.

DR. SARVER: Nava Sarver from the NIH.

Concerning the preclinical development, this is more a question to the panel, other than animal models which are rather difficult to access and not all investigators have access to this expensive resource, what is the situation with looking at other preclinical models, such as explant model, tissue modeling, three- dimensional, culture models, something that would allow you to ask a specific question.

Clearly, I mean, there are limitations in those models but at least there are more less expensive and there are more accessible, and a lot of questions especially in the pharmacogenetics, and even some pharmacogenetics questions can be addressed.

And I think that if these were looked at a little bit more closely and

some of them were to be developed if they are not already available, the development, the preclinical development can perhaps accelerate a little bit faster.

And so it is more a question as to what is the status of this venue?

DR. SERABIAN: Again, I think, as I have said before, I think it is a total database that would serve to supplement but I do not think we are at the stage right now, especially with gene transfer, to say in vivo animal models are not going to be necessary. I think that is a crucial point.

But I think what you are saying can definitely supplement and can potentially down the road, who knows, but right now I do not think you can say, no, it is too expensive, we cannot do it.

DR. O'REILLY: I am concerned, though, the in vitro assays in the field of angiogenesis are pathetic to put it bluntly. Basically you are taking an endothelial cell and asking it to behave not like an endothelial cell anymore and then expect to make conclusions that are going to replace in vivo models as a supplement to some of the in vivo with sort of a rational attempt to explain things. I agree it is useful but I do not think that at least in the current state we can rely on in vitro assays of angiogenesis because there are not any.

The three-dimensional tube assays, you could take an inhibitor of angiogenesis or a stimulator of angiogenesis and get the exact same effect in some of the three-dimensional assays. For example, endostatin can cause scattering of endothelial cells but in other factors scatter factor can also scattering of endothelial cells and they have the opposite effect on angiogenesis.

So I am concerned that, as I say of getting to a point where there is widespread use of in vitro models to try to explain things are going to lead things down the wrong path. And the best example of that is the TGF beta. TGF beta is among the most potent inhibitors of endothelial cell proliferation in vitro. Yet in vivo, with maybe one exception, all of the literature suggests that TGF beta is amongst the more potent stimulators of angiogenesis.

And so I guess I am getting a little worked up because I am very concerned with what I see with some of the -- in the field of the inhibition of angiogenesis about dramatic conclusions being made based upon in vitro data without the focus being on in vivo validation.

DR. SARVER: I think it depends how you define in vitro. I am not talking about tissue culture modeling. I am talking more about tissue -- like three-dimensional tissue organization or something where you have --

DR. O'REILLY: They are even worse.

DR. SARVER: -- where it may make -- well, that is what I am addressing to the panel. I do not know if this --

DR. O'REILLY: That is a huge problem in the field of angiogenesis, is that there are not great in vitro systems or nonanimal systems for studying it.

DR. ROBERTS: A couple more. Go ahead.

DR. MARBAN: I think it depends on what you are targeting. In vitro models can be extremely useful in vector refinement, for example. You do not have to go in vivo to -- at least in the first stages of vector development to

design inducibility or to test tissue specificity as long as you have good in vitro surrogates for the particular tissue of interest.

There are certain models -- angiogenesis is tough because it is inherently a tissue organ phenomenon but there are certain things that you might want to do involving modulation of electricity, for example, in which it is highly appropriate to use a monolayer model to ask the very difficult question of what do you do. It is not obvious which transgene would be the best.

So for vector development and transgene selection and optimization I think in vitro models are absolutely essential. It would be foolhardy to go straight in vivo is my personal opinion.

DR. ROBERTS: I agree. So I think that basically in terms of looking at safety it is difficult but certainly in the development I think everybody agrees, and we have to agree, that that is the way it was and that is the way most things are. That certainly is an opportunity to go back to look at it as Eduardo said.

We are going to have one more comment and then I think we will go to lunch.

DR. MARTIN: Thank you. Tyler Martin from Valentis.

I would like to make a quick comment regarding a comment Dr. Marban made earlier today about the rescue strategy, particularly for transiently expressing systems like nonviral or adeno. It is very much like traditional drug development in that the product just has a long duration of action. Those are not permanently integrating systems where you basically do not have a rescue

strategy. The rescue strategy is you wait for the half life of your drug to dissipated.

The second comment I would like to make relates to a comment Dr. Nemerow made earlier this morning about the utility -- the predicted utility of preclinical safety data as we move into the clinic. And since we are talking here about selection of different vector strategies I think it is important to point out that nonviral systems have a greater predictive utility because there is not the interspecies receptor variability factor to confound the utility of the preclinical model.

And then the last thing just as a final comment, since Amy showed data this morning that half of the current cardiovascular trials involve nonviral vectors, the next time we have this meeting I would prefer to see during our vector discussion a nonviral representative presenting some data.

DR. ROBERTS: Thank you.

I think with that we will retire to go to lunch. Be back here at 1:00 o'clock. Lunch is upstairs in the cafeteria.

(Whereupon, at 12:13 p.m., a luncheon recess was taken.)

* * * * *

AFTERNOON SESSION

TRANSGENE SELECTION

DR. ROBERTS: I welcome everyone back after lunch.

We are going to get started following the same format. We will have our speakers tell us what we need to know and then we will discuss it in panel.

And recognizing that we have got a long afternoon, and some of you may want to get home tonight, and recognizing that I think we will get started with our first speaker. It is Dr. Isner, who will give us a talk on the safety profile of VEGF gene transfer for angiogenesis and restenosis.

Jeff?

SAFETY PROFILE OF VEGF GENE TRANSFER

FOR ANGIOGENESIS AND RESTENOSIS

JEFF ISNER, M.D.

DR. ISNER: Thank you very much, Bob.

(Slide.)

I want to thank the organizers for allowing me to participate in this symposium. I think given all of the various issues that as investigators, FDA, RAC members and so forth, that we all are faced with, we all have the impression that our own position is always much more difficult than anyone else's but obviously this is a complex process and a complex series of goals that we are after.

(Slide.)

I think that when we look at the issue of safety considerations in cardiovascular gene transfer, as we are discussing today, there are two basic premises that may be appropriate to consider. The first is that the data to date, small as it is, is promising and, albeit not definitive, the results are, that have been demonstrated in the preclinical and the Phase I human clinical trials, are certainly at the very least intriguing promising. And I think that is important because if there was not any hint that these kinds of strategies were going to be productive then it would clearly not be worthwhile to spend the amount of time that all of us are spending this day and many other days considering all these safety issues.

(Slide.)

And I thought it would be useful to just quickly point to a couple of these areas and why we think there are some promising hints in these early trials. I mean, this is a young woman who came to us with this large area of gangrene in the forefoot, exposed tendons. This had increased over the last six months despite conservative therapy. She had been recommended to undergo a below the knee amputation.

And this is what the foot looked like four months later after intramuscular VEGF gene transfer. I mean, I would submit the chance of this happening on the basis of just chance alone is almost negligent -- negligible and so I think that the -- when you see these kinds of events as many of us have seen, I think that is encouraging.

(Slide.)

This gentleman presented with a sensory neuropathy that extended to the level of the knee. Over the next several weeks following VEGF gene transfer that sensory neuropathy resolved completely.

(Slide.)

And in a series now of 23 patients that were prospectively -- 23 consecutive patients that were prospectively evaluated with nerve conduction studies, in most of these patients, not all of them, there have been objective signs here indicating improvements in nerve conduction studies following gene transfer for critical limb ischemia that constitute yet another piece of objective evidence to make one think that there is some value to these approaches.

(Slide.)

In the case of the heart, this is the result of a very small pilot study that was performed at our institution using percutaneous VEGF gene transfer. Patients were randomized to either -- this is a single blind study so that the investigator knew what the patient was getting. The patient did not. And so that is the caveat. But nevertheless these are the control patients. There was a clear placebo effect over the first 15 to 30 days but after 30 days these lines diverge and patients that were treated with the mock procedure, no gene transfer, start to have a return back to their baseline symptoms, whereas the patients that received VEGF gene transfer continue to improve out to one year.

(Slide.)

And objective evidence that VEGF gene transfer improved myocardial perfusion was gained in that small study using a couple of objective

tests, both spec scans, to look at myocardial perfusion.

And just as an example of that, here is a patient, and if you would just focus on this bottom series of images, this is the test that was performed before the first procedure. And what one should see here is completely homogeneous distribution of the radioisotope in all walls of the left ventricle.

And as you can see, we are only seeing perfusion of a very small amount of the tissue there and that is indicated in yellow.

And then when the patient is submitted to pharmacologic stress, the perfusion disappears all together and so that in this case the patient develops global ischemia.

Now the patient is subjected in this case to a mock procedure, randomized to a mock procedure, and so he comes back 90 days later still with no improvement in his clinical status and these scans are performed again. And you can see again there is absolutely no change here. There is still under perfusion at rest. Gets much worse with stress.

Now the patient crosses over to VEGF gene transfer and is restudied 90 days later and you can see now a marked enhancement of perfusion, both at rest as well as with pharmacologic stress.

(Slide.)

And interestingly in this patient, one of a series of a patients that was also studied with a companion type of imaging strategy, and we do not have time to go into the technical details of this but this is the same patient now who had a big defect in the lateral wall. You just saw that on the spec scans.

If this patient was looked at with the so-called NOGA mapping there is a large red zone here indicating an area of hibernating myocardium prior to gene transfer. The patient, as I said, was randomized to the mock procedure and so that large area of hibernating myocardium persists. Now the patient gets treated with VEGF gene transfer and 90 days later, consistent with what was observed on the thallium scan, that large area of dysfunction has been reduced to a small area of dysfunction suggesting that there are again another piece of objective evidence of improvement in myocardial perfusion.

(Slide.)

So I think those are the kinds of events that those of us involved in these trials have observed and I am sure we will hear more about that with the subsequent speakers. And I think that is what provides the basis for pursuing on with these clinical trials in the future.

(Slide.)

Now the second premise is that patients with cardiovascular disorders, which have thus far comprised the targets for gene transfer, are severely disabled and at risk for loss of limb or life.

And I think this is an important issue to keep in mind because we are not dealing with a benign disorder. We are dealing with the sickest group of cardiovascular patients. This is a skewed group of St. Jude's patients who have little hope for other -- for improved prognosis because they are -- at least as they have been studied to date -- not candidates for conventional revascularization, refractory to any available medical therapies.

And so if there are deaths in this population of patients, it should not be entirely unexpected.

(Slide.)

And just to show that, here is some data that was taken from a publication recently looking at patients with critical limb ischemia and trying to document their natural history.

If you look at the patients with critical limb ischemia six months after they were first seen, who were suitable for or failed revascularization, conventional revascularization using angioplasty or bypass surgery, six months later 25 percent were cured. That was the good news but 25 percent of these patients at six months were dead. Twenty-five percent were alive but had lost a limb. And about 20 percent of these patients continued to have symptoms that were unimproved in response to conventional therapy.

So we have obviously targeted a group of patients that are quite ill and the same is true of patients with chronic myocardial ischemia who are no option patients. That is not candidates for conventional revascularization.

(Slide.)

This is some very contemporary data published last year in the New England Journal of Medicine in one of the randomized TMR trials, that is trials of laser revascularization. This data really formed the basis for this technology being approved. This is now commercially approved and available. And yet in this group of patients you can see that with the therapy itself by 12 months there was an attrition here of approximately 20 percent, at least that was

the survival free of major cardiac events.

If you looked at the crossover group, 40 percent of the patients had had some major cardiac event. And if you look at the patients who got medical treatment without crossover, almost 85 percent of those patients at 12 months had experienced morbidity or mortality related to their cardiovascular disease.

(Slide.)

If you look specifically at the mortality you can see here that with TMR in the group initially randomized to medical therapy the mortality here was seven percent and the group that did not get the therapy 15 percent, in the group originally randomized to TMR a mortality rate of about 15 percent.

So with a therapy or without it, we are dealing with a group of patients here that is at high risk for loss of limb or loss of life.

(Slide.)

And in that context I wanted to just summarize the data from our own experience. This is the mortality for all 85 patients with myocardial ischemia that we have been involved with in a trial of cardiovascular gene transfer. This is the total mortality now with a cumulative follow-up of over three years. You can see here that of the original 30 patients treated with VEGF-1, 28 of those are still alive. Although one of those patients has gone on to have a heart transplant. Of the 30 patients treated with VEGF-2, 29 of those patients are still alive.

And in the group of 25 patients that have been treated in either a single or a double blind randomized trial of catheter based gene transfer, there

have been no deaths, although one patient in this group has now gone on to heart transplant because he was part of a double blinded group we do not know what he received.

But if you look at this composite mortality over three years in a group of 85 patients, I think it compares very respectfully with the data that I just showed you and at least as a bottom line indicates that the decisions that have been made by the regulatory agencies have perhaps been appropriate in considering all of the safety issues, which we have looked at in some detail this morning, and I want to look at in a little more detail right now.

(Slide.)

So what are the specific risks and what is the clinical experience to date with some of these risks that have been presented? Well, one actually that was not touched upon much this morning but was listed in the series of questions was the issue of structural and functional integrity.

And I believe what this is alluding to is a study that was published by the group from Regeneron in Nature Medicine several months ago in which they showed that VEGF gene transfer using an adenoviral vector produced -- either with an adenoviral vector or in transgenic engineered animals that were over expressing VEGF, that there was an excessive amount of permeability that was inferred to be the result of death in several of these animals.

(Slide.)

Well, what is the clinical experience with VEGF gene transfer with regard to edema? I think that the worst of that experience is summarized in this

slide. This is taken from a paper published by Iris Baumgartner in our group in The Annals of Internal Medicine several months ago in which we summarized the development of edema in patients undergoing VEGF gene transfer for critical limb ischemia. And this edema was graded, as you can see here, as either Grade 1, 2, 3 or 4. Most of the patients that developed edema fit into either Grade 1 or Grade 2. That is either the forefoot or extending up to the ankle.

(Slide.)

And as you can see, this was most common in patients who had advanced critical limb ischemia and was seen less often in patients with rest pain. It occurred overall in about a third of these patients and most often in the patients who already had an established ulcer or a nonhealing wound.

The good news is that in all of these cases the edema resolved promptly in response to conventional outpatient oral diuretic therapy without any sequelae. There have not been any instances in which there has been evidence of edema in the case of myocardial gene transfer. Either one was able to identify it directly or even by inference when we look at the mortality or morbidity associated with that.

So I think that if you look at the animal studies they were associated with nearly industrial levels of VEGF, far higher than we ever see circulating in any of the patients studied to date. And I think that the clinical experience here is a little bit more reassuring than some of the animal data.

(Slide.)

The issue did come up this morning regarding diabetic retinopathy,

what has been recognized ever since the first RAC meeting in which we presented our protocol as an issue and a potential concern.

(Slide.)

And here I think we do have again some meaningful data. We have now a group of over 100 patients that have received VEGF gene transfer in a variety of protocols.

These patients have been followed by a group of independent retinal specialists unaffiliated with our institution who have performed fundoscopic examinations before and at yearly intervals after gene transfer. such an example is shown here.

(Slide.)

This is a patient who was identified as having mild background diabetic retinopathy at baseline and the same picture now persists two-and-a-half years later.

(Slide.)

If we look at the total of 129 patients that have now been followed in this fashion you can see that the fundoscopic examinations that were done at baseline indicating that 85 were normal and that these were -- 18 showed evidence of background diabetic retinopathy. There were another 26 diabetics who had normal fundoscopic examinations.

And at their most recent follow-up most of these patients, almost all of them, over one year follow-up, you could see that the distribution here has now changed and that distribution reflects the fact that there has not been a single

individual identified thus far in whom there has been a change in their fundoscopic examination. So once again I think that clinical experience, small as it is, to date is reassuring.

(Slide.)

Now there was a lot of discussion given this morning to the issue of VEGF in particular promoting progression of atherosclerosis and some of this is based on work, as mentioned, was published by Karen Moulton with Judah Folkman several -- about a year ago, I guess, now in Circulation showing that administration of endostatin in a high cholesterol mouse, the APO-E knockout mouse, managed to reduce the development of atherosclerosis in the ascending aorta of those mice using this potent angiogenesis inhibitor.

(Slide.)

Well, that was not exactly the same as giving an agent that promotes angiogenesis and seeing whether or not that would be associated with progression of atherosclerosis. But that study has been done in four separate animal studies, at least from our laboratory, all of which have been published and one of those is shown here.

And the effect that one sees in animal studies when one denudes an artery and then administers VEGF directly at the site of our arterial injury, the maneuver that is intended to promote the development of neo intimal thickening, what one sees is not that VEGF promotes atherosclerosis but, in fact, it is just the opposite. That it actually inhibits the development of neo intimal thickening by accelerating re-endothelialization.

So that in this series of studies this is showing representative examples of animals that constituted the control group, balloon injury, then gene transfer of Lac Z as a control, and the other group of animals that got balloon injury followed by VEGF gene transfer. And you can see the white here indicates the extent of re-endothelialization and as early as three days there is about 80 percent reconstitution of the endothelium in this balloon denuded site. Whereas, at two weeks in the control there is still only about 80 percent re-endothelialization that has occurred and somewhere between five days and week re-endothelialization is virtually complete. This was shown to be functionally recovered as well.

(Slide.)

So that the transfer of the VEGF gene in this case operates essentially as an endothelial band aid. It reestablishes functional endothelium and secondarily shuts off the underlying proliferative processes that result in neo intimal thickening. The data for that is shown here.

(Slide.)

Again these are the control animals in which you see that administration of VEGF led to reduced neo intimal thickening compared to the controls which was continuing to increase out to 28 days.

(Slide.)

And the same has been demonstrated, in fact, with stents and in two separate studies. One involving administration of VEGF protein. The other involving administration of the gene. Again as naked DNA. There was at both

14 and 28 days a marked reduction in neo intimal thickening within the stent compared to the control that got Lac Z gene transfer.

(Slide.)

Now the information that we have about this issue is not limited to these animal studies. We have had the opportunity to study our first 13 patients beginning back in December of 1994 that underwent VEGF gene transfer via an intra-arterial route. And so an inherent part of that gene transfer is balloon injury. We used a balloon to deliver the VEGF gene, which inherently disrupts the endothelium, and then these patients were followed by angiography and intravascular ultrasound six months later.

This is a typical example of what we saw. No evidence of atherosclerosis here. This is an intra-vascular ultrasound image. Here is the probe. This is the lumen. There is no evidence of any neo intimal thickening at any one of these sites along the entire segment that was exposed to balloon injury and that was the case in all 13 of these patients, including four who actually had the VEGF gene delivered to a site that contained atherosclerotic foci.

(Slide.)

And most recently we have now had the opportunity to complete enrollment of 30 patients in a Phase I trial, which has taken advantage of these preclinical studies, and the strategy here is patients who were undergoing balloon angioplasty of the superficial femoral artery for claudication following balloon angioplasty undergo VEGF gene transfer to promote re-endothelialization. And you can see here the incidence at latest follow-up.

This is up to three year follow-up now of restenosis, which is 20 percent in the group that received arterial gene transfer. And that is compared to results that were just published in Circulation last month for brachytherapy where a similar group of patients treated with brachytherapy had an incidence of about 25 percent and angioplasty alone, restenosis of almost 55 percent at follow-up with 12 months.

This does not prove that this strategy works to prevent restenosis but at the very least it suggests that we are not making matters worse or accelerating the development of atherosclerosis.

(Slide.)

Just two final issues on which there is not much data yet but I think that because they were brought up I wanted to just briefly mention them. One, of course, the potential for VEGF to promote or any of these angiogenic strategies to promote cancer. I think that is obviously something that is going to take a long term follow-up to decipher.

And complicating that is the fact that we are dealing with a group of patients by virtue of their age who are naturally at risk for that complication.

(Slide.)

And the most dramatic example of that is a patient shown here who got VEGF gene transfer in our restenosis protocol, underwent an aortic ultrasound study the following day to be evaluated for an aortic aneurysm and that examination just fortuitously disclosed a renal cell carcinoma that had been previously silent and undiagnosed.

Now if the patient had not had this ultrasound study performed the day after VEGF gene transfer fortuitously, if that ultrasound study had been performed six months, twelve months later, then of course the question would have been raised about whether the gene transfer had something to do with this tumor and that is, of course, the hazard that we are dealing with in this population of patients.

(Slide.)

Finally, the issue of unintended neo vascularization that I know Matt will talk about at great length in just a few minutes so I will not go into any of the data that he and Helen Blau published in this paper from which this photograph is taken from. But I will say that at least to date using a different strategy of gene transfer, that is naked DNA, and to my knowledge adenoviral gene transfer as others such as Dr. Crystal have used, I am not aware that in any of the preclinical studies and certainly in none of the clinical studies has there been any evidence of this kind of angioma formation seen when a low dose of VEGF for a shorter period of time was utilized.

(Slide.)

Finally, I just wanted to -- sorry. One question again which was not discussed this morning, which individuals with cardiovascular disease constitute appropriate participants for early trials of gene transfer. I think it is apparent that we have started now with patients, these so-called no option patients, who represent an extreme in terms of cardiac pathology.

(Slide.)

However, I think it is fair to consider that as our experience over time increases with either a gene product or that gene product plus a particular vector, or most of all with the gene product, the vector and now a route of -- a form of delivery that we find to be safe, that we may ultimately find that our threshold for treating these patients is reduced as a function of time.

And so right now we may be appropriately limiting some of these trials to these severely disabled patients. But over time if these curves are borne out, we may have the opportunity to extend this therapy in a systematic fashion to less severely disabled populations of patients and I will stop there.

Thanks for your attention.

(Applause.)

DR. ROBERTS: We will move on with our next speaker and finish the three speakers and then come back to the panel before we take any questions if that is okay. So we will go on to our next speaker who will discuss VEGF expression levels: Functional vessels versus deleterious effects, Dr. Springer.

VEGF EXPRESSION LEVELS: FUNCTIONAL VESSELS VERSUS DELETERIOUS EFFECTS

MATTHEW SPRINGER, Ph.D.

DR. SPRINGER: Okay. Thank you very much.

(Slide.)

I think Jeff Isner gave a very nice demonstration of why there is so much interest and excitement about the prospects of angiogenic gene therapy. In

my research over the past two years I have taken a close look at the physiological effects of VEGF gene expression at high levels in the mouse, and my talk today is going to focus on two different issues of interest to this symposium.

Number one, what are the deleterious effects that can result from too high levels of VEGF gene expression? And the vessels that are formed by VEGF, are they real vessels? Are they mature vessels, functional vessels if we take a very close look at them?

And I am going to be showing pictures of some pretty dramatic deleterious effects and so I want to stress at the outset that I do not believe that these data mean that VEGF gene therapy should not be pursued. Simply that it is very important to know what can happen if you deliver too much VEGF in these ways.

(Slide.)

So I should mention then that this is work that I have done in the lab of Dr. Helen Blau at Stanford University and the gene transfer technique that we use there, in general, is different from what you have been hearing about today so far. Rather than directly inject viruses or DNA into muscle, we implant skeletal muscle precursor cells called myoblasts that we have already genetically engineered and cultured in this case with retroviruses.

