started in small animals, we have moved to large animals, and we have moved to patients, and these are the cells that we knew the most about, and we have had to say you know what, we missed a lot, and we have had to go back and take the safety and functional effects that we have seen and reevaluate them in all of these animal models again, and I think that should be a lesson for going forward.

Is it time for randomized trials, and, if so, who? The think the questions we have to ask is, is it safe, who are the right patients, what are the appropriate endpoints.

I show this every time. If we do this wrong, we are really going to doom what I think is an exciting field. I don't want to be standing up here talking to you about the gene therapy lessons that we could have learned and didn't.

I think what we can learn from gene therapy is in 6 open-label trials, they were all positive, and 4 out of 5 randomized, double-blind, control trials, they weren't. Is that because patients got better care, or because there was a better placebo effect? Well, we won't really know until we do the randomized trials, but we need to under-promise and over-deliver, not conversely.

These are the people who did all the work, and I thank you.

[Applause.]

DR. RAO: Thank you, Doris.

We are open for questions.

Q&A

DR. ROSE: Dr. Taylor, I enjoyed your talk, but I must say, as a clinical cardiologist, I just have difficulty in envisioning how these myotubules, that presumably result from the injection of the myoblasts into scar, increase systolic wall thickening, which is what I would accept as evidence of improved regional function.

Unfortunately, many of your images weren't showable, but was the increase in thickening that you showed on that on that one graphic slide, was that from your larger animal models as opposed to your smaller animal models?

DR. TAYLOR: No, that was from rabbit.

DR. ROSE: You mentioned early in your talk about a disconnect between the numbers of cells and the functional improvement. Is that true for the myoblast injection? In other words, there was probably a range of responses, I would imagine, some had perhaps better systolic thickening

improvement than others, and by histologic examination, were there more myotubules or were the myotubules oriented in a different direction than the animals that perhaps had less improvement in systolic thickening?

DR. TAYLOR: Have we quantified that unequivocally? No, because it is very difficult to quantify the number of cells in an infarct and know that they are actually myoblasts in the cells you injected because we are giving autologous cells.

One of the rate limiting steps in this field is having good markers for the cells, and that is one of the reasons we went to iron, so that we could actually stain our sections later for Prussian blue and look for iron and say, okay, these are the cells we injected. We shouldn't see that in normal cells.

So, we are starting to now look and try to answer that question. What we do know is that with a number of studies that have been done by a number of groups, the number of cells that you retain in the scar after you inject them is about 15 to 25 percent of the cells you initially put in there probably. On a good day, maybe 30, 40 percent of the cells you put in there, but that is rare.

What we also know is that numbers of cells can die over time, but we also know that these cells can proliferate over time, and when we look for proliferation, we in fact can find that in these scarred regions.

I don't actually think that we are seeing evidence of long myofibers necessarily. We see cells that line up with each other and connect with each other, and we believe that these cells are being mechanically induced to contract.

We know when you stretch muscle, it contracts. Do I think they are electrically coupled with the rest of the heart? No. Do I think they are mechanically coupled? Yes. What we see is we measure left ventricular pressure going up, we see pressure plateau, and right at the time that pressure within a few milliseconds after pressure plateaus, we see our crystals move in that region of the heart, and then pressure decreases, and then we see that again.

We can track that beat after beat after beat in the scarred region. We can go from negative work loops where the rest of the heart is being pushed by the remainder of the heart to positive work loops when we put the

cells--actually, I have got it backwards--negative in this direction versus positive work loops, that actually correspond to systole.

So, I believe that there is wall thickening and actual contraction going on in that region, and I think our sonomicrometry data are the most convincing data.

Is it possible to get the image to show, so I could try to show one of the functional wall thickening images, so you can actually see that? If you just give me the slide thing back, I will try.

I am going to try this first.

So, this is, in theory, it is beating here. No, apparently I can't, maybe you can, but we are able to actually show thickening and I will pull my computer out to show anybody later who wants to see it.

You can actually see in the sham-treated animal, here is the scarred region and only in the very center of the scar is it not thickening anymore. The scar was from about here to here, by contrast.

The scar of the sham-injected animals over here is not thickening at all. Here, we only don't

see thickening right in the very center of the scar, and we used contrast enhancement, so we took 10 slices through the rabbit heart, in a long axis view, we used contrast, and only where we saw contrast gadolinium did we call that infarct, and then measured thickening in that region, and we did that in a blinded way.

We are pretty convinced that we see wall thickening in that region.

DR. BLAZAR: You made a good point that the larger animals allow you to assess function much more directly and extrapolate to humans. With the smaller animal models, you have a higher throughput. So, the question is, what is the data that says that the smaller animals extrapolate, the large animals extrapolate to the human as you go through all of these different examples, because one would hear your presentation and think that it really just should be restricted to large animals minus a few, more esoteric.

DR. TAYLOR: What I can say is we have made measurements in rabbit and pig for many, many years, and I swore we would never use mice or rats, and then we decided that we wanted to start making comparisons with bone marrow derived cells, and we

didn't have the markers to isolate those cells in rabbit or pig, so we went back and did the same experiments in mice, and we got the same results.

So, now I have to bite my tongue and show mice data even though I swore we never would. I am convinced at least with MRI, that we can make meaningful measurements that show us the same sort of thing.

DR. BLAZAR: Although you also made a point as to the dose, location, et cetera, it would seem that that is going to be extremely difficult.

DR. TAYLOR: It is very difficult. That being said, what we do is we inject the cells in the center of the scar in a mouse. Do I believe we get the same percentage that we get in larger animals? No. We really don't know what number we actually get in.

We have started doing some biodistribution studies to try to answer that, and we are mostly doing those in rabbit and pig, because I have no confidence for the numbers we get in mice. In terms of doing stem cell studies, though, I think it is critical that you use something where you can clearly define the cells.

DR. ALLAN: A little bit of a follow-up,

which is use of nonhuman primates. Somebody mentioned that there were some primate studies. This morning, I think somebody just referred to them.

DR. TAYLOR: Right, with bone marrow cells.

DR. ALLAN: Is it with bone marrow cells? Because you can use those markers on many of the nonhuman primates, you can look at stem cells.

DR. TAYLOR: Right.

DR. ALLAN: Maybe to use that model as a step between "large" animals and humans, because anything that you have derived that looks promising could through that nonhuman primate.

DR. TAYLOR: I think that is good point, and we have actually started collaborating with some people in California who do nonhuman primate studies. I can tell you that they are God-awful expensive, they are hard to do, and I personally find them hard to do.

Sometimes we just have to get over it, but I think if the pig data are good enough, I would rather stick with pigs, and the fact that we can use some of the same stem cell markers has made me focus more in that area.

DR. BORER: That was a very interesting presentation. I have a question that sort of falls into are we looking at the data the right way or oversimplifying box. You can't argue with success, and I believe that some contractility is probably better than done and perfect is the enemy of good, and all that stuff, but, you know, you have put in skeletal muscle cells, and they contract, and yet to get useful work from the heart, forced generation is just part of the equation, you have to have forced transmission, as well, and I haven't heard anything yet about remodeling at the extracellular matrix and regeneration of dystrophin ECM hookups, and whatever, that might demonstrate that we are actually developing a forced generation/forced transmission system.

I don't know how important that is ultimately, but it makes me wonder, the fact that we haven't heard about that, and I am not sure that I could expect it would happen, that we are actually looking at the data the right way, so could you talk a little bit about that?

DR. TAYLOR: Sure. The one piece of data that we do have, that I think addresses that, is the compliance data where we looked at changes in

strain with the different cell types, and that begins to address matrix and what is going on in that matrix - does it look at signaling, does it look at MMPs, does it look at any of that? No.

I think the bottom line is when we started this 15 years ago, our goal was to show it worked, and then once we showed it worked, we thought we would go back and figure out how it worked.

As soon as we showed it worked, God and everybody wanted to do it clinically, and so we are having this discussion rather than understanding how it worked, which we have had to develop in parallel. I think we are still catching up in terms of trying to develop, trying to understand the mechanisms by which it works.

Five years ago, at American Heart, Michael organized a session, and there were probably five or six people talking about this. If you look at American Heart now, there are two days of people talking about this. People didn't believe it five years ago.

Now we have a critical mass in the field and we can start asking those questions, and I think the data will emerge over the next couple of years.

DR. RAO: Dr. Ruskin.

DR. RUSKIN: Doris, you described a very significant prolongation of action potential duration in the myoblasts from about 20 to 120 milliseconds. That suggests a change in iron channel expression. Do you have any information as to how that came about?

DR. TAYLOR: Gus Grant told me it did? No.

DR. RUSKIN: I will buy that.

DR. TAYLOR: Short answer is no except that we know that we started seeing a plateau phase which wasn't there before, and we didn't change the rate of rise of the action potential. Do I know any more than that? No.

Have we looked for channel markers? You know, here I am saying, well, the only thing I will believe that if you tell me it's a cardiocyte, is a channel marker, have we looked for channel markers? No, because we did all of that in rabbits, and the darn markers don't exist.

Are we now trying to figure that out in some of these other animal models? Yes, and I think that is where some of the mice genetic models of changes in electrical activity in the heart may

actually be really useful in terms of trying to dissect what myoblasts can do.

DR. HARLAN: You had a great quote from Einstein. I will have to give you a quote I have from Osler, where he talks about stern iconoclastic spirit that we need, and that you reflected.

My question is I think I misunderstood, you implied that there was not the cellular specificity that people assume, that you have seen some contractility with myoblasts, but also with bone marrow. I wonder how extensively you have studied that with other cell types.

DR. TAYLOR: We have looked with myoblasts, we have looked with bone marrow stroma, and we have looked with bone marrow mononuclear cells, and we see an improvement with all of those. We haven't gone back and dissected the bone marrow mononuclear cell populations, we are starting to do that now.

Other people have seen the same thing with MAPC cells. So, have we dissected that in detail? No, but by the criteria that we have used, which is sonomicrometry and MRI, we see the effect, and yet we don't, when we look at histology, we don't see the same degree of muscle formation with all of

those different cell types, which begins to argue that mechanism is more complicated than we thought.

DR. HARLAN: Let me ask it this way. Are there cells that you have looked at, that you inject, where they don't work?

DR. TAYLOR: Fibroblasts.

DR. HARLAN: Fibroblasts don't work.

DR. TAYLOR: Fibroblasts actually improve compliance, but make systolic function worse in our hands.

DR. RAO: Dr. High.

