SPEAKER 1: Good afternoon. Welcome to CTSA Grand Rounds. This is one of the series that is introducing research, especially translational research that's going on within our new hybrid centers. And today we're featuring research that's related to and going on within the Center for Regenerative Medicine. And this will be a joint presentation by two of our leading investigators in the cardiovascular disease areas. And I think you'll find this is very exciting how we are really now taking things from the laboratory and moving them onward and into patient care, really at the forefront of the field.

So the two speakers are Dr. Rob Simari, originally, I think, from Massachusetts, and Dr. Amir Lerman, originally from Tel Aviv, Israel, who've come together and will be talking about these projects. Both are extremely distinguished investigators in their field. As usual, we'll not give a long introduction except to say that both are consultants in the division of cardiovascular diseases, and both are professors of medicine.

Dr. Simari is also Chair of the Cardiovascular Cell Therapy Research Network, which is a national network sponsored by NHLBI, the National Heart, Lung, and Blood Institute. And his main research interest is in fundamental understanding of the molecular basis of vascular disease, and using this mechanistic knowledge to develop new biological therapies.

Dr. Lerman is also Director of the Coronary Physiology and Imaging within the Cardiac Catheterization Laboratory. And he has a broad spectrum of interests related to clinical studies and cardiac cath lab. So this is from patients. But then also focusing on the role of endothelium in vascular disease, and then theilial-derived factors in vascular disease, and then using physiological organ bath and other systems to study basic mechanisms in depth of cardiovascular disease. And a portent theme here, I think, is that bringing the most important mechanistic basic science discoveries right up into the clinic and the patient.

And I think Dr. Simari is going to begin. So Rob, thank you.

DR. ROB SIMARI: Thank you. It's a pleasure to be here representing both the CTSA and the Center for Regenerative Medicine, and to have my friend and colleague share the podium with me today. We're going to talk today about cell therapy for heart and vascular disease. Amir and I are engaged in a program for valvular development as well. We're going to save that for another talk at another time. So today we're going to really focus on, if you will, the right part of the translational spectrum, that part which is already into the clinic, in late phase clinical trials. And really, it complements many of the early phase aspects of the regenerative medicine program for the heart. But today we're going to focus on the right side of the translational spectrum.

Every talk in novel therapies should give someone understanding of what the unmet needs are to develop new therapies. And for heart failure, particularly heart failure following myocardial infarction, in spite of all the progress that has been made in the prevention of atherosclerosis and the early treatment of myocardial infarction, heart failure still remains a significant problem that has not declined over the years, probably from a balance of saving patients that would have otherwise died during heart attack who now have heart failure, and a combination of the increasing aging of the population. But there are still unmet needs in the treatment of patients following heart attack to prevent heart failure.

And so we think about how we might be able to use cellular therapies for patients who have ischemic vascular--ischemic LV dysfunction. We have two options. One is to identify patients who are high risk really in the post infarct period and to deliver those cells to prevent subsequent maladaptive remodeling. And the other would be to wait and see which patients of that high risk population actually maladaptively remodels and treat then.

The opportunities really do affect how we select our cell types, since in early infarction times we really have to use a cell that's available, off the shelf, or a cell that could be harvested and isolated quickly. Whereas in a chronic patient, we would have maybe longer period of time to isolate or develop cells. The strategies that I'm going to speak about this afternoon are really focusing on the first. They're the furthest along, and that I'd like to share with you. How do we try to prevent heart failure in patients who are at high risk following myocardial infarction?

The fear really started in 2001 when in, really, a landmark, highly controversial study, Piero Anversa demonstrated that in mirroring studies of myocardial infarction in which a coronary artery was ligated, bone marrow was harvested, and c-Kit positive cells were isolated from the bone marrow and then delivered into the myocardial scar, one could find pockets of repopulating cells within the scar compared to the scarred control group. These animals survived longer, they had stronger hearts, and the thought was that these bone marrow cells had trans differentiated, if you will, into myocardium.