And because myoblasts will fuse to preexisting multinucleate muscle fibers, in essence, donating their nucleus to the cytoplasm of that muscle fiber, if we have transduced the myoblasts to express VEGF, then after we implant them in the muscle, the muscle itself will now express VEGF.

(Slide.)

So let me briefly go through what we have seen here then. So we are using a very strongly expressing retroviral vector, MFG. And when we implant myoblasts that express both VEGF and the marker gene, beta galactosidase, in the muscle, the initial response after a couple of weeks is an infiltration of cells seen here in two neighboring sections. These cells are not the myoblasts themselves because they do not stain blue. Here the muscle fibers that the cells fused into are staining blue.

Immunostaining with antibodies against endothelial cells and macrophages shows that those are the primary cells that show up. The endothelial cells that stain with PCAM and other markers and the macrophages as identified by F480.

(Slide.)

Now with time these implantation sites swell and grow, and so after about a month-and-a-half the implantation sites have now grown and formed large hemangiomas in the leg. Everything on this slide is the same magnification. So this is the implanted leg. This is the nonimplanted leg of the same animal. And these are control legs implanted with cells only expressing beta galactosidase. So it is specific to high levels of VEGF over expression.

The hemangiomas themselves are blood pools, vascular sacks surrounded by tissue.

(Slide.)

And if we take a closer look at that tissue we see that the tissue

itself lined with endothelial cells that are positive for PCAM, which is the dark blue stain in these pictures. So the tissue itself is lined with endothelial cells.

Another way of looking at that is that the vascular channels are lined with endothelial cells. This is a low powered picture of the hemangioma stained for PCAM. These are high powered pictures. And here is the corresponding H&E picture.

(Slide.)

And if we look even closer with florescent microscopy, we see that not only are these vascular channels and the tissue that surrounds it lined with endothelial cells, here PCAM is red florescence, they also have a layer underlying the endothelial cells of smooth muscle, as shown here in green staining for smooth muscle actin, which is a smooth muscle marker. Where the red and the green overlap is yellow.

And this is essentially identical to what you see when you stain normal blood vessels, shown here for comparison, with these antibodies. The endothelial layer, smooth muscle layer. So the hemangioma tissue has the architecture and components of blood vessels. It is just disorganized and essentially gone out of control.

(Slide.)

Now this is the picture that Jeff just showed. We got together with Randy Lee who is a cardiologist at UCSF to put these cells expressing VEGF into the heart to see what would happen there.

And first just to convince you that, yes, indeed, you can put

skeletal muscle myoblasts into the heart, these are control cells expressing beta galactosidase. They have engrafted into the heart. They have not become heart but they are there and they are happily expressing their transgene after several weeks.

(Slide.)

This low powered picture of a heart implanted with VEGF expressing myoblasts -- they are also expressing beta gal -- shows that the implantation site stained in blue here is characterized by again hemangioma with blood pools. And the cells that surround the blood pools stained positive with the PCAM antibody.

PCAM, by the way, is CD31, for those of you who do not know the nomenclature.

(Slide.)

I should mention, by the way, that we were not able to follow these mice out more than a couple of weeks because they ultimately died of cardiac failure presumably.

The last variation on the theme that I will show you, and I apologize to the people who are right up next to the screen, it is disturbing to see this 10 times larger than you are, but we did a collaboration with Gonzalo Hortelano at McMaster University and Donna Boulet, who is a veterinary pathologist at Stanford, and we encapsulated these cells in allogeneic capsules, microscopic allogeneic capsules, which allowed us to implant them subcutaneously and intraperitoneally.

And I do not have time to show you all the histological data but, in a nut shell, control capsules if a mass of them was put under the skin formed a slight bit of inflammation that did not change over time. When the cells expressed VEGF the masses became big, bloody and pulpy and continued to grow.

Now remember that this is VEGF being expressed continuously so as the VEGF has continued to be expressed these masses continue to grow.

When the capsules were put IP, intraperitoneally, control capsules had no effect at all. The VEGF capsules gave a large amount of inflammation, angiogenesis, a lot of edema and there was extensive vascular leakage into the peritoneum.

(Slide.)

So if I have to sum up what I just told you in six words in an acronym, it would be "Too Much VEGF is Bad For You." This is not really all that surprising as a concept. I mean, you can probably kill yourself by taking too much aspirin but aspirin is not considered a dangerous treatment.

And that then is the point that by knowing what can happen when you have too much of something like VEGF being expressed that should be able to allow us to establish a framework where we can see what the good therapeutic window for this treatment is.

And that would bring us then to the other point that I want to discuss today, which is these vessels that VEGF can induce, can we get the vessels without hemangiomas associated with them? And the vessels themselves,

are they mature vessels, are they stable, are they functional, that is continuous with the circulation, do they leak or don't they?

(Slide.)

So to deal with this, I want to take you back into the leg and let's take a much closer look at what is going on. This is a two week implantation site where myoblasts expressing VEGF have been implanted into the muscle. PCAM in this picture is stained dark blue and the regular tissue is just pink. And you can see that at the implantation site the myoblasts are here, this is surrounded by a zone of PCAM positive cells, probably capillaries and potentially individual cells as well. Outside of that is that is another zone with larger caliber vessels and then the rest of the muscle is essentially unchanged.

There is really no change in the number of capillaries in this muscle over controls.

(Slide.)

And if we take an area like this and enlarge it, it gives us the opportunity to see that these are large caliber vessels in a region where there are not really any capillaries left, and this is interesting because it is tempting to think that maybe these large caliber vessels were derived from the capillaries that used to be there.

(Slide.)

We can take that one step further by staining for smooth muscle actin. Here smooth muscle actin is green, PCAM-1 endothelial cells is red. Here is the implantation site and those large caliber vessels are actually all covered

with a very thick smooth muscle layer like an arterial. So we have then the possibility that preexisting capillaries have been stimulated by VEGF at the implantation site to grow in size and to mature and to become covered with smooth muscle cells.

(Slide.)

And there was a recent commentary in Nature Medicine that predicted that this kind of treatment would not cause what is called arteriogenesis, the formation of muscular arteries. This data would actually seem to suggest that you do, indeed, get mature vessels from VEGF treatment.

(Slide.)

Now let's take a step back down the ladder of expression. If we do not have that much VEGF, if we look at sites where there has not been huge hemangiomas where for whatever reason less of an effect has been stimulated, here is an implantation site. Cells are fused into the muscle. This area here is a mix of myoblasts and endothelial cells. Look at the smooth muscle actin vessels. They are in clouds around the regions of endothelial cells.

As a matter of fact, if you expand this you see the endothelial cells or capillaries here. There is no smooth muscle actin staining there. The smooth muscle actin vessels are clustered around that region.

(Slide.)

And to go all the way down, here is a needle track. This is where a few myoblasts crept back up the needle after implantation. There has been a slight bit of fusion into the muscle there. This has triggered the formation of a

few extra capillaries, no pathological vessels here, and even at this level there is this cloud of smooth muscle covered vessels. It is not here. It is not here.

(Slide.)

Okay. Now the last question. What are these vessels? Are they leaky and are they continuous with the circulation? Well, they are leaky. We know that because if we inject florescent dextran into the circulation we will see it in regular capillaries but it completely disappears in VEGF induced vessels.

So by perfusing the mouse vasculature with a suspension of microscopic beads, florescent beads that are shown here in blue, these beads are small enough so that they fill up the vascular space but large enough that they do not leak out of VEGF permeabilized vessels.

And down here -- pardon me -- down here at the VEGF myoblast implantation site you see that most of these structures are actually perfused by the beads, they are thread-like structures and are, therefore, capillaries.

There are also some structures here that were not perfused by the beads. Now some of them might just by chance have not been perfused but I think some of those are actually individual cells. And what we are in the process of doing really for the past half year was using confocal microscopy to take a closer look at these implantation sites.

I have here a tissue section, 3-D reconstruction of a tissue section that I can rotate, just to show you what is there. So this is -- it is like a piece of paper turning around because it is a thin section but you can still see by following something like that -- this is stained with PCAM so that is the outside of an

individual cell, here is three cells right there, here is a cell here and there are also thread-like structures.

(Slide.)

So I think that implantations like that are characterized by both real capillaries and individual cells that again may derive from these endothelial precursor cells that everyone is talking about.

(Slide.)

So to summarize then, VEGF -- this was the heparin binding isoform -- caused local expression, a local response in both kinds of muscle, subcutaneously, intraperitoneally. And, by the way, this is not ischemic muscle.

If VEGF continues to be over expressed, hemangiomas can result that have the architecture of blood vessels. The site of VEGF over production is surrounded by concentric zones of capillaries or endothelial cells and larger caliber smooth muscle positive vessels.

Angiogenesis and what appears to be arteriogenesis in this case can be achieved without pathological vessel formation or hemangiomas at low VEGF levels. This argues that having delivered too much there really is a point where we need the other people who maybe are not delivering enough or delivering just right amounts.

The capillaries induced by VEGF are leaky but are continuous with the vasculature, which was controversial before.

And, lastly, and this is basically the mantra that we have been saying for the past couple of years, regulated expression of a gene such as VEGF

is probably the best way to get safe reproducible angiogenic gene therapy.

We have been experimenting with regulatable vectors that have been behaving beautifully in culture and have not been behaving beautifully in the animal. Hopefully, at some point we will be able to show that this actually works.

(Slide.)

So I want to quickly say again that this is work done in Helen Blau's lab and we have very fruitful collaborations with Randy Lee at UCSF, Gonzalo Hertolano at McMaster University, and I guess I will take questions later after Michael O'Reilly has talked.

(Applause.)

DR. ROBERTS: Thank you. We go on to our last speaker before the panel.

This person has got the other problem. He does not like all those blood vessels. He wants to inhibit them.

Dr. O'Reilly will tell us about inhibition.

ANGIOGENESIS INHIBITION

MICHAEL O'REILLY, M.D.

DR. O'REILLY: Thank you very much.

(Slide.)

I actually do like blood vessels quite a bit. I just like them when they are under control and regulated, which I think actually we are all trying to do. I am just trying to prevent angiogenesis in tumors where I think actually part

of the problem in balloon angiogenesis is not so much the microvessel density that results but the rapid turnover or the persistence of angiogenesis and the lack of control, which I think is why many of these gene therapy strategies seem to be working so well is that you have a better chance of controlling things so you can turn on angiogenesis when it is needed and then it goes off and you can restore the normal harmony.

I think that gene therapy -- the fields of gene therapy and angiogenesis have quite a bit in common but probably the most relevant thing is the fact that we all are able to seem to provoke the media to a frenzy and give us unreal expectations. But looking at the accomplishments that I have seen today, you folks in the field of angiogenesis stimulation are heading -- making improvements in leaps and bounds compared to sort of the trickles of improvements we are making in the field of angiogenesis inhibit in cancer. And I think that is very impressive since I have always thought that gene therapy was a bit of a fight against evolution. Trying to get cells to accept viruses or DNA which they have probably evolved to try to spit out as soon as you put it back in. So congratulations to everyone who has been involved with all these projects.

(Slide.)

This slide illustrates what I am trying to prevent and you guys, I guess, in terms of the angiogenesis part are trying to stimulate. And it just is a slide that -- or an eye of a patient. Tony Ademus gave me this slide, an ophthalmologist in Folkman's group who actually has his own lab now -- showing just how critical angiogenesis is for the process of tumor growth.

You can see this tumor growing here in the normally avascular cornea and this tumor has had to overcome the relative inhibition of angiogenesis that is seen in the cornea, stimulated angiogenesis, and if looking at all these vessels it is interesting to notice how closely associated the tumor cells are with the vessels.

It also brings up a point which has been brought up before as to whether or not the gene therapy with VEGF or other angiogenesis stimulators would exacerbate this process. And I suspect that the effects particularly with VEGF that was targeted to vessels in the heart or a peripheral vessel would not because these tumors actually are producing huge amounts of not only VEGF but other stimulators that are all acting locally.

I think with many of the angiogenesis stimulators the consensus or the data suggests that the inhibitors act globally or can circulate and act at distant sites but the stimulators may act primarily where they are produced and that actually is supported by the fact that in most cancer patients when you remove a large primary tumor you actually do not see an effect on the metastases.

It is only the minority of patients that have that and I would suspect that if the tumors or if the stimulators being produced by these tumors were able to stimulate angiogenesis at distant sites when you removed the primary tumor you would expect to see a regression of the metastases.

And, in fact, the opposite happens more commonly and it is very rare. I think in maybe about five percent of renal cancer patients do you see the effect where you remove the primary tumor and the metastases go away.

So it means that I think it is going to be a small minority of patients that there would be a concern of local therapy with an angiogenesis stimulator would lead to accelerated growth of distant metastases or microscopic disease. I do have some data that supports that indirectly a little bit that I will show in a few moments.

(Slide.)

And angiogenesis, as you have heard, is the result not only of stimulators or inhibitors but of the balance between the stimulators of inhibitors. That actually is to me a very simple concept but seems to be one that a number of people have a hard time grasping and focusing only on the stimulators or the inhibitors. But I think the field of or the area of using gene therapy to treat peripheral vascular disease and coronary artery disease illustrates this concept very well based upon the work that was presented of Dr. Moulton and also the work that has been presented showing that the stimulators can prevent or can improve circulation after myocardial infarction or in some of these ischemic limbs, suggesting that perhaps strategies that incorporate both the use of the stimulators and the inhibitors might be best for some of these diseases.

I can envision perhaps someone coming in with the problem of peripheral vascular disease or coronary artery disease and having ischemia and needing a stimulator, and then perhaps getting -- to fix the problem and then perhaps getting the inhibitors to prevent the problem, and it is going to obviously be critical to define what is going on and to try to best integrate both sides of angiogenesis, the stimulators and inhibition, not only to treat peripheral vascular

and cardiovascular disease but also in the treatment of cancer.

(Slide.)

And I have discovered a number of angiogenesis inhibitors. One of which is angiostatin and angiostatin is a fragment of plasminogen.

(Slide.)

I am just going to go through endostatin which is a fragment of collagen 18.

(Slide.)

And finally an antiangiogenic conformation of antithrombin, which physiologically anyways, seems to result from the cleavage of the antithrombin-3 molecule, although you can also induce what is called the latent confirmation of antithrombin. This was work defining these confirmations of antithrombin by Robin Carroll and I found that this anti -- this cleaved form or this latent form, these different confirmations, all are antiandrogenic. Suggesting that in this case it is the confirmations that is important.

The reason I showed these three inhibitors is not to beat my own drum, although I am good at doing that sometimes, but actually just to show a concept that in all cases these inhibitors are not expressed directly. Instead they are fragments of other proteins or they are released or they are activated by enzymatic activity, which suggests that in the plaque -- in the atherosclerotic plaque, at least this suggests to me that perhaps what is happening initially is these inhibitors are being mobilized in small amounts to prevent the process.

As the atherosclerotic plaque progresses you get hemorrhage into

the plaque and some thrombosis, which then could mediate inflammation and activation of a number of enzymes. Those enzymes could be releasing more of these inhibitors and the inhibitors could then lead to the ischemia. So it becomes a vicious cycle where the inhibitors have a positive role at one point, followed by a negative role, and I think that is where you need the stimulators to overcome perhaps the over production of these inhibitors.

(Slide.)

But then after you have done -- you are done stimulating the vessels and revascularizing, then perhaps strategies that try to restore the inhibitors back to their normal levels might be prudent as well.

Again it is speculative but it does fit or it is at least consistent at least in my view with the fact that all of these angiogenesis inhibitors are fragments that you might expect to be forming in the atherosclerotic plaque.

(Slide.)

But in any event I wanted to shift gears and just show some of the potential of angiostatin and endostatin. Angiostatin and endostatin have been very effective against a wide variety of mouse tumors. So far we are still in the -- doing clinical trials with these agents and although some of the results have been very encouraging, lack of toxicity and some responses in a few patients, who have been able to stay on the drug for long periods, we still have yet to graduate from the mouse studies to patients where we belong. But in any event it does show that these angiogenesis inhibitors, as I think some of the stimulators might be best used, is in combination with each other.

I am just showing that angiostatin and endostatin, this human angiostatin and endostatin made by EntreMed can fully regress these tumors and hold them versus angiostatin and endostatin alone. At comparable dose we are not able to do that.

I wanted to shift gears and just actually show some results that I actually found while working with some of these angiogenesis inhibitors and some metastatic models of cancer. And I will conclude if you bear with me for a minute with some points that I think are relevant to the questions that were raised earlier about whether or not some of these angiogenesis stimulators when they are given with the gene therapy will result in the accelerated growth of dormant tumors.

I was looking at over the past several years a number of different patterns of metastatic disease that can be seen in patients on developed mouse models of at least three of the patterns. In one pattern the primary tumor seems to suppress the growth of this metastases. This is a patient who presents with the primary tumor. Clinical work up, no evidence of metastatic disease, remove the primary tumor and the metastases start growing rapidly. It only happens in about five percent of patients and I have developed a mouse model of that and actually that is where we -- I was first able to find angiostatin and subsequently in other mouse models of this phenomenon found endostatin and the antiangiogenic confirmation of antithrombin.

Noelle Belk and her colleagues have found that tumors can mobilize fragments of thrombospondin and do the same thing.

The more common pattern is the primary tumor and the metastases growing concurrently. A model which I am still trying to work on. There is an experimental model where you do tail vein injection of metastatic cells where the primary tumor or the metastases seem to suppress the primary tumor, the so-called unknown primary.

And finally this pattern actually, which has led to some work that suggests that strategies using angiogenesis stimulators to revascularize areas of disease may not result in the growth of metastases is this model here.

It is a model where I had implanted B-16 F-10 melanoma or a variant of B-16 F-10 melanoma in mice. In most of the mice the melanoma would form lung metastases that would grow either while the tumor was in place or when the tumor was removed but I noticed that in a couple of mice I had developed -- there was a variant where when I removed the primary tumor the mouse was apparently cured. Remove the tumor, wait two years, the mice die of old age, and they have no clinical evidence or no visible evidence of metastatic disease.

(Slide.)

But I thought the metastases should be there and so this just illustrates the mice. These are mice -- they are melanoma resected about a year previously. You can see here not a very cosmetic job. You can see why Folkman -- I was initially when I joined Dr. Folkman's lab, I was a surgical resident. Subsequently I switched over to radiation oncology. I think Dr. Folkman might have been a little more enthusiastic in his support of that based upon seeing some

of the work I did on the mice and the area here where they did not heal exactly right.

But in any event these mice will go on to die of old age or would have gone on to die of old age with apparent a cure of their melanoma. Very much like what could be seen in patients. The patient who has a tumor removed and then is disease-free or supposedly disease-free for many years but has recurrence 20, 30 or even 40 years out after surgery in some cases.

(Slide.)

If you look at the lungs, the lungs look completely normal. These are mice that had had their primary tumor removed a year previously, eight months previously. We have since done the same thing up to two years after surgery. The lungs look completely normal. No evidence of clinical metastases but if you look here histologically there are microscopic metastases present throughout the lung.

(Slide.)

And this model is very exciting because I think it might be useful in the study of dormancy in patients. The patient who I described earlier who presents with a cancer, has it treated, is apparently disease-free or enters what is called disease-free survival only to have recurrence many years later, and that is actually very perplexing what is going on there. Why does the disease stay dormant and then again why does it start growing?

But when I was asked to give this talk I thought that some of the studies that I did trying to get these metastases to grow are relevant to some of

the questions that were posed earlier. And what I found was that if I injured the lungs of these mice repeatedly by using a 30 gauge needle and poking through the chest wall of the mouse about 20 times -- the mice are not a big fan of me unfortunately since I do horrible things to them but in any event if I did that I found that not only would metastases start growing in the lung that I injured but they would also start growing in the contralateral lung. Suggesting that it was some soluble factor.

And so I tried a number of different things and the first agents I tried were angiogenesis stimulators given the fact that I am angiogenesis centric as most people who have trained with Dr. Folkman are. But in any event I used vascular endothelial growth factor giving it systemically via intraperitoneal injection at high dose for up to three to four weeks at a time and had absolutely no effect on these metastases. They all stayed dormant indefinitely despite the fact that I had treated the mouse with basically -- excuse me, vascular endothelial growth factor. Did the same thing with basic fibroblast growth factor. I have also done the same thing with interleukin-8 and all three of those angiogenic factors had no effect whatsoever on this dormant disease.

And then I read some work by -- read some work suggesting that in sites of inflammation you could see accelerated tumor growth and that was due to TGF beta. I tried TGF beta. TGF beta did not work. But I also thought that perhaps it was TGF beta or inflammatory response plus an angiogenic response and so when I gave TGF beta plus basic fibroblast growth factor, the metastases grew explosively.

(Slide.)

So the point is not so much that the FGF might be causing this. I think the FGF alone, as I said, had no effect. It was only when FGF is given with the combination with inflammatory cytokine, TGF beta, was I able to see accelerated growth of the metastatic disease.

(Slide.)

I have another model of dormancy where I found the same thing. Using endostatin given at high dose with cycle therapy, this was some work by Thomas Boehm and Tim Broder and Folkman and I did and published a couple of years ago where we were trying to see if an angiogenesis inhibitor would induce drug resistance.

Using endostatin we found that there was no evidence of drug resistance. In fact, in these mice, the Lewis lung carcinoma, fibrosarcoma or melanoma. We allowed the tumor to grow. With endostatin the tumors regressed and came down. With endostatin therapy we did the same thing for several cycles and no resistance. But a very surprising find, one we are still trying to figure out, is after a point after six cycles of therapy for Lewis lung, four cycles of therapy for fibrosarcoma or two cycles of therapy for melanoma, the tumors did not come back off therapy.

The mice all still have active disease at the site where the primary tumor was but it persists as small microscopic dormant nodules indefinitely off therapy.

(Slide.)

And I found that the same strategies I had used for that melanoma model where the melanoma metastases worked in this case. This is an example of the melanoma after treatment with endostatin. Off therapy for about half a year microscopic disease is still present. In fact, the mice, unless we intervene, will go on to die of old age.

(Slide.)

But if I do either TGF beta plus basic fibroblast growth factor, or injury the tumor by moving it from one site to another, I will get the regrowth of this metastatic disease.

Again, as with the metastases, excuse me -- with this primary disease. As with the metastases,the use of an angiogenesis stimulator alone, basic fibroblast growth factor or vascular endothelial growth factor had absolutely no effect whatsoever. It was only when used with TGF beta and basic fibroblast growth factor in combination.

(Slide.)

And these are the mice after TGF beta plus basis fibroblast growth factor. You can see the area where the dormant tumor was. In fact, you can still see a little bit of scar tissue in these two mice where the dormant tumor had been and you can see a recurrent melanoma after TGF beta and basic fibroblast growth factor.

I think the key to relate this to some of the questions about would these angiogenesis stimulators when given alone accelerate the growth of occult disease, I think these data suggest that that would not be the case, that the

regulation of dormancy is more complex and so the use of an angiogenesis stimulator alone at least in these murine models had absolutely no effect.

(Slide.)

And in conclusion angiogenesis inhibitors. As I said, you all seem to be making much more progress or more rapid progress than we are since there are a number of angiogenesis inhibitors now in clinical trial, Phase I through Phase III, and we still do not know how to use the angiogenesis inhibitors. And, in fact, although there have been some potential successes in the clinic, it is still far from obvious at least to me how angiogenesis inhibitors will be used in the treatment of cancer and other diseases.