DR. HIGH: I want to ask one question to try to reconcile some of your data preclinical studies with some earlier clinical work that we heard about.

Was that in rabbits or pigs that you said that you needed at least 10^8 cells to see an effect, was that rabbits?

DR. TAYLOR: That was rabbits--no, 3×10^7 in rabbits, 10^8 was in pigs, I am sorry.

DR. HIGH: Okay. Then, the 10^9 cells that are being injected in the clinical study would be roughly appropriately correlating in terms of--

DR. TAYLOR: Right, we actually see a better effect with 3×10^8 cells in pig. We

haven't gone up to 10^9 cells yet although we have plans to do that. Philippe can tell you, you start dealing with massive numbers of cells, and when you are doing this in an autologous way, and you are dealing with cells that you have to keep at low confluence, it gets out of hand pretty quickly.

DR. HIGH: You said that in, is it the pig studies, about 15 to 20 percent of the injected cells are retained?

DR. TAYLOR: That is actually not data from my lab, that is data from other groups, and that has been in some pig studies, and I believe in some--I know of pig, and I can't remember what else.

In our hands, in rabbit, we see a little bit higher than that, on the order of 20 to 25 percent, but that has only been in a few studies with indium-labeled cells, so I don't trust those numbers yet.

DR. HIGH: Is that known to be a function of time after injury?

DR. TAYLOR: Actually, what we found is that the longer we wait after injury, the easier it is to get cells to hang around in the heart, that if we inject cells at 2 weeks, we get fewer cells

retained than if we inject cells at a month.

What we also know is if we inject cells in normal heart, they all go in the cardiac vein and get carried elsewhere, that the junctions in the myocardium are so tight that it is really hard to get those cells into the normal heart.

Again, it gets back to injection. We have come up with a way where we inject the cells, we see a bleb, we wait for the bleb to go away, we inject more cells, but that is just empiric, because it works for us. Do we know how it is being done by other groups? No clue.

DR. SCHNEIDER: Doris, you summarized nicely both the cellular complexities and the technical complexities that are involved here, and I wanted to comment about one in each of those categories.

You talked about cryoinjury versus coronary artery ligation, and I wanted to agree with your comment that relatively little of the work in the field is being done with ischemia reperfusion injury, which more closely resembles the clinical situation particularly in an era of stenting and reperfusion therapies.

A further complication there, though, is

that much as was learned over a period of years in investigations of stunning, it may be necessary to distinguish between open-chested ischemia reperfusion injury and the chronically instrument close-chested animal that undergoes ischemia reperfusion injury, which is something that a few labs have been able to develop as a means to minimize potential artifactual effects of the surgical procedures.

DR. TAYLOR: Right.

DR. SCHNEIDER: With respect to cellular heterogeneity, you talked about the possibility that SP cells in the skeletal muscle population might be important. Michael Rednicke has identified in skeletal muscle, and investigators at Indiana have, as well, a scar-positive, LIN-negative, CD34-negative, CD45-negative population that lacks any ability to undergo hematopoietic differentiation and very closely resembles the scar-positive cells we found in adult heart.

DR. TAYLOR: I have seen data from the University of Minnesota like that, as well, and I also should say that Johnny Heward at Pittsburgh has found that as you increase the passage number of cells, and these are actually old data, from '98

I believe, that as you increase the passage number of cells in vitro that you see differences, I think passages 3 and passage 5, or something like that, give you much better functional results in engraftment than passages 1, 2, and 4, and the desmin staining of those changes.

So, I think we probably are selecting for different cells.

DR. SCHNEIDER: Along those lines, do you know if the scar-1-positive fraction goes up or goes down in your skeletal muscle cells over time?

DR. TAYLOR: Scar-1, we haven't look at scar-1 in our population of cells. We have looked primarily for SP cells. What I will say is that we grow our cells a little differently than most people. As you know, we make an explant and actually allow our cells to grow out from the explant, and we are getting a much higher percentage of more immature cells than other people as a result, and I think that is because we are not throwing them all away when we do the filtration and enzyme digestion, and those kind of things, and that may impact some of our functional data.

DR. RAO: We will take on last question. Dr. Simons.

DR. SIMONS: Doris, you mentioned that up to 90 percent of cells that they injected in the heart die soon thereafter. So, the cells that are still there, do they need to be concentrated at a certain per sort of square area of the cell, or can you spread them as much as you like, so is it really a mechanical effect or is it something that the cells make, because you are making a point that it matters where you actually inject them?

DR. TAYLOR: What we have found is that we can do three parallel injections, and we get the same effect as if we do one injection, as if we do a star-shaped injection, so we spread them out in different--I mean we have given them under different geometries, and we see the same effect, the same dose of cells.

What we don't know is how many die under each of those conditions.

DR. SIMONS: So, if you can spread them around, that would imply that that this is probably not a purely mechanical effect?

DR. TAYLOR: I think there is absolutely some truth to that. I don't think it's purely mechanical. I also don't think--that is what I said when we use these muscle cells, there are

multiple populations of cells in there, and I think different ones have different effects.

Absolutely, I think mechanism is an open question, we really don't know.

DR. RAO: In the interests of time, I guess we move on. Thank you, Doris.

I apologize for not recognizing members of the public, but this is the part of the meeting where we have to give priority to the committee members in terms of questions. People in the audience can address the committee in the open session.

Dr. Itescu.

Preclinical Models - Hematopoietic and Mesenchymal Cell Therapies for Cardiac Diseases

DR. ITESCU: Thank you very much for inviting me here today. As if the talks haven't been complex enough, I have got the difficult task of speaking for 30 minutes and covering a variety of animal models, as well as a variety of cell types, so I hope it will be cohesive enough.

The issues to consider in this field, I have tried to address some of them here in this slide in terms of small animal models or large animal models, and it is the precise

characterization of cell type and population to be used to define the cell source and process for isolation to determine if there is a need for ex vivo culture and expansion to identify the mechanisms of action for inducing cardiac repair, to identify appropriate animal models, in other words, small versus large, species-specific versus those that use human products and those that involve acute versus chronic ischemia. These are all very, very important questions.

Finally, experiments that address dose-ranging studies for functional correlation and toxicity, and the last question which has not been touched on yet today, which I will at the end of my talk, is that between autologous versus allogeneic products.

The adult mammalian bone marrow contains two stem cell populations at least. The one that we are most familiar with are hematopoietic stem cells defined as being CD34-positive, and more importantly, CD45-positive.

These cells form blood-forming elements, such as monocyte and macrophage lineage cells, these account for about 10 to 20 percent of the CD45-positive fraction, and more recently, cells

that are endothelial progenitor cells or angioblasts that express these markers plus several others such as AC133 and c-kit.

The second population, the non-hematopoietic stem cell fraction is typically CD45-negative and CD34-negative, and at least three cell types have been defined within this fraction - the mesenchymal stem cells, which really is poorly termed as stem cells since these cells are defined based on their in-vitro culture characteristics. The way they are isolated is very crudely, very grossly based on density properties and plastic adherence. In fact, the population cells that is pulled out initially is very heterogeneous.

A second stem cell type is the MAPC that you have heard about. These cells are potentially more homogeneous in nature, however, the fact that they are dependent on negative immunoselection means that again we don't really know what the cell type that is ultimately derived is, and the cell culture conditions are very laborious and require low density for outgrowth.

Finally, there is a third population of mesenchymal precursor cell or progenitor cell which can be defined on the basis of several surface

markers and can be selected by immunoselection, positive immunoselection freshly from bone marrow, and we are using these cells currently in my laboratory, and I will touch on them a little bit towards the end of the talk.

In respect to the hematopoietic stem cell fraction, the CD34/CD45 fraction, there is high frequency within the bone marrow compartment, as I have mentioned, 10 to 20 percent of macrophages, 1 to 2 percent of the CD34 progenitor cells that can be freshly isolated without any requirement for in-vitro culture and expansion, and are well characterized for many years with established isolation protocols.

In this slide, I tried to summarize the interaction and cooperation between the CD45-positive and 45-negative subsets in terms of vascular network formation, so that in the bone marrow, the primordial circle stem cell may give rise to the CD45-positive/34-positive endothelial progenitor cells, which egress to the embryonic organs where you get the initial vasculogenic small, thin-walled capillary development.

At the same time, the CD45-negative parasite fraction derived from a mesenchymal

progenitor cell produces a variety of factors including VEGF, FGF, angiopoietin, and SDF-1, which can interact with these vasculogenic capillaries to give rise to the more mature vascular network through a process of angiogenesis.

In addition, the parasites may also migrate and give rise to the smooth muscle outer wall, so that you really have this cooperation between both cells and factors to give rise to the permanent new vessel formation.

With this as a background, I will like to address several studies that have looked at how angiogenesis per se can improve cardiac function. This study from Kobashi [ph] and Collins in 2000, I think was the first to demonstrate or one of the first to demonstrate that whole bone marrow in a small rodent model could induce transient angiogenesis and improve cardiac function.

You can see here the implantation of whole bone marrow into the ischemic myocardium of a rat gives an increase in local production of VEGF protein within 24 hours, but by 7 days this protein production is gone, and in parallel, the angiogenesis that is occurring in the heart is again transient, maximal at 1 week

post-implantation, gone by 4 weeks.

So, I think this particular model gives us some pause and suggests that the whole bone marrow approach may not be a way to give rise to permanent vasculature. Others have used this sort of approach now in larger models, in pigs. Work from Kemiharder [ph] and colleagues demonstrated that in a LAD-ligated porcine model, again whole bone marrow implantation gives rise to angiogenesis. This is within 6 weeks post-implantation, you can see improvement in collateral flow associated with some degree of improvement in function.

In parallel studies they published about two years ago now, the same group demonstrated that in a more chronic ischemia model in pigs using the ameroid constrictors, they were able to again induce angiogenesis using mononuclear cells from bone marrow, as well as mononuclear cells from peripheral blood to a lesser extent. You can see improvement in regional blood flow and improvement in global parameters of cardiac function including ejection fraction DPDT, and a reduction in the ischemic area.

But again, all of these studies were done in the setting of an acute ischemic or perhaps more

subacute ischemic model in this scenario, and the animals were followed up for no more than 6 weeks, so if we take that into consideration that in the smaller rodent, the neovascularization and the cardiac function improvement was transient, but I think the 6-week follow-up in these larger models is really not adequate especially in a pig where the physiology much more closely resembles that in man.

The next approach is to look at perhaps subsets of some of these cells, and I would like to show you some work on endothelial progenitor cells defined by surface markers.

