SPEAKER 1: So good afternoon. We will continue today this interface between regenerative medicine and CCaTS. And regenerative medicine, in many ways, is an example of how here, at the Mayo Clinic, we translate very fundamental knowledge ultimately into clinical applications. And so there will be a great opportunity in listening today to Dr. Behfar to highlight essentially what we mean by this translational space, and how a discovery ultimately is being developed into a clinically applicable product or maybe even service. And this is how regenerative medicine is being rolled out at Mayo Clinic as an integrated discovery translational application algorithm.

And so in that setting, we are very, very pleased to introduce Dr. Behfar this afternoon. As many of you know, Atta has been at Mayo for a number of years starting essentially his medical training, and then moving on into his PhD training. And then later, also his residency as part of the clinician investigator program followed by cardiology fellowships. And throughout this whole essentially decade plus of being at the Mayo Clinic, Atta has developed very unique tools that range from tools applicable at the bench but also tools with the idea of ultimately developing a product that has this translational value.

So today, I think what is particularly telling is that it's no longer sufficient in many ways to just stop at this initial excitement of the discovery of the bench. And I will let Atta maybe develop that story a little bit more for you because I would like us to all collectively I think start thinking, what are other areas within regenerative medicine and beyond that we can really take advantage of of these technological breakthroughs in terms of a translational paradigm? So I would like also to thank Dr. Windebank as a critical link between regenerative medicine and this whole translational space. And without further ado, we'll let Atta walk us through this journey of how we take discovery and bring it to the patient.

ATTA BEHFAR: Andre, thanks so much for that introduction. Thank you all for attending this session. Just with my disclosure, some of the topics we'll cover here have been patented by Mayo Clinic. In terms of learning objectives, the first objective I think of this talk is really to highlight cardiovascular regeneration for you, show what's been ongoing in terms of the global effort to introduce cell therapy to our patient populations, and then maybe focus it a little bit more to something more close to home-- what we've done at the clinic here to essentially position ourselves globally in that space.

Once we've established that, I'll highlights some of the efforts that we've made in trying to now optimize cell therapy for the heart. How can we potentially improve cell retention or cell delivery to myocardium? And then lastly, I'd like to really highlight efforts of the future. In other words, how can we use all the science that we've established up to now to take a discovery all the way to the patient and apply it to new cardiovascular problems?

So the cardiovascular regenerative effort when you look at regenerative medicine as a whole here in the institution, is just the component. It may be the most advanced component in terms of we started it first. But really this is a total body effort where we're looking at all of these different systems within the body and trying to find regenerative tools to try and address chronic problems that aren't currently treatable. So far, because of this effort, we actually have a broad array of therapies that are currently under way in our clinical practice through the effort of a multidisciplinary team here at Mayo which spans from discovering how the cells can be useful, to how to grow and scale up cell based technology, to applying it to the patients.

In focusing on the heart, I always like to start with this slide, which really shows that despite all of the different medications we provide our patients or all the efforts that we've made to curb acute injury to the heart, really over the last several decades the incidents of heart attack has really remained the same. But what really changed is what we can do about a heart attack. In the 50s and 60s, you observed the patient and hoped they didn't die. In the 70s came either medications, such a streptokinase or techniques such as angioplasty where we could go and open up blood vessels. And going all the way to the last decade, we've found a very sophisticated stent technology that can rapidly restore blood flow to the heart.

And so because of that, the rate of angioplasty as a treatment for heart attack has gone up. What has that done? It's reduced the incidents of death with heart attacks. So we can dramatically reduce the number of people that die coming in with a heart attack. However, we create an epidemic of heart failure in doing so. So people that survive their heart attack go on to suffer from heart failure. So really when we get these patients then into the clinic, we are left with telling them that overall you have a one out of two chance of dying in five years. And you have to be on these medications to palliate your symptoms.

And as patients advance in their disease, we have to offer them additional devices to further palliate their-- excuse me symptoms. And ultimately, in the latter stages of this disease, the heart no longer works. So either we can offer transplants, or offer mechanical circulatory support devices such as LVADs to try and further mitigate the symptoms, or bridge them to a heart transplantation. Now, heart transplants simply trading one illness for another. You trade heart failure for immunosuppression. And again, you start this cycle back of 70% survival in five years.