However, those data had been questioned mechanistically. The candle had been lit, and the field took off. And so, between 2001 and September 2006, there were three randomized, controlled clinical trials published in the *New England Journal* based upon mouse studies that had been published five years earlier, an amazingly rapid translation based upon two factors, in my opinion. One is the unmet need, and the second is that the cardiovascular field tends to be early adopters. And so this combination of factors led to these three clinical trials, which had actually quite mixed results, but got to the level of being published in the *New England Journal.*

The controversies that surrounded both the mixed clinical trial results and this controversial paper that was published in 2001 really focused on the unclear mechanism of any potential benefits. Piero Anversa and his colleagues originally suggested that the way these cells worked was that cells were delivered and integrated into the tissue, and differentiated into cardiomyocytes that would help with the process of cardiac function following their homing and integration. But that was questioned, both in preclinical studies as well as in clinical studies, of whether this actually took place. And there's been a broad spectrum of mechanisms that have been hypothesized, including the fact that delivered cells might fuse with resonant cells and then have survival benefits, that they may produce paracrine factors, shown in the middle here that either stimulate vascular effects through angiogenesis, or arteriogenesis, or activate endogenous stem cells that might be existent within the heart. And then also it could be that they might differentiate into cells that are not cardiac myocytes, into endothelial cells or smooth muscle cells that would help the vasculature support a functional improvement in cardiac function.

So we had a really strong burst of studies in the early part of the first decade of the 20th centuries. And we were left with uncertainty of mechanism and uncertainty of the right approaches for stem cells following heart attack. When a number of these studies were done-- and as a field is tempted to do, take a lot of little studies and generate a single meta analysis. And this was the first to have multiple meta analyses that have been done in this field. When we look at the ejection fraction change, shown here in aggregate-- this is the global left ventricular ejection fraction-- between patients and randomized trials who received cell therapy, or control. The aggregate bar here is to the left, showing a small but statistically significant improvement in left ventricular ejection fraction in patients who received autologous bone marrow cells following heart attack. And these results were suggested that the improvement was small but definitive, or might be potentially definitive, and it led to a further larger series of trials, some of which I'll speak about today.

So in 2006, the safety profile had been quite encouraging that cells could be harvested and delivered. There were these modest effects seen with left ventricular function. And interestingly, the experience was mostly outside the United States-- actually, mostly in Europe at the time. And so the federal government set a few panels together to look at what's the right approach in the United States to participate in this field. And the NHLBI, the National Heart, Lung, and Blood Institute, established the Cardiovascular Cell Therapy Research Network to perform early phase studies in cell delivery following heart attack.

And so in 2007, a group was established using the clinical research network mechanism that had been used in the pulmonary branch, but not necessarily in the cardiac branch field before, to put together a group of centers across the United States, including Texas Heart Institute, University of Florida, Cleveland Clinic, Minneapolis Heart Institute, Vanderbilt University, with additional recruitment satellites, including Amir Lerman, who led the satellite here at the Mayo Clinic to recruit to these trials. They were being coordinated out of the University of Texas School of Public Health, and I've been honored to chair this group for the last for the last six years. And the goal of this group was to do early phase studies in patients who have had heart attack or have chronic left ventricular function dysfunction due to coronary artery disease.

And so in the last two years we've published three papers in *JAMA* highlighting the results, two of which I'll share with you today. These trials looked at autologous-- so cells coming from their own patient that were harvested at the time following infarction and re-infused into the infarct area within a few days after myocardial infarction. The time trail was aimed at defining the timing of delivery since most of these studies have delivered cells within the first week. And the late time trial was to see whether we could extend the benefits out to a later time point when patients, A, might have resolved their acute infarction and be stable enough to have these procedures done, and B, might be transferred from other centers into the clinical trial network.