Isner and colleagues originally described these cells demonstrating that endothelial progenitor cells were present in the bone marrow, were released to the circulation under certain signaling and present from growth factors under ischemic conditions, and that these cells can incorporate into regions of ischemia.

So, we asked the question if one could identify these cells in the adult marrow of humans, could they potentially improve function through a process of neovascularization.

The model that we use in my lab is one of

using the incompetent nude rat, which is an athymic rat model that lacks T cells and B cells, continues to have some degree of natural killer cell function, in other words, it is a linking model, but it is able to tolerate certain types of human cell injections, particularly human cells that have not been in vitro cultured and expanded.

Our objective was to cause a permanent occlusion of the anterior descending left coronary artery, in contrast again to other models that perhaps are using the reperfusion type of model. Our objective was to induce an infarct and see whether we could then protect the subsequent territories of myocardium still at risk in the periphery.

We mobilized in the progenitor cells from healthy human donors using GCSF, think that, in fact, in the future this would have been a way to move towards clinical trials, in other words, being able to harvest large numbers of progenitor cells.

In our studies, we then immunoselected these on the basis of surface markers, CD34 and CD117, injected these into the tail vein of acute ischemic rats to see whether they homed to the myocardium.

I would just like to pause for a minute to say that given the recent paper in the Lancet, I think a week or two weeks ago, I think that certainly raises a question about how such cells are going to be accessed in large numbers if they are to be used at all at the time of an acute myocardial infarct because I think under a local anesthetic, a bone marrow aspirate gives rise to extremely few numbers of these types of cells, and you really do require either large harvesting or large numbers to be mobilized, and I think at this point in time, GCSF is not the agent to be used post-infarct.

In any case, the point of our studies were to see whether intravenous administration at the time of acute infarct would lead to selective homing to the myocardium, and we have met these trafficking pathways based on the type of chemokine expression that occurs post-infarct, and we found that B cells selectively migrate to the peri-infarct region, where they within two weeks induce both incorporation into vessels, and this is staining with antihuman CD31, and you can see cells that are previously labeled with dye/Dil, incorporate into vessels of the peri-infarct

region, but in addition, a very dense induction of angiogenesis at the more distal areas as defined by rat-specific anti-CD31 antibodies.

So, again, akin to the type of data shown by Dr. Epstein, it appears that these cells are able to not only incorporate and induce vasculogenesis, but presumably secrete a variety of antigenic factors.

From a pathophysiologic perspective, we see that when we induce a vascular network at the peri-infarct region, you can see these large capillaries. There is nice viability of the cardiomyocytes at the peri-infarct region as opposed to these apoptotic cardiomyocytes. You see reduction in scar formation and clearly a viability and survival of the myocytes.

But in addition to that, we were quite surprised to see that as early as five days post-neovascularization, there seems to be induction of cell cycling by endogenous myocyte-like cells, and you can see these by confocal microscopy small cells of the peri-infarct region that express alpha-sarcomeric actin in blue, but more interestingly, in yellow, the staining with the rat-specific anti-KR67 antibody, which

recognizes cycling cells only of rat origin. You can see lots of these type of small cells in the peri-infarct region only in the situation where we also have neovascularization being induced by the human cells.

Within two weeks, in these same areas, what you see is that the cells that express sarcomeric actin now express troponin, and you can see an example of that large mature differentiated cardiomyocyte where the nucleus continues to be in cell cycle, and we see about 8-fold increase in numbers of these type of cells at the peri-infarct region that has received the human progenitor cells.

We think that the cells or the new capillaries are secreting factors that are presumably inducing cycling of these cardiomyocytes, and we are looking at a variety of anti-apoptotic genes that are increased in expression in these cells including redox-related anti-apoptotic genes.

The end result is a very significant salvage of the anterior wall of myocardium. You can see that here, and you can see in this scenario obviously a very dramatic left ventricular scar

formation and aneurysmal formation here.

We can show a dose-dependent reduction in scar formation at the left ventricle as we increase the number of progenitor cells that we inject.

Now, others have published recently that perhaps the CD34 cells may have the ability to become multipotential, perhaps transdifferentiate, however, the data are fairly scant, and I would say that given that only 1 in 7 healthy animals demonstrated HLA human troponin co-staining, I think at this point, it is open to debate whether, in fact, these cells do transdifferentiate or whether these cells have the capability to simply fuse with the donor cells.

Nevertheless, whatever the precise mechanism, it appears that hematopoietic stem cell injection, either intravenously or intramyocardially, does result in significant global improvement in cardiac function, as we showed here, at least 30 percent improvement in ejection fraction, which is permanent. This was 15 weeks of follow-up post-LAD ligation, and we can look at fractional shortening or DPDT and demonstrate similar sort of things.

In this scenario, you can see that there

are other cell types that we use as controls, CD34-negative cells, saphenous vein endothelial cells, and what this sort of study shows is that you must always use different cell types when you are trying to evaluate a particular therapy. It cannot just be compared to saline or existing control. You have got to demonstrate specificity in the product that is being tested.

Moreover, the question that we asked was what about if we combine this type of an approach with existing therapies, because really that is the question you want to address, and the example of restenosis with stenting is just one type of combination therapy between a new product and existing therapies.

What you see here is if we combined CD34 cells with both ACE inhibitors and beta blockers in the same rodent model, we had very significant synergy in outcome, and histologically, the reason for that was really not because the two products worked in a similar way, but because they had very separate mechanisms.

The ACE inhibitors and beta blockers had no impact on neovascularization, but, in fact, prevented fibrosis in the posterior wall as they

are known to do, so we have two very different mechanisms of action working in synergy, but it could have just as easily worked the other way around, and that is why we did the experiment.

It could have been that by reducing wall strain or reducing fibrous replacement, we may have reduced the drive for endothelial cells to induce neovascularization and improve cardiac function.

So, what can be concluded from preclinical data using non-cultured cells? The objectives that one should look for when you do these kind of studies include identifying the predominant mechanism by which a given cell type induces cardiac recovery.

A comparison of efficacy of one given cell type with others, identification of the tissue distribution of that cell type following the preferred mode of delivery, unique short- or long-term risks associated with the preferred cell type, unique risks associated with the method of cell acquisition or isolation. I have given you the example of GCSF administration, and perhaps alterations in efficacy or safety following coadministration with standard therapies.

Now, I would like to shift attention a

little bit to the second population I want to talk about, the non-hematopoietic stem cells, the CD34-negative, CD45-negative fraction.

These cells are rare. They account for less than 0.01 percent of bone marrow cells, and they do require, for this reason, in-vitro culture and expansion.

I apologize about the complexity of this slide. I will just take you through it a little bit. I mentioned these three types of mesenchymal lineage cells that people are working with. The so-called mesenchymal stem cells are more likely committed progenitors, but the point is that they are tolemerase-negative.

The multipotent adult progenitor cells are MAPCs, tolemerase-positive, and the stromal or mesenchymal precursor cells are also tolemerase-positive. These cells may be related to each other, but all three types of cells give rise to mesoderm lineage cells. The MAPCs have also been shown to give rise to endodermal and ectodermal cells. It appears that the mesenchymal precursor cells can also give rise to endoderm and ectoderm.

With respect to mesoderm, which is really

what we are interested in primarily here, cardiac muscle and smooth muscle lineage cells can both be differentiated from the mesoderm, but also these cells can give rise to other cell types with mesodermal lineage.

The so-called mesenchymal stem cells are cells that are derived from whole bone marrow and then isolated by simple density centrifugation to a particular layer. These cells are then taken from the interface, put down on plastic, adhered for 24 to 48 hours, and then the cells that continue to adhere are then cultured again fairly crudely with generally fetal bovine serum for weeks at a time.

So, if you understand how this process is initiated, you understand that really, the isolation of these cells is so crude that you are starting out with a very heterogeneous population simply based on density characteristics, and that the true multipotential cell within this fraction probably is not more than 1 in 1,000 to 1 in 10,000 of the cells you are starting out with.

In any case, after, say, 10 to 14 days of culture and passage, you see the sort of monomorphous type of fibroblastoid phenotype, and you see the cells that have survived this period of

culture are fairly homogeneous in terms of the type of markers that are being used.

There are specific antibodies that can define surface characteristics of these cells, SH2, SH3, endoglin, and I am not sure what SH3 actually defines. But in any case, these cells, after two weeks in culture, become fairly homogeneous.

I borrowed this slide from Dr. Epstein, his recent paper in Circulation Research, which shows that these cells, after several passages in culture, demonstrate production following induction of ischemia of a variety of factors that are associated with both angiogenesis and arteriogenesis, and in a nice model or rat hind leg ischemia, you can see--I think you showed this slide already--demonstration of improvement in perfusion following MSC infusion in these cells in ligation of the hind leg artery.

But in addition to secretion of pro-arteriogenic factors, the interest in these cells lies in the fact that they seem to have multipotential capability in that under appropriate culture differentiation conditions, they can be differentiated to adipocytes, chondrocytes, and osteocytes.

Work now since at least 1999 has demonstrated that if you use appropriate inductive signals in these cells, in this case, 5-azacytidine to demethylate the cells, but there have been reports of other agents that can do similar sort of things, you can push the cells toward a cardiomyocyte-like lineage with appropriate marker expression and electromicroscopic criteria consistent with BT myotubes.

Probably the best study to date to demonstrate that these cells can actually do something in vivo is work from Victor Dzaus' lab published last year in Nature Medicine. Here, we are talking about again rat mesenchymal lineage cells cultured in the way I have just defined with density separation and long-term culture with bovine serum, fetal calf serum, and what you see is after injection of these cells into the peri-infarct region of rats following LAD ligation, these cells appear at least phenotypically to acquire markers of cardiomyocytes, so that they express myosin heavy chain, cardiac troponin, sarcomeric actin, and connexin 43. This is within two weeks of implantation.

However, the majority of these cells just

don't survive when you put them in. They die after early transplantation. Causes appear to be ischemia again, competition for oxygen nutrients, inflammatory and oxidative stress in the post-infarct myocardium, loss of survival signals from cell to cell or cell matrix interactions, and probably lack of tolemerase activity and self-renewal capability by these cells, and I said to you earlier that these cells just don't express tolemerase, certainly when they are in culture.

The work by Mongi, et al., in fact, showed that if you genetically modify these cells with an AKT transgene, you could significantly prevent these cells from dying following implantation, significantly reduce the apoptotic index, increase survivability, and overall improve function almost to the level of the non-infarcted animals, quite impressive, but it required essentially making them resistant to death or apoptosis by AKT overexpression.