So with that, regenerative medicine in this disease serves as a unique opportunity to really try and expand the lifespan of our patient population. We've tried using stem cells for heart disease for now a decade and a half. The first generation approach used bone marrow. So what was done was that bone marrow aspiration was made.

The mononuclear cells, so these are the white cells and the bone marrow, were concentrated. And these cells were essentially delivered down the coronary arteries at the time of heart attack. And a multitude of patients have actually received this type of therapy largely due to the fact that in the initial trial-- so in the mid 2000s there were several trials that actually showed very nice data that this worked. In other words, that there was some benefit to the patient's heart function. So the squeeze or the heart improved, and there is actually benefits to their survival. So when they receive this type of therapy, there was a mortality benefit. And they lived longer and better.

However, when additional iterations of these trials were made, including two that were carried out here in the United States, they were neutral. In other words, this first generation approach where we just take a mixture of cells from the bone marrow and acutely deliver them to the heart didn't appear to help our patients when we tried these here in the United States. Now, there is some contention between the initial trials and the people that organized those first trials and the latter trials in that the manufacturing of the cells was a little bit different between the first and the latter trials. And so now, there is this big European effort, 5,000 patients in a phase 3 trial that's going to use those original approaches. And see if really we can definitively prove whether or not this first generation approach works.

And when we look at cell therapy, if we want to move especially beyond the first generation approach and target our cell based therapy such that it can potentially be more effective for the heart, we have to look at the mechanism of benefits. So when we think about a stem cell that's transplanted into the heart, it can really do just a variety of things. One thing it can do is secrete factors to make new blood vessels, or it itself can become new blood vessel. Second thing it can do is if you're giving it at the time of heart attack, it can secrete factors that simply protects the heart against injury. It can potentially reduce the immune response to injury. In other words, mitigate the kinds of immune cells coming into the heart after injury to potentially reduce scar size. It can potentially promote endogenous cardiac regeneration, or the cells themselves can potentially become cardiac cells.

To this end, going beyond this first generation approach to new generations of cell therapy, several efforts have now been made both here at Mayo Clinic and other institutions where we're trying to utilize these hypothesized mechanisms of benefit to really establish new cell based therapies that could potentially be efficacious for our patient populations. And these include whether we use standard of care in terms of coronary interventions with certain types of cell therapy, potentially conditioning the heart-- several studies have used extracorporeal shockwave therapy so that the heart becomes more receptive to cell based therapy, selection of particular cell populations either out of the bone marrow or out of the heart itself, or what we call a next generation cell therapies.

This is either conditioning the heart to really perform a particular action within the heart. In other words conditioning them let's say to just treat heart failure. Or to really harvest themselves from a particular location in the body so that it has a very specific organ action. In other words, if we derive them from the heart, the hypothesis the hypothesis would be that the greatest amount of benefit from that stem cell would be on the heart itself.

And so these new efforts, whether the cells are derived out of the heart itself or whether we get them out of the bone marrow and condition them to become cardio regenerative, we call next generation cell based therapy. And for the rest of my talk, I'm really going to emphasize some of our work here where we've taken cells from patient bone marrow and reconditioned these cells to maximize the therapeutic benefit we can see in our patients.

So when we look at our patient populations starting this project, and let's say you take a particular cell lineage out of the bone marrow-- in this case, mesenchymal stem cells-- and we take these patient derived stem cells and we deliver them into models of heart failure-- so animal models of heart failure that are immunosuppressed. What we notice is that the stem cells from patients with heart failure in very rare instances may have this unique capacity to regenerate the heart. But in the vast majority of cases have absolutely no repair potency. In other words, you can put these cells in the heart, and you get no benefit whatsoever.

What was unique about this initial effort, was that there were very rare individuals who harbored stem cells that actually had this regenerative potency that was very reproducible. And so we were able to subject those stem cells to high throughput analysis, and identify that they had huge up regulation in their capacity to see their environment. In other words, if we put these cells in a highly cardiogenic environment, they started to adapt adopt a cardiogenic phenotype. If we put them into a vasculogenic environment, they started to adopt a vasculogenic phenotype. Whereas, the cells that didn't have this regenerative potency, just stayed stuck as stem cells. They were sort of trapped or sequestered as stem cells, and couldn't really become the plastic cells that we need to repair the heart.