So there are some shared features that we used in the network. We used a processing element for these cells, referred to as Sepax, a system from Biosafe, and it's a system that uses closed preparation of bone marrow mononuclear cells. We thought it was really important to use double blinding, and that required us to use some sort of blinding agent to make plasma look like bone marrow. And in this case, we got approved to use autologous blood to spike the albumin to make it look like a bone marrow source, so that the individuals who are delivering the therapy were not blinded. We decided to used a fixed cell dose, and to use regional and global cardiac MR as the primary endpoints, since they're the most sensitive ways to detect cardiac function. We had centralized data coordinating centers, cores, and we're doing long term clinical follow-up. This was the system that we used.

As we got into the bone marrow field, we realized that the open preparation of bone marrow for bone marrow transplant is really a thing of the past. And it's really, now, if you get a stem cell transplant, you get cells mobilized and isolated through magnetic separation. And so the idea of using this open Ficoll and removing this through with a needle just didn't seem to be rigorous enough to do in a multi-center trial. So we engaged the Sepax system here from Biosafe, in which bone marrow is added to the top, the centrifugation through Ficoll is done, and the cells are isolated automatically. And it can be done in the same day.

We thought that it was important to use a system that could be translated more broadly across the network. And in fact, we've used this system shown here with some studies from Danny Spoon. In my lab we've compared the angiogenic capacity of these cells compared to counterparts using an open Ficoll system, and find that in this reperfusion model in the mouse, looking at hind limb ischemia and angiogenesis, that the two cell populations are equivalent. So we think this is a rigorous, reproducible system for isolation of bone marrow mononuclear cells.

So the first study that we performed and published was the late time trial. And the study was designed to assess the safety and efficacy of bone marrow cells delivered two to three weeks following infarction, on left ventricular function. We treated 87 patients with first large myocardial infarctions, and we followed them for 24 months. We isolated and delivered 150 million bone marrow cells or placebo in a 2 to 1 ratio delivered by intracoronary infusion.

As I go through this talk, I'd just like to point out a couple things that we might do differently. So if you're thinking about trials in the future, you might think differently. 2 to 1 randomization was used because we thought that it might help us enroll quicker, because patients have 2 in 3 chances. It was a killer because, ultimately, it decreases your control group. And it really made it quite difficult, you'll see. Small control group with lots of variability is a real problem. We won't do those 2 to 1 randomization anymore, even if it takes us a little bit longer. The primary endpoints were global and original function measured by MR.

And so this is what happened. Patients come into the cath lab with their index AMI. They get a coronary intervention with stent placement. And if their ejection fraction's less than 45%, they could be randomized and in this time frame. Then they'd come back two to three weeks later, they'd get their baseline cardiac MR, bone marrow aspirations done in the morning. So patients come in, get their cardiac MR, get their bone marrow done, and are treated later in the day with either intracoronary treatment with their bone marrow cells or placebo, and then they're followed for six months. So there was a 2 to 1 randomization between cell product and placebo.

So because it was first myocardial infarctions, it was a younger group than is often treated. It was in the mid-50s, mostly white, not by intent, and mostly male, not by intent. But these are the populations we saw. You can see that the qualifying EF here by echo, before they came in, was in the mid-30s. And their peaks CKs were quite high. This is a population that would be having a high risk of heart failure following myocardial infarction. They were treated early from the ER, [INAUDIBLE] from chest pain. The door-to-balloon times where were consistent with current treatment strategies.

We isolated bone marrow in all patients. So even the placebo group had a bone marrow aspirate done, another important part of doing this randomized double blind study. We isolated 150 million cells. They were all viable. You can see that when one looks at stem cell markers such as CD 34 and 133, it's generally about 2 and 1/2 percent of 34, and about half of that having the more primitive marker CD 133. And were able to grow endothelial colonies and from these tissues as well as stromal-- as well as other colonies.

So we had six-month primary endpoint, but this is the safety data. We had one non-cardiac death. I just want to remind you, these are patients who have been followed six months after their primary infarct. We had one non-cardiac death, one reinfarction. You can see the event rates are very low, and although numerically different, this is twice as many people in this group than this group. They were not statistically different. So there didn't appear to be a safety signal in this population.