I will just move along to the second population of cells, the MAPCs. These cells, as I mentioned, are defined based on negative expression of all known markers, CD45 and many other known markers of lineage-specific differentiation, and

following long-term culture, these cells have been shown to be capable of differentiating to a variety of tissue types requiring cocktails of various cytokines and differentiation factors.

In particular, following activation with VEGF and implantation into appropriate medium, in this case, tumor model in the scid mouse, these cells appear to be able to incorporate into first neovascularization, and you can see they can induce networks of capillaries, neocapillaries really, in wound-healing tissues, so they may contribute to vascularity.

With that as a backdrop, Gallegos and colleagues at the University of Minnesota decided to look at whether again fresh non-differentiated MAPCs from dogs could be implanted into an acutely LAD-ligated model and could perhaps improve function in some form.

The model was to induce again a complete LAD ligation in the dog, autologous cells were taken from the marrow, they were expanded for about four weeks, and then four weeks later, injections were put directly into the myocardium.

What they found was that a month post-injection of the cells, there appeared to be

increase in myocardial perfusion reserve defined as perfusion under stress relative to perfusion at rest compared to the controls, and this is in four animals.

But significantly, although there was some improvement in regional myocardial contractility within the infarct area and the peri-infarct zone, defined as circumferential shortening and radial thickening, there was actually no improvement in global function of cardiac measurements as defined through systolic improvement by fractional shortening or ejection fraction.

Now, just to the last area that I would like to touch on, and that is the possible use of cultured allogeneic stem cells for cardiac disease. The issues to consider here are whether or not cells constituitively or under inductive conditions express surface molecules, surface HLA molecules, and co-stimulatory molecules, secondly, whether stimulation of recipient T cell responses occur in vitro, and, thirdly, whether these cells might induce inflammatory responses after in vivo injection.

This work may or may not be familiar to many people here, but many labs now have completely

demonstrated that mesenchymal lineage cells from humans, from primates, and from smaller animals routinely actually do not express Class II HLA molecules until they are induced by gamma-interferon. They certainly do all express Class I, but in addition to that, do not express a variety of co-stimulatory molecules, such as CD40, CD80, and CD86.

All of these molecules are absolutely critical in inducing T cell immune responses when you transplant cell or an organ between individuals.

What I want to show you here is that mesenchymal stem cells clearly do not induce a T cell response following standard mixed leukocyte reactions in vitro. This, you can see in comparison to allogeneic mononuclear cells, which induce a vigorous T cell response.

In addition, even activation with interferon gamma to upregulate Class II HLA has no effect on mesenchymal stem cell induction of T cell responses, where you can see the potency of gamma-interferon at inducing allogeneic responses if you do it to dendritic cells or to allogeneic mononuclear cells.

So, there is something very special about these cells, that they do not seem to express co-stimulatory molecules, they do not seem to induce an allogeneic T cell response at least in vitro, and moreover, they seem to have the ability to suppress ongoing immune responses.

What you can see here is that this is now a third party mixed leukocyte reaction where you have T cells from one donor proliferating. This is to allogeneic mononuclear cells, to allogeneic dendritic cells, or to PHA, and you can see that if you add third party mesenchymal stem cells, they will suppress this proliferative response, and this suppressive effect is in a dose-dependent manner.

Additional studies have suggested that, in fact, part of this suppressive effect requires cell-cell contact inhibition, and part of it requires secretion of a variety of anti-inflammatory cytokines, such as TGF-beta, but the precise mechanism at this point has not been defined.

The only human trial that I am aware of using allogeneic mesenchymal stem cells to date is one where allogeneic stem cell transplants were performed with third party allogeneic mesenchymal

stem cells, and I understand the results of those were better engraftment and reduction in graft-versus-host disease, suggesting again that the third party mesenchymal stem cell, not only does not induce an immune response, and is presumably not itself rejected, but is also beneficial in outcome.

But just to go back to the cardiac studies now, using that again as the backdrop, Brett Martin and colleagues from Ceros Therapeutics--and these are two slices that he has given me--presented at the American Heart Association last year, their findings using allogeneic mesenchymal stem cells from pigs in acute myocardial injury, and this is now in a reperfusion model to persecute myocardial infarction, what they was generate a panel of allogeneic porcine mesenchymal stem cells that are matched at blood group antigen, they used very large numbers of these cells distributed over the infarct zone. We are talking about a reperfusion model without any immunosuppression.

What you see here is that within two weeks of implantation, the cells were still present, so they hadn't been rejected. You can see the presence of cells two weeks later. However, these

cells did not differentiate to cardiomyocytes, so they did not induce a rejection episode, but they also did not appear to really do much in terms of integration or differentiation.

With respect to function, what you see is actually improvement again in diastolic parameters, as we have heard earlier, with increasing diastolic wall thickness at various time points including well beyond six weeks of study, and a reduction in N-diastolic pressures, however, again no improvement in systolic function, which is again consistent with perhaps alterations in matrix, alterations in ground substance, but no real effect on contractility or myocytes.

This table, what you see is really a summary of the type of the cells that are being used of mesenchymal lineage. The MAPCs, so far we have seen only modest efficacy in cardiac models in dogs in the absence of predifferentiation in vitro.

Mesenchymal stem cells have shown good function in rats, but modest only in pigs, and mesenchymal precursor cells are currently being investigated. We think they may be far more potent than either of these two because you are able to isolate them by surface characteristics when they

are fresh.

The final slide here is that I think many remaining hurdles is the message. The appropriate clinical indication needs to be defined. The more proof of principle animal studies, we have got to determine optimal doses for efficacy, careful registry of adverse events in our animals, let alone humans.

We have got to optimize the ex vivo culture process, which I haven't even talked about. The autologous versus allogeneic question is critical because it is going to impact both on the process and on the cost of this whole therapy, determine the best route of administration and really how do you improve engraftments, we have heard all about that before.

Thank you very much.

[Applause.]

DR. RAO: Thank you, Dr. Itescu, for a really nice summary and trying to help keep us on time, as well.

In the interests of trying to get us back on track, I am going to ask people to limit their questions to things which are directly relevant to the talk and if there are specific burning

questions on this issue.

Q&A

DR. CUNNINGHAM: I just have one question. I was curious, in all your rat studies you published in Nature, did the rats die, and what did they die of, you know, the ones that didn't get to the endpoint of the experiment, were there any adverse events that you noticed?

DR. ITESCU: You mean unrelated to the initial surgery? We have something like 30, 40 percent death rate from the initial surgery, but pre-cellular implantation, but after cellular implantation is what you are asking me, we carried the animals out. There was no real adverse events that I am aware of in these studies.

DR. SCHNEIDER: Terrific presentation. A quick question. What are the surface markers that you use as most indicative of the MPC?

DR. ITESCU: They may very well be related to the cells that you are looking at in mice, but the markers that we look for are not human-specific, so 3G5, Mak-18, so interestingly enough, markers that define human parasite-like populations in vivo. In fact, it appears that these cells are present in perivascular locations

throughout the body, so we are using those markers to pull out the cells from different locations to purify them and expand them.

I would actually be very interested in seeing whether there may be some correlations between these human cells and the cells you look at in the mouse.

DR. KURTZBERG: You made a passing comment as you were speaking that you thought GCSF wouldn't be safe after an MI. Can you expand on that?

DR. ITESCU: I think in the recently published study, just to summarize my take on that study, a randomized study where one group received GCSF subcutaneously to mobilize the endogenous population of marrow, another group received GCSF subcutaneously to mobilize and then had the mobilized cells harvested, not immunoselected, and I think the cells were then delivered by I believe intracoronary routes, whole unfractionated cells.

The conclusion of the study was that the patients that received the cell therapy had a significant improvement in cardiac function, whereas, the group that received GCSF alone did not.

However, in both populations, the study

was cut short because of this complication, but I think if you pooled both populations, 7 out of 10 patients developed significant restenosis at the site of the stent implantation.

Now, these are bare stents. This is the pre-repamycin days. I think the anticipated restenosis rate would have been maybe 25 percent at most in that population. I think even with repamycin, you would expect to reduce the rate--if 70 percent is right--diabetic patients actually have a restenosis rate with repamycin stents still of about 20 to 25 percent, 50 percent without repamycin, so these patients are more severe than diabetics are.

I wouldn't anticipate that you would be able to lower that to below 35, 40 percent, and that is obviously totally unacceptable.

Now, the question is why is it that GCSF was associated with this high restenosis rate, and I think we can all make speculations, but I think at least one possibility is that the cells that are mobilized, CXER-4 positive cells from the marrow, many cell types express CXER-4 including smooth muscle progenitor cells, and I think that is probably the simplest explanation, that there are

smooth muscle progenitors that are mobilized, that migrate to the site of the stent, and I am not sure how one can get around that actually, but that is just a guess really.

DR. TAYLOR: That was great. I have one quick question. You didn't mention this except that in vitro, a lot of these cells can become a number of mesodermal cell types, and I think there are some data in rats that showed early on that if you inject these cells in the center of an infarct, they, in fact, at times become adipocytes or chondrocytes or osteoblasts, or something like that.

I wondered if you would comment on what cells you think are likely to do that and whether all of them are.

DR. ITESCU: Obviously, that is the worst case theoretical question in this whole field. What we don't want is to develop bone in the middle of their hearts.

I am not aware really of a lot of studies that have demonstrated that sort of abnormal differentiation. Certainly, adipocytic differentiation has occurred, I am not sure that I have seen bone differentiation.

It really depends on how well defined the cell population is, I think, and to what extent the cells have been predifferentiated or to what extent they may be still very multipotential. It is not clear to me whether you need to start with a cell that is very multipotential or that is fully differentiated, and perhaps the culturing process where you are pushing the cells, and again using fetal calf serum I think is an unfortunate--it is the only thing that as been done to date--but it is I think probably the worst way to be culturing cells, because you don't know what is in your culture medium, you are pushing these cells to many different lineages.

When we look at these cells following--very few studies have been published that have looked at this--but we have looked ourselves in this way, and you can see that after three or four weeks of culture, you can see cells that express markers of mature smooth muscle cells, of mature, some bone differentiation, some differentiation to cartilage.

Whether or not that is relevant when you put the cells back in, whether there is going to be differential potential for outgrowth of one cell

type over another, I think is really a totally open question, but I would push towards putting cells that are less differentiated in rather than more differentiated, because I think the less differentiated cells have got the ability to still proliferate, to be pushed towards the appropriate lineage under appropriate inductive signals that may still be present in the heart.