I would like to thank you all for your attention and I guess we will be answering questions as a panel.

(Applause.)

PANEL A DISCUSSION OF SYMPOSIUM QUESTIONS

DR. ROBERTS: Okay. First I am going to turn it over to my co-chairman to direct the panel.

DR. FRIEDMANN: Okay. First, let me invite the audience or panel members to direct some questions to the speakers that we just heard if there are any issues that arose which we did not have a chance to raise questions during their talks. And then we will get to the rest of the panel and the comments on their questions.

Yes, please?

DR. FERRANUS: Tony Ferranus from the NHLBI.

The question is for Dr. Springer. Do you have any quantitative numbers about the amount of VEGF protein that you have expressed in your system so you could sort of put a straw line and say this is actually too much protein expression that will cause these effects?

DR. SPRINGER: I have figures but I will not say that they mean anything. Let me explain that.

When we do the transfers we can measure VEGF in the serum at approximately forty picograms per ml if I recall correctly in the general serum. But what we had noticed is that when we take blood from near the hemangioma if we are isolating tissue, hemangioma tissue, the dead animal bleeds from next to the site, which does not usually happen. When we collect that blood the levels of VEGF are elevated more like 200 picograms per ml.

And I am not really sure what to conclude from that, which is why I have not said anything strongly about it. What is the VEGF level that is actually within the hemangioma? We do not actually know that. What it looks like is happening is that the heparin binding form of VEGF is made and initially bound and used because early on we do not see any blood. Later on we do. And I have a feeling that the VEGF we see in the bloodstream is actually just leaking out of the hemangioma, that is my guess.

In the other systems that we have done, the encapsulation experiments, for example, despite the massive dramatic effect that we saw, there was no VEGF in the blood, which backs up my interpretation of that.

Presumably if we used a nonheparin binding isoform of VEGF we

probably would see it in the blood of an earlier stage.

DR. _____: It is more of a comment. I would like to expand on Dr. Springer's presentation. We have used the tat system to regulate the expression of VEGF in the myocardium in work mostly carried out with Eli Kishet's lab in Israel, and I think it points out that the abundance of the gene product and the timing are very important.

For instance, if you induce the over expression in adult animals for only two weeks you get angiogenesis but these vessels regress if you then turn off expression. So they are immature. If you continue the expression you start getting smooth muscle markers becoming positive. And if you continue expression for over a month or two, you actually start getting a cardiomyopathy with so much angiogenesis going on that it is very deleterious and the mice actually die.

So I think it really does point out that it is important to be able to control the abundance of some of these very potent growth factors if one really wants to get a therapeutic response and not get a deleterious response.

DR. SPRINGER: I agree.

DR. FRIEDMANN: Could I remind the people in the audience to please identify yourselves when you ask questions for the record.

DR. ENGLER: Engler, San Diego.

Dr. Springer, there have been a number of publications about the types of vessels that one sees with over expression of VEGF. Harold Dvorak at Harvard sees vessels that look a little bit different than your's. You seem to see

vessels that look pretty normal but you say they are leaky so I have two questions regarding that.

First, could these myoblasts be over expressing other growth factors as well as VEGF just because they are myoblasts and somewhat more primitive cells and they are implanted in a region and are interacting with the tissue? Other growth factors then would be cofactors for the VEGF.

And, second, how do you know the vessels are leaky since VEGF just by itself causes leaky vessels? Maybe the vessels are normal and they are just leaking because there is high VEGF in the region.

DR. SPRINGER: Well, let me take the first question first. The work that Harold Dvorak did that he published at the beginning of this year, I believe, he saw vessels that did not look like the regular vessels that I showed but they looked very reminiscent of the hemangiomas, what he calls mother vessels.

His interpretation of what -- in his paper he actually saw things that looked quite a bit like our hemangiomas. His interpretation was that you start off with a large vessel and that endothelial bridges come in and bisect that vessel into smaller compartments, which would be the tissue dendrils that I saw in the hemangioma.

It is a possible explanation of what we see and I do not think there is all that much difference between what he sees and what we see.

In terms of the leakiness -- well, no, in terms of the myoblasts expressing other factors, you have to remember that after the myoblasts are implanted they are no longer myoblasts. After they fuse into the skeletal muscle

they are now muscle fibers that are expressing VEGF.

So in that sense it should be very similar to what happens when you inject plasmid. How Dvorak put in his adenovirus in the muscle, it did not transfect muscle fibers. It transfected the peripheral cells in the muscle. So that actually was a different system than what we are looking at.

In terms of the leakiness of the vessels, it is one or the other. I do not know that they are really any different. The VEGF is made in the muscle. A lot of new vessels are made. They are almost all VEGF induced and those vessels are leaky. I cannot say what it was doing to the regular vessels in the area because you cannot see them anymore. There are so many new vessels there so I am not really sure how to answer that last question.

DR. GREENBLATT: Jay Greenblatt, National Cancer Institute and Recombinant DNA Advisory Committee.

I guess my question is to Michael O'Reilly. Yesterday there was some discussion about tumor formation, tumors which have arisen in patients given vectors containing -- encoding VEGF and beta FGF. It was not clear to me that your experiments were directly relevant in that these patients are not being given VEGF or beta FGF. They are given a vector which can migrate throughout the body and actually possibly locate within the tumor or within circulating endothelial cells or precursors, which can enter the tumor.

And I wonder what your thoughts about that possibility being able to cause -- promote growth in dormant tumors.

DR. O'REILLY: Well, I think in the study I did it was not

injection of basic fibroblast growth factor or VEGF into the tumor. It was actually systemic administration by repeated intra-peritoneal injection. So although it does not answer the question, at least in that model -- in all four of these systems I have not seen the effect of basic FGF or VEGF to have any effect on these dormant tumors.

And with regards to whether or not there may be some patients that that could happen, I am sure there are a small -- or I suspect there are a small percentage. I just do not think it is going to be the majority. I mean tumors are quite good at producing excess amount of stimulator and I do not know if necessarily the small amount or relatively small amount that would come say from a virus that had gotten out of the target area and migrated to an area of occult disease would be enough to get the process going.

The other reason why I am not as concerned is that most tumors need sustained angiogenesis. That it is not enough just to add VEGF and then stop therapy. They need to have VEGF continually so I think that -- particularly with some of the limitations of gene therapy currently where you cannot often get prolonged expression that might actually be a benefit in terms of preventing some of the potential side effects.

But as I say the main reason I am not as concerned is that clinically tumors produce lots of growth factor that get in the circulation and really the clinical patterns at least -- except in a small percentage of patients -- really are not consistent with the hypothesis that circulating angiogenic factors can accelerate the growth of tumors at distant sites.

In fact, all the data suggest that the stimulators only work locally or primarily work locally.

DR. FRIEDMANN: Can I just follow-up on that and ask what do you infer from the mouse B-16 model notoriously responsive as it is with respect to the need in other animal systems for sustained and high levels of expression of more than one growth factor?

Can you confidently say --

DR. O'REILLY: Oh, I did not mean to imply that but if you look at the tumors the vessels are turning over rapidly. I mean, when I look at a tumor they have actually -- all tumors have more vessels than they need. And in many cases those vessels are turning over rapidly. They have high endothelial proliferation which is what suggests to me not just melanoma but pretty much -- certainly with almost any animal tumor and most of the human tumors that have been looked at, the two -- it is the turnover that distinguishes angiogenesis in the tumors.

DR. FRIEDMANN: But isn't that still --

DR. O'REILLY: And so that the tumors have more vessels -- actually tumors are actually inefficient in their angiogenesis. They stimulate it too much, which suggests to me that they need that turnover for some reason. Probably more for a paracrine effect that the endothelial cells when they turnover can make up to 60 factors that could potentially directly stimulate the tumor cell. That is why -- as I say, I think that the reason the tumors are turning over their vessels rapidly is more for this paracrine effect and, in fact, most tumors are over

perfused and have very inefficient angiogenesis.

DR. FRIEDMANN: But the conclusion from the mouse study is that you need simultaneous and sustained levels of both growth factors.

DR. O'REILLY: Oh, in this case -- I am not sure in the case of those models that the TGF beta is functioning primarily as an angiogenesis stimulate. TGF beta has a number of functions. The problem with TGF beta is you can have any hypothesis you want and you can find two papers to support it even if they are directly contradictory to each other, the hypotheses.

So my thought is that TGF beta may in part be increasing angiogenesis that leads to the growth of those tumors but may also be having some immunomodulatory effect or some yet unknown effect that initiates the process and then perhaps the FGF helps sustain the process. I am not sure. In that model the mechanism is unknown.

It has been a frustrating project because I can do lots of experiments that further define the phenomenon but finding mechanism has been particularly difficult.

DR. FRIEDMANN: So if there were some disease stage at which there was an increased level of TGF beta, for instance, and one were to add --

DR. O'REILLY: Potentially.

DR. FRIEDMANN: -- and one were to add the second function like VEGF --

DR. O'REILLY: Yes. So I do not think that it is only one event or one factor will initiate the process. And actually the project was inspired -- in

terms of the -- the first thought in terms of why I sort of injured the lungs and then led to using the TGF beta based upon some of Mina Bissel's work and later TGF beta plus FGF was a patient I had seen who had had a sarcoma of his arm resected about ten years prior to when I had seen him and had had no evidence of disease.

And he was kicked repeatedly in the arm -- he was carrying a child apparently and had a sarcoma form at the site. And obviously it does not happen all the time that trauma leads to regrowth of dormant disease but anecdotally there are a number of stories that suggests that that could be at least the initiating event. That is why I think that the -- to get dormant tumors or to start growing more rapidly, one event is not enough. You need probably a series of events.

DR. FRIEDMANN: Have you tried kicking your mice instead of sticking them?

DR. O'REILLY: Actually there were studies where to activate dormant liver metastases someone apparently banged on the chest wall of a rat with a hammer as the function of trauma and that did the same thing.

DR. FRIEDMANN: All these sort of word of mouth instances of children kicking the leg and then sometime later coming in with bone tumors in the region. Is that beginning to --

DR. O'REILLY: It happens enough to make -- or at least it happened enough to give me the idea to look at it in this system and it worked. Again I do no think it is one event that leads to it. There is probably a series of events that all come together that lead to escape of these dormant tumors, which

again is why I think that sort of a single therapy using the angiogenic factors for gene therapy probably is not enough to really initiate this cascade in the majority of patients.

DR. _____: Like football players.

DR. FRIEDMANN: I do not know but on a pediatric ward the incidence is high of children with osteosarcoma and there is often a history of athletic injury or something.

DR. BOYD: Alan Boyd from EuroGene in London.

This is a practical question. In designing clinical studies with VEGF what baseline screen should we be performing? What follow-up should we carry out?

So as an example with Dr. Isner's work in the eyes, should we now be planning prospective eye studies just in case? I know the results were very favorable but it is a small number. Should we be looking for those sort of things?

DR. FRIEDMANN: I think we will come to that extensively later as part of the clinical design discussion but there is no reason not to answer now.

DR. ISNER: I think in the case of the eyes even though this initial data is encouraging, the thing that makes it easy to continue to monitor that is that the examination that is required is very straight forward and it is not particularly expensive. And I think it becomes much more difficult when you consider this issue that we are talking about here of tumors and, you know, how extensively do you screen the patient and how do you monitor them, for how long and so forth. I think that is something that I will be interested to see what the

other panelists have -- what their ideas are about that.

DR. CSAKY: One issue that I am a little bit concerned about, we actually did two Phase I studies here at the NIH where we monitored patients who were getting infusions of recombinant VEGF for coronary and peripheral vascular disease, and we did extensive eye examinations on all those patients. And fortunately we did not find any problems in terms of development of complications but the problem is when you look at the group of patients that you are going to be treating, I think diabetics for the most part, their disease progresses very slowly even in the normal condition. So even if there was a stimulatory effect, it would be very, very slow and fortunately we have very good treatments for that.

So I do think that diabetics, especially if they have pre-proliferative disease where we would put them at high risk of developing neo vascularization just by natural history, I think those patients should be screened fairly closely but the group of patients that I am more concerned about are those patients with age related macular degeneration. Because there it can be very -- more rapid in its progression and we do not really have good treatments, and we have some treatments that are marginal in their effect but we really do not have good treatment.

And I think there is a case where we have to be a little more careful and again I think we have to screen patients. Although most of the data suggests that there may not be an effect. I think that is a group of patients that if we identify them in a study that they should be monitored closely as well.

DR. ISNER: Carl, is there data available as to how well these gene products cross the blood ocular barrier?

DR. CSAKY: Right. So for the most part in the normal animals -- so again one of the problems with let's say doing an animal study with looking at systemic effects in the eye is that there is blood retinal barriers that prevent access per se. In disease states that changes. In diabetes that changes. In age related macular degeneration that changes. So that now you have got a change in that dynamic.

So potentially especially with AMD where you have the choreocapillaris being exposed, and that is the site where the neo vascularization occurs, you could -- there is no reason to suspect that you are not getting a VEGF effect or potentially a VEGF effect at that site.

So again it is the fact that in the human condition all the animal information just is not applicable.

DR. MARKERT: Louise Markert, RAC member.

I have a question for Dr. O'Reilly. I am not entirely sure that the model you described of latent disease is what I worry about with respect to VEGF potentiation of tumors. You have these mice with latent tumors and the immune system is just asleep and all those T cells are doing nothing and NK cells doing nothing about those little cells that they should have gotten rid of.

Well, I may be standing here and there may, in fact, be some little -- a few cells somewhere in me trying to form a tumor and I may have T cells or NK cells there producing all these little factors to try and kill those cells. And it

may be that in that situation with the immune system creating all these little factors plus the VEGF being given, maybe that would be enough to cause a problem.

Could you comment on that hypothesis?

DR. O'REILLY: Actually it is an interesting hypothesis. First of all, I am beginning to think that everybody has in situ cancer. If you look hard enough you find it and there are a lot of studies that suggest that. The studies of prostate cancer that if you go through the prostate with a fine tooth comb and you get old enough and you are a man you are going to get a prostate cancer. It is just that the minority of those actually grow, which suggests that perhaps the reason there are some of these angiogenesis inhibitors out there waiting for me and others to find is that they are having this protective function of keeping in situ disease in situ.

In terms of the immune reaction to these, these are the mice -- many of these mice in these studies were all immunocompetent. They had perfectly active T cells, perfectly active macrophages that were functioning normally, and yet still had this dormant disease that could not be eradicated.

But, as I said, these were studies that initially we thought the mice were cured. It was only after taking a lung and spending -- staying up all night going through it one section at a time that I was able to find this dormant disease. So it was much more widespread.

And the immune system did not seem to be T cell dependent since I did the same experiments in nude mice, SCID mice, and beige mice, which were

deficient in NK cells, which is again why I think it is not only the immune system or angiogenesis. It is not -- I do not think it is any one system that is regulating this. I think it is the interaction probably of a number of control mechanisms that are keeping these dormant, which is probably bad for me to say as an angiogenesis person that it is not just angiogenesis but that is the way I am starting to think.

But again you are right. I agree that these are mouse models. They are not directly translatable but still at this point I would rather have a shot of VEGF than a cigarette in terms of its cancer causing potential.

DR. COLEMAN: Coleman from Valentis.

A question for Dr. Springer. If I am not mistaken the cells that you used in your ex vivo delivery strategy were C2 cells or were they endogenously derived?

DR. SPRINGER: No, they were primary myoblasts.

DR. COLEMAN: They were primary myoblasts. Okay. I think it is still important to note that a caveat to interpretation of you data is that during the fusion process those cells will up regulate endogenously in an autocrine manner, IGF1 and IGF2, so at least transiently until fusion has taken place there are going to be other growth factors present.

And I wanted to pose as a question to the panel, you know, what potentially is the endogenous tissue milieu in the target organ a determinant of a response to the angiogenic factor? I think one of the most interesting things about Dr. Dvorak's data if I recall those data correctly is that different tissues

responded differently in terms of the persistence of the vessels to the injection of the adenovirus expressing VEGF.

And I am aware of some data that Keith March's group has collected and at least reported in abstract form showing that the concentration of angiogenic factors in pericardial fluid decreases with age.

And the other point I would bring up here is that I am sure we are all well aware that at least in the animal models of acute ischemia that endogenous VEGF is up regulated and recently there was a paper in the New England Journal showing that in ischemic myocardium VEGF and HIF-1 alpha were up regulated in the ischemic zone but not in the well perfused zone of the myocardium.

So, you know, is there potentially a way to screen patients for those that are going to be more responsive to the therapies and identify the target patient population that way?

DR. SPRINGER: I guess I can start. That is quite a mouthful and I am not sure if I will be able to remember everything. But the two main comments I wanted to make about that, with regard to the myoblasts transiently expressing other growth factors, that is a very good point and it is something that we should keep in mind. It also is why the controls are so important because the controls go through the same growth factor up regulations, et cetera, that the VEGF myoblasts do.

We cannot rule out the idea that whatever growth factors are being transiently expressed play a synergistic role with VEGF. That would be difficult

to rule out.

The other thing is getting back to Harold Dvorak's work. His paper really had a lot of parallels with our's and it is true that depending on the tissue that he put the VEGF gene into, the results were very different. They all started with formation of mother vessels and then depending on the tissue the vessels either degenerated into smaller tangles that resolved themselves or they formed muscular vessels that we saw, the arterogenesis.

So tissue specificity makes a big difference and actually the biggest difference, I should point out, between Dvorak's system and our system is his is transient because he used adeno. We have continuous expression in our system.

There was one thing you said at the end. What was the last point?

DR. COLEMAN: (Not at microphone.)

DR. SPRINGER: Right. And actually before I turn it over to the rest of the panel I did want to comment about that because most of the work done with VEGF in the past has been with ischemic myocardium, ischemic peripheral limb muscle, and everything that we have done in our studies has been with nonischemic muscle and nonischemic tissue.

We have been doing work with people in cardiology at Stanford to use ischemic hind limb models similar to what Jeff Isner and others have used to see what the effect is and we actually have been having a difficult time doing that because the endogenous revascularization keeps coming up before we can see the effects of our VEGF. So I cannot really say much more about that.

DR. DAWSON: Kevin Dawson, Baltimore.

In the early growth factor work there is pretty convincing evidence that the angiogenesis proceeds up concentration gradients and from that standpoint it makes sense that systemic delivery of the VEGF protein would not produce angiogenic effects in that the effects that particularly Dr. Isner described related to hypertension or to edema would be the type of systemic effects that we would see from systemic VEGF.

What I am wondering is whether or not our concerns should be not systemic VEGF but systemic delivery of the vector because we have very good evidence that delivery of the vector through the cardiac tissue causes systemic delivery of the vector and that maybe our concerns should be not the VEGF being uptaken in the local tissue but that the low probability of event that the vector is taking up in the tumor or in some other potential target would cause local concentration gradients of VEGF and that is where our problems would actually appear.

DR. FRIEDMANN: Yes.

DR. SERABIAN: I just want to say that is one reason why we do ask for biodistribution studies of the vector, too, to see where it does go. It is important.

DR. DICHEK: Maybe this is an opportunity for Jeff to clarify something about the systemic distribution of VEGF.

I guess I went to a presentation by your group at the AHA where I think the bottom line was that you should measure serum VEGF or you should

not measure -- you should measure plasma VEGF and that they looked very hard and could not find systemic VEGF but I saw you presenting serum VEGF.

So what should we be looking for in the blood and how should we be measuring it and is it really distributed systemically?

DR. ISNER: So I think the answer is when we did our initial studies as somebody showed, Rob Simari showed a slide this morning of serum VEGF levels that were measured in a series of patients that were reported by Iris Baumgardner. At that time we were measuring VEGF by looking at samples of serum. It subsequently became clear from a number of laboratories that if you used serum you may over estimate the magnitude of VEGF expression because as platelets clot they release VEGF.

And so it was then recommended that one use plasma to make those measurements and that is what we have used subsequently in our current studies. And so it seems at least that theoretically plasma levels of VEGF ought to be more consistent and reliable.

Now looking at the experience both with serum and with plasma, you do get elevated levels of VEGF. They are in the picogram per milliliter range. And they are very short duration so, you know, you typically see something going up and peaking at about seven to twelve days and coming back down by roughly 18 to 20 days.

But those levels, as I say, are really quite low and so that again the notion is that if the systemic distribution is transient, short-lived and at low level, that should help to minimize the risks of all of these various considerations that

we are discussing here today.

DR. _____: This is a question for Dr. O'Reilly.

One of the things we grapple with is whether the VEGF is a cause or effect in tumorigenesis. Now I was trying to find out from you if there are any animal model, tumor animal model where you have VEGF gene delivered and then you can study whether that actually de-accelerated the process or do you know of any model?

DR. O'REILLY: You mean of a mouse that is VEGF negative?

DR. _____: Yes.

DR. O'REILLY: It is lethal. VEGF knockouts were lethal. In terms of tumors that are VEGF negative, there are tumors that do not produce VEGF in significant quantity and make other growth factor -- other angiogenic factors and do fine. There are also tumors that have been transfected or normal fibroblasts that have been transfected with the gene for basic fibroblast growth factor that will become tumorigenic without at least tumor derived VEGF.

The problem with most of those systems is that tumors are quite good at inducing normal stromal cells to produce VEGF or mobilize VEGF and so, in fact, much of the VEGF or at least some of the VEGF that is stimulated in tumor growth is not from the tumors. It is from the surrounding stroma that has been induced to produce it by the tumors.

So I guess that was a long way of saying no, I do not know the answer to your question but I do not think it can be done, at least technically could be done adequately to prove or disprove the hypothesis.

DR. FRIEDMANN: Dr. Russell?

DR. RUSSELL: Yes. I just want to harp back again to this question of transgene expression monitoring because it is clear with VEGF that low levels are good, high levels are bad. The clinical data looks pretty exciting but, you know, it is embarrassing that here we are without any direct evidence that the gene is actually expressed in these patients without any knowledge of what the level of expression is if it is expressed.

And, you know, if we do detect circulating levels of VEGF then how do we know it is from the transgene versus endogenous? So there is really a need for some kind of an innovative thinking about how to address this question.

DR. ISNER: I think there is definitive evidence that the gene is being expressed. I mean, now in, you know, a lot of patients.

DR. RUSSELL: What is the evidence?

DR. ISNER: Well, I would submit that the evidence is that if you look at the levels of circulating VEGF before gene transfer and then several days afterwards you see a rise in VEGF levels that are not seen in control patients, for example, that receive an intra-muscular injection of saline. We have done that study. And then you see that VEGF level come back down to baseline within a period of a week or two.

So although the levels that are seen are low, there is a clear and statistically significant increased followed by a return to baseline. And while that may not be, you know, ideal, I think it would be better, of course, if we could harvest tissue and look more directly at the local of expression, which is clearly

likely to be far higher. Unfortunately, you know, there are practical limitations to doing that but it seems to me that those circulating VEGF levels should be reasonable surrogates.

DR. FRIEDMANN: What would you like to see, Steve, for proof of expression other than for rises?