So here's our primary endpoint. And the bottom line is that there was no difference in global left ventricular function between the group that received bone marrow on the left or received placebo on the right, compared to baseline. Couple of things to note here. These baseline numbers are about 10% higher than the echo numbers that got the patients into the trial. There's two reasons for that. One is it's two weeks later, and the second is that EF by MR is about 10 points higher in general than by echo. So these numbers are quite different, but there was no difference between the placebo group and the treatment group in terms of global function.

When we looked at original function by cardiac MR, the same thing could be said. If one looked at the infarct zone, there was no difference. And if one looked at the border zone that was adjacent to the infarct zone, there was no difference. So the fact is in this trial, based upon these data, delivering cells two to three weeks following myocardial infarction had no improvement on left ventricular ejection fraction or global or regional left ventricular function. When we looked at things such as volume indices or infarct volumes, there were no statistical difference. Infarct volume went in the direction of a treatment effect, but was not statistically significant.

So what's the implication of this trial? Well, two to three weeks may be too late to deliver cells in myocardial infarction to have an effect on left ventricular dysfunction. It may just be that we're outside the window. Was it under-powered? Well, the first data sets I showed you from the randomized trials, at the time we designed the study, the best cardiac MR data we had was from one of the German studies that suggested a 5% difference in effect size. And so we powered the study based upon this 5% difference. The latest meta-analysis that have been done suggests this difference is 1.8%. So we were grossly under-powered to detect a 1.8% difference, if we wanted to detect a 1.8% difference. I would suggest that that's probably not biologically meaningful, even if we wanted to test it.

So lessons from this study are that's a rapidly moving field, and some of the underpinnings of the clinical trials really change during the time it takes to complete the trial. So while we were working in the bone marrow field, there were a lot of basic scientists developing new opportunities. But nonetheless, this is where the clinical trial science was at the time.

And to keep in mind that the public and the field, , investigators within the field have very high expectations and often unrealistic expectations. And a lot of the studies that were done in Germany, I would posit, are done by believers. They're done by people who are certain of the results before they start. And to get it into dispassionate hands in a federally funded trial is a completely different story. And so I think the field had very high expectations. And I think the data from well-designed studies, whether it's positive or negative, can advance the field. But clinical development is really non-linear, and so we're working our way through this.

Now, this is from Kelly Noonan. If only stem cells were this easy. You know, here's a couple of snowmen who are-- it's going to happen to all of us today. But the fact is it's a very difficult field. And so we also-- a companion study with the late time study was the time trial. Now, the time trial was working in a time frame which the studies in Germany and Europe had suggested there was an effect on cell delivery on LV function. And so we were trying to identify whether there was a sweet spot within that first week in which cells might have its greatest effect.

The reason for defining the sweet spot is that in a practical way, can it be done while patients are hospitalized, or do they need to come back. And so optimal timing for cell delivery had not been ever directly tested in a prospective trial. And we know that the heart is rapidly changing following myocardial infarction in the early time after MI, with cytokines, different inflammation resolving, which may create an optimal window. And remember that our patients are producing their own product as well. And as the inflammatory state changes in a patient following myocardial infarction, their bone marrow is changing at the same time. So not only is the target changing, but but the product's changing.

So we felt it was important to try to sort out in the first week what would be the optimal time. And all the trials before, essentially, had delivered within seven days. But no one had ever prospectively randomized. And there had been some data to suggest that within the latter part of that time was the beneficial and post-hoc analysis. As I mentioned, late time showed no benefit at two to three weeks.