There may be more differentiation towards maybe a cardiomyocyte lineage, but at least towards a smooth muscle lineage, so you might get some degree of arteriogenesis, as well as I think it is the more undifferentiated cells that are the ones that produce the very rich supply of arteriogenic factors.

Just getting back to what I was saying, the very undifferentiated cells express markers really of parasites, so if you are thinking of parasite implantation, it is one way of maintaining viability of endothelial cells and integrity of the endothelium, and maybe it is a way of building the vascular network.

DR. RAO: This is the last question.

DR. BLAZAR: The issue of transdifferentiation in vivo is striking that you

can get cells there and they just sit there, and I think this has been seen with a number of non-hematopoietic cell sources, MSCs being one.

I guess the question is whether there are inhibitory signals that are present that prevent differentiation in vivo under certain conditions, and has anyone ever taken these cells back out of the heart and show that they can, in fact, be induced to differentiate in vitro? Do we know anything about the inhibitory factors present at the site that are precluding differentiation?

DR. ITESCU: Those are obviously great questions, very important questions, and again I think it speaks to having a good understanding of the surface markers of these cells because if you know what they express, you can do the sort of experiments that you are suggesting. You know what you are putting in, and then you can actually take them out again based on maybe immunoselection.

We are actually trying to do those type of experiments to ask exactly those questions, but I think in many ways you are limited then to human cells, because many of the well-defined surface markers, there just aren't enough reagents. Perhaps mouse cells is the only other. The mouse

system is the one that is well enough developed and the human system, and other than that, we are really missing reagents where you can do those type of experiments. DR. RAO: Thank you, Doctor.

DR. HARLAN: Can I still ask a quick question? You were careful to say so in your talk when you were talking about allogeneic cells, and you said at least in vitro, these allogeneic cells don't activate T cells, but I just wish to emphasize the point that in vivo, it is so much more complicated than that, and the presence or absence of B7, I thought Bruce maybe was going to speak to this, you didn't make the point, but I think it is important to emphasize that whether or not a cell in vitro stimulates a T cell response is really a very poor predictor of whether that will be rejected in vivo.

DR. ITESCU: Well, I am not sure I agree with that at all actually.

DR. HARLAN: Then, we will have more discussion. I saw the pig data, that is interesting.

DR. ITESCU: I am actually a transplant immunologist. This is what I do, I take care of

cardiac transplant patients, and I am going to tell you that 12 months ago, when I first heard about these sort of data, I was extremely skeptical, along similar lines to what you are saying, you know, is it an in vitro artifact.

However, you go back and you see how many labs are reproducing these data, which is really what I am surprised about, and I can tell you that the mixed leukocyte reaction is about the best single assay that we have in transplant medicine to predict allogeneic rejection and allogeneic sensitization.

In the old days, it used to be used routinely for kidney transplant selection, donor-recipient selection, and it is still the best assay. I am not sure whether it reflects whether these cells are going to be accepted long term in vivo, but it is certainly a very good marker for biology. I don't know what it means, but it is routinely being reproduced using these type of cells.

DR. HARLAN: To say it is the best, and I won't disagree with it, is fine, but it is still a very poor predictor of in vivo function, and it goes back 30 years to the two-signal model,

thinking if you could get rid of the antigen presenting cells within a graft, that you would take, and it seems to work in rodents, but it just doesn't work in higher animal models.

Bruce, do you want to step in?

DR. BLAZAR: I think there is the point of somewhere in between because clearly, there are cell-to-cell contact phenomenon that happen in vitro with regulatory T cells. You can show a TT-dependent inhibition of responses throughout TGF-beta, and depending on the models in vivo, those either are true or they fall apart.

We know in matched sibling donor transplants, mixed leukocyte reaction culture does not predict graft versus host disease. So, I think that if you do see suppression, it is encouraging to try to go forward for in vivo, and that is probably as good as you are going to do, but it doesn't necessarily mean that those mechanisms take place in vivo particularly as those cells themselves change in their own ability to elaborate cytokines or express other molecules.

DR. ITESCU: I agree entirely and I am saying the exact same thing, that I think it is a phenomenon that is reproducible in vitro, it's an

unusual and unique scenario. Whether or not it has implications in vivo remains, but I think at least it ought to be tested, and I think it may have implications on dendritic tolerance-inducing mechanism, which truly is a different way of thinking about this all together.

It may have nothing to do with the ability of these cells themselves to escape surveillance in the periphery, but perhaps they end up in the thymus, and they may actually be able to reeducate the immune response, but I think all of that remains open to test.

DR. RAO: Last comment.

DR. NEYLAN: A very quick transplant-related question, again, maybe bring some of these last discussions to a very practical safety concern.

That is, given the ability of these allogeneic cells to abrogate or reduce the immune response of the host, is it possible that the migration and homing of these cells may differ to autologous cells in a way, maybe akin to the micro-chimers and observations of solid organ transplantation, that potentially pose a safety risk to the use of allogeneic cells, homing to or

disseminating and finding a welcome home in other tissues.

DR. ITESCU: I think that is a fair point, in other words, if they are immunoregulatory and you are injecting them and they find their way into the thymus, for example, will they induce a state of tolerance to an exogenous antigen that the patient sees at exactly the same time, which is the concern whenever we use an immunoregulatory new drug.

Now, there are a couple of studies actually that have just recently been published that suggest that, in fact, while they induce tolerance to themselves and maybe tolerance to an alloimmune reaction, they don't seem to have induced tolerance to an exogenous pathogen, for example.

Now, again, that is two studies, more work needs to be done, but I think the question is fair, it's an absolutely valid point, and obviously, you have to worry about that when you do your studies and follow up the patients very closely.

DR. RAO: We will visit it tomorrow. We should move on.

Dr. Taylor.

From Mouse to Man: Is it a Logical Step for Cardiac Repair?

DR. TAYLOR: It is a logical step because I think it raises actually the issues that we are just talking about, whether or not data we get from rodents actually can be translated to humans or to larger animals.

I also want to say actually, in terms of an apology, I realized when I sat down that one of the reasons my last talk was so disjointed was that the version that was up here was not the version that I had on my computer, so it was a kind of foreign talk to me, so I apologize and hope we will do better this time.

From mouse to man, is it a logical step? I am going to start with another comment from Ghandi, which I have had on my office door for the last 10 years about this field, which is, "First, they ignore you, then, they laugh at you, then, they fight you, then you win."

I think it raises the point that we are somewhere in the continuum in this field, and it is time for us to start asking the hard questions, so that we can have the fight and then win.

As we are talking about moving from mouse

to man, I think we have to talk about cell type, and I think I would be remiss if I didn't say that I think cell type depends on what we are trying to do here, and that if we are trying to look at chronic ischemia or hibernation, we are probably looking at cells that are more likely to induce angiogenesis, such as the cells you just heard about - bone marrow mononuclear cells, angioblasts, some subpopulation of stromal cells, growth factors, or maybe even myoblasts plus growth factors, but if you want functional repair and contractile cells, you are either want cells that are contractile, such as skeletal myoblasts or cardiocytes, maybe cardiac stem cells, or you want bone marrow cells that can become contractile cells.

But the most important issue probably in this whole field, which is why I think we are going to have to talk about moving from mouse to man is probably this arrow, and the fact that we need both angiogenesis and myogenesis if we are going to have an appropriate outcome, and that, in fact, it may be not just one cell type, but multiple cell types that we end up needing for cardiac repair.

Unfortunately, as we are looking at these

cells, we don't have the opportunity to do those anywhere except in rodent at the present or in humans, so we are going to have to move from mouse to man at least with many of these cells unless industry provides us with the tools that we need to do the studies in between, because right now we really don't have the capability of moving to a larger animal model.

I guess I want to start by asking the question what the appropriate preclinical animal models are and how quickly can we move forward by saying, you know, I presented this slide a minute ago, we had 15 years of preclinical data in rabbit and dog and pig and rat, mouse, and sheep, and we thought myoblast transplantation was safe, effective, and feasible.

But we missed a lot of things, and we missed--I apologize, I thought there was another part down here, we are off to a great AV start, but that's okay--so what did we miss? We missed the fact that these cells might be electrically incompatible with the remainder of the myocardium.

We missed questions about location of injection, we missed questions about some of the dosing phenomena, we missed a number of things in

our early preclinical models despite the fact that we used both large and small animal models.

So, the question then really is what do you really need to do and when do you move to clinical studies, and I am going to give my jaded perspective for a minute and say that I think sometimes you move to clinical studies because your institution wants you to and kind of forces you to either because there is a financial incentive or that there is no such things as bad PR, but I would like to say that the appropriate time to move to clinical trials is when the data warrant it and that again we have to underpromise and overdeliver.

So, if we believe that every cell injected seems to work, and that thus the heart is easier to repair than we thought, let's take, for example, the possibility that that is really the case, and if that is the case, when do we move to the clinic.

I guess I could start by saying we have already moved to the clinic, but that being said, if we look at the clinical data, does it support the fact that the myocardium is easier to repair than we thought.

Well, yes, everything works, but none of the clinical cell studies are placebo controlled,

and 8 patients or even 53 patients can show you anything, especially when follow-up is short and we aren't considering age, gender, or heart failure status.

I want to get back to these, and I will in this talk, that we haven't talked at all about factors like age and gender and how they may be really relevant, and there is a reason that drug trials involve thousands of patients with at leave five-year follow-up.

Also, most of the clinical cell studies out there were designed as Phase I safety studies as they should have been, yet, many of these claim efficacy despite the fact that they were either revascularization studies or had other co-treatments involved, and I think it really raises questions about what we need to do.

So, if we believe that the myocardium is easier to repair than we thought, what does that tell us about moving to clinical studies? I am sorry, those two slides are actually backwards. If every clinical cell works, what does that mean?

I think it means that we have no clue how they work, whether they create angiogenesis or myogenesis, unloading of the heart, recruitment of

stem cells or whatever.

What questions does that raise? It raises questions about patients, which raises the same questions about injury models, preclinical injury models. It raises questions about dose and timing of cells, which then have to interact with the injury models. It raises questions about route of administration or location of the cells, and how we measure the outcome, and those all affect which animal model you can choose.

Well, the genie is out of the bottle, as the people at Mayo have said. Clinical trials have started, so what do we do? I think we educate people about what the appropriate situation is.