DR. RUSSELL: I do not know. I mean, I just think it is a critically important expression. You know, as I was saying, it is the gene -- in this situation it is the gene product that is the drug. And we give it in a prodrug form that is variably converted into the actual gene product and we really are, you know, in our infancy as to how we actually determine what the efficiency of that conversion is.

I think maybe linking the expression of the VEGF to some marker gene product that could be measured in the serum could be the appropriate way to get definitive information.

DR. ISNER: That would be much more useful and elegant. I can tell you just in case it is reassuring at all that we have looked at a couple of other physiologic markers, if you will. For example, if you look at the edema studies, the development and resolution of edema as was published in that Annals paper perfectly parallels the upswing and return to normal in the VEGF levels that were sampled.

And in another study looking at mobilization of bone marrow derived endothelial progenitor cells there is a similar associated rise and fall in circulating EPCs associated with a rise and fall of the circulating VEGF levels.

So, you know, there is some evidence there, although I certainly understand why it would be ideal if we could, you know, confirm that with some other marker studies.

DR. _____: Plasma levels would not reflect the tissue levels. You know, you really do not have any monitoring from that. I mean, the heparin binding sites and so forth, plasma levels may be zero while the tissue levels may be quite high.

DR. ISNER: That is true.

DR. DICHEK: You know, Jeff, my recollection of that data that your group presented at the AHA was that they looked at a tremendous number of data points and groups and that there was only one data point in one group where there was a very, very marginal difference in serum or plasma VEGF.

And that -- I just was not convinced by that data and maybe you have other data -- maybe Rob, I know, was chairing the session and Simari -- from that I was not really completely convinced that there was detectable plasma VEGF. Maybe you have some other data.

DR. ISNER: Yes. Well, two of those studies -- four of those studies now have been published and, you know, I mean I would refer you, I guess, to those four published studies which, you know, clearly showed a rise and fall.

I think one of the points that Ben Friedman made at that presentation was that in an individual patient, you know, if you look at the group as a whole, there was a rise and a fall and most patients went up and down. In

individual patients there may not be a detectable rise or the rise was certainly quite variable. Again always in the picogram per milliliter range but quite variable. So linking clinical events to the exact absolute level of VEGF was difficult to do.

But I would be happy -- I can -- I would be happy to show you that data.

DR. FRIEDMANN: Bob?

DR. ROBERTS: I wonder if at this point it would be a good idea to have someone spend a minute on talking about the different types of VEGF, the ones that bind to heparin and the ones that are soluble and do not because I understand from some of the people in the audience that there is quite a difference in terms of what you would expect, whether they are one or the other, and who would like to just give the ABC's of that?

DR. ISNER: So Ron Crystal will be talking shortly about VEGF-121. I am sure he will go into some detail about that. There are basically three isoforms of the VEGF-1 or VEGF-A gene, 121, 165 and 189. There are a number of others but those are the three with which there has been the most experimentation performed.

And as the number goes up so does the number of amino acids and the heparin binding property of that isoform. So 121 diffuses most widely. It is not to any serious extent. Heparin bound 189 is extensively heparin bound and diffuses not much at all. 165 is sort of in the middle. There are other VEGF genes, VEGF-2, VEGF-B, C and D. But those are the three isoforms that have

been used with VEGF-1. And in terms of which -- there is contrary data in terms of isoform specific functions or consequences.

We did a study several years ago, which was published and showed that in the rabbit ischemic hind limb model 121, 165 and 189 all appeared to have identical consequences in terms of promoting angiogenesis. There has been some other data in some genetically engineered mice suggesting differences in the effect on the heart between 121 and 165. But that is, I guess, the short version.

DR. FRIEDMANN: What is the significance at the heparin binding domain and is that fully intact in the intermediary structure, the medium length one? And, in fact, that is of course one of the questions that we have asked the panel to think about. How does the physiology, the pathophysiology of ischemia, either peripheral or in the heart lead one to the choice of one or the other of the VEGF isoforms? How do you decide which one is likely to be most effective?

DR. ISNER: I think that is a great question. I would guess that to some extent, you know, it has been empirical. I mean, we started out -- the first gene that we had access to was 165 isoform and that seemed to work well in the animal models and so we just proceeded with that.

DR. FRIEDMANN: The main structure of the protein is well characterized so that one knows where the different functional properties of the --

DR. ISNER: Yes. Right. I think for the isoforms of the VEGF-1 gene it has been very well characterized, and the development of a number of mutants and so forth. But I think that relating the kinetics of cleavage, for

example, of those isoforms and relating that to functional outcomes -- I think that is not so clear. It is not so clear that -- how those differences in length and cleavage may lead to different outcomes when those differing genes are used for these kinds of strategies.

DR. FRIEDMANN: And again the heparin binding function, what is the significance of that?

DR. ISNER: Well, initially there was some thought that if you -- you know, you could argue it both ways. If you had something that was avidly heparin bound then when you injected it locally most of it would stay there locally and maybe for a long period of time.

DR. FRIEDMANN: The protein?

DR. ISNER: Yes, the protein or the gene product for that matter. When the gene was produced by the cells in which it was transferred, gene product might remain locally and for some period of time. With 121 maybe the opposite would happen. But I do not think there is a lot of data to again suggest that those theoretical notions have had clearly identified, you know, predicted consequences.

DR. FRIEDMANN: Do you think that it has anything to do with the mechanism of cellular uptake of extracellular VEGF? Is it related to binding sites, to receptors on the cell?

DR. ISNER: To my knowledge, there is no evidence of that but maybe, you know, Ron might want to -- is Ron around? Ron might want to comment on that. I am not aware of that kind of data.

DR. FRIEDMANN: We will catch him later.

DR. _____: I have a comment about that, Ted.

DR. FRIEDMANN: Yes.

DR. _____: Garen Neufeld for the VEGF-A gene anyway, Garen Neufeld has shown that EXON 6A and 6B have separate functions. One is primarily heparin binding and the other is proteoglycan binding. Those are usually both expressed in 165 so that --

DR. FRIEDMANN: Is that proteoglycan binding the heparin domain?

DR. _____: No. Two different domains. Okay. It is EXON 6A and 6B and you can separate those functions with one heparin in one case and without in the other. But what the importance of that biologically is, is not clear.

But there are some interesting experiments that were published a year ago with FGF which seemed to support a hypothesis that Andrew Baird has had for a number of years that the teleologic purpose of these binding domains is to keep the growth factors locally so that when you have local injury, local tissue healing, you can get local production and not have them have systemic effects.

So there was a transgenic mouse model that was developed that over expressed FGF with an eye specific promoter and those transgenic mice were blind because they had some excessive growth of blood vessels and endothelial cells in the eye.

They then engineered the heparin binding domain out of FGF so that it was no longer heparin bound. Those transgenic mice then had multiple

systemic congenital anomalies. Suggesting that Baird's hypothesis is right and that the functionality of that binding domain is to keep the growth factors local. And I think that was the theme that was reflected all morning in talking about delivery to the organ and trying to get a growth factor to be more concentrated in the organ of interest than in other places in the circulation.

DR. FRIEDMANN: Bob, do you have a question?

DR. ROBERTS: No, that was my point. I mean, that certainly I think that has been the prevalent idea and I think that I had spoken with someone this morning who was from Valentis, I think, I saw him earlier, who had indicated to me that I got the impression that when it does bind to proteoglycans or to the heparin-like that it was active and it appeared to increase the concentration and there should be some evidence to indicate that you had a better response when you had binding to either of those but I take it that that gentleman is not around now.

DR. BYRNE: I just have a comment that I think all the gene therapy protocols make use of a local -- of a region of delivery which generates a depot of cells. Those cells are bound to have a gradient and whether that gradient is high because there is tissue binding or lower because there is active secretion in the protein is dependent on the binding properties of that protein.

So, you know, I imagine the gradients at least in some studies, I think, that have been done with secreted proteins in the lung is up to three orders of magnitude. I do not know whether you have any data from VEGF studies in skeletal muscle which demonstrate the difference between tissue level expression

and plasma.

DR. ISNER: No, but I will tell you the best study that I know of in that regard is a paper that was published by a Japanese group in Cancer Research about three years ago or it was Cancer about three or four years ago, and they looked at patients who were undergoing removal of glioblastomas and they measured the VEGF levels that were circulating in those patients at the time of surgery and then measured the amount of VEGF that was in the tumor.

And the amount of VEGF that was in the tumor was about 100-fold greater using an ELISA assay than what was circulating systemically. So I think that is consistent with what all of you are suggesting.

DR. FRIEDMANN: Let me just suggest, we have ten minutes or so before our scheduled coffee break. I wanted to invite the panel and then the audience also to look at some of the questions that we have asked the panel at least to consider. We have gone over some of the issues but there are several that we have not discussed. We have asked them to think about what features of transgene structure function are relevant to the selection of specific transgenes in clinical models. We have asked them to think about what it is about pathogenesis that might drive them to selection of one or the other transgene.

What the expression of those transgenes might be on critical -- let's say -- cardiac functions such as conductivity and electrical activity and contractility? And that is one area that we have not had much discussion about today.

So let me ask if there is anyone on the panel or in the audience

who has either has information on or comments about the effect of gene transfer vector delivery to the development of hemangioma or tumors in the heart as a result of these kinds of manipulations as followed by assays for tests of electrical activity and contractility. What do we know about the effect of the manipulation on these properties of the heart?

QUESTIONS FROM THE AUDIENCE

DR. SPRINGER: I am sorry to say that in our own heart study we cannot answer that question because the mice had all died by the time that we got around to examining the hearts.

DR. FRIEDMANN: Are they feasible questions in the mouse model?

DR. SPRINGER: I believe so. Yes, I believe so but I think that I should probably defer to Jeff Isner on that.

DR. FRIEDMANN: Are there any data from larger animal studies? What do we know about the heart as a pump?

DR. ISNER: I think Eduardo was going to comment on that issue of electrical.

DR. BYRNE: I can make one comment in a naturally occurring disease model in dogs that get hemangiomas that affect myocardium. They do die of heart failure and whether that is because they have diastolic dysfunction or high cardiac output, it is not clear but there is a naturally occurring animal scenario similar to what you are suggesting.

DR. ISNER: The electrical system aside, I mean I think the one

thing I can comment on and I mean I -- this is, you know, I think important to understand. We have looked at larger animals. We have looked at mice. We have looked at rats. We have looked at rabbits and we have looked at pigs. And there is probably all together, you know, 750 to 1,000 animal experiments there where animals have gotten in some cases ten times the dose of VEGF at least in the case of naked DNA that we have administered to patients.

And we have never ever seen anything resembling an angioma or hemangioma in any of these studies and that was with fairly extensive sectioning of the hearts and/or the limbs. And I think that --

DR. FRIEDMANN: Is that with one particular isoform of VEGF?

DR. ISNER: That would include experience with the VEGF 165 isoform of the VEGF A gene and it would also include a large body of experience with VEGF-2, a different gene but another VEGF family member.

DR. FRIEDMANN: So for other angiogenic -- either induces event of angiogenesis or inhibitors of angiogenesis or whatever, for other such functions one would want that kind of information presumably?

DR. ISNER: I think we have also again with the folks at Genzyme looked at HIF-1, the protocol that Alex was describing in both again rabbit hind limb models and the swine cardiac model and have never seen anything resembling an angioma and I suspect that Kirk was about to say that he has probably not seen the same thing with basic FGF.

DR. _____: I was going to address your question mostly about cardiac arrhythmia but we have not -- I have been doing this since 1993 and

have done -- if I have not done 1,000 pigs it would surprise me and I have never seen a hemangioma and we have used VEGF and FGF in combinations in a variety of different settings. Mostly in the setting of myocardial ischemia I might add. It may be different when you put this into a normal milieu without other corresponding growth factors.

In terms of arrhythmia, with the adenovirus vector by the intracoronary route, arrhythmia is not a problem. The -- I think the most persuasive data that we have in that regard, we always monitor --

DR. FRIEDMANN: So with --

DR. _____: With intracoronary administration --

DR. FRIEDMANN: -- intracoronary.

DR. _____: -- of an adenovirus. The most persuasive data perhaps is data that we have in which we have put in 300 times the highest dose that we have used in the clinical studies and then did Holter monitoring for 14 days subsequently and counted all the beats.

DR. FRIEDMANN: In pigs.

DR. _____: In pigs. And there is not a shred of evidence of tachycardias or premature ventricular or atrial contractions. They were treated with both saline, with transgenes -- with adenovirus encoding transgene and with adenovirus encoding EGFP. So it just does not happen and I think the clinical data would support that as well.

DR. FRIEDMANN: Kirk, then how would you interpret the results demonstrating in this myoblast transplantation study?

DR. _____: Yes. There was another study --

DR. FRIEDMANN: How do you see that?

DR. _____: There was another study that is not widely recognized even in your own article that Bob Cloner published about a year before your's where plasmid VEGF was used in an infarct model in a rat and they also found hemangiomas.

DR. SPRINGER: Actually I wanted to ask about that because, Jeff, you were on that paper, too, right, the Cloner --

DR. ISNER: Yes.

DR. SPRINGER: -- paper. I did not mean to cut you off. I just wanted to sort of jump in.

DR. _____: So I do not know if that is a peculiarity of the model or the result of enormous local doses of VEGF into the --

DR. _____: Yes. I think there are two really different situations. In the Cloner study at least there was corresponding ischemia there so you had other growth factors up regulated that could work in concert with VEGF. In this study I do not think there is any ischemia present.

DR. _____: No, there is not.

DR. _____: There is another source of this, though -- there is another source of this that we should mention and that is work by Ian Koppoulous at Regeneron and I cannot remember where they published their work. But when they over expressed VEGF using a cardiac directed transgenic mouse model they find these -- the phrase "wispy vessels" came into the lexicon

of angiogenesis after that paper.

And his tenet is that monotherapy with VEGF or probably any growth factor does not make normal vessels but, of course, the pitfall with those studies is never talked about, is that those animals were not ischemic. So, you know, they did not have the corresponding up regulations of a variety of other factors which added together were not enough perhaps to cause angiogenesis but when you put an exogenous gene in they were and then vessels could be quite normal.

The vessels that we make in the hog are normal. They appear histologically normal and they persist. They are persistent vessels. So these comments earlier about how there has never been a vessel or any evidence it lasts long, that is nonsense. That just belies an unfamiliarity with the literature.

DR. ISNER: Just -- I had a slide and I did not have a chance to show it because of time related to the Cloner study and what Bob had done was he had asked us for some plasmid DNA because he wanted to see if administration of VEGF at the time of an acute infarct in a rat would salvage ischemic myocardium. So he took the same dose of VEGF that we have been using for the rabbit. Obviously a much bigger animal. 500 micrograms and injected it locally into one spot in the right ventricle of the rat after it had been made ischemic. And he did see, as Matt and Kirk suggested, the development of angiomas that were similar to what was described or Matt just described.

However, when that study was redone with half the dose, at 250 micrograms, there were absolutely no hemangioma formation observed. So I

think the lesson from that is that it is a function of dose and the length of expression. I think that if have high doses and/or persistent duration of expression that is when you start crossing the line and incurring the risk of some of these other complications.

Does that make sense?

DR. SPRINGER: Yes. I think it is all very consistent with the idea that it is dose specific and that -- and, by the way, I do not want it to seem like we were slighting the Cloner article, we had already written a paper when that came out, which is why it was not in our's.

DR. UNGER: Ellis Unger from Center for Biologics at FDA. Formerly of Cardiology Branch, NHLBI.

We looked at a number of growth factors in ischemic myocardial models, predominantly dog, and in the late '80s actually we found evidence of angioma formation in dog hearts with LAD amaroids. This was with FGF-1, acidic FGF applied in combination with gortex sponges. This was published in '91 in Circ Research and there was one example of a tumor. I would call it a tumor that almost looked like a leiomyoma in the anterior wall of the heart.

I believe that is the only example of FGF-1 and that is the only observation of that effect but certainly we observed it.

DR. SPRINGER: I will just comment on that. One of the reasons that we decided to use VEGF instead of FGF or other growth factors is that we were worried about pleiotropic effects and we wanted something that would be as specific as possible and that was certainly one of the reasons that we felt that

FGF or something like that would probably have a larger chance of getting some other kind of tumor.

Interestingly, they have been doing work recently with FGF that has not been showing tumors as far as I know but that was the rationale.

DR. UNGER: We used FGF-2 in probably a couple hundred dogs after that point and did not observe tumors.

DR. FRIEDMANN: Can you bring us up-to-date on the difference between the FGFs?

DR. UNGER: FGF-1 --

DR. FRIEDMANN: 1 and 2 and how many others there are.

DR. UNGER: Well, there are many FGFs. I mean, those are the two. Basically the best known of the FGF family. That is about an hour talk which I could not give with updated information. There are definitely differences. There is FGF -- there is -- actually I do not know whether one of you would like to talk about or will be talking about FGF-4 or FGF-5.

DR. FRIEDMANN: Okay.

DR. UNGER: It is --

DR. FRIEDMANN: We will hear about that.

DR. UNGER: It is too much.

DR. FRIEDMANN: Let's have one more question from the audience and then have a break.

DR. _____: This was actually going to be a comment and I am not in the research lab. I am a clinical electrophysiologist. I thought I could

comment on your question on arrythmia genesis.

I think there would be two separate types of concerns. In terms of brady arrhythmias, some of the models particularly of direct injection show extensive fibrosis and so a concern if you are injecting around either the sinus or AB node would be that that level of fibrosis could inhibit function of those nodes and cause brady arrhythmias.

In terms of tachy arrhythmias, it depends on a couple of things. One of the biggest concerns in terms of fixed reentry is a site of conduction block and so if you are creating any sort of scar or any sort of obstacle in the myocardium like a hemangioma then the potential exists for conduction around that which could set up a tachycardia. One of the best examples of this would be tachycardias around scars after cardiac surgery.

The reason I bring that up is because those are tachycardias that we do not see for ten to twenty years after the surgery and so it would be difficult to assess those risks in short-term studies.

DR. FRIEDMANN: In terms of some of the large scars?

DR. _____: Scars of one or two centimeters.

DR. FRIEDMANN: In crucial areas.

DR. _____: No. Usually these are atrial arrhythmias related to scars from the cannulation for cardiopulmonary bypass and so the scars are the size of the cannula which are maybe a centimeter and they just exhibit changes over time that leave people predisposed to these arrhythmias.

Other types of arrhythmias would be dependent on the choice of

transgene and for the transgenes that we have discussed today there are not obvious concerns but one of the things that has not yet been discussed is the myocardial gene therapy literature and there is a growing literature on beta adrenergic receptor or beta adrenergic receptor kinase gene therapy. And any type of manipulation of the beta receptor system could be expected to have arrythmogenic side effects if not controlled.

DR. FRIEDMANN: You would expect that?

DR. _____: Right.

DR. FRIEDMANN: Dr. Marban?

DR. MARBAN: I apologize for having stepped out during the discussion or the question earlier but I think it is worth noting that there are three fundamental factors which promote arrythmia genesis in any situation. One is tissue level heterogeneity. The myocardium consists of a couple of syncytia of cells which are spot welded to each other electrically so that the influence of one cell is transmitted for approximately a millimeter. So one cell influences a core of perhaps 100 surrounding myocytes.

Anything that makes the electrical behavior of the syncytium heterogeneous promotes the possibility of unstable reentry and the precipitation of potentially lethal arrhythmia.

Another is slow conduction.

If there are regions in the tissue that are marginally coupled to each other in which conduction is slow from one cell to another, that is a potent arrythmogenic phenomenon. In fact, Glen Fishman, who is going to be up here

momentarily as part of Panel B, has produced a mouse model which intentionally produces patchy coupling and slow conduction, and it is highly arrythmogenic.

Altered repolarization would be a final pro-arrhythmic phenomenon.

And, in general, cells that are living on the edge have highly altered repolarization properties.

I cannot imagine a more pro-arrhythmic recipe than to put in, you know, sprouting little vessels into marginally -- otherwise marginally diffused myocardium. And certainly quantitatively you could take a matrix like that and argue quite convincingly that you are worse off from an electrical stability point of view than having a completely solid infarct that is not full of little canals.

So I think in the general category of unintended consequences one would have to say that this is a highly nonlinear system. It is a complex system and one in which you could easily imagine it is an unintended consequence arrythmia genesis or pro-arrhythmic effects as a result of vessel sprouting.

It would be a situation where there would be a clear counter example to the general truism that more vessels are always better.

DR. _____: Eduardo, I have always shared your concerns about the homogeneity issue but I have to say when Ron Crystal gave his first presentation to the RAC and talked about the potential effects of gene transfer to areas of the heart, he started out with a picture of transmyocardial laser revascularization where, you know, an investigator took a laser and put 50, you know, blew 50 holes in the heart. And Ron basically said, "These people do

fine." And I had to scratch my head and say, "Gee, you know, that is interesting."

So I would be interested in your thoughts on maybe, you know, when you burn 50 scars into the heart focally why those people end up doing -- they seem to do okay from the standpoint of rhythm.

DR. MARBAN: If the tissue is already dead it is not going to make any difference if it has 50 holes in it.

DR. _____: Let's say it is not though.

DR. MARBAN: Well, I think the devil is in the details and if you purpose -- if you have 50 channels that produce such burn injury or such tract injury that you effectively uncouple them from their neighbors, they are not going to influence their neighbors. You can imagine a situation where you produce kind of a deadly matrix effect and it is always easy to explain why things happen to go right but what we are looking for here is reasons that they might conceivably go wrong. Right?

DR. _____: No, I certainly agree but I am astounded actually at the lack of deleterious -- of the apparent lack of deleterious effects of the laser revascularization.

DR. MARBAN: Some of these things might not be immediate consequences. They might be long-term remodeling consequences. Just as if you give a drug and it wipes out PVCs, everybody feels happy that the Holter looks good and it is only when you do mortality data that the patients seem to be dying more frequently.

DR. ROBERTS: But I would say also about the laser to the heart

that I am not sure that it has undergone the scrutiny to prove that you had that many new vessels growing out into tissue that was alive and if you look at that versus the placebo group that data certainly is not available.

DR. ISNER: Eduardo, I am just wondering converting that sort of -- those concerns into a practical approach to things, what would your level of concern be in terms of how intensively one should be looking, you know, for that kind of consequence clinically in these patients that were treating these protocols?

DR. MARBAN: I think what I would recommend specifically is not throwing the baby out with the bath water but rather looking quite carefully at electrical stability as one endpoint. It would -- and the way to do that would range from the easy and noninvasive, getting 72 hour Holters, to actually at least in some selected patients looking for -- looking invasively at inducibility of arrhythmias using electrophysiology protocols.

That would be kind of a safety monitoring issue that would not necessarily need to be done routinely but would be looking for deleterious consequences in a subset of patients.

DR. ISNER: Do you have an idea about what time point would be ideal to carry out that kind of study?

DR. MARBAN: Well, a priori one complex issue is that cannexin cells are coupled to each other by gap junctions and the principle electrically conducted proteins that make up the gap junctions are cannexins. Cannexin turnover is quite rapid so that the lifetime of a given cannexin is measured in tens

of minutes, if not hours. So you can imagine a very, very quick electrical remodeling but by and large electrical remodeling follows tissue remodeling.