So the time study was similarly designed, in which patients come in with their index infarct, their echos less than 45%. They're randomized during the hospitalization to either a three-- day delivery or a seven-- day delivery. And at that time they get a cardiac MR, both at three days and at seven days, so that we had resetting of the baselines for both populations. And then treatment with delivery of the bone marrow dripped through a catheter that's placed at the site of the prior stenting. They received 150 million cells, and the primary endpoints were at six months. So the steady aim was to assess the effect of the cells at day three versus day seven, in a two by two design, in which patients either receive placebo or cell therapy, and the primary end point being this change in global and regional left ventricular function.

So the patients are very similar to the group that I showed you before. And I'll just point out a couple of things that really affect small studies, and we live and die by ends in these small studies. Here's the number of patients that were treated in both groups. So you can see, we used the 2 to 1 randomization strategy, which gave us very few placebo patients in each group. And you can see that there's seven more placebo patients here than here. And the fact is that we had seven patients drop out before they had cells delivered, and so we added those seven patients back. We added those seven patients back to randomize seven more, and they are randomized to the three day group. So you know, it's-- when you deal with small numbers-- and the other thing I would point out is with small numbers in the placebo group, you can see, here is-- this is chance. We got zero diabetics in one placebo group. These are the kinds of things that can kill study design with small numbers. So just pointing out that trying to keep our ends up and trying to randomize 1 to 1 is something we'll do in the future. But the population was very similar to late time population, with their EFs by echo in the mid-30s.

So we isolated again 150 million cells, and we've analyzed them very carefully. And these are the primary endpoints. This is global ejection fraction in the placebo group on the right, and on the treatment group on the left. And there was no difference in global ejection fraction measured. There was no difference between the two groups, but there was an improvement in both groups between the time they were treated and six months. This is for the total population. And when we broke it out by three and seven days, there was no difference between the three day treatment group versus the seven day treatment group, as shown here. So all patients got some improvement, but treatment did not have an impact.

And when we looked at original function by MR, the same thing could be shown. So again, unfortunately, like late time, there was not a signal with delivery of these cells. And again, infarct volume went in the right direction, but we were significantly under-powered to detect changes in infarct volume. Again, we looked at safety outcomes and deaths. We had one non-- again, a non-cardiac death in these two patient populations. We have not had a cardiac death, thankfully, and no difference between the populations in terms of events.

So our conclusions are that intracoronary delivery of these autologous cells is safe in moderate and large infarcts, but has no beneficial effect in terms of global and regional left ventricular dysfunction.

So what are the implications of the study? Well, maybe there's no effect of bone marrow cells on LV function in the United States. Maybe it's a United States effect. It's possible. There have been large studies that, you know, the US effect is different. And a lot of those come down to the standard of care that's being applied at the same time. So one could imagine that, all things being equal, if the care was less and the risk was greater, maybe there would be a benefit.

Maybe this is regression to the mean. We see with new therapies that the benefits are greater, and the longer they are tested, they come down. Maybe this is a differences in the patient population. Or maybe it's the way we isolated the product. We don't believe it's the case but it's been suggested by some. But maybe there is no effect. Maybe the effect is so small that we'd need a huge study to detect it. And we're just-- we would need about a thousand-patient study to detect a 1.8% difference. And I think that's money that's not well spent. So maybe we were just under-powered.

Or maybe EF's not the thing we should be measuring. That's a surrogate for function, and it's a surrogate for patient effects. So maybe we should be doing something other than measuring ejection fraction. Maybe we should really be following these patients clinically. Now, this is from the riparian eye study by Andreas Zaire, who, when asked to publish his paper in 2006 the *New England Journal,* was asked by the editors, we want all the clinical outcomes. And what they found was quite surprising, in that there were statistical and numerical differences between the bone marrow group and the control group at one year follow up, including things like target revascularization, myocardial infarction, and death.

So maybe we shouldn't be measuring EF along maybe we should be measuring outcomes. At two year follow up, this is a Kaplan-Meier curve for event-free survival. The bone marrow cell group did significantly better than the placebo group for major adverse cardiac events. So maybe the cells don't necessarily have a big effect on EF, but maybe they're causing patients to live longer.