So in order for us to really be able to take the vast majority of patients and have their stem cells be therapeutic, we needed to have a three pronged approach. One approach was to really identify the right patients to target. So what's the disease that we're looking at and how do we want to target it? Where is the biggest need for our patient populations? Once that's established, how can we now take these ineffective cells and develop a biotherapeutic that can become regenerative? And once that's established, how do we actually get it back to the patient? How do we give this to the patient in a way that would be therapeutic?

So that's how we started this cardiopoietic stem cell technology. And the way we approached it was simply by looking at these super regenerative stem cells and realizing that the ones that were regenerative were really mimicking what we see with embryogenesis-- and in particular cardiogenesis. In other words, when we take a very plastic stem cell, like an embryonic stem cell, and subject to differentiation we can very easily derive vascular cells or cardiogenic cells. But we couldn't with the non reparative cells. So we thought, what if the signals that we see in the embryo that push particular cells towards the cardiovascular lineage could be harnessed to push R cells, the non-repetitive cells, towards that same phenotype.

And in order to understand how that works, we derive these artificial embryos. These are called embryoid bodies. And these are simply derived by taking a fixed number of stem cells and forming a sphere. And then they differentiate into these embryoid bodies which beat-- you can see little beating areas here and here. And the beating is simply due to the fact that the ventral endoderm, the slayer, signals to the pre-cardiac mesoderm right here. And so if we could figure out how to augment this signaling, we could potentially decipher the proteins that are needed for this to occur. And so one of the experiments that we performed identified TNF alpha as one of these priming factors. TNF alpha dramatically up regulates the cardiogenic secretion out of the endoderm, and pushes the means that are more towards the cardiac phenotype. And so when we derived embryoid bodies again, almost the entire field was now beating.

So the signals that are secreted by the endoderm, primed and unprimed, could be subtracted from one another. And the key elements needed to induce cadiogenesis could be deduced from that. And we did that experiment, and essentially identified a group of factors which we call cadiogenic cocktail, that could be formulated in recombinant fashion and were capable of driving cells potentially to the cardiac phenotype. And we tested this first on embryonic stem cells. So these are pluripotent embryonic stem cells up here. This is what happens to them when you remove lift. Lift keeps them pluripotent. In other words, keeps the, plastic. If you remove that, they just start going into every direction. In other words, they can become any cell in the body.

But if we remove lift and then subjected them to this cardiogenic cocktail, we were able to derive this intermediate population-- this cardiopoietic population, which highly expressed very particular cardiac transcription factors. And if those cells were purified and were again formulated into one of these cellular spheres, we could derive a cardiosphere. So all the cells differentiated into cadiomyosites. And they looked like this if you disrupted the cardiosphere.

So that appeared pretty promising. In other words, we now had identified factors that could drive at least the most plastic cells towards the cardiovascular lineage. Next question was, could we get these non-reparative cells that never expressed cardiac transcription factors when we put them in priming environments to do the same if we now subjected them to this guiding cocktail? And indeed, we noticed that that was the case. So we could at least derive a phenotype that was more similar to those rare patients that had the reparative stem cells. This process is now being repeated on I believe 300 or 400 patients. And uniformly, it works.

The first step we did then is once we derived these cells that we called cardiopoietic-- we called them cardiopoietic simply because they adopted the same phenotype that we could get from a cardiac intermediate cell from embryonic stem cells. So it gave us a really unique model. We had naive cells-- so cells that did didn't repair, and we knew that repair. And we had the same cells serving as the source for these prime cells that at least hypothetically should repair. And we could actually do head to head studies in animal models. So patients matched studies into mirroring models of heart failure. And what we saw was that, indeed, [INAUDIBLE] dramatically up regulated the ejection fraction of these mice, and was correlated with this particular phenotype-- this cardiopoietic phenotype.

All of those studies were done in the mid 2000s. By 2009, we were able to with the pre-clinical work get regulatory approval to move to a phase 2 clinical trial, which we called the C-CURE trial. This is using cardiopoietic stem cells for heart failure. This was a multi-center trial focusing really on the late stages of heart failure. So these are people that would in a short period of time need that mechanical circulatory support device. And this video really highlights how the trial was carried out.