So if you put in a vessel and you now start to regenerate myocardium around it, I would expect that the chance of deleterious consequences would be pretty closely related to the increase in vascularity. So whatever the time course of that is I think would be an appropriate time course.

DR. FRIEDMANN: What kind of transgenes should we be afraid of in this -- in a tissue which is marginally stable and waiting to demonstrate electrical aberrations? Let's say that we believe the empirical data that the VEGF and maybe other agents that induce neo vascular formation in that region do not destabilize there. But what kind of transgenes should we be wary of? There was a comment about beta adrenergic receptors. What other transgenes would you fear?

DR. MARBAN: The comments that I have made are applicable to anything that induces mechanical -- that induces a change in the microarchitecture of the myocardium so they are quite generic for all transgenes that would cause vessel sprouting.

You could of course imagine transgenes that would be -- that would add insult to injury by having paracrine effects but I am actually limiting my comments to the best case scenario and not to worse case scenario.

DR. FRIEDMANN: Dr. Dichek, and then we will have a quick coffee break until about 25 after.

DR. DICHEK: I would just like to raise a point that I alluded to in

my presentation, and that is the issue of whether any of these interventions are increasing survival. I would doubt that based on what we expect from angioplasty and bypass surgery in many cases where we believe ischemia improved quality of life but do not improve survival.

The idea that we are balancing risk against benefit I think is very important and I wonder if there are individuals who are expecting survival benefits and what their rationale is. If those rationales are not real firm and turn out not to be borne out, whether we should be talking about saving lives with these therapies and whether that is really the best way they should be presented.

I open that up to anybody who would like to respond to that.

DR. FRIEDMANN: Okay. Let's have a break. Let's reassemble at 3:30 flat.

(Whereupon, at 3:16 p.m., a break was taken.)

DR. PATTERSON: If everyone could take a seat and be quiet, please.

I would also like to take the opportunity to ask you to fill out the evaluation forms. We want these safety symposiums to be as useful as possible to the participants and the only way we can do that is if we get constructive and critical feedback from you. So please take a couple of moments to fill out the feedback forms and hand them in before you leave.

Thank you.

SAFETY CONSIDERATIONS IN CLINICAL TRIAL DESIGN:
STUDY CONTROLS; INFORMED CONSENT;
AND THE SELECTION, MONITORING, AND
FOLLOW-UP OF RESEARCH PARTICIPANTS

DR. FRIEDMANN: Okay. Let's move ahead then. This is the last session which has to do with the safety issues with respect to design of clinical studies and particularly with respect to questions about controls, selection of controls, selection of patients, subjects, research subjects, how they should be monitored and for what.

And the first speaker in this session would be Kirk Hammond from UC San Diego and he will be talking about the design of clinical studies involving adenovirus into the heart.

CLINICAL TRIAL DESIGN AND MONITORING
KIRK HAMMOND, M.D.

DR. HAMMOND: Thanks, Ted.

Since Ted sort of brought it up, I may as well admit that I am the scientific founder of Collateral Therapeutics but I have never been an employee of the company, although I do have a consulting relationship with them. I am a full-time professor at UCSD.

(Slide.)

I want to talk rather fast because I want to try to cover the questions that were in the material as well as give enough of a view of the preclinical data to let you know how it was that the clinical trial that we did was

done. In other words, it has to be based on the kind of safety and biodistribution data that the preclinical data provided.

But, first, I want to talk about the rationale for this kind of treatment. And by the way, we are not doing it to prolong life. Although, in theory, that could happen. And how one selects the patients. And then in the last few slides we will talk about the clinical trial design and what the important factors are.

(Slide.)

Okay. To start with, which patients should be enrolled in angiogenic gene therapy trials?

(Slide.)

Well, I think you could ask this question: Does angiogenic gene therapy potentially treat a serious or life-threatening condition, address an unmet medical need, or reduce morbidity and side effects of available therapy? And I think that answers to these questions provide guidance regarding patient selection for clinical trials.

(Slide.)

What about serious or life-threatening? Is angina serious or life-threatening? Well, every minute another person dies of coronary artery disease in this country. These patients often only have 30 or 40 percent stenosis on coronary artery but have plaque hemorrhage. It is the most common cause of acute myocardial infarction and death.

So clearly angina is a serious condition. Each year a million

bypass surgeries or PTCA/stent procedures are performed in the U.S. and the prevalence of angina is over seven million patients.

(Slide.)

What about an unmet medical need or reduced morbidity or side effects from available therapy? Well, despite the proven role of a coronary artery bypass graft surgery, morbidity and mortality remain high in some patients. With respect to stent there is less morbidity and mortality but restenosis remains a problem. Many patients are unsuitable for either of these procedures and medical therapy can be ineffective or intolerable in many patients. So you do really have several factors that would suggest that there is an unmet medical need and that you would get either reduced morbidity or reduced side effects.

(Slide.)

So we see ultimately whenever this may happen, I am not going to wager exactly what year this will take place, but I see the therapeutic options for cardiovascular disease to have in addition to drug therapy and bypass surgery and PTCA/stent, the possibility of angiogenic gene therapy. And even though on these diagrams, these are independent therapies, I think that in many respects as time goes on they will be complementary.

(Slide.)

The manner in which the company that I am the scientific founder of and that is doing clinical trials -- by the way, the only placebo controlled and double blinded clinical trial that is complete in terms of Phase I/II trial. This was done by nonsurgical delivery of the adenovirus into the coronary artery and it is

depicted in these cartoons for those of you who are not familiar with this technique.

But there is no real cut in the skin. There is no thoracotomy. A catheter is placed into the femoral artery and it goes up into the coronary arteries where the adenovirus is delivered. It is delivered into major conduits that feed the heart muscle, the left and right coronary arteries in the case of IMA grafts or vein grafts. It is delivered there as well.

The advantages is that it is nonsurgical. It can be performed at the time of diagnostic procedure and it is suitable for patients with impaired LV function in whom a surgical procedure would be risky.

(Slide.)

I will not go into this very much except to pay obescence (?) to my favorite vector, the adenovirus, despite some of the nasty comments, which I have heard earlier in the day. It is a wonderful thing. We have a central library on the UCSD campus that is patterned after the adenovirus. Those of you who have been there will know that. It is a double stranded DNA virus that is easily manipulated. You can make replication defective derivatives. It infects nondividing cells making it ideal for targeting cardiomyocytes which are terminally differentiated and the mutagenesis insertion is highly improbable.

(Slide.)

Here is a quick shot of pig myocardium transmural section five days after delivery of 10^{12} virus particles showing that this is a nuclear tag Lac Z showing very good gene uptake and expression. This is a high powered view of

the same animal. There is no inflammation. Even when you look at CD4 and CD8 marker antigens for cytotoxic T cells you do not find them.

This kind of data are why the FDA embraced this study as much as they did and allowed us to be first in injecting this vector into human coronary arteries.

(Slide.)

This is an amaroid occluder which is a hygroscopic material that slowly swells. You place this around the coronary artery in an animal model and because it slowly swells it does not cause much of an infarct but it does completely occlude the vessel and meanwhile endogenous collateral vessels, not due to gene therapy, just endogenous collateral vessels form, but these vessels are inadequate during times of stress. So you have normal resting flow and function but in times of metabolic stress the pig sees food. Its heart rate and blood pressure goes up and it thinks it is going to eat. Then the pig will get ischemic.

So it is a very nice model of angina. So angina in pigs.

(Slide.)

When we look at one of the parameters, basically percent wall thickening, when the heart is ischemic it does not thicken well and you can actually see this on echo. As a cardiologist -- by the way, I am not a molecular biologist, I am a cardiologist. When you image human patients with heart disease and they are under stress you can see these wall motion abnormalities and you can see perfusion deficits with contrast echo.

We used the same technique in these large pigs. And when you

pace the heart at 200 beats per minute, the function which normally is around 60 percent, goes to about half that. These animals received adenovirus encoding Lac Z by intra-coronary injection and then were studied again two weeks later, and they had the same deficit in their function. In contrast, these 16 animals who received adenovirus encoding FGF-5 had greater than a twofold increase in their function to a degree which was statistically indistinguishable from normal function. These studies were done in '93 and '94 and, believe me, it completely blew me away.

By the way, these were blinded studies so that the people doing the examination and the measurements had no idea which gene which animal had received.

A similar increase in perfusion is noted in the circ bed which normalized the ratio to completely normal. The paper was published in this journal and the company was founded shortly after that.

(Slide.)

Now some of the limitations are how long does the effect last because I have just shown you data two weeks after transgene delivery. Are the vessels normal? That has been a recurring theme today. Are there -- is there angiogenesis at distant sites? And what about the fact that everybody knows that whenever you use adenovirus in any venue you always get inflammation.

(Slide.)

Pardon my bitterness. I have just been fighting this battle for seven years and sometimes I cannot help it.

Here are some data that show the percent wall thickening with the clinical material that we are using. This is an adenovirus encoding -- this is a human adenovirus 5, E1 deleted, encoding human FGF4. And these are the numbers of animals that were studied sequentially here and the weeks after therapy. In the pretreatment state they had a very poor thickening and that doubled two weeks later. And then there was no recrudescence in that function clear out to 12 weeks. We killed various animals along the way to look at transgene expression in a sequential manner.

So this would suggest that the kind of improvement in function and flow that we got at two weeks likely stayed substantially longer than the transgene would be expected to stay and that also would suggest but does not prove that those vessels are functional.

(Slide.)

With respect to the features of the angiogenesis, I referred to this a little bit earlier, the enduring reduction in ischemia at 12 weeks suggests that new vessels are long lasting. By the way, when pigs are that old, they get so big we cannot keep them. Pigs are very strange. They continue to grow almost until they die. So a two year old pig weighs four or five hundred pounds. So by the time they are 12 weeks after this therapy they are over -- well over 110-120 pounds so they just get too big to keep.

(Slid.e)

The wall thickness to diameter ratio of the new vessels is similar to that seen in untreated animals through a wide range of vessel caliber. We divide

them into deciles and looked at all of them in a blinded manner.

Gene transfer is not seen in the arterial walls with our reporter genes. The reason I mention that is because it is in these muscular walls where you usually see atherosclerosis and we are not getting gene transfer there.

In order to get gene transfer into large muscular walls you need double balloon techniques and lots of dwell time and high doses of virus, which usually leads to inflammation. We are just blowing this stuff in. There is nothing that is stopping it from going through. The level of entry probably is at the coronary endothelium.

(Slide.)

These features that I have mentioned --in addition, FGF is detected in the heart but never detected in the plasma, and I will show you that a little bit later, would suggest that angiogenesis and atherosclerotic lesions is unlikely. We cannot disprove it but very unlikely.

(Slide.)

here I am going to show you some ELISA FGF4 detection data for the FGF4 protein. These were animals that were treated with doses from three to sixfold the highest dose that we delivered in our clinical trials. And then we sequentially sampled blood both before treatment and then sequentially after between three days and 21 days.

And we cannot detect the protein in any of those samples.

There is one here where there was a one positive that is just above the level of sensitivity of the assay and then there was this peculiar one here that

also is just around the sensitivity of the assay in an animal that had never received it. But by and large this is no detectible FGF4 that is available.

The sensitivity of this assay, however, does not exclude the possibility of less protein being present but are not being able to detect it and that may be important, particularly if that lower level of protein has a biological effect. We cannot disprove that.

(Slide.)

However, we can get some lessons from the in vitro data shown here in which we took recombinant FGF4 protein and looked at thymidine incorporation in HUVEX cells and determined that below 100 picograms per ml, these were five day incubations, we do not see a significant increase in proliferation.

(Slide.)

The detectability of the protein is quite easy in the heart even though you never detect it in the plasma. The FGF4 and FGF5 are easy to detect in cardiac homogenates. And that is shown here in a treated animal. We see it both in the anterior descending and the circ bed. In untreated animals we do not. We do not find it in the liver and the eye. We have done 18 organs of 30 animals and this is a consistent finding where we do not find it in any of the noncardiac organs.

(Slide.)

The reason for this is not entirely clear. However, these data show an example of PCR which we use to detect the adenovirus DNA in

biodistribution studies. And again this is a 30 animal study where we looked at animals of each sex sequentially at five days, four weeks and 12 weeks after gene transfer by -- at three different doses of the virus, and I will show you some of those data in tabular form in a moment. But here is the plasmid. You can see it in the heart. You do not see it in the eye or the liver or the diaphragm. There are some nonspecific bands here that you can detect. They do not show up. Here is one here.

(Slide.)

They do not show up well on this particular slide but you can see them on the actual gel but they are higher than the FGF4 band. We just do not find the -- and this is at a dose of 10^{11} virus particles.

Now before people fly off the handle, I will say this, that when you give 10^{12} virus particles intra-coronary you find substantial number of positives in noncardiac sites. So there is a threshold effect and remember that we are dealing with very large animals here so these animals are probably 70-80 pound animals with high blood volumes and, you know, billions and billions and billions and billions of cells in their body where this virus is getting distributed to.

When you give 10^{11} we found one positive in the spleen. We do not find it in the eye. It is rare to find it in the brain even at high doses. And there are the data.

(Slide.)

Here is the sensitivity. Now these sensitivities are pretty good for

the most part with some glaring exceptions. Now the way we do the sensitivity is the way it ought to be done and that is we spike the homogenate of an organ with the virus particle, with a given number of virus particles, and then we extract the DNA.

Most people, which is cheating, I think, extract the DNA and then spike the virus. If you do that you get your sensitivities down to vanishingly small but that is not what you are after in a clinical trial. You are looking for the virus particle DNA in the organ and not the other way around.

So these are pretty solid data and they have been repeated a number of times.

(Slide.)

So, in summary, five days after intra-coronary delivery of adenovirus encoding FGF4 at 10^{12}, which is 30-fold the highest clinical dose that we have used, adenovirus DNA was detectable by PCR in several extra cardiac organs. But RT/PCR in those positives was negative so was protein expression. Rare PCR positives and samples after 10^{11} virus particles were given by intra-coronary delivery.

FGF4 protein was undetectable in plasma and we believe that these things together reduce the theoretical risk of promoting tumor growth, which has been a major theme of this day.

(Slide.)

Now with respect to inflammation we have looked high and low for inflammation in a variety of different doses and we have not found it. One of

the things that we did was we used CD8 and CD4 antibodies to recognize cytotoxic T cells. Using the spleen of the pig as a positive control, which is ladened with both CD8 and CD4 expressing T cells, we know that we can detect it.

And then looking in animals that got no adenovirus, here you see one positive cell, which is sometimes seen in normal hearts. This was an instrumented animal.

And here at two times 10^{11} adenovirus we do not detect anything.

We have looked at a substantial number of animals. This is just representative animals that I am showing you and in addition we have even gone up to 10^{12} virus particles and checked out the same thing. And an independent laboratory looking for evidence of inflammation has not seen it.

(Slide.)

Now let's go to screening patients and detecting potential toxicity in the last few slides. What I am going to do is sort of model what the clinical trial that we just finished enrollment on, which is a Phase I/Phase II dose escalation trial with intra-coronary delivery of three times 10^{8} to 10^{11} virus particles of adenovirus encoding FGF4 in patients with stable Class 2 to 3 angina.

These are not patients at death's doorstep. These are patients with other options. About 30 percent probably would not have been good candidates for revascularization but about 70 percent might have been.

Age less than or equal to 70, ejection fraction initially greater than or equal to 40 percent, and we excluded people with current or prior malignancy,

retinopathy, unstable angina, liver disease, immunotherapy or PTCA/stent within six months. This is a double blind randomized placebo controlled trial.

The consenting patients fulfilling entry criteria by interview underwent physical exam, chest x-ray, thorough blood analysis, PSA, pap smear, mammography, fecal blood checks, urinalysis and endoscopy, which was done serially. They then underwent cardiac catheritization if they met these criteria and if they met criteria for the coronary anatomy and had two consecutive exercise treadmill tests that did not differ by greater than 25 percent they received the agent.

Now the reason for that last criteria is to make sure that you are looking at patients who do not have a lot of variability on treadmill testing because that would obscure the data.

(Slide.)

With respect to assessing potential toxicity, we did a lot of things and I do not mean to -- because I am in a hurry and because there is a lot to do here, do not let that make you believe that we were not anything but vigilant. In fact, when many other trials in gene therapy in general were being shut down because of the calamities of a year ago in November, we had a safety profile and have continued to have a safety profile on a reporting characteristic where we were not put on clinical hold through the whole episode.

We have had a very good relationship with the FDA in that regard.

We did interviews and physical examinations, electrocardiography, blood analysis, particularly looking at alterations in liver function or evidence of

myocardial injury, we used troponin as well as CPKNB and we also did some studies on the adenovirus antibody titers and there was a 12 month follow-up.

(Slide.)

Now I am going to generalize this to the clinical trials that either have been published by Dr. Crystal who will be talking soon and by Dr. Isner as well as the data that we have in our trial, which have not been published as yet, and out of those three trials there have been actually now I see from Jeff's previous slide this number should be about 182. They have been followed between one and three years. And there was one death that occurred within 24 hours of gene transfer. This was in a thoracotomy delivery protocol. There were six additional deaths in total that occurred well after gene transfer and unlikely related to therapy.

And if one looks at the sort of composite, most of these patients, as Jeff just told you, were patients with no options and so at least half of these patient populations, and that is being very conservative, probably much greater than half, had no options, and those people had about a 15 percent annual mortality.

Now if you go down to the other extreme, just age matched people, with minimal coronary artery disease, that is about a three percent annual mortality. If you average those you would expect about a nine or ten percent average mortality, which would mean that you would expect 34 patients who have died just by virtue of that is the way the disease goes.

And certainly we did not see that. We saw six. So the perception

in the press and the overall perception it seems to me is not supported by the facts. This is not an experimental protocol that is associated with a high death rate. So I will just make that point alone with this trial. I am not trying to suggest that this is prolonging life. All I am saying is that it looks quite acceptable with respect to mortality.

(Slide.)

Now in my last slide here I want to just review with you some of the major advances in cardiovascular disease therapy over the last 50 years and I will be brief and to the point with this.

First was cardiopulmonary bypass or extracorporeal membrane oxygenation. This had a very high mortality rate in the early years. It was developed in 1955.

Bypass surgery developed in 1967 had a very high morbidity initially and it took greater than 15 years to show any survival advantage with this therapy and even now that seems to be survival advantage that is there for ten years and may not be there for 20.

PTCA in '77. Initially there were very few suitable lesions and extremely high restenosis rate. As much as 50 percent within six months.

And thrombolysis developed in 1980 initially had poor results in hemorrhagic stroke, which confounded the early studies, and no survival benefit was proven until years later when the N in the studies was up to 12,000 patients.

I think that there is a couple of things, a couple of points that can be derived from this slide. The first is that it is lucky for our patients that these

developments were allowed to continue even though their early days had some problems. Imagine a world now taking care of patients like I do where you do not have bypass surgery, you do not have stent, you do not have thrombolysis.

The second issue is that by comparison to these things, the early days of angiogenic gene therapy looked pretty good compared to the early days of these various advances.

I will stop there.

(Applause.)

DR. FRIEDMANN: Thanks, Kirk.

Since we are a little behind let's keep up the procedure that we have started now and that is to finish the talks and then have the questions directed to the speakers as well as the panel when we are finished.

The next speaker then will be Ron Crystal from --

DR. CRYSTAL: Dr. Hammond, are these Dr. Hammond's?

DR. FRIEDMANN: Ron Crystal from what used to be called Cornell and now is the Wilde School of Medicine. Is that right?

DR. CRYSTAL: Wilde-Cornell.

DR. FRIEDMANN: Wilde-Cornell.

DR. CRYSTAL: If you have $100 million you, too, can be --

DR. FRIEDMANN: I will think seriously about it.

(Laughter.)

DR. FRIEDMANN: He is going to bring us up-to-date on his studies on cardiovascular gene transfer.

CLINICAL TRIAL DESIGN RELEVANT TO SAFETY ASSESSMENT IN CARDIOVASCULAR GENE TRANSFER TRIALS

RONALD CRYSTAL, M.D.

DR. CRYSTAL: Thanks, Ted.

(Slide.)

What I would like to do is just review some concepts and then show you some data that tries to focus on some of the issues that have been brought up at this symposium.

The risks to cardiovascular gene therapy can be divided into several categories. First, we have the problem, as we have heard, of administration of therapy and this can be by thoracotomy, with or without bypass, it can be by catheterization, or in the case of peripheral vascular disease by intramuscular injection directly.

Then we have the risk to the host responses to the vector, whatever that vector is, and these can be local responses or systemic.

And then we have transgene responses. We have the problems with VEGF or the issues of leak. We have the problems with fibrous growth factor, theoretically fibrosis. We have the problems with these growth factors of both the issues of retinopathy and malignancy.

(Slide.)

Now in terms of trying to design protocols we have a challenge in dealing with cardiovascular disease. Our population is older. The study

participants have complex, life-threatening diseases, often with inexorable down hill course. They have comorbidities, including malignancies and ophthalmologic disease. And we have to, as I said, use invasive procedures like thoracotomy and catheterization to administer the drugs.

(Slide.)

Now what are the risk related design strategies that we can build into our protocols? Well, one thing we can do is consider the issue of avoiding systemic delivery and I will show you a little bit of data about that. And then we can try to avoid specific risks. Now there are sort of routine risks of any kind of therapy involving these kind of patients, particularly when you are doing thoracotomies or catheterization.

We have the risk of malignancy, the ophthalmology risk, the mortality and, of course, then I want to talk a little bit about biologic markers.

Since it was brought up this morning relating to pharmacokinetics relating to these vectors, I thought I wanted to show a little bit of data relating to some assessments we have made of intra-coronary versus direct myocardial administration.

(Slide.)

This is not to make the argument that the talk that you just heard by Dr. Hammond is not the way to do it. It is rather just to point out the difference in pharmacokinetics because I think the doses that are being used by us, Dr. Hammond and all the others in the cardiovascular gene therapy trials are well within for adenoviruses -- well within the safety parameters that are now

known clinically for these vectors.

In thinking about how to deliver gene transfer vectors one can give them intravenously, you can give it intra-coronary as you have heard, or intra-myocardially, you can do it by epi-cardial administration or as in trials that are now starting through endocardial through catheter strategies.

(Slide.)

And the most direct way to look at pharmacokinetics is to look at the drug itself and the drug, of course, in the case of adenovirus is a ball of proteins together with some DNA, and using TaqMAN quantitative PCR one can readily detect now down to one to two copies. The methodologies are linear over five or six logs and you can easily measure the numbers of -- the molecules of your vector per cellular genome and that is the way this data is presented and it is comparing in the pig the intra-myocardial, that is epi-cardial administration. These are three different pigs compared to intra-coronary administration of the same dose.

And each of these dots that you see for each pig represents a different part of the left -- the free wall of the left ventricle that was assessed.

And so you see a number of things. First of all, there is considerable variability. You see the variability is not in the test. The variability is in the delivery.

The second thing is when you look at the relative amounts that you see in the myocardium when you administer the same dose, in this case intra-myocardial, it is divided into ten doses, delivered in the -- on the myocardium

throughout the free wall of the left ventricle, intra-coronary as a single dose but it is the same total dose, is that the relative ratio when you take all of this data and average it is about a 26-fold difference. So you can achieve 26-fold more delivery to your myocardium if you go to the direct intra-myocardial route.