As a matter of fact, the latest meta-analysis, done by Buddhadeb Dawn from the University of Kansas, showed that the greatest difference between the group was really all-cause mortality and cardiac deaths. That's a 60% reduction in all-cause mortality and cardiac deaths in the group. Now, I would point out that we didn't see any cardiac death, and we saw very little mortality. So it may be a population issue.

So there is a large Phase 3 randomized trial in Europe called the BAMI trial that is going to use 20 European sites, and mortality is going to be the only endpoint. So they're going to randomize patients to cells or no cells. Then they're going to account the bodies at the end of two years. And the European governments have said, you don't need a placebo because as far as they know, no one's faked their death on the placebo group. So they've said, we don't need a placebo. We're just going to count the bodies at the end of two years.

And this study is powered to detect a 25% reduction in mortality at two years. Now, I've got to tell you that I think this is probably over-estimated by two- or three-fold. But the proponents would say we're under-- we think we'll get a 50% to 60% reduction. So being small might be in their favor. So they're looking to start this study any time. It will keep the Europeans busy for five years doing this study for myocaridal infarction. So the end game of all these bone marrow trials will be evaluated, and it'll be probably about five years. So stay tuned.

So the prevention or treatment of left ventricular dysfunction is really not the only clinical viable target for cardiovascular cell therapy. And within the network, we're looking broadly. We completed a study in LVAD patients. Were starting a study for peripheral arterial disease in claudication. And we're using a broad range of products, including mesenchymal stem cells, allogeneic products, and a cardiac progenitor cell population, c-kit positive, that really hearkens back to the original mouse studies that I showed you. So I think the field is broadening greatly, and we were funded for another seven years to continue these studies. That

The concept of targeting the vasculature more directly is really a segue into Dr. Lerman's talk. The idea that one can induce angiogenesis in ischemic tissue, either in the periphery of the coronary artery disease or in the coronaries, is really an area that's quite exciting and really leads into a clinical trial that he is the PI of at Mayo. So, Amir.

DR. AMIR LERMANI: Thanks, Robert. Thanks for the introduction. It's a pleasure for me to be here and share this information with you. And actually, Rob laid a great foundation for this talk. This is an effort to extend the concept of cell therapy to another indication, and it just showed that there is interest in that. And you need the collaboration, more than just NIH that Rob was able to bring into Mayo, but also from industry.

So this is a study that is currently ongoing with the center study, that is funded in majority by Baxter. But at Mayo, there is a great support from the Center of Regenerative Medicine, and I would like to recognize Kelly Noonan, who is the study coordinator that was on the time and currently on the study.

So this is a group of patients that are very challenging to treat. These are patients that carry different kinds of titles-- the non-revascularized patient, the non-option patient, multiple different issues. So these are patients that have coronary disease and continue to have chest pain and ischemia, in spite of optimal medical therapy. And multiple studies showed that even if you applied the best medical therapy, about 30% of the patients continued to have chest pain that compromised their lifestyle.

From a definition standpoint, if you have patients undergoing interventions such as bypass surgery or stenting, this is also a technical definition. When you look at the angiogram, you say, technically, I cannot help this patient from the facility that would think that I have now. For multiple reason-- maybe the vessel is too small, maybe the distal vessel is too small, and maybe you don't see a specific area that you can target. If you ask people who are on the ward how many patients in their practice are falling into that category, it's a very tricky question. Because interventionists don't like to admit that there is an entity they call non-revascularized patient. But it's like asking the surgeon how many normal appendix they take out. So in a sense, most of the population agree that you have around 20% of the population in the cath lab, more 50% to 20%, that actually are falling into this category.