So the first step was much like those initial first generation stem cell trials. We harvested bone marrow, but unlike those trials, we got rid of all the cells except for the as mesenchymal stem cells. We grew these mesenchymal stem cells up to 100 million cells. And then we subjected them to that guiding cardiogenic cocktail, which is this magical yellow solution there. The cells then started to adapt this cardiopoietic phenotype by showing particular expression. And we grew those cells up to a billion cells. So then a billion cells were delivered through a percutaneous approach. That means we go through the femoral artery, up around through the aortic valve into the heart, and we directly injected these million cells around the scar which we could map and identify.

For efficacy, these patients were followed up for about six months. And what we saw was that their ejection fraction actually improved by about 7%. And was correlated with a shrinkage in the size of the heart. And more importantly, these patients started to be able to walk longer distances. In six minutes, they could essentially walk a football field length further, which in the heart failure area is a big deal to us. And this is just a non-animation version of that same data. So ejection fraction went up 7%. And it was significant. Although, these are all efficacy signals because this is a phase two trial. The size of the heart appeared to get smaller. That's very important for us. That means we're getting reverse remodeling. And we were able to get the patients compared to control to walk about a football field length further.

So this study really serve as the basis for the next study, which is the CHART trial. Now, CHART-1 was a European study-- or is a European study. It started last year. We've recruited about 130 patients in the study. The objective is to get 240 patients. It's a two arm study. One is standard of care with a sham procedure double blind. The other is standard of care with these cells-- these cardiopoietic cells. And here we're actually looking to go beyond just looking for safety signals. We're actually looking to see whether or not these cells work in a large population. Similar population to the phase two trial-- so we're looking at very sick heart failure patients. These are patients who've suffered from a heart attack and have subsequently developed heart failure. And for the most part, it is being carried out in Europe and also in Israel.

CHART-2 trial we're very excited about here at Mayo because this came after the FDA approval to do the PHASE III trial here in the United States. Mayo is going to participate in this trial. Again, we're going to do another 240 patients primarily in North America. There may be some additional countries included. The FDA likes simplicity. So for the US based trial, that walking distance, that six minute walking distance, which is really one of the factors that we use to assess how our patients are doing on a every three month basis when they're diagnosed with heart failure, is the criteria that they wanted us to track for the primary endpoint.

However, we're still going to look at echo parameters like ejection fraction and heart size. We're going to do exercise studies looking at VO2 max and other parameters. And of course, as with all of the trials, safety is a paramount consideration. So as we got towards this bigger trial, as we got to look at potentially doing a PHASE III trial, One of the concerns that we had here at Mayo and also our colleagues in Europe, was that the current technology to deliver stem cells to the heart is pretty inefficient. We did the study to sort of prove a point. We wanted to see how many cells would stay in the heart if you were to give them down the coronary arteries, or if you took a needle like your standard syringe, and directly inject the cells into a beating heart.

And what we realized was that it's very, very little. If you take 600 million cells, you get around 60 million cells that remain. If you directly inject into the heart, you get around 20 to 30 million if you put them down the coronaries. So when you're growing up a billion cells to then end up with such a small number is I would call suboptimal.

So we have a recent publication where we actually describe a new catheter design. And simply, this catheter rethinks the needle. In other words, the project said OK the straight needle approach isn't working for us you know it's been around for $0.3 I understand but it's just not doing it for us when we want to deliver stem cells to the heart. And so we use computer modeling techniques to come up with a better design. And the best design we could come up with was this curved needle. And instead of having an end hole, it would have side holes. So it make sense. Right? If you have a straight needle you're injecting, you get this big pocket of cells under high pressure in an organ that's squeezing. So you pull the needle out and you have a straight trajectory for the heart to squeeze all the material right back out. With a curved needle, you don't have that straight line. And with the side hose you don't have one pocket. You have a distributed cell surface.

And so we were very excited at least with the computer models. And we through the help of a company called Creganna, produced this catheter, which is called the curved needle catheter or a C-cath. And essentially, it features a curved needle. And I don't know if you can see, these are animations showing big holes, but they're actually pretty tiny holes on the side. And what we do is essentially use intracardiac ultrasound and biplane fluoroscopy to let us know where the scar is in the heart, and to guide our catheter positioning. And using that, this is a porcine heart just to highlight how we can position these cells. And this is just blue dye injected into these hearts. We can essentially position the catheter in a very particular spot and deliver our cells.