That is all data at one hour. So that is immediate.

(Slide.)

If you look at 24 hours basically you see the same thing. Here is molecules of vector per cellular genome. This is one hour, 24 hours. Here is intra-coronary. It drops off about 90 percent or so over the first 24 hours and it does not matter how you administer it but you pretty much maintain that same ratio in terms of delivery.

(Slide.)

Now how about systemic when you administer the vectors in this way? This is comparison. Again quantitative PCR. This is data at 24 hours after administration of the percentage of the total vector that has been recovered.

We see some interesting things. All of this data is in the pig and for anybody who has worked in the adenovirus gene therapy field, and you have done murine studies, everybody knows that 90 to 95 percent of the vector goes to the liver. But look at this data in regard to where the vector goes.

So the yellow is intra-coronary and the orange is intra-myocardial. And I think Dr. Roberts pointed out that when you administer these vectors we know they go elsewhere, they certainly do no matter how you do it, but there are differences so that if you deliver it intra-myocardial you clearly get more in the

myocardium than you do if you give it intra-coronary.

That does not mean this is not enough. I am sure it is. But you get more if you do it by epicardial administration.

But you also get it systemically. You get some in the spleen. You get some in the liver. But look at the lung. In the pig if it goes systemic it goes to the lung and not to the liver.

Just keep in mind in terms of humans we do not know whether humans are closer to pigs or whether they are closer to mice and so it is something to keep in mind in terms of our development of our safety studies.

(Slide.)

Now for the remainder of this talk, this is also my conclusion slide because what I would like to -- the major point I would like to come away with is that at least from our experience and I think everybody else's, is that really the concept is that in this patient group, in this kind of indication, that to evaluate risks to gene therapy, you have to have a control group. And I want to convince you from the data that we have generated of why that is the case.

(Slide.)

First, what are the major adverse events that we saw in the clinical trial that we carried out? This is major adverse events other than death. I will come to death in a moment. Following myocardial administration of adenovirus vectors for coronary artery disease.

And so here were three groups that we evaluated. The two major groups were the gene therapy groups. That was a group that was adjunct to

bypass and coronary artery bypass surgery and then there was a group of 15 individuals -- 16 individuals that had minimally invasive surgery.

And these groups were pretty comparable in terms of their comorbid indices.

And they had some major adverse events defined on a scale of zero to four of a three or a four. And as you can see per individual it was about 2.1 or 1.0 and 84 percent of them were within the first week and 38 percent in that group.

But we also had another group because I was interested in following some of the parameters, particularly VEGF in the plasma, and so we got another group. These were not matched in any way. It was just consecutive eight CABG patients with no gene therapy and they had 1.1 major adverse events per individual. Most occurring in the first week.

And so clearly these patients in this case of CABG clearly have a lot of adverse events and that is relevant.

(Slide.)

Then there is the issue of malignancy and these are the malignancies known to develop during the trials and the trials are now all closed and so this is sort of the total data in terms of all the -- up to the point that it was recently closed.

So the groups are the bypass, the people who had coronary bypass surgery, together with our -- I am sorry, this should be the -- yes, this is together. The coronary artery bypass patients plus the minimally invasive surgery. That is

our group who had gene therapy.

We also had a control group that was the group that we followed along that I just showed you. There is a group of patients with peripheral vascular disease that got gene therapy and there is a small, only four individuals, individuals that were followed as controls.

And they were followed for a various amount of time and clearly we followed most of these patients with the ones with gene therapy for a total follow-up of 48 patient years and the number of malignancies are over here. And we did have three malignancies in this group and compared to this control group and again it is not a match control group. It just happened to be a control group we were drawing some blood on but they had a malignancy also.

And then when we calculate out the number of malignancies per patient year, it turns out in this group it is .06 and in this group it is .12. I think that helps point out the fact that you need controls. I am not arguing that there is more or less malignancy in either group. It is just that clearly to sort this out we clearly have to have an appropriate control group.

(Slide.)

And I am sure most of you know of the GenenTech trial where they used VEGF-165 protein, where they had a total of 160 or 70 patients, but there was a second group of 102 that they recently reported in Circulation. There was a subgroup of 102 patients in that trial that were followed in 13 centers and blinded one year follow-up. And they followed, among other things, malignancy. In their VEGF high dose group there was zero malignancies. In their low dose

group there were three malignancies. In their placebo group, eight percent had malignancies. Again pointing out that in this group of patients who were older, who were prone to malignancies, you have to have control groups.

(Slide.)

We also in our studies followed retinopathy in our patients. This was before and then at six months. And this is the CABG group before and after and the number of individuals. And then the retinopathy was scored on a none, mild, moderate, severe, so a scale of up to three. This is the group who were bypassed before and after. This is the minimally invasive surgery with no bypass before and after. And peripheral vascular disease.

We saw two individuals in this group that had a slight worsening of their retinopathy and this individual went from mild to moderate. This individual had diabetic retinopathy -- proliferative diabetic retinopathy requiring laser surgery beforehand. And this other individual that is here that had a mild increase had a hypertensive retinopathy. Is that associated with the therapy, our therapy, or is this the natural history of these patients?

There ar two ways you can approach this in terms of design. You could say, okay, we are going to exclude everybody with hypertension and we are going to exclude everybody with diabetes. If you do that, it is going to be awfully hard to find patients because that is the group of course clearly who are most obvious to enroll in these kinds of studies. Or you can do control groups and I would argue strongly that the appropriate way is to do control groups.

(Slide.)

This is the mortality. If we could focus that. I am not sure I know how to focus it here.

That makes it worse. If the projectionist could focus it, that would be great.

In any case, like Dr. Hammond reviewed, this is basically the same. These are all kinds of trials that are in the literature. This is the one year mortality, coronary endarterectomy, TMR open label, TMR randomized, TMR medical control.

It is fine. Leave it. Thank you.

And so these are all cardiac -- and this is the numbers -- or next to the number are people in each of these trials.

And so, as Dr. Hammond pointed out, they range anywhere from five percent mortality up to 25 or more percent and our mortality was in here at one year. And this is the PVD studies. You look in the literature for peripheral vascular disease, the mortality for one year runs everywhere from two to three percent up to almost 30 percent. And for the group, and I think Milt Pressler will probably talk about this, was clearly low.

So again the important concept is having a control group. That is a historical control.

(Slide.)

Finally, I thought I would mention biologic markers because in this field in prior meetings there have been discussions of biologic markers and I just thought I would share a little bit. Of course, we all can use routine CBCs,

chemistry panel and so on. And, of course, the obvious place is to look in blood.

I just want to show you a little bit of data relating to transgene product in terms of cytokines.

I did not bring along the data relating to VEGF levels in our gene therapy patients because we cannot detect them and that we talked about last year and we published this. So, like Dr. Hammond, we cannot detect the transgene to any significant extent in the plasma.

(Slide.)

However, we have looked at VEGF levels in CABG patients. Just patients getting routine cardiac bypass surgery and this is VEGF levels in their plasma, citrated plasma. This is in picograms per ml pre-incision, pre-bypass, immediate post-bypass in day one, two, three and four. And as you can see interestingly the group is sort of going up in terms of their VEGF. So if you have CABG, one of the things you are doing with bypass surgery is you are inducing your own VEGF endogenously.

But the most interesting thing in terms of this group, this is --

(Slide.)

-- the mediastinal fluid VEGF levels in CABG patients. This has nothing to do with gene therapy. As you know, in bypass surgery there is always a catheter that is left in the mediastinum and the pericardium to drain it. So we looked immediate post op at six hours and 24 hours at VEGF levels and this is in picograms per ml. And on the average it is over 2,000 picograms per ml.

So if you have CABG what you are essentially doing is dumping

huge amounts of VEGF in your mediastinum.

And so the point that I am trying to make with all this is not that gene therapy is safe or not safe but rather that we need control groups to be able to evaluate the parameters that we find.

(Slide.)

And, finally, let me just share with you some data relating to cytokines and what I thought would be interest was Interleukin-6 because that was talked about a year ago at this meeting. It is a multifunctional cytokine produced by a wide variety of cells.

An important regulator of acute phase responses. Elevated serum levels of IL-6 are observed within 24 hours following adenovirus vectors administered to experimental animals and it has been suggested as a marker of antiadenovirus vector host responses of early responses.

So let me show you a little bit of data from various trials.

(Slide.)

This happens to be from one of our cystic fibrosis trials and this is Interleukin-6 in picograms per ml in the serum. And at the top are individuals who are -- this is our cystic fibrosis down below. This is repeat administration to individuals with cystic fibrosis and as you can see IL-6 goes up.

This is a group of normals that we administered another vector to and it goes up in those individuals.

(Slide.)

This is our cardiovascular trials receiving the VEGF vector and

you can see the levels go up between 100 and 1,000 in most of these individuals. This is in the bypass individuals. This is over time. And these are individuals getting the mini-thoracotomy.

So you could say, well, it does go up and that is consistent with an acute phase response.

(Slide.)

And this is peripheral vascular disease of a small group of patients in that trial. Here is Interleukin-6 levels and you can see it goes up. And you could say, well, that is consistent with the hypothesis that maybe adenoviruses induce these things but then we went ahead and we have done some controls.

(Slide.)

And so this is just two groups of controls. On the left-hand side are Interleukin-6 levels in patients getting bypass surgery and no gene therapy. These are individuals getting bronchoscopies. There are normal individuals. And other studies that we do, we are getting lavage and brushes but no gene therapy. And the levels you can see are the same.

And so the procedures that we use also induce these kind of markers and again this is arguing strongly in our studies that we have to do controls.

(Slide.)

These are the peak serum IL-6 levels following administration of adenovirus vectors to humans versus the procedures used to administer the vector. I have combined here all of our studies, intra-dermal, intra-bronchial,

cardiac, intra-tumor, everything combined. This is IL-6 levels in serum.

And this is the dose. As you can see there is no dose response at all and here are a group of controls. These are procedures without gene therapy. Bronchoscopy and CABG and so on. And you can see there is no difference.

That does not mean there will not be markers we can find but it argues strongly that what we need and I think all of us have now gotten to the point in these gene therapy trials that we now have to begin using controls.

Of course, we want controls to help us get assessments of efficacy but we also need our controls to assess whether or not there is risk and whenever we can I certainly would strongly recommend that in our trials we design in appropriate controls to evaluate the adverse events that we see in our trials.

Thank you.

(Applause.)

DR. FRIEDMANN: Thanks, Ron.

Let's finish off these presentations with Milton Pressler from Pfizer in Ann Arbor.

The Executive Director of the Clinical Research Cardiovascular Program.

CLINICAL TRIAL DESIGN AND MONITORING

MILTON PRESSLER, M.D.

DR. PRESSLER: Now let's see. I may need your help, Kirk.

(Slide.)

First of all, I would like to thank Dr. Patterson and the NIH and the

FDA for organizing this symposium and for inviting us to participate. I am the last speaker today so some of the things that I will talk about have been discussed by others but we would like to at least discuss some of the concepts about doing gene transfer trials.

(Slide.)

And if you will permit me for a moment to wax philosophical, I would like to discuss a little bit about the situation in the public discourse. And many of us here are physicians. We took the oath of Hippocrates that says, "First do no harm." But doing no harm in the modern era may also involve not neglecting our patients.

So a corollary credo is do not fail to do good.

In diseases with great morbidity and mortality, like advanced atherosclerotic disease, failure to act, failure to try new therapies means that we as a society view suffering naturally to be more acceptable than making a prudent attempt to relieve that suffering.

So what we would like to discuss here is prudent attempt.

(Slide.)

And to begin I would like to give an overview of the development process because as one of the other speakers talked about today, this is development, not just research. So in Phase I, the first phase of development, one has to do some exploratory tolerability and this is where a number of the trials are presently, is in assessing the tolerability of a given new treatment.

After one, together with others, decides that a treatment is

tolerable, then we move on to dose exploration and trying to prove the concept. And as Dr. Crystal mentioned, this is a perfect time where controlled studies can guide that proof of concept as well as establish what is the optimum dose.

Once the dose is known then we must settle upon proving the efficacy and assuring that we have a sufficient number of people in the population and a breadth of that population that we can assess safety.

(Slide.)

So what are some procedures to optimize that safety that seem to be important not only in the trials that we are doing but in others?

I think multiplicity of review is important. This -- the review by many different organizations and eyes provides that overlap that makes sure that things do not fall through the cracks. Starting with our regulatory agencies, the FDA and NIH, but also at the institutional level. Local ethics and biosafety committees provide their review and approval.

And then lastly and integral to the process of conducting a safety trial is the individual investigator's judgment because it is only that investigator on the scene taking care of those patients that knows really what is best.

Careful adherence to good clinical practice, including through site monitoring and scrutiny of source data.

And, lastly, an extra step that we feel is important is commission of an independent safety committee to review results and safety on an ongoing basis.

(Slide.)

Now, as Dr. Crystal presented, the natural history of advanced atherosclerotic disease is that some of these patients die during the course of follow-up even with the usual care.

And in this particular slide we have tried to summarize a group of studies in patients with advanced coronary disease using those that were -- here I am sorry that little bit of ditzels there -- using those who were being studied for transmyocardial revascularization as perhaps a comparable group where the patients have few options and are unresponsive to conventional care.

And what one sees in these studies is that the one year mortality ranges from around 11 percent to 23 percent.

(Slide.)

So predicting from natural history in a population of advanced atherosclerosis we can say that mortality will occur in 11 to 23 percent of those patients followed for a year. We also know from knowledge of the disease that irreversible events, myocardial infarctions will occur. And even despite our best efforts at prescreening, cancer will occur.

The reason for this is that cancer is very prevalent in an elderly population that is subjected that has heart disease. So one must consider the age of these patients and their multiple co-morbidities.

Furthermore, even without a gene product the length of follow-up determines how many cancers you will find.

So this reiterates what was said by some of the previous speakers. The importance of controls, controls, facilitate interpretation of safety and

efficacy data. It is also part of the contract that we have with participants that the results be meaningful and add new insights.

Controls also account for changes in usual care as Dr. Hammond was mentioning. Cardiovascular disease is changing rapidly. There has been continuous improvements ongoing in the care of these patients and how will we know whether the benefit is due to improvements in the background treatment versus the therapy itself.

And, lastly, controls account for inter-patient variability as long as there is adequate numbers from the entire population.

(Slide.)

So in the last part of the presentation I would like to go through an example of a gene therapy or a gene transfer trial in patients with severe coronary disease.

(Slide.)

What are some inclusion requirements that we might advocate? Well, first of all, we think that it is important to pick patients who are severely symptomatic. These patients should be unresponsive or poorly responsive on maximal medical treatment comprising multiple conventional drugs.

We think that the disease should be documented to be multi-vessel and that they not be a reasonable candidate for conventional revascularization.

Lastly, we think it is important in this population to document that they have ischemia on exercise testing at moderate stress. Many patients who have had prior revascularization become cardiac cripples. They get chest pains

for multiple reasons besides just myocardial ischemia.

(Slide.)

Some important exclusions. Some of which have been already discussed. This is our take. Advanced stage, certainly greater than 80. The comorbidities begin to pile up and the likelihood of tumors developing increases.

Our personal cut off has been to have a left ventricular injection fraction less than 25 percent because in these patients there are often poor candidates for bypass and we felt that was a good determinant of where the risk of heart failure became significant.

Recent acute coronary syndrome or infarction. Unprotected high grade lesions we also think are important in this initial population because it takes time for the angiogenic effect to occur.

Having an uninterpretable electrocardiogram or inability to exercise also we feel is an exclusion because those patients cannot be evaluated later on.

We also advocate that those patients who have either a very poor immune status or a heightened immune status at this particular stage ought to be avoided but as more experience is gathered that should be reevaluated.

Lastly, those who have underlying chronic renal disease or known cancer, and this last element is something that we have to deal with every day. We have specified that those who had been cured or had a localized cancer might be eligible but what about someone who has had a malignancy discovered five years ago? Should they be excluded? Is the risk too great?

(Slide.)

Some safety assessments. In addition to the usual clinical assessment and scrupulous monitoring of adverse events, we have employed the American Cancer Society based cancer screening. Ophthalmologic exam and especially exclusion of those with proliferative retinopathy and macular degeneration.

Serial electrocardiograms. Again attempting to survey for those who might develop an arrythmogenic state after treatment. Echocardiography in the immediate post-dosing period and adenoviral immunity.

(Slide.)

So, in sum, five points in approach to safety and development. Selecting initially for highly symptomatic patients unresponsive to conventional treatments. Multiple levels of oversight by government, institutions and independent entities of both protocols and adverse events excluding those at higher risk of extra cardiac problems while learning where the risks are in terms of neo vascularization.

Control groups for contemporaneous comparison of reported adverse events and lastly periodic review of the risk/benefit ratio.

Thank you.

(Applause.)

PANEL B DISCUSSION OF SYMPOSIUM QUESTIONS

QUESTIONS FROM THE AUDIENCE

DR. FRIEDMANN: Thanks, Dr. Pressler.

So now let's have the audience or panel members direct questions or comments to the speakers that we just heard and afterwards I would like to go around the table, in fact, again because the panel is different.

Why don't we do that now? We have a lot of new members of this panel who were not here earlier for the other panel. So let's go around the table and maybe when you introduce yourself, if you could just add another sentence saying what your particular area of expertise is and why you are here.

DR. WALTON: Mark Walton. I am in the Center for Biologics, the Division of Clinical Trials, and I participate in the FDA review of clinical trials of cardiovascular biologic products.

DR. UNGER: Ellis Unger, also in the Division of Clinical Trials, the same branch, and before I was at the FDA I spent 13 years or so at NIH basically trying to grow blood vessels in the heart primarily in animals but also was involved in Phase I -- a couple of Phase I studies.

DR. SRIVASTAVA: I am Sudhir Srivastava, Chief of Cancer Biomarkers Research Group in NCI. My expertise is cancer biomarkers and screening.

DR. SPRINGER: I am still Matt Springer from Stanford University and I guess my area of expertise on this panel is not being a doctor.

DR. PRESSLER: I am Mil Pressler from Pfizer Global Research and I am here as an interested sponsor.

DR. MEISEL: I am Alan Meisel. I am from the University of Pittsburgh where I am the Director of the Center for Bioethics and Health Law.

MS. KING: I am Nancy King from the University of North Carolina and also a member of the RAC and I am an ethics type interested in subject selection and decision making issues in gene transfer research.

DR. MANN: My name is Doug Mann. I am a cardiologist from Baylor College of Medicine and my area of expertise is inflammatory mediators in heart failure.

DR. FRIEDMANN: Larry Friedmann, National Heart, Lung and Blood Institute, epidemiology, clinical trials, bioethicist.

DR. CSAKY: My name is Carl Csaky. I am at the National Eye Institute. I am a retina specialist and I also direct a lab on ocular gene therapy with a particular interest in diabetic retinopathy and age related macular degeneration.

DR. CRYSTAL: Ron Crystal, Wilde-Cornell, with interest in -- particularly in vivo gene therapy with adenovirus vectors.

DR. ROBERTS: Bob Roberts, Baylor, Molecular Genetics and Cardiology.

DR. PATTERSON: Amy Patterson, NIH, Office of Biotechnology Activities. Our office is a locus within NIH for the oversight of gene transfer research. We also provide the administrative and staff support to the Recombinant DNA Advisory Committee.

DR. FRIEDMANN: Ted Friedmann, UC San Diego, and a member of the RAC.

Okay. So questions or comments from the audience or from panel

members towards speakers?

Dr. Engler?

DR. ENGLER: Engler, San Diego.

I would like to take a few minutes to respectfully submit a very different point of view than what I have been hearing.

I would certainly agree that control experiments are absolutely necessary but these are human experiments who put -- we put patients at risk. And we need by the principles from Helsinki, Nuremburg, and the Belmont Report, we need to assure these patients that we will get meaningful results from these trials.

They are volunteering and putting themselves at risk. The benefit is probably in most of their cases not going to be to them. Some of them are getting placebo. And they want to be -- we should be able to assure them -- the investigators should be able to assure them that we are going to find meaningful results.

So this is really a clinical experiment. The clinical experiment had better have a good chance of answering the question that we are asking.

Now if we were to enroll patients that are refractory with a high underlying incidence of morbidity and mortality, it is going to be very difficult to draw conclusions about toxicity and about efficacy.

And so I think that it perhaps is a mistake to take the patient who is on death's door, the patient who is having rest angina, the patient who has got the most severe pain, give them something in a noncontrolled fashion when there is

no control and try to draw a conclusion because there is a very powerful placebo effect, both for angina and for peripheral vascular disease. Very powerful.

So I think that we not only have to have controls but we have to select patients for the trials that give us the best chance with the fewest number of patients at risk of drawing a meaningful conclusion about both safety and about efficacy.

And I was on sabbatical for a couple of years at Collateral Therapeutics where we designed the agent trial that Kirk Hammond talked about and in designing that trial our thinking was along the lines that I just presented. We decided that we needed to look at chronic stable patients who are likely to do well for weeks or even months on their own with stable angina so that if our therapy had toxicity or made things worse we could detect it.

Also, we thought if we were putting patients at risk looking for toxicity, if we are going to treat 40 or 50 or 60 patients, which we thought we would need to do to find toxicity in these patients, that we might as well look for efficacy and do a placebo controlled blinding randomized trial and exercise the patients and have a chance of finding efficacy endpoint as well. So that is the way we designed the trial. We did not use refractory patients.

We also figured that angiogenesis was not going to grow a new left anterior descending. It is not going to grow a new IMA or a new saphenous vein graft. What it is going to do is increase or stimulate the natural collateral growth that occurs, which for reasons that we do not understand is turned off in these patients. It is going to stimulate the natural collateral growth and augment it.

Sort of kick start it. So the patients have to have a patent conduit vessel.

So one of our entry criteria was that each patient enrolled in the trial had to have one vessel that was at least less than 70 percent stenosis, not severe disease, not extensive disease. That is what made the most sense to us and that is why we had that as actually an exclusion criteria.

So we really took a very different tact than what some of the members have been suggesting and we started this about four or five years ago. And as Kirk said, as he showed on the slide, our entry criteria were EFs greater than 40, not sick patients, one vessel with 70 percent patency or better, and chronic stable patients, and that makes more sense to me in designing a clinical trial.

So I would hope that the members of the RAC would think very carefully about what types of patients they want to enroll in these trials both from an ethical point of view as I have pointed out but also from the point of view as to, you know, what can we learn from the trials. Can we really advance the development of these products and in what patients are they most likely to work? I think that an angiogenic factor is not likely to work in a patient that has got 95 percent stenosis of three vessels.

So I guess that is a little contrary to what some people have been saying but at least it has been my point of view and it has been the philosophy behind the agent trial which just finished enrolling double blind fashion and we will soon see the results.

DR. FRIEDMANN: Well, that is a terrific way to start the

discussion and so let's pick that up. We have people on the panel who have thought about the same thing, both people doing studies and people thinking about how to do studies.

So please let's get some response.