So they estimate that, depend on the definition, up to 1.8 million Americans suffer from what we call the non-revascularized patient and continue to have chest pain. Secondary to the elderly population, we have every year about 50,000 to 100,000 cases. And also it's an increasing problem in Europe, and this is the estimation of how many patients are in Europe. They have very poor quality of life. They require a lot of medical attention and re-hospitalization. The outcome is variable, and I will talk a little bit about it. And there is a poor success of novel therapies. And there are multiple attempts in the last several years to treat these patients without success. So advanced investigation therapies are emerging as a potential option for improving patient outcome and quality of life.

One of the-- wouldn't say mistake, but one of the concepts that led investigation into this area was under the assumption that these patients have higher mortality. So most of the studies that were designed use end point of mortality as their endpoint. And that's one of the reasons that these studies failed. Because if you look at these studies, one of them from Rob Simari in 2008, in our population at Mayo, and data from our colleague Henry, Tim Henry, from Minneapolis, who both are dealing with this population. This patient population do not die very often, fortunately. And even if you look at their mortality, about 50% of their mortality is not cardiac. So if you have a situation as Rob suggested in his talk, that you have a situation with low mortality, it's very difficult to fix. And that's one of the reasons that some of the studies failed, because they used this one as an endpoint.

But what is characteristic of this patient? They have prevalence-- it's very prevalent. And if you look at this study of 200 physicians, about more than 2000 patients, and they use the Seattle angina questionnaire, 29% have more than one episode a week of chest pain. And you look at their angina frequency, they have poor quality of life the more angina they have. And 24% have decreasing quality of life and decrease in physical limitation. And it also was strongly associated with frequency of depression. And they seek a lot of medical sources.

So when you apply a new therapy, you need to look at the balance between the benefit and the risk of each therapy. If you have a therapy that is very invasive and have a lot of risk, the benefit may be less. So you need to balance what kind of therapy used in this kind of situation.

One of the concepts that is known, and that led some of the investigation into this area, is the fact that we know that the lack of function of the microcirculation of the myocardium, number or function is associated with increased cardiovascular event in patient after a Mayo patient with ischemic heart disease. So this summarizes about four studies looking at invasive and non-evasive studies, showing each one of them that the presence of microvascular dysfunction, obstruction, or lack of microvascular, is associated with event. So the presence of microvascular disease is associated with increased cardiovascular event. So that's led investigation to think that maybe if we increase the microvascular structure, function, or number, we will be able to reduce this event.

So there are several tries to use that. They initially want to treat this patient, tried to induce angiogenesis by injury. So they use laser and injure the myocardium from the hope that this myocardium, as we see in Micro CT images here, will grow vessels and improve the profusion. The results were negative, majority of them. There were some reduction in angina, but it was not successful. And there were some think that one of the reasons it was denervation, and it was placebo effect. So this concept did not pan out clinically.

Then there are multiple studies using what we call angiogenesis studies, that use multiple agent to potentially increase the number of vessels. So eight different agents were used. It was safe. It was blinded, the studies, but the results were very modest, and if at all, didn't show significant effect. One of the majority was that there was a lot of [INAUDIBLE] of the placebo group. So it's very difficult to compare. So here we have another two concepts to increase angiogenesis that did not pan out to be clinically efficient.

Currently, the fact that maybe we can apply these cells as Rob described, and from the hope that there [INAUDIBLE] bone marrow cells will differentiate to endothelial progenitor cells with the CD34 positive as a marker. And this says express CD34 markers, specifically, have been shown in previous clinical and preclinical studies to support neovascularization and cardiac proliferation. So the concept now is to take these cells and try to apply to the myocardium in these patients with non-revascularized option.

So the background to this study started from in animal studies, in a rat model of myocardial ischemia, and a treatment group with a control and low in mononuclear cells , high mononuclear cells, and CD34. And they look-- first of all, they show the capillary density, which showed the angiogenesis, showing that the CD34 groups have a higher density of capillary in the myocardium in this model. When they look and compare one of the parameters of ejection fraction, again, they show in the same group, with the CD34, there was significant increase in ejection fraction and reduction in myocardial fibrosis. So this animal model demonstrated the application of CD34 directly into the myocardium was associated with increased angiogenesis and improved myocardial function.