There are a number of my clinical colleagues that I have talked with, and it scares me a little bit, who don't even realize that if you are going to use bone marrow derived cells, that the FDA needs to be involved if you are going to put them in the heart, and I think that is an issue that we really have to address.

I think we need to require enough preclinical data, and then I think we need to quit rewarding people for doing it wrong. What do I mean by that? Well, what I mean by that is we have

to quit doing science by the Washington Post, as you said earlier, and we have to quit focusing on the fact that this is a multibillion dollar market every year, and focus on the patients instead.

So, what do myoblasts tell us about moving forward? Well, as I said, what we knew and what we missed is that there are electrical events, the route of administration wasn't clear, location and timing wasn't clear, culture medium, we thought we knew, but it didn't turn out to always be the case.

Autologous serum has been reported to be safer than non-autologous serum. Different vehicles have been used to deliver the cells and been associated with different outcomes, and we don't know anything about biodistribution, and we really didn't look.

So, what issues are there? The issues are safety and efficacy obviously, and as we move up this continuum, we can address these issues differently. Safety obviously involves cells, delivery, dose, and we have to do that in relevant models.

Efficacy involves the right model, acute MI potentially, looking at various cell types in a side-by-side way. We have to begin to

differentiate between diastolic heart failure. I think the preclinical data that exists so far suggests that everything works to begin to improve remodeling and diastolic effects including cells that don't work in systolic heart failure.

Systolic heart failure in our lab at least, we know fibroblasts don't work, stromal works less well than some other cell types, and I think the issues are open questions that have to be addressed.

If you then believe that the issues effect are impact cells, delivery, and effect, how does that translate to the animal models? Well, in small animals, I think we can ask questions about the cells. We can ask questions about deriving the cells, we can ask questions about markers for the cells, we can ask in vitro questions.

I think as we begin to move towards delivery and effect, we have to move up the animal continuum. Delivery, we really can't do in small animal models, relevant delivery, we can't do. Effect, again, I don't think we can do in small animal models, we have to do it in larger animal models.

In addition, when we start looking at the

mechanism of effect, to some degree, if we are looking at angiogenesis, we can measure capillary density in small animals, but it is very difficult to get adequate measures of relevant vessels or perfused vessels in small animals, and I think we have to move up the continuum.

In terms of myogenesis, we can get some data about ex vivo in isolated heart preparations and whether or not there are gross improvements in contractility. We can begin to measure wall thickness, ejection fraction, and you can certainly do exercise studies in small animals and move up the continuum, but in terms of electrical compatibility and mechanical compatibility, you are never going to get it done in a rodent model, you have to do it in large animals.

So, I think it is only relevant to work with small animals when you have no choice about the cells, but as you start to measure the important parameters of physiology, you have to move up the model.

So, what are the possible effects of these cells? I think they can cause unloading of the heart or reverse remodeling simply by altering the mechanical properties of the scar. They could

possibly engraft and become muscle and contribute to contraction.

They can obviously potentially form vessels or secrete factors that recruit cells that improve blood flow. You know, if you think about a lot of the data we have seen so far, it is possible that the cells we are putting in are doing nothing but recruiting bone marrow derived cells and actually ramping up endogenous repair.

It is possible that the cells have a paracrine effect and either change the cytokine supply to the scar or the remainder of the heart, or recruit other stem or progenitor cells.

Another possibility that is not really talked about, and again you can do these studies in small animal models better than large animal models, is fusion with existing cardiocytes. It is possible that the cells we inject actually fuse with cardiocytes that are hibernating and save those cells, and thereby contribute to contraction.

If we are going to talk about the different possibilities, cell delivery and effect, and which animal models to use, I want to briefly say that we have to define our populations of cells, and that is easier in some animal models

than others.

Again, when you start looking at myoblasts, fibroblasts, and other cells, being able to do that in rodents is much easier than being able to do that in larger animals. I think I have beat that horse, so I won't keep saying it, but cells, I think are studies we can do in rodents.

You have seen this slide before where we begin to talk about how the cells might work and whether or not we have an effect on reverse remodeling or growing new cells, and whether or not we think we can do those studies in large animals or small animals. I would submit that we are going to have to do those in large animals because we can't really measure remodeling and reverse remodeling in all of the new cells in the small animal models.

Now, we took the approach that if we are going to start moving back up this cascade of events, that might, in fact, take a combination of angiogenesis and myogenesis, and it might take cells plus genes or multiple combinations of cells.

Initially, we looked at cells plus genes by virally infecting myoblasts with VEGF adenovirus, and we measured the effect of those

cells on capillary density in peripheral skeletal muscle and a hind limb ischemia model, and these are old data, so I am not going to spend much time on them other than to say that we found that myoblasts had a relatively significant effect in terms of increasing capillary density greater than VEGF virus alone or MT virus, but when we overexpress VEGF in the myoblasts, we got back up to about 75 percent of the control without the side effects that we saw with VEGF virus alone, angiomas.

So, we decided to move into the myocardium, and these are data done with cells that overexpress another angiogenic factor where we looked at MRI, and I apologize, this is percent change and ejection fraction, and our historical controls, and these really are historical, they are not done at the same time, the active study was myoblasts versus angiogenic myoblasts versus historical shams.

We found that we increased capillary density significantly in these animals. These were studies done in mice. We couldn't do these studies actually in rabbit because rabbit cells don't have the cell surface receptors to actually take up or

be transfected with some of the viruses that we were trying to use, so that becomes a problem.

But we believe that angiogenesis was more important than to promote cell survival or proliferation, and we also looked at Victor Dzaus' data, I wanted to show briefly. You saw it when Silviu presented it, that if you overexpress a survival factor AKT in bone marrow stromal cells and transplanted them into the heart, survival increased.

So, we know angiogenesis increases function, increasing survival increases function, so if angiogenesis helps and increasing survival helps, why use a gene, why not use a mixture of cells.

We based that on data that we have gotten now in our hands where we were able to show that if we gave cells from young apoE animals, gave bone marrow cells from young apoE animals to apoE animals that were fed on a high fat diet, normally develop pretty bad atherosclerosis, that we could actually prevent this atherosclerosis.

We took, we actually have now begun to take these cells and deliver them in combination with myoblasts to look at effects on function. I

don't have a slide, but I can tell you that we are beginning to see better effects on function with a combination of cells and with individual cells alone. We couldn't do these studies except in mice, so there are times when mouse cells are relevant.

We did another study where we began to look at bone marrow mononuclear cells. These are data that were just presented at the ACC last week, where we actually infused mononuclear cells into the circulation of animals where we created a vascular injury.

We are able to show in our sham-treated animals or animals treated with other cells that you had neointimal proliferation, that we were able to prevent with bone marrow mononuclear cells.

So, again, we have now started the approach of delivering these cells in combination with myoblasts to see if we can have a more dramatic effect on not only myocardial repair, but on vascular repair, as well.

So, what are the other factors that are likely involved that are going to affect the model we use? The timing after injury, whether or not we can really grow old animals that replicate the six

to seven years that are needed, the type of injury, it is not probably going to be feasible to do dilated cardiomyopathy studies in mice and inject significant numbers of animals, plus when we start trying to treat ischemic and chronic and acute animals, mice, the issue we have to consider is we have got a 1 mm infarct, we got a 1 mm infarct, and the cells are microns in diameter.

Those cells are the same size essentially in rat, rabbit, pig, human, they are not much different in size, so the whole geometry of putting those cells in and getting an improvement is going to be much different than you are going to see in larger animal models.

There are two issues that really don't affect what animal you choose, but they are not being discussed at all, and those are gender and age.

Most of the preclinical data that we have published are in female rabbits, so we went back and started doing studies in male rabbits, and what we found is that male myoblasts die under conditions where female cells survive, and that is true both in vitro and in vivo, and that really surprised us, and we had to go back and begin to

reevaluate what we think is going on here, and we are just beginning to follow up on that, but I think it is an interesting point that we are going to have to consider going forward.

In terms of age, all of the studies we have done have been in old animals, but that is rarely the case. It is rarely the case that old mice are used in these studies, that old pigs are used in these studies. In fact, typically, people use young pigs because they want to keep them small, and don't want them to grow significantly over the duration of the studies.

I think the numbers and kinds of cells that you can obtain are going to be very different. Other than cells, we have to consider the culture conditions, and we can't ignore the fact that autologous serum has been touted as one reason that there is a Spanish study where there aren't any abnormal electrical events even though there have been in all the other human studies.

So, the injury models currently that we are using don't match the patients, and I think we are going to have to really think about that going forward. We don't have heart failure models, we just don't. Nobody is using heart failure models,

but every patient is a heart failure patient.

As I said before, I apologize, in terms of whether or not we can use small animals, I think for an isolated heart prep, mouse and rat are fine, but as you are going to start doing physiologically relevant studies, you have to move up the animal continuum, but there are limitations there, as well, so we have actually chosen, and I think more and more people are choosing, to use sonomicrometry or cine MRI. Fortunately, you can use that for all of the animal models that have been proposed so far.

I am not going to show those data.

I actually want to end with two slides that show something that I have tried to gather from the clinical data that exists, but I think they make a point.

We are talking about different animal models, but we are also talking about different cell types, and people are constantly saying how do myoblasts and bone marrow derived stem or progenitor cells compare.

The point I want to make is they don't. If you look at the studies that exist so far--and these are clinical studies, not preclinical

studies--if you look at myoblasts, the dose varies widely.

If you look at bone marrow derived stem or progenitor cells, the dose is significantly less and the physiologically relevant cells are a very small subset of those, as you just heard.

Moreover, the patients differ greatly. With myoblasts, the patients are from greater than one month to end-stage heart failure, but with the progenitor cells, the patients are 3 to 9 days post-MI or have refractory angina.

The delivery methods differ significantly. They are intracardiac for myoblasts, surgically or percutaneously. They are intracoronary for the bone marrow derived cells. Yet, people are trying to compare the outcomes from these, and I think that is true, not just clinically, but preclinically, as well, as people are trying to make the argument for their cell type.

So, until we are doing side-by-side studies with the same cell type and the same animal model, I really think we can't draw conclusions about what is going on.

So, the questions that I think are really out there are: Is there a best cell? I don't

think we know that there is yet.

Should we use just growth factors? I think the GCSF data would suggest not yet, we don't understand it well enough.

Is there a future for biologic devices? Probably. Are we going to be putting cells on stents? I don't know, but we probably are going to be putting them on patches and other devices in the very near future.

There is a real question, dose, timing, and patients, probably, but our models need to mimic that.