DR. PRESSLER: I think that there is a fair amount of synergy with what you are saying and what we presented. The idea of a patent vessel was put forward in not having an unprotected LAD. If the LAD is unprotected then one does not really -- there is risk there to that patient that is too pronounced.

In terms of the assessment of the patient's symptomatology, we have relied a lot more on exercise testing to give our guide of our patient population than just what the practitioners are reporting in terms of symptomatology. We think that at this early stage it is important to pick patients who are relatively severely symptomatic but through the exercise test alone the time on that treadmill one can eliminate those who are -- who barely can get on the mill before they have to come off, and those who go so long that you wonder if they are not a marathon runner.

So I think it is really more in terms of how you set up your exercise test parameters that are some of the determinants about your success rate.

And then I think in terms of doing controlled studies I think we agree it is important to do controlled studies in order to be able to put into perspective not only the efficacy but also the safety.

DR. UNGER: I would just like to add I agree largely with Dr. Engler and have thought about this for many years and, in fact, in the first

attempt to basically engender collateral development in animals involved a model where I brought in a systemic artery to the heart. This was the internal memory artery. It was essentially a Weinberg procedure.

The idea was that in the patient population you would like to be able to treat, which is patients with no options, you probably do not have a patent vessel. And so the idea back then was to bring in the systemic artery because that, in fact, was patent.

So I think I agree. I mean, if you use patients with severe triple vessel disease, no patent artery, you have probably no chance of succeeding.

The other point, the point about placebo controls, in terms of detecting biologic activity, there was a study. Many of you are familiar with it. It was a myocardial laser study that was recently presented at the TCT and AHA by Mary Leon and that was a 300 subject three arm study where they compared basically subjects who had laser holes made with the low density, high density or they did not have laser holes generated.

But it was truly a double blind study and there was a remarkable two functional class improvement across the board in all three groups of 100 subjects, including the group that received placebo.

So the take home from that study, I believe, is that if you take a subject with very few options and you give them the option of participation in a trial with a laser or a gene, you can probably expect a two functional class improvement, which is very sobering, I think.

DR. ROBERTS: Doug?

DR. MANN: I wanted to follow up on some of Bob's comments and some of Eduardo's comments. I think that the discussions have talked a little bit about heterogeneity but no one has really talked about the issue of remodeling on top of heterogeneity. I think that it is improper to look at a patient with triple vessel disease and an EF of 55 percent and make the same comparisons to a patient with triple vessel disease and an EF of 20 percent.

I think that early on in these clinical trials probably people would be better off choosing patients with normal or near normal left ventricular ejection fractions just to try to minimize the problems of heterogeneity and re-entrant arrhythmias. Because the remodeled is ventricles is dramatically different than a normal ventricle and so I think, you know, Bob's way of phrasing this and looking at the cutoff criteria are actually right on the mark.

DR. ROBERTS: Yes?

DR. MEISEL: I would like to say something from an ethical perspective as well. I think there is a lot of wisdom in the suggestion. One of my concerns about any kind of investigation that uses the sickest subjects is that they are the most compromised, I think, in terms of voluntariness of the participation.

The problem that I see, however, is that if you use patients who are doing relatively well, as you suggest, when you go to get informed consent from them, if you are really going to do informed consent in a serious way, one of the things that I would be concerned about is why would they volunteer if there are accepted therapies that stand a pretty good chance of working for them.

So are you going to be able to accrue subjects into a protocol who

are -- who have reasonably good options that are not experimental?

So I find this to be a real dilemma. I am basically sympathetic to your suggestions and yet I wonder if they will work.

DR. ENGLER: It does make it difficult but I think it is the only fair and right thing to do and it took us two years to enroll the number of patients that we did, and that may have been part of the reason.

But another -- to follow-up again on your point that you are throwing back to me, is this, the person who gets the informed consent from the patient has to really be both detached and beneficent and has to really tell them what the pluses and minuses are and has to really tell them that they are volunteering for an experiment, that they better not count on getting any benefit themselves from this because it is unproven and we are not sure if it works, and you might get a placebo.

We know from psychological studies, as you ethicists know very well, that most patients actually think and here, they think, "Oh, well, I am going to get the active drug and the doctor would not be giving this to me if it did not really work." But, you know, that is maybe what they think but we really should try to inform them that that is not the case.

And for that reason I think that the people that have the strong interests in the trial, you know, should not be the physicians getting the patients in. We need to have objective clinical investigators who are not connected with the people sponsoring the trial, who can be objective, get the informed consent, who have to be enthusiastic and say, look, this is an important experiment, this

could revolutionalize medicine, we do not know if it works yet, you know. You have got some disease, you could have an angioplasty. It might make you better. It probably would make you better and relieve your pain but if you are willing to wait 12 weeks to participate in this experiment, here is the pluses and here is the minuses.

And there are people out there that want to do that and those are the kinds of people that should be in a clinical trial. Not a person who has unrealistic expectations of getting better because those are unrealistic. We do not know that.

MS. KING: Can I add something here?

DR. ROBERTS: Yes. Before you do, I just want to make clear we are now sort of entering into the panel discussion to try and answer the first question because I think it is very appropriate, and I just want to make one quick comment.

I think that what we have heard today is based on people who have severe diseases who are infrequently not responsive to at least most of conventional therapy and that is how they got into the trial.

And now Dr. Engler has proposed that we should take the other group of chronic documented coronary artery disease, symptomatic probably with normal ventricular function, as Dr. Mann has proposed, and so I want to carry on the discussion with those two models because I think one thing we can do at one point is ask everybody who would disagree rather than who would agree with those two. And whether, of course, you can get the people in those trials is one

thing but if we could would it be a reasonable thing.

Go ahead.

MS. KING: Okay. First of all, I think that Dr. Engler might as well just come up and take my seat because you have already said all of the things that I was planning to say and I am really glad to hear them from somebody in the audience.

But I think that there are ways in which the subject selection issues and the informed decision making issues overlap and they overlap exactly here because, as has already been pointed out, the issue of what potential subjects understand when they come into a trial is really critical and where they are starting from affects the framework of their understanding.

And one of the things that I want to just say is that as a nonscientist, you know, listening to a forum like this is really fascinating. Of course, I am already in information overload and I only understood about ten percent of it so I do not know how the rest of you are who understand a lot more of it.

But what is fascinating is that an interprofessional discussion like this highlights uncertainty, it highlights what is unknown, it highlights differences of opinion like where is the best subject population to start from in early phase clinical trials. And you never see that kind of stuff in the consent form.

Instead what you usually see in the consent form is something like the following statement: "You have been offered the opportunity to participate in

this research because you have a disease that cannot be treated by standard therapies."

That is a true statement but the implications of that statement for potential subjects are arguably very different from the implications for researchers who are setting up the trial. And I think that it invites the therapeutic misconception.

And so I think we need to think very seriously about starting with subjects with less serious disease who do have other options and who are in a position to either postpone or to be -- to undertake treatment that does interfere with the investigational intervention and who can really make a decision to be involved in research that is not likely to benefit them but we hope is going to gather good enough data to benefit folks in the future.

Because really the researcher's two primary duties, it seems to me, are to minimize risk to subjects and to maximize the value of the data that are gained. That is really what the risk/benefit calculus is and there are a variety of different ways to achieve that and I think these two subject selection models are two very different ways of achieving those ends.

How you get there, as somebody said earlier, the devil is in the details where you start with each specific trial.

DR. ROBERTS: Go ahead.

DR. AGUILAR-CORDOVA: Yes. Estuardo Aguilar and I am also a member of the RAC. One of the issues that has come up today and has come up at various RAC meetings in the past is the use of controls. And we had

various presenters explain how important these are. As a scientist as well, I have nothing but support for that statement.

However, having incomplete data is sometimes more dangerous than having no data at all so, therefore, if one is going to have controls within these studies, one should be very careful to design the study in such a way that those controls are meaningful because if one has only very small numbers and then makes grandiose conclusions even as to trends that are being portrayed by those numbers, if they are not significant, they can be very misleading to the patients, to the scientific audiences when presented, and to the investigators themselves.

So I think it is very critical that if one is going to have controls and one is going to propose to have controls that the power of those groups be significant enough to have statistical --

DR. ROBERTS: I think that is a key issue and a good point because in the second group that we are talking about, I am not sure it is going to be ethical to use them as a control when they are candidates say for angioplasty or surgery or both. And maybe you can go ahead and address that ethical issue.

DR. WALTON: I would like --

DR. ROBERTS: Go ahead.

DR. WALTON: I think I would like to comment on that, which is if I understand what you are saying, that it is enrolling a group of patients to be the control arm or who get randomized to be the control arm --

DR. ROBERTS: Yes, right.

DR. WALTON: -- if they have other therapeutic options and uncertainty about the ethics of that.

I think that is no different than enrolling patients for -- whom get randomized to the active arm in our early stage studies where we have no confidence that there will be any benefit at all.

DR. ROBERTS: But you put the control then into active therapy versus you are comparing active accepted conventional therapy to gene therapy if you do that. But here we have got people with chronic coronary artery disease, symptomatic, who are candidates, let's say, for angioplasty or surgery. And you are going to randomize those and at the moment if I read it correctly we are not randomizing them to gene therapy versus conventional therapy. It is going to put them in a controlled group say with medical therapy perhaps but how do you deal with that today?

DR. HAMMOND: I think one of the keys here is that at least in the trial that I presented that Collateral had done with Schering, patients did not have unstable angina. There was no clear indication for surgical revascularization or revascularization of any kind. And yet they had anywhere from Class 2 and then later on Class 4 angina but they were not unstable and they were not preinfarction. So it was not as though we were depriving them from a traditional revascularization.

DR. ROBERTS: But we would be in the case of what we have talked about here or could be, I suppose.

DR. ENGLER: Bob, I do not think so. I was not proposing that

we randomize left main patients. I think patients that have a clear benefit from bypass surgery would be -- in terms of survival, it would be unethical to withhold that. You do not let a patient with left main disease sit in the CCU over the weekend, you know.

DR. ROBERTS: You said people with chronic coronary artery disease, symptomatic, meaning intermittent chronic I assume --

DR. ENGLER: Right.

DR. ROBERTS: -- who are otherwise doing well but many of those patients today in many places would be considered for angioplasty.

DR. ENGLER: Well, of course, they would but that procedure does not prolong the life. It does relieve their angina. These patients all have an angiogram in advance and one of the exclusion criterias in any trial like this ought to be if there is life-threatening disease, that is left main or several paroxysmal three vessel disease that is bypassable, the patient should go to bypass surgery because you are giving them the choice of something that works versus something that is unknown. I do not think that is ethically right. But for patients that have one or two vessel disease and chronic stable angina, they use four or five nitroglycerins a week, these procedures do not make them live longer.

DR. ROBERTS: Yes, but, Bob, you know in the real world they want something to relieve those symptoms that is rather than taking medicine and I think --

DR. FRIEDMANN: Could someone help me understand, please,

what the word "control" means in a Phase I study?

What is a control in a Phase I study? And what are they intended to --

DR. HAMMOND: Well, in the study that Bob is alluding to, the Collateral trial, the patients received material and the patients and the investigators do not know whether the material has active agent in it or in this case saline.

DR. FRIEDMANN: But I am thinking of what a Phase I study is. A Phase I study is a study aimed to understand toxicology -- toxic effect?

DR. HAMMOND: Right, but the toxicology requires, I think, a control group. It is mysterious to me that that is not a requirement for some of the reasons that we just said because the classic example are those eight patients or so that in the protein infusion trial that had cancer -- that developed cancer there were all in the control group. And so had those -- without a control group in that study, it could have been -- you know, the wrong conclusions clearly could have been drawn.

Any time something bad happens to a patient that got a gene therapy, of course, it is due to the gene therapy. And one would assume that even as an investigator that you need the controls just for that reason.

DR. AGUILAR-CORDOVA: Could I follow up on the original question?

DR. ROBERTS: Just for clarification. In you trial did you give a vector without the transgene?

DR. HAMMOND: No.

DR. ROBERTS: No.

Go ahead.

DR. AGUILAR-CORDOVA: So the original meaning of the question was not on the ethics of putting them on one therapy versus the experimental approach because you are absolutely right, whether they are in the experimental arm or the placebo arm, at this point in time one has to assume that it is the same.

The point is that if you have five subjects or eight subjects in one group, any number that comes out of there in a Phase I study, if it is not statistically significant, you might as well not have the number. And if you have created any kind of discomfort or risk to that patient and yet the number is not statistically significant, then what is the purpose of that number and why would you put that patient through that?

Even though it might make you feel better that, oh, well, some of the control subjects also had this and, therefore, it is not related to therapy. One could possibly get that data somehow else.

And if the numbers are not significant, they are not significant. It might make you feel better but --

DR. ROBERTS: Well, I agree. So what you are saying is that if you are going to do Phase I that you should have significant numbers for statistical analysis. On the other hand, I think in Phase I there is also observations made with respect to the dose and other things that certainly may

not require the same numbers from a statistical point of view.

DR. AGUILAR-CORDOVA: Absolutely. And in those cases then should there be control arms for those studies or should those studies just be done with one single arm? And when historical numbers be also potentially meaningful?

DR. ROBERTS: I think what Dr. Hammond is saying to you is that he would prefer to have some control even though it may not be a perfectly defined control where you are giving the vector without the gene and so forth but he feels that it should be a control even in that situation.

DR. HAMMOND: Yes, I think if it is the 21st beta blocker you are right but if it is angiogenic gene therapy I do not think that is -- you know, maybe we would have a different view.

DR. AGUILAR-CORDOVA: But if you have five patients, one -- five in one arm and five in the other, it is still not significant. So that it is not a meaningful number.

DR. HAMMOND: No. Clearly in order to get a real idea of the incidence of events you need to do studies with hundreds and probably thousands of patients but that is not going to be done with angiogenic gene therapy in a Phase I or a Phase II trial. That is the problem.

DR. AGUILAR-CORDOVA: It depends on the differential that you are working with.

DR. HAMMOND: So I do not think the alternative is not to do any controls.

DR. AGUILAR-CORDOVA: Not to do any controls. To do significant controls once you get to where you are assessing efficacy.

DR. ROBERTS: All right. I think that I want to move this along. We are still in this mode of operation of selecting patients and we have got these two groups. One I think that is running into some trouble but Bob Engler feels it can be done. And the other one is the group that people are working on with some probably lack of uniformity because I think we heard today some people had ejection fraction in the 30s and others in the 20s.

And so I am not sure that we will resolve but I do think that -- let me put it this way: If one can choose patients with chronic coronary artery disease and minimal or otherwise symptoms, and that they can be, indeed, treated with gene therapy versus a control, and that control will not include angioplasty or surgery because if it does I think you have not got the control we want. Let me say to the people who are sitting up here, would you think the time is right to do such a trial if you could?

DR. CRYSTAL: It depends very much on the -- what you are looking at. It depends on the agent, which changed, depending on the question. It depends on the patient group. It depends on the question that you are asking.

I would suggest that this discussion has to be superimposed on that. That is there are some trials that are going to start with five people or ten people and severely ill people because it is very analogous to doing chemotherapy initial trials in cancer. You do not start with people. Everybody knows that if it is going to work you are much better off having earlier phase

patients but there are the ethical risks and they are usually -- they are always started in late stage patients.

I would suggest the same thing is true here. There is going to be some trials and some agents that you are going to start with small trials that are going to be uncontrolled and then they evolve. What I was suggesting in my talk was that for some of these trials they are evolving to the point where we need controls to evaluate them but there are going to be other trials where we are still going to want to use late stage patients and they are going to have small numbers to begin early observational data and then you run into the late stage.

So I see it as a gradation. There is no absolutes.

DR. FRIEDMANN: Can I make a plea that we be very careful with our own descriptions of what we are doing and not be too facile with the phrase "gene therapy studies", studies of gene therapy. What we are talking about is studies of gene transfer which may or may not be therapeutic. We should reserve the word "therapy" for those instances in which we really either demonstrate or really do expect therapy.

DR. ROBERTS: I guess the intent is to be therapeutic.

DR. FRIEDMANN: Of course. But we can delude ourselves even in discussions like this.

DR. ROBERTS: I guess, well, it looks like from what I have heard and what you are saying is that there might be a select group out there, Bob, that you can sit down and have two days at the NIH and tease it out and there would be perhaps a group with chronic disease that are -- have normal ventricular

function or near normal and that you could find that group. And I guess I am going to leave that because it will take very detailed analysis to pull them out.

The next question I would ask --

DR. ENGLER: But we already did it.

DR. ROBERTS: Yes. Okay. Then that is possible.

The -- but we have to evaluate it when we see it.

The other issue is what people should be screened to be excluded, whether it is the severe or the moderate? Who should be excluded? Should we have an ophthalmaologist look at these people or should we make sure that diabetics are excluded, et cetera? And who would like to take that one on?

DR. CSAKY: Well, I think the issue of exclusion has to do with benefit. I mean, if you have -- if you are evaluating something and you feel like there is a benefit then clearly the risk in terms of retinopathy or blindness is taken into consideration. But if the risk is very, very low and the benefit is high then you would go ahead and treat those patients.

I think from at least the evidence to date suggests that at least there is no clear acceleration of retinopathy or even age related macular degeneration but the numbers are very small and clearly I think in all trials it would be very helpful if for no other reason to gather the data to have these patients evaluated by an ophthalmologist and on follow-up just so that we can start to evaluate (a) are there high risk patients that might progress that we do not know yet, and that is true for all therapies.

I mean, we are constantly screening patients and we have identified

through the years patients that are at higher risk that we did not know this beforehand. So there may be a subset of patients that will be subsequently at higher risk. Perhaps patients who do have high risk diabetic retinopathy.

And so I think it is important that all these patients be screened.

I think a priori if it is not a limiting factor, I probably would exclude patients who have high risk diabetic retinopathy or who have severe proliferative retinopathy at this stage of the game because I think you are potentially putting them at risk. And if you do not need those there is no real reason to include them.

And I think the same thing has to do with patients who have severe or any form of neo vascular macular degeneration. So I think all these patients should be screened and I think there is a subset of patients that should be automatically excluded. And I think the rest of the patients should be followed and the data should be gathered.

DR. ROBERTS: And I guess that that is probably in keeping with a lot of trials that have been done over the years that most people would realize that this time it might be good to keep those people out until we know more information or where it is going.

Yes, go ahead.

MS. KING: Can I add something just from sort of a guiding principle standpoint and you all can tell me whether this makes sense in terms of the facts that you have a better command of. It seems to me that there is two things at issue. One is -- and I am not sure that it is only a very small subset of

studies in which it is appropriate to enroll as subjects patients with less serious disease. It seems to me that there is this duty to minimize risk to subjects but in addition there is two other things that have to be considered.

One is that you have to look -- eventually you want to end up with an agent that you are going to use in a broader population than you are starting from and people have been talking about that as a consideration. How do you think about that as you are going through the phases of research.

But then there is also this issue that has come up a lot, which is you have to be able to distinguish the effects of the disease from the effects of the intervention and it seems to me that that is really fairly key and one of the things that happens inadvertently when you always start with very sick subjects is that you cannot make those distinctions and it becomes very easy to say, well, you know, we are really minimizing risks by using the sickest subject populations because we know that we can expect a certain number of deaths. We can expect a certain amount of morbidity and that masks a lot of things.

So I think if there are ways to tease out those distinctions it really may require making different choices about first subjects.

DR. ROBERTS: I guess that point was brought up today and I think that was also Bob's point of going to a group where they are not that sick and you can tease out both the safety as well as the efficacy. And I appreciate that point so go ahead.

DR. PRESSLER: Could I make a comment, though, about that?

If one picks a population where there are existing conventional

therapies that are readily available and those are implemented during the trial, which is so often the case, then you have a trial that is uninterpretable. And what are the ethics of doing a trial that then comes out with a nonmeaningful result?

So part of the desire at least initially, not once concept is proven but initially when one does not know if the concept is going to work or not, that if you pick -- especially in advanced coronary disease, if you pick patients who are good candidates for bypass or angioplasty, at least in this country, they will cross over into those treatments and then the data is uninterpretable.

MS. KING: Well, I agree that that is certainly a problem. My only point is that there are a lot of ways to end up with uninterpretable data.

DR. FRIEDMANN: Can I ask what did we learn -- ask a question of the ethicists in the room. What did we learn from the OTC study in Philadelphia and the choice of subjects in that study? As you remember there was an issue with regard to picking the sickest OTC patients, the infants who were likely to die, or picking less severely ill patients, older ones that had already shown that they can cope with their disease? What did we learn from that as an ethical principle? Not necessarily from the results of the study but what principles were resurrected in that and what did we learn from it?

MS. KING: I think actually that study is a pretty good illustration of the points that I am trying to make, which are that the initial argument that the best first subjects would be very ill infants in hyper anemic crisis was problematic on a number of bases, but most importantly it seems to me that it would have been very difficult to distinguish the effects of the intervention from

the effects of the disease in that study population.

And in addition the very great temptation is to make the biggest bang for the buck argument and say, gee, if this works, it will help these patients the most and that is why we should do it.

And that allows you to sort of forget the "if" because it has not been tried yet and it certainly is not proven to work.

When you move to a population of subjects who are more stable then you uncover some hidden things and the tragedy of that case was that it basically uncovered that either some aspects of the design or the way that the design was implemented were simply too risky and maybe the answer for that particular trial is if you start with less sick subjects you are going to have to be so much more exquisitely careful about minimizing risks that you may need to get sent back to the drawing board more often and redesign studies so that you do not run the risk of really creating harms that are very visible.

And in that case they were much more visible than they would have been if a different subject population had been selected.

DR. ROBERTS: So as a principle does aiming the studies at patients that are most likely to benefit, does that phrase mean anything?

MS. KING: It does not mean anything to me in early phase studies.

DR. ROBERTS: In Phase I studies.

MS. KING: Yes.

DR. ROBERTS: Because it implies --

MS. KING: It implies that --

DR. ROBERTS: -- that you are expecting therapy.

MS. KING: Yes.

DR. ROBERTS: Larry, you wanted to make a comment?

DR. FRIEDMANN: Yes. I just wanted to kind of expand a little bit on what Dr. Crystal said. I think there is too much emphasis on what a single study might teach us and I think we need to accept the fact that there is going to be an evolution and that the early phase when we do not know a whole lot about something may imply one sort of patient or one sort of control.

Later phase implies other sorts of patients and other sorts -- or as we learn more about the interventions. Similarly whether the kinds of controls or kinds of patients should be broad and very general versus very narrow. Again I think we are making a mistake if we think that a single study is going to address all of those. It has to be a strategy of learning over time and modifying as we learn more, whether it is the kind of patient or the kind of control or whether or not we exclude certain things.

DR. ROBERTS: We are going to leave out selection of patients except for one pointed question and that is we want to know if you want to exclude the patient with the tumor how many CT scans or blood tests do you do? Do you do this exhaustively? Who has got a suggestion? A good quick solid answer.

DR. UNGER: A quick question, which is in VEGF in vivo trial, how many of those tumors would have been detected? How many were solid

tumors that would have been detected prospectively by running their bowel, doing a CT of the abdomen, chest?

DR. HAMMOND: I think the answer is those patients were screened when they entered that trial.

DR. UNGER: ACS screen, right.