And in doing so, we were able to dramatically improve the retention rates that we were getting with this old fashioned straight needle. We went from around 10% to around 30% or 40% depending on the study without really causing any more injury to the heart. That was a big concern of mine was that if we have this curvature in the needle are we going to cause a bigger triponin leak or more injury to the heart per injection. And we didn't see that.

This is an animation essentially demonstrating the fact that when we do deliver biologics to the heart using this catheter, you can get really long term in vivo retention. We did a study really looking proof of concept at whether or not viruses delivered with this catheter could remain in the heart. This is six months after delivery of a virus carrying the sodium iodide symporter. And this is the heart. And as you can see, it's not supposed to glow at all. And it's glowing really bright. So at least you know this new design of catheter allows us to get long term in vivo retention, opening the door to new potential projects, which weren't available with the old catheter designs.

So finally, having now gone from really a fundamental discovery at the cellular level and figuring out along the way, over a decade or more, how to take that discovery and apply it to a human being. We now, are trying to address the other big problem that I highlighted in the beginning. The problem that when you come in with the heart attack and you survive, you have this huge vulnerability towards heart failure or death. Right now, when we bring our patients into the cath lab and they're suffering from a heart attack, essentially one out of four of those patients is going to do poorly. The rest actually do pretty great. And that's great news. But one out of four MI's either dies within the year, or has really life threatening heart failure within a year.

And so we wanted to understand why. And this is all a big effort in our regenerative medicine program to take what we call the cookie cutter approach to medicine. In other words, you come in with a heart attack, we're going to give you a stent. And you're going to do great. That's the cookie cutter approach. Right? That's not personalized. What we want to do is individualize it. So yeah, you absolutely need a stent, and you need that to survive. But is there something else that you might need that will prevent you from either dying or developing heart failure?

This is a study that really underlines the concept that I'm trying to share with you here. It came out in 2013 in the New *England Journal of medicine.* And it highlights that after 2005, with us continually trying to get these patients into the cath lab faster, and faster, and faster, we haven't actually made any impact on mortality. In other words, there was a threshold of benefit up to 2005. And now, all of that effort to continue to be fast doesn't help anymore. We've really hit the ceiling of benefit with getting the patients into the cath lab in a timely fashion. So there must be a molecular basis to why this 1/4 of our MI population does poorly.

And that's actually been a big question for us recently. We wanted to know why. And so what we did was we took patients coming in with a heart attack into our cath lab, and we thought well, you know, when they come in with a heart attack what that means is that they have a clot in their coronary artery. Right? And all of the blood downstream of the clot is just sitting there collecting information from the heart as it's getting injured. Right? And so if we could get that blood that's just stagnant and sitting there collecting information, and understand its protein content, we could potentially real time predict who's at risk for catastrophic injury or long term damage from their heart attack.

And so that's what we did. One of the things that you do at the time of heart attack is when someone comes in with a thrombus, the blood clot would be right here, you pass a wire through that clot. And then you put a thrombectomy catheter over the clot, and you just simply aspirate. And when you aspirate you get all the blood that's downstream of the clot and the blood clot all coming out. And you restore blood flow.

Well, what are we to do with that blood? We throw it in the trash. We don't need it. Well, we decided to store it. We prepped it, and we subjected it to pure proteomic analysis. We did this on a cohort of patients that were coming one after another to the cath lab. And when we look at them clinically, they all pretty much look the same. Demographically they look the same. They have the same amount of triponin rise. Their heart function looks the same. But when we follow them prospectively, we had that 1/4 of the population that ended up doing very badly. Half of them died. And the rest of them had a big ejection fraction drop. And so we were able to then separate the samples into highly vulnerable patients and protected patients.

And so now, you could essentially do proteomics analysis comparing the protected the population, that coronary thrombus aspirate from the protected the population, from that obtained from the vulnerable population. And that really identified 19 factors that were different. Out of all the proteins we looked at, only 19 were different. And out of the 19 that were different, there were only two that were nearly absent. So two proteins that are absent in the vulnerable population of patients compared to the protected. And those are TGF alpha and NAP-2. And so what we wanted to do was deliver these into the myocardium. But proteins are a lousy entity or biologic to put into the heart because it will disappear within a matter of seconds. Right? And so we worked to develop a biomatrix that was injectable that could polymerized at 37 degrees. And this is a bioprinted