I will stop there.

[Applause.]

Q&A

DR. RAO: Thank you, Doris. I wanted to start off by asking you one question first. That was that implicit in all of these talks throughout has been the fact that it seems to be important when choosing a model that you have to have the right markers. That really is because you are doing syngeneic transplants. You want to put the same animal species cells back into the animal to do the follow-up.

Nobody, at least in this field, seems to

think that you can immunesuppressed mouse and use human cells directly, or do some equivalent otherwise, and that that is reasonable.

Now, is that true? That is important because this is going to be really important in the future. I just wanted to get your feel for that.

DR. TAYLOR: I think that is fairly accurate, and I think the problem has been that we haven't had the opportunity to really measure the effects of these cells in mice very well until the last couple of years.

Until the last probably year and a half to two years, you couldn't do MRI in a mouse reliably. You certainly couldn't get a good enough image to measure regional versus global function.

Sonomicrometry was hard to do in a mouse. The pressure volume catheters weren't quite up to snuff, and so the ability to make those measurements weren't true. Moreover, most of us believed early on at least that autologous cells were more likely to be clinically accepted, and patients certainly liked the concept of getting their own cells better than the concept of getting somebody else's.

So, I think we chose those cells because

they made sense to us clinically, and we were technically limited by our ability to make the measurements with other cells.

In terms of using human cells in immunocompromised rodents, I think we can do that now, but whether or not--I think it is an open question about whether or not we are going to get the best functional outcomes.

DR. ITESCU: Can I just maybe add a little bit to that? You know, we use the immunocompromised rodents pretty well, but I think what we are learning as we move forward is that even the so-called immunocompromised rodents are not fully immunocompromised, and you have really got to start understanding which kind of lineages in their immune system remain active, and what impact does that have on the cells you are putting in.

We are now at the point where we are adding cocktails to try to remove even the residual immune function in these kind of animals.

On the other hand, I think that if you are trying to use a cocktail of immunosuppressive agents in a normal animal, I think then you are going to run into the problems of what effects all

these drugs have on the cells that we are putting in, are they inhibiting differentiation, are they inhibiting function. There is a whole range of issues that I don't think we want to get into.

DR. RAO: Absolutely, that is important.

DR. HARLAN: I will make a comment on that, and then I had a question. I agree, in the islet transplant field that I know best, for instance, in order to correct a mouse with human islets, you need about 1,500 islets, whereas, you need about 400 rodent islets, and presumably it is because there are species differences in the factors that support the growth of those cells.

Then, I agree and appreciate your talk, but I would extend it in two ways. I think a theme of your talk was that the large animal models in general tend to be better than rodents in predicting things, and I think that is true, but I wish to point out that all models are models.

In our transplant studies, we did things in primates, testing various immunotherapies in different systems, and it worked beautifully and failed miserably in the clinic, so even large animal models, even using nonhuman primates, are models, and they have variables that are hard to

predict.

DR. TAYLOR: I would add one comment to that, which is all of us are looking at progenitor cells and the effects of these progenitor cells on cardiovascular function, and we know that the animals we are treating are relatively acutely ill even if we have given them heart failure, but patients are very ill.

They have had things impacting their progenitor cells for years, that we know nothing about, and it is not just aging, it's drugs, it's other things, so the cells we get out and the cells we put back in are going to be very different than cells we get out and put back in, in animals that we have only made sick for a month or a year.

DR. HARLAN: And because we don't know all the factors that made people sick, generating that perfect animal model may be an impossible ideal, that's the only point, I think we are agreeing.

The second thing is, though, that you didn't mention is in any model, and when you go to the clinic, I think it is important and we started the day that way, identifying the patients for whom existing therapies just have failed, so that is one way where you can go. When you have got nothing

else to offer, and you have the patients in a bad position, then, I think it passes the threshold.

DR. TAYLOR: But those aren't the models that people are using.

DR. HARLAN: Well, we will talk about that tomorrow, I agree.

Then, the third point, I say this in jest, and I don't buy it, but I like your Ghandi quote, and I will cite another philosopher, W.C. Fields, who said, "If at first you don't succeed, try again; if it fails again, you might as well give up, there is no sense being a damn fool about it."

I don't agree with that, it's just the other take.

DR. RAO: Dr. Epstein.

DR. EPSTEIN: I just wanted to make a point, which I think is important because I sense that one could very easily come to the conclusion without thinking it through further that a large animal model is the really only valid preclinical model.

I think Doris sort of made this point, but it might have been lost. It depends on what you are looking at. For example, if are interested in myogenesis, I would agree, ultimately, you have to

go to the large animal model because the small rodents, mice, become difficult to draw conclusions about, but if you are looking at angiogenesis, the mouse model is much better, I think, than a large animal model.

We have excellent ways of measuring perfusion now, superb ways of measuring perfusion, and what comes up in our laboratory a lot is if we give an intervention that has an effect, and then we want to see whether we could further enhance that, like stromal cells and then genetic engineering of stromal cells, in a pig model of myocardial ischemia, it starts out with 85 percent of normal.

If you get it up to 95 percent of normal with your first intervention, you have no room to look at the next step, whereas, with mice, that could be modulated much more easily, and we are able to demonstrate a primary effect and then an additional effect on it.

So, I think, you know, I wouldn't like the FDA to go away with the conclusion that you have to do an efficacy model in a pig or a dog to go to the clinic. Again, it depends what you are looking at.

I will make a point about the

immunosuppressed animals. Angiogenesis, an intrinsic component of the angiogenic process is inflammation, so if you put in cells in a model that is immunocompromised, it is a laboratory model where you have taken away one of the normal modulating influences, so you have to be very careful using an immunocompromised model at least to look at a process in which inflammation is a very critical component.

DR. RAO: A point well taken.

Bruce.

DR. BLAZAR: Doris, I wanted to ask you, since you have reviewed all of the preclinical data, and you had the tenet that the data should drive the studies, what of the preclinical data is sufficiently compelling that this would have driven the studies to go forward, are the models done so far incomplete to be able to decide on appropriate studies?

I know we are going to talk about that tomorrow, but since you have reviewed in two talks this issue--

DR. TAYLOR: I think there is certainly a lot more preclinical data from myoblasts than there are for some of these bone marrow derived

progenitor cells. I think that is in part because we have known about myoblasts since 1961 and we have know about these bone marrow derived cells for the last five or six years, so it is not surprising.

I think myoblasts have been used in peripheral models, they have been used in cardiac models of injury, they have been used in large animals, they have been used in small animals, and there is a confluence of data, all of which say if you give these cells, the animals get better.

I think most of the cardiac models were relatively acutely after injury, within a month after injury, but nonetheless, they said if you use these cells, the animals get better.

I think what is starting to happen in the bone marrow mononuclear cell field and in the bone marrow stromal cell field, and in even the MAPC field, is that we are seeing isolated studies with cells that are called a given thing, but aren't necessarily defined the same way.

The way one group defines an EPC, and the way another group defines an EPC may be very different, so there is not necessarily a confluence of data yet, and you can't necessarily even compare

some of the preclinical studies because the cells are very different, or we don't know what the criteria are for those cells.

So, I think that what has to happen is we have to know what the definition of the cells are, and that is only now becoming true for endothelial progenitor cells.

DR. BLAZAR: So, a corollary of this question is, is it pretty well established by those of you in the field, what the bar is that you have to get over.

It seems the way you have described this, it's a systolic function bar that is--no.

DR. TAYLOR: I think it depends on what you believe the mechanism of action is for the cells, and I think again we don't know that, but it looks like what is coming about generally is that for bone marrow derived cells, the goal may be angiogenesis, and for muscle-derived cells, the goal may be myogenesis, and those have different criteria and different preclinical studies that I think you ought to do.

DR. BLAZAR: But that presumes for clinical applications that you know exactly the pathophysiology of the lesion you are trying to

treat.

DR. TAYLOR: I think it begins to argue for timing after injury.

DR. BLAZAR: Okay.

DR. RAO: If anybody has a really brief comment, otherwise, we will take Dr. Mulé and then Dr. Schneider.

Do you have a quick comment?

DR. MENASCHE: Go ahead. I just had a comment, but I can wait.

DR. MULE: I would just like to follow up on Bruce's questions. This has been an incredibly frustrating afternoon for me from the standpoint that I think the presentations have nicely pointed out the strengths and weaknesses of small animal models versus large animal models.

Overlaid on that is the fact that none of these models really are good models for the actual disease state in humans. What I was hoping to hear this afternoon was taking the strengths and weaknesses of each of the models and maybe laying out, hopefully tomorrow, what an ideal, if we could go that way, what the ideal recommendations would be for the field to help the clinicians, such as Philippe, in conducting the next generation trials.

That is the real concern to me, is that at the end of the day, the horse is out of the gate, and Philippe and others are going to be conducting these trials rather rapidly, and I think we need to help them to establish some guidelines as to whether or not we should abandon animal models, move on to the clinic, and design the clinical trials in such a way that we get the best scientific data available and the best clinical situations that can be defined, and define the endpoints of the clinical trial with the appropriate placebo.

So, I just wanted to lay out that my deepest concern is that years from now, we will be using the same models that you have very nicely summarized, small animals, large animals, fully aware that these limitations continue to exist, and whether or not those data that are generated over the next several years will help Philippe and others to characterize how we should go forward in conducting these clinical trials. It is just some comments I had.

DR. RAO: I think you are echoing what the FDA is feeling, I guess, right now.

Dr. Schneider.

DR. SCHNEIDER: Doris, you know that I share your general cautionary note and share many of the specifics, but let me disagree with two specific points that you made about the lack of utility or impediments to the use of the mouse.

One of them that you singled out is the impediment to using the mouse as a model for studying the electrical connectivity of donor cells to the host environment, which has become an issue of prominence because of the issue of ventricular tachycardia.

I think Loren Field has shown very convincingly you can use 2-photon microscopy to study the propagation of action potentials on the epicardial surface, you can study the propagation of calcium transients in the mouse heart with grafted cells, so that is a non-issue as of this year.

DR. TAYLOR: I completely disagree, but we can have that discussion--

DR. SCHNEIDER: Perhaps so, but Circulation doesn't, and there is adequate peer-reviewed data out there that shows that it is technically feasible with some esoteric instrumentation.

The same thing I think is true in terms of the issue of mechanics. As you say yourself, cine MRI levels the playing field across all of these species, and at a few centers, cine MRI is even being combined with spam, so that people can do finite element analysis in the small mammals.