DR. HAMMOND: That is my --

DR. ROBERTS: Yes.

DR. UNGER: So, I mean, Dr. Isner showed us a patient who, you know, the day after they were dosed, you know, here they do an MRI of the abdomen and there is a tumor. Obviously that is one subject.

DR. HAMMOND: That would not have been picked up in the usual screen.

DR. UNGER: Right. So my --

DR. HAMMOND: That particular tumor.

DR. UNGER: -- question is if a more intensive screening were done, would we be able to -- I mean, obviously we would prevent some of these. The question is cost benefit and I wonder -- I mean, based on case reports I have read -- if some of these, you know, many of these would not have been picked up and maybe ACS is not enough. Maybe we should be doing more and I would like to throw that out.

DR. ROBERTS: All right. We have thrown it out and we have got about one minute to answer that one.

Anyone -- are either of the people who are at the mike, are you

going to answer it for us?

Okay. An NCI point of view?

DR. SRIVASTAVA: Yes. I just wanted a few questions for Dr. Hammond. In his study he excluded all the neoplasm and then I wanted to find out what was the rationale because most of the time the neoplasm has a long natural history and your trial perhaps may not be that long. So I wanted to know what was your exclusion criteria?

DR. HAMMOND: Cancer ever except basal cell and then pretty thorough screens, paps and, you know, fecal, blood and mammography, and PSA. But any cancer, any history of cancer at any time they are out. They are not in the trial. Does that answer your question?

DR. SRIVASTAVA: So what was the rationale? Why did you exclude them? They are not likely to benefit from your treatment?

DR. HAMMOND: I mean, this was -- remember this was the first ever intra-coronary delivery of an adenovirus.

DR. SRIVASTAVA: Okay.

DR. HAMMOND: And so, you know, we wanted to make sure that the potential risks of this would be kept to a bare bones minimum.

DR. ENGLER: Maybe I could answer that briefly. We were aware of the data from Judah Folkman's lab for many years that the tumors could be occult and be dormant. And so we were afraid that if a patient had a resected tumor they could have a dormant and we could actually make that patient a lot worse. And for that reason we excluded patients with any history of cancer.

I still think that that is smart.

DR. ROBERTS: So bottom line is that anyone that has any suggestion of cancer picked up by any means you would exclude them but the question, I guess, still left in the details is how far you will go to screen, and I think that probably that is a detail we will have to leave unless someone has got some quick answer.

DR. SRIVASTAVA: So I just want to -- NCI does not have any guidelines for cancer screening. Everyone is following ACS. We used to have guidelines but no longer. We no longer endorse. The U.S. Preventive Task Force has guidelines for cancer screening. So either of those two or both -- both of them should be applied.

DR. ROBERTS: So one or both of those would be what we would recommend at the moment to follow those guidelines.

DR. SRIVASTAVA: Which one I will recommend? Personally, not from NCI, I would recommend that ACS is being used more frequently and the Preventive Task Force is more conservative in terms of frequency of screening.

DR. ROBERTS: Okay. All right.

I will turn now -- go ahead. Sorry, you have been waiting.

DR. MARTIN: That is all right. I have another question about Phase I clinical trial conduct. Basically the question -- Tyler Martin from Valentis.

The question goes to Milt. You know, you said one of the critical

things that in your opinion was critical to conducting a Phase I gene therapy trial was to have an independent oversight safety committee and, you know, we have been doing -- on the industry side we have been doing Phase I clinical trials for 40 years, the literature goes back to Gahad in '61 and so on and so on. And that has never been the way we have conducted Phase I clinical trials.

So my question for you is what do you think about the hypothesis testing and Phase I trials is different in gene therapy than all other drug development or should -- or has your organization decided that for all Phase I clinical trials you do, regardless of the therapeutic area or the method of delivery, that you can have an independent oversight committee?

Thanks.

DR. PRESSLER: No. I was specifically referring to gene transfer trials and one might make an analogy to other areas where it is a forefront technology. We do not have routinely independent data safety monitoring boards until larger studies are done. But in this particular case we thought that it was a prudent extra step.

DR. MARTIN: So you would agree, Milt, that the principles are, in fact, effectively the same and that perhaps this is a position that Pfizer may have chosen to take but it is not necessarily the rule you would apply across the universe of industry sponsored Phase I trials?

DR. PRESSLER: Right. That is right. Not across the universe. Just we felt it was a prudent step in this novel area.

DR. ROBERTS: Yes?

DR. RUSSELL: This is just a plea again for better pharmacokinetics analysis in Phase I and II clinical studies because, you know, we have heard again about two fairly large studies with VEGF and FGF. And at the end of these studies we are going to have no direct evidence that the gene was expressed in the tissues in these patients.

We all believe that the genes were expressed but there is no direct evidence for that. The gene product cannot be detected in blood. And I think, you know, ideally the pharmacokinetics data that we would get from Phase I studies would tell us which tissues they are expressing in the gene, when does expression come on, how high does it go, how long does it last, when does it come back down to zero to guide repeat dosing and so on and so forth.

And I see this as a serious problem because if the treatment does not work then does that mean that this transgene is completely ineffective or does it mean that the vector did not efficiently deliver and express it?

DR. ROBERTS: So I take it from that that you would like for them to measure the protein in those tissues to see if it is expressed, is that right?

DR. RUSSELL: Somehow, yes. But perhaps by expressing marker protein that is secreted into the blood, whose expression is linked to the transgene expression, or perhaps by developing some other kind of innovative methods to try to determine whether or not the transgene is actually being expressed, because that information is completely lacking in these studies.

DR. ROBERTS: Who would like to have a crack at -- the last crack at that?

DR. SPRINGER : Actually the area of noninvasive imaging of gene products is getting very, very interesting right now and I think that is really the reason why. When we do the animal studies we can sacrifice the animal and take as much tissue as we want and analyze it but what you really want to be able to do is follow gene expression in a human subject over time. You do not want to be punching biopsies every day.

So I can leave it with that that there are MRI based and PET based noninvasive imaging methods that are being worked on. I do not think they are quite there yet but I think that will get us to where you want to be.

DR. ROBERTS: So that means that things like MRI imaging at the moment is not feasible but probably will be and is rapidly moving in that direction. Is that fair?

DR. SPRINGER: Well, it is feasible. It is just not quite as -- the resolution is not quite there.

DR. ROBERTS: Yes. Right. Well, that is what I mean, put the whole picture together. But that if we were to do good careful studies in the animals and look for the protein in those tissues, it seems to me that while we are in this limbo phase of waiting for resolution, that would be a very reasonable story and certainly should not deter from going ahead to do the appropriate trials.

Is that a fair statement?

DR. HAMMOND: It is a fair statement and keep in mind there are noncardiovascular gene therapy trials where clearly the transgene protein that is secreted into the plasma has been detected for a long, long time. I mean, AAV,

hemophilia trials are, you know, among those. So it is not correct to say they are not followed.

The problem here is that it is the heart and the problem also is that plasma does not equal heart so you can have nothing in the plasma and a lot in the heart. And then the levels of virus that we use in our studies may well be beyond the level of detectability even if you, you know, did it in a pig. I mean, that little virus that we are using is awfully hard to detect.

We are using surrogates, more time on a tread mill, better perfusion, better function. Those things are very real surrogates because what they translate to is people feeling better and being able to do more.

DR. RUSSELL: But that is not the surrogate for gene expression because we have heard about the problem with placebo effect of any therapy in this situation.

DR. HAMMOND: I am not arguing that it is. I am just pointing out the impediments to your getting what you have been demanding all day.

DR. _____: I would like to follow up on a theme that was just articulated. Even if you were to succeed in developing an assay, which by no means is easy for growth factors, if you were to develop an assay that can detect in any reliable manner the biologically active form of the protein, you still are left with a very incomplete story because it is not the protein alone that is doing it if it is doing something. It has to do with receptor expression, receptor occupancy and downstream events post receptor occupancy.

So just by measuring the circulating level of the protein in the

plasma you might be left -- not only are there localizing issues but you have a very incomplete story as to what may be happening in the heart post receptor occupancy, especially in the context of VEGF protein -- family of proteins, you have both VEGF1 and VEGF2 sharing the receptor.

How much of VEGF2 binds the receptor in the heart when VEGF1 is already bound? What does VEGF1 do to VEGF2 occupancy? How does that change post receptor events?

DR. ROBERTS: I assume that it was meant that you would like to measure the protein in the setting of all the other functional analysis such as the treadmill and so forth. And I think that would complete the story from that point of view in that -- he has just left -- but if we could measure the protein and those functional tests, I think that would complete the story from that point of view. You would know at least that it is expressed and if we can do it in a variety of tissues to know is the gene expressing its end product in tissues other than the heart when we are dealing with cardiovascular disease?

So I think that was what he had in mind.

Now I have got a couple of specific questions. In monitoring the heart both for safety purposes and efficacy that there are lots of things we can do today. We can do noninvasively echocardiogram. We can do the exercise testing to look for that sort of thing and the routine.

The question that was brought up earlier today about the electrical stability.

And one quick question, I guess I can address Eduardo and anyone

else who wants to do it clinically, that it probably is not practical to do EP testing and that Holter monitoring is about what you can have together with the routine EKG. But is it feasible or should it be recommended to do some EP studies in Phase I or Phase II?

Eduardo, do you want to --

DR. MARBAN: That is a tough one. If you look at the long view and in an appropriately powered trial you can satisfy an unbiased observer that there is no increase in sudden cardiac death, then I think all of the theoretical worries can be laid to rest.

But you are asking a more difficult and perhaps more appropriate question at this stage of development and that is whether to -- in the context of Phase I -- purposely look as hard as you can for adverse effects related to electrical instability.

It depends on the specific trial design and the expectation -- I think you have do a reasonable prediction of what the risks are likely to be and it is not a question that I think can be answered glibly.

It might be actually an interesting question for numerical simulation and there are some decent electrical models now of whole heart conductivity that are biophysically detailed and rely on massively parallel numerical simulations. And it might actually be instructive to use these quantitative models of cardiac excitation to predict just how much heterogeneity induced in the context of neo vascularization it would take to provoke an arrhythmia in an otherwise stable substrate.

A good -- that is an interesting topic and one that I think would have to be studied in a very deliberate manner before a good answer could be provided. I cannot give you a yes or no answer on that.

DR. ROBERTS: Doug, do you want to --

DR. MANN: I have one comment and then a question for Eduardo.

I mean, your case report forms pull out sudden death versus pump failure. Okay. So I mean most of that -- at least if the case report forms are set up so that you could pick up sudden death versus a nonsudden death. And do you think there would be any utility for single average ECGs or T wave given that they are both predictive for events in patients with ischemic coronary disease as a way of predicting sudden death or at least heterogeneity?

DR. MARBAN: No, I do not think so but true believers might.

DR. ROBERTS: That is a good answer. I like that answer. I mean, my only point I would say -- I am not -- I do not want to put down either of those methods but I think neither of them at the moment has found its robust or otherwise defined role as we would like to and I think it would be a little bit difficult to use them in a situation such say this. That would be my answer but certainly they are noninvasive techniques and ones that could be used if they did give you the sensitivity.

I would be concerned about the sensitivity at this moment.

Did you want to come back on that, Doug?

DR. MANN: I just -- I think that at least in heart failure studies

they have been reasonably predictive for ischemic cardiomyopathies rather than dilateds and it is just a simple way of looking at something to begin to address some of the issues you brought up.

DR. MARBAN: I am just worried that you might get false reassurance from a negative result. The CAST trial is instructive. People -- based on conventional wisdom, every reasonable prediction based on Holters and EP studies at the time, the drugs that were used there in a prospective randomized trial of ventricular arrhythmias were thought and predicted to benefit mortality or at least not worsen it. In an appropriately powered trial they actually were found to kill people.

There is a lot of examples in the electrical literature of counterintuitive results and when you are doing something as potentially messy as gene therapy I think one would err on the side of caution and over documentation in Phase I.

So I think in that general philosophical vein, yes, you want to study patients as thoroughly as possible. But doing an EP study on every patient is a little bit akin to doing a whole body MRI on every patient before they get FGF. Maybe that is appropriate and maybe it is not.

DR. ROBERTS: One other comment I should make, and we deliberately did not tell you this earlier today, that everything you are saying is going to be published and so that is why we wanted you to be very candid. Now you will probably be more cautious.

Dr. French has promised to publish this so it will be put together.

The -- I think that probably I would like to get some idea about two things. Follow-up studies. It is true that most of the data that we have talked about has been short-term and so how extensive should we be in the follow-up first and foremost about safety?

I mean, should everybody have total body scans at six months or one year or what have you because we are looking at this neoplasia, we are looking at possible tumors, and that is the two things that has come up. The information seems to be that it will herald itself in other ways and we do not have to look as hard to find that as we do perhaps those other incidental findings if they are incidental.

So I would like to have some discussion on how far you are going to go looking for tumors in follow-up.

DR. UNGER: May I take that?

I would say that long-term follow-up is important but obviously the detection is not too much of an issue because if someone is going to have a tumor it will show up eventually. So in terms of the types of studies that have to be done, not much is needed. But one of the major concerns about all of these agents and gene agents is the potential for systemic neo vascularization.

And the argument about crossing the blood-brain barrier not withstanding, the retina offers a perfect opportunity to view blood vessels and take pre and post pictures. It is very, very difficult to assess blood vessels in a three-dimensional structure, which is basically every other structure in the body.

The retina is two-dimensional and it lends itself beautifully to

analysis of blood vessel numerical density and it is perfect. So for the question of whether there is systemic neo vascularization, I think a follow-up, and this is not an FDA position, this is my scientific clinical judgment that the retinal follow-up is essential for the issue of systemic neo vascularization.

DR. CSAKY: I would just add, though, that in the patients that we have evaluated for retinal neo vascularization, I think it is adequate. You know, it is the same thing. You can do floracine angiography. You can start doing extensive testing for neo vascularization.

But I think it is important in guidelines, in our guidelines, and some of the studies that we did at NHLBI, we simply did observation and photography for documentation. I think at that point -- at this point that is probably adequate.

If we start finding positive results, that is if in some of these trials you do start to see perhaps an incidence that is higher than the natural history then you might want to pursue it further and start to investigate is there something going on.

But I think at this stage it is probably adequate just to get -- you know, have a retinal exam, have documentation by photography at follow-up and probably at several months, at three, six months, and perhaps a one year follow-up after the gene insertion would be adequate at this point, I think. And then review that data in relatively non-high risk eyes and see if there is an increased incidence. If there is not, then assess it from there and then go to the next level.

DR. ENGLER: I have a follow-up. When we were looking four

years ago I consulted with a number of ophthalmologists and what they said to me was that the detection of proliferative retinopathy or pre-proliferative retinopathy enough is easy to do without a photograph.

So they said that we did not need photographs unless the patients had pre-proliferative retinopathy.

If they were at the stage of pre-proliferative and up then it would be very important to have photographs to look for the details of whether or not we were actually changing the course.

Would you agree with that or are you saying to get photographs of every single patient even with a normal exam and no diabetes?

DR. CSAKY: Well, in the study we did here we took photographs and there have been some studies to show that the sensitivity on photographic rating is just perhaps a little bit higher than on individual examination but probably not that critical.

So I would agree that if you had a retina specialist -- and again I think it depends on the level of expertise of the examiner. If you have an optometrist examining the patient versus a retina specialist, there is obviously going not be a level of sensitivity that the investigator is going to be able to detect.

So I think there is a couple of issues. Clearly if there is a normal exam, perhaps not. But again at this stage where you are just gathering data it would also be a question of -- unless there is a cost, which is not that high, I would almost argue for why not collect it at this state when we are really in a data

gathering mode so we can always go back and examine it retrospectively.

DR. PRESSLER: Just as a comment on retina photographs. In trying to implement this over multi medical -- major medical centers, it has been a major impediment just to meet the standard of the retina specialist reviewing -- an independent retinal specialist reviewing those photographs.

So our experience has been that it is -- although it sounds very straight forward, if one has expertise to evaluate retinal photograph for proliferative retinopathy, meeting that person's expectations is not trivial.

DR. ROBERTS: I am going to turn it over to my co-chair and I think we are up to the last topic to say something about the consent form.

I want to thank everyone for a terrific day. I have to leave. And I also thank the opportunity of being here. Thank you very much and I will turn it over to Dr. Friedmann to continue.

DR. FRIEDMANN: Thank you. Have a good trip.

DR. ROBERTS: Thank you.

DR. FRIEDMANN: Well, it is getting late and I think we are all running out of steam but there is one issue that would be very helpful to all of us certainly on the RAC and to other people trying to design clinical studies.

That is how we reflect this increased wisdom and increased information plus maybe increased uncertainty to the study participants in terms of the consent process, the consent documents.

What do we say to them or what -- at the end of today would we design consent documents any differently from what we would have done earlier

today? What do we say about tumors? What do we say about the likelihood of other vascular disease, either preexisting or induced by the manipulations? Are there any suggestions about rethinking the information that should go into a consent document?

MS. KING: Let me say something really general, which is that it depends.

DR. FRIEDMANN: It depends on what you mean by "is" right?

MS. KING: Yes. But, you know, I do not think you can do this by saying there is boiler plate out there. You have to say we have got to be very careful and do this on a study by study basis and it is going to differ depending on, you know, all the things everybody has already outlined.

I think it is extremely important and this is actually a comment about follow-up as we were just discussing. One of the issues that is always really clear in research is that subjects can drop out of a study at any time. That has profound implications for intervention studies in which there are long periods of follow-up.

If subjects think they are patients they are much more likely to drop out after they have got the intervention because it is either going to work for them or not. If subjects understand that they are -- that there are long-term issues that we do not very much about, whether it is in specifics having to do with angiogenesis or in general things like the number of unknowns then they may understand that follow-up is especially important for their own health but that it is also especially important in order to make a contribution if they are made

partners. If the financial burdens to them of follow-up are minimized, which may be considerable in very long follow-up. If everybody, as they have been talking about, looks at noninvasive monitoring techniques and develops better ways of doing that kind of follow-up.

So there are some general kinds of things that consent forms need to be better about in terms of really telling subjects what to expect from the entire experience. And what they are really getting involved in order to do.

DR. FRIEDMANN: Well, the next protocol you get then in the RAC that deals with angiogenic approaches to disease, whether it is cardiac disease, cardiovascular disease, tumors, whatever, are you going to be looking out more for -- are you going to be demanding some information, some comment about long-term oncogenesis, long-term vascular disease? Is that something that you are going to expect?

MS. KING: Sure. And I think actually a lot of consent forms already have some information so there are models out there but in every category of gene transfer there are some things that should have more emphasis than they currently do in the consent form and also in the consent process.

DR. FRIEDMANN: So no new principles from the discussion today. More and better of the same.

DR. MEISEL: Can I say something about that? I think I would like to repeat the remarks that are probably responsible for my having been invited to serve on this panel, and that is there is a very important difference between consent forms and the consent process.

The consent form is not informed consent as I hope you know but in the event that you may not, I need to say it and I certainly need to emphasize it even if you do know it already. Consent forms should document consent but they should not be the vehicle for conveying information and I really think that the most important thing in the whole discussion of informed consent is to emphasize that probably it would be better to ditch the phrase "informed consent" and talk about subject education.

And, as Nancy said earlier, I think that one of the most fundamental things is for subjects to know that they are subjects, not patients, and that this is a long-term participatory process and that it is a partnership and that any benefit that the subject might acquire, at least in Phase I or other early studies, is purely accidental and incidental but it certainly was not the intended purpose of all this and that they really do not stand -- they are really not intended to benefit by it. If they do, so much the better.

DR. ENGLER: I recently heard Jesse Gelsinger's father talk, probably some of the others in the audience did, too. He gave a 40 minute talk or so entitled, "Jesse's Intent." And it was very interesting. He was very clear that his son's intent was to be a volunteer subject in a trial that might help others with a disease.

And so one of the things that I think that many investigators make a mistake of is when they come to that question that says "risk/benefit ratio" they think about the benefit to the patient who is enrolling in the trial.

And I have never thought that. I thought that that was relatively

minor and relatively unimportant and sometimes should not even be considered because if it is really an experiment or a trial, the patient should not expect any benefit.

The real reason to volunteer for a trial is what good you might do for society and I think that needs to be emphasized in informed consent and there will be a videotape available of Jesse Gelsinger's father giving this talk. It is fantastic and your eyes will not be dry when it is done but the incredible thing is that this kid actually had the right intent. He knew that he was not going to benefit.

DR. MARTIN: Tyler Martin from Valentis.

I would like to make one quick comment. I made it before to Ms. King. I would like to ask the same thing of Alan. Do you discriminate between unknown benefit and no benefit? If the answer is no, do you discriminate between no risk and unknown risk? Because I think there is a very different thing. Because if we accept that efficacy of a product is an intrinsic characteristic of a product at a given dose given in a certain way then a patient in a Phase I trial will have potential benefit if, in fact, the product is determined to be infective or to be effective. It is unknown at that point in time but it is not necessarily known.

DR. MEISEL: Well, I think in any clinical trial a major part of the discussion has to be on uncertainty because if there were not a high degree of uncertainty we probably would not be doing the trial.

And that goes for benefits and risks and it is very important, I think

as Dr. Engler said, to emphasize to whom the benefits are most likely to accrue. And that is one of, I think, the fundamental features of clinical research especially in the early stages, is that the risks are assumed by the subjects but the benefits are more likely to accrue to society in the long run.

I mean, ultimately it may be to the subject or to people in the subject's class but in the immediate future it is not likely to be or at least the subjects need to be told that.

And I really do think that the therapeutic misconception is very, very strong. It is a very strong part of psychology and it has already been said here today. The people go into these trials with the notion that even if they cognitively know it, that there is an underlying affective belief that, well, it will. A secret belief that it will benefit me. And I really think we need to do something to try to dissuade people from believing that.

We may not be successful but at least we will have done the right thing in attempting to dissuade them in that.

DR. FRIEDMANN: Okay. Let me take the opportunity then -- unless there is something urgent from the audience or from the panel -- to thank everyone involved. Thank the audience for coming. Thank all the panelists for what I think has been a very helpful and a very constructive day.

I started out the day saying that I thought that this field was in many ways typical of the gene therapy conundrums that we faced but presented particularly difficult and complex clinical situations, opportunities and obstacles. I think that we have proven all that today but the discussions have been very

constructive and very helpful.

Certainly I think it has helped me think about how I am going to read the next set of protocols. I hope it has helped the rest of the RAC.

Of course, speaking for myself but I hope I am reflecting Amy's feelings, too, that it has been a very useful session and thank her, of course, for all of her incredibly hard work to piece this together.

I thank the NHLBI for their participation.

And remind you that all the organizers, NHLBI and OBA, would be delighted to get your feedback either on the forms that are in your package or by e-mail. We need to know if we have had an effect on the way you think about the field and if there are areas that we missed in this session today that you think are important for OBA or for Amy or for any of the other people to be aware of. Put them down in e-mails or letters or whatever.

Have I missed anything?

Our deepest thanks to all of you and to the panel and let's go skating home. I guess it is not too icy outside. Thank you again very much.

(Whereupon, at 5:52 p.m., the proceedings were adjourned.)

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