It raises the question, how do you apply the cells? How do you provide the cells? There are several methodologies. In the time and late time protocol, we provide the cells in intracoronary fusion. There are several studies that show that there is a difference between the way you apply the cells. So this is, for instance, intramyocardial application, which carry more risk, but may be more effective in some specific incidents. And then you have intracoronary, intrastitial retrograde through the coronary sinus. For a particular application, the direct application of the cells into myocardium may be relevant. And in my study, they show that the application more directly, it's true that it carried a little bit more risk, but it's possible that if you identify the area its risk and apply directly to this area, then you will see a beneficial effect.

In order to do that in humans, we have a system called the NOGA system. This is a invasive methodology that is used to map, and it has two phases. We actually introduce a catheter into the left ventricle, where we-- in this patient known to have ischemia-- and mapping the area with electrical activity and mechanical activity. And the principle is that this is the electrical activity of the left ventricle, and this is the mechanical. And this is how we use the mapping. So we look at the discrepancy between viable myocardium and severe wall motion disturbances that suggest the presence of hibernating myocardium. And if you identify this area that are at risk, the concept is that you need to inject directly into this area.

So the first study was a Phase II clinical study of the same intramyocardial autologous CD34 cell therapy for refractory angina. And this was a design of the study was 167 patient, the placebo group, but mainly there are two cell group that received the cells-- the low dose and the high dose. And these patients were followed for safety, efficacy, and again, instead of looking at event, we look at barometers of quality of life, which is exercise time at three, six, and 12 months. And we have MRI at six months.

So these initial studies show that in the patient that received these cells, there was significant reduction in angina frequency at six and 12 months. So first of all, quality of life improved in these patients. It's also interesting to see that there is no difference between the high dose and the low dose. That, again, may raise point that were raised about cell therapy, that sometimes the effect is not directly from the cells, but rather they're from the media of the cells. This study showed that the effect of 12 months on the angina episode per week, again, consistent with the reduction of chest pain in these patients. Exercise duration increased significantly at six and 12 months. Again, no difference between the high dose and low dose, indicating that the injection of these cells to this specific area with hibernating myocardium improved the exercise capacity of these patients.

So based on this study showed that in 167 patients there was significant improvement in angina and exercise time in this population. Based on this study, they moved to the next phase of the study, which is currently ongoing, look at the efficacy and safety of the target intramyocardial delivery of these cells for improvement of exercise capacity in the subject. So the primary objective is to evaluate the treatment with these CD34 cells for improving functional capacity as measured by change in total exercise time, as an exercise test. This is the inclusion and exclusion criteria to these patients. Usually these patients have multiple procedure on optimal medical therapy. They can not perform very good on the exercise test. And they have evidence of ischemia.

This is the design of the study. There is randomization, about 400 patient. They're getting active cells with the intramyocardial injection and active control. And then they are being followed, up to five years after the mapping. So this is the stage that we do. We first of all give to the patient injection with a granulocyte colony stimulation factor to increase the bone marrow production of these cells. After that, we apply apheresis to obtain these cells. The mononuclear portion containing the CD34 cells is processed via Isolex CD34 cell selection. And we take these cells and inject them directly to the area of ischemia on hibernation guided by the NOGA system. So there are several stage to this procedure.

Post-injection follow-up, we have visits at week 2, 5, 3 and 6 and 12 months about safety, angina frequency, quality of life, exercise test, and MRI follow-up on these patient. Based on this study, there are plans to continue this project, to extend it to Europe with this patient population and potentially start the extension of this study, Phase III, in the next year or so.

At Mayo, we have a specific clinic that we see these patient, and it's located in St. Mary's. And it's a chest pain and chronic physiology clinic, where we're target in seeing specifically patient that we do not have any other options for reduction of their angina and ischemia. So if you have any need any for information, you can welcome to contact us. Thank you for the opportunity to present.