So, to me, and I share Dr. Mulé's frustration, I tend to focus myself on a different issue, and that is not whether the small mammal is adequately achievable for the endpoints that we want to study, but is the small mammal adequately predictive of the pathophysiology we want to study.

There, the issues include the fact that neither the small nor the large mammal is done in an aged animal against a background of diffuse atherosclerotic disease.

DR. TAYLOR: I want to just comment on that looking at electrical connectivity is not the same thing as looking at ventricular tachycardia or arrhythmias, and that it is going to be difficult to do those studies when the heart is beating at 600 beats per minute, and that is I think one of the main issues, as well as the fact that most electrophysiologists tell you that the larger geometry is much more conducive, of the human

heart, is much more conducive to electrical abnormalities than the smaller geometry of a rodent heart, so you are not going to see the same properties even if you have an injury there.

DR. SCHNEIDER: Again, it becomes an issue of whether it is a predictive biology, not an issue of whether it is technically achievable.

DR. TAYLOR: Sure.

DR. MENASCHE: To some extent, Dr. Mulé has anticipated my comment. What I wanted to emphasize from a clinical standpoint is that regardless of the animal model we are going to use--and I have advocated large animal models, as well--there is not a single animal preparation which can realistically model the very complex situation of the patients we are dealing with.

There is not a single model which can reproduce the situation of a 70-year-old person with Class III heart failure, two previous bypass, seven angiopathies, collaterals, and so on, so at what point we really have to be prepared to move forward and to go across the gap.

Now, considering skeletal myoblasts, we have clear evidence regardless of the limitations of the models that the technique can be implemented

easily in patients.

Number two, we have reasonable evidence that it is safe provided some precautions are taken. We don't fully understand the mechanisms, but many interventions are currently using patients without an extensive understanding of the mechanisms. I think it took years before people understood how aspirin was working.

So, as we are continuing to try to understand the mechanisms, I think that given the huge population we have to deal with, and the number of patients without any option, it is critically important and timely appropriate now to move to the efficacy studies, and we are certainly looking for advice and help for designing the study in such a way that they can draw meaningful conclusions and answers to the two fundamental questions, does it improve function in the areas where cells are put, does this improvement have an impact on the clinical outcomes.

DR. TAYLOR: Philippe, the one comment I would make to that is I think you are absolutely right, we don't have good animal models, I don't anyone in the room will argue that.

I think the thing I would add to what you

said is you mentioned no-option patients, but those aren't the patients that are involved in some of the studies that are going forward, and I think those are the patients who should be involved in the studies going forward, and I hope that is one of the things that will emerge from this, that it may be responsible to go forward in those groups of patients, it may not be responsible to go forward in some other groups without more preclinical data.

DR. RAO: I would like to just remind everyone that part of the discussion is not specific to one particular cell type, but is in general, and maybe the conclusion may be that it is just simply we have to have differing criteria depending on the cell type or the model, or whatever, when we discuss it, at least its options, and that may be as far as one can go.

So, keep that thought in mind, that nothing that you hear necessarily means this is absolute or anything in that fashion.

DR. ITESCU: Along those lines, I think the point you are making is exactly right. Some cell products are based on characterization of the surface phenotype, based on many years of immunoselection with markers only present in humans

and not present in any other species.

Some cell types have been characterized by very different biologists, who have used functional outcomes and functional criteria. So, you are talking about apples and oranges really, so you can't have the same clinical models or preclinical models for those, so you have got to define appropriate models for each of those cell types, because they have been characterized from totally different perspectives.

DR. RAO: I thought we might use that to start the conversation tomorrow, yes.

Everybody looks like they need a break, so we will take a short 10-minute break.

[Break.]

DR. RAO: Welcome back, everybody.

We are going to change topics a little bit and talk about devices now. It is finally going to be the turn of Dr. Jensen to talk on cardiac catheters.

Cardiac Catheters for Delivery of Cell Suspensions

DR. JENSEN: I am going to go ahead and get started because we are running a little bit slow here, and I am also going to try and see if I can catch up a little bit in terms of time.

My name is Nick Jensen. I work in the Division of Cardiovascular Devices in FDA's Center for Devices and Radiological Health, and I have been asked to briefly introduce the cardiac catheters that have been used for delivery of cell therapies intended to treat cardiac disease.

My presentation today will be limited to potential questions that relate to the interaction between cell therapy suspensions and the cardiac catheters used to deliver these therapies.

Further, the questions that I list today are among the standard questions that we currently suggest to all sponsors either of cell therapy INDs or for these investigational catheters.

As examples, we will discuss two types of cardiac catheters that have been used to deliver cell therapies to the heart.

First, is infusion of cells into a coronary artery during a balloon occlusion of the artery. Second, is needle-tipped injection catheters designed to permit percutaneous transendocardial injection into the myocardium.

Again, these simply represent the methods and catheter types that have been most commonly used to date, and they are again meant to provide

useful examples for discussion. I think it is hard to predict what types of devices, what delivery methods may be used in the future.

The first method, infusion of cells into a coronary artery offers potential advantages that include simplicity and ease of use. As has been mentioned earlier today, this method may not be suitable for all types of cell suspensions. Potential limitations include the potential requirement that infused cells be able to migrate from the vasculature into the myocardium, and a potentially increased risk of embolization or microembolization if some types of cells are infused using this method.

Published case series using this method have demonstrated preliminary clinical feasibility both when used within hours to days following acute myocardial infarction, and this is often following emergency stent placement at the site of thrombotic occlusion.

It has also been used in patients who suffer from chronic myocardial infarction and ischemia.

In the studies reported to date, balloon catheters have typically been used to temporarily

occlude the coronary artery proximal to the treatment region. The desired cell suspension is then infused into the artery distal to the inflated balloon either using a lumen within the balloon catheter, typically, a guidewire lumen, or using a separate infusion catheter placed lateral to the balloon catheter, such that it lies between the inflated balloon and the artery wall.

Use of balloon occlusion permits infusion of cells at pressures that exceed coronary artery pressure, and it has been hypothesized that increased infusion pressures may provide benefits that include increased dispersion of cells within the vasculature, increased adhesion of cells to the vascular endothelium, and increased migration of cells across the vascular endothelium and into the myocardium.

This familiar illustration from a publication by Strauer, et al., illustrates the use of a coronary artery balloon catheter to infuse cells into a region of acute myocardial infarction.

In this illustration, the balloon catheter has been inserted into a large artery, directed retrograde through the aorta, then, into a coronary artery, and then directed distal within that artery

to the site of acute thrombosis.

Following inflation of the balloon to obstruct the artery, a syringe is used to infuse cell suspension through the guidewire lumen of this catheter and into the coronary artery distal to the balloon.

Finally, although it is not obvious in this illustration, in this clinical study, the balloon was inflated at the site of acute thrombosis, and more specifically, at a site where a coronary artery stent was placed as an emergency treatment for the myocardial infarction, and that is potentially important because balloon deployment within a coronary artery stent can largely protect the artery from one potential concern that we will discuss briefly today, and that is the potential for damage to the artery caused by balloon inflation and subsequent stretching of the artery.

Studies of this cell delivery method reported to date have commonly used balloon angioplasty catheters to occlude the artery. The catheters are originally designed to stretch the lumen of an occluded fibrotic atherosclerotic coronary artery to a specific diameter that has been selected in advance by the treating physician.

They are also used to expand coronary artery stents and again to prespecify diameter. Although these catheters were not originally designed for occlusion of an artery, they can be used for that purpose, followed by cell suspension and, additionally, as noted, if these catheters have a central guidewire lumen, that lumen can then potentially be used to infuse the cell suspension.

Potential considerations when you are using an angioplasty catheter for this purpose include the following. First, contact between the catheter lumen materials and the cell suspension can potentially adversely affect either the viability or the functionality of the infused cells.

Additionally, those cells may contact various lubricants that are commonly applied to the guidewire lumens during manufacturing for the purpose of facilitating guidewire passage.

As a note, we are not aware yet of published reports that have examined whether catheter lumen materials may adversely affect viability or functionality of cells, however, this published animal study cited in the slide evaluated cardiac delivery of a transgene by an adenovirus

vector.

The investigators found that some lumen materials tested for use in a prototype needle injection catheter, the second type of catheter we will discuss today, adversely affected both the viral activity and viral transduction.

They also found that a change in lumen materials was sufficient to completely eliminate these adverse side effects.

Additional considerations. Number two. A second consideration when balloon angioplasty catheters are used for this purpose, infusion of cells, is that the balloon was originally designed to stretch a coronary artery, in other words, to controllably damage the artery, and for this use, it must instead be used to occlude that artery, hopefully, without damaging it. This is potentially important.

The degree of artery wall stretch that is typically created during balloon angioplasty will also subsequently induce arterial stenosis due to multiple mechanisms. We think it is therefore essential that safe methods for balloon inflation be developed and demonstrated if you want to use an angioplasty catheter for this purpose.

Importantly, development of safe methods for this new use could be complicated because balloon angioplasty catheters have widely varying pressure diameter relationships, in other words, compliance can vary greatly between different catheter models, so therefore the methods that are developed for one catheter may not be applicable to another one.

A third potential concern related to this is the potential that concentrated cell suspensions may clog the catheter lumen.

A fourth concern is that the lumens and connectors of angioplasty catheters are primarily designed for passage of a guidewire. They may not have been tested for the ability to sustain high pressures that can occur during infusion of concentrated cell suspensions.

The second type of catheter we will discuss today are needle-tipped injection catheters. This method of cell delivery also offers potential advantages. Notably, first, the ability to directly inject cells into desired myocardial locations. Second, the potential for use with all types of cells.

The investigational cell delivery systems

developed for this therapy consists either of a catheter or of a system comprised of a catheter plus delivery sheaths, that include a retractable distal injection needle. None are approved currently for sale in the U.S., however when we consider these new devices, it may be useful to note that some design requirements, and thus, potentially catheter characteristics, may be similar to requirements for other currently marketed cardiac catheters, potentially including both cardiac electrophysiology ablation catheters and endocardial biopsy catheters.

More specifically, all three types of catheters or catheters plus sheaths would generally require a steerable or deflectable tip in order to facilitate direction of the catheter tip to various endocardial locations, and all three types of catheters must be sufficiently stiff to permit the user to maintain stable contact between the catheter tip and the moving endocardial surface of the ventricle.

These illustrations are from a publication by Dr. Perin's group, and they illustrate one, investigational needle-tipped injecting catheter. The photo on the left illustrates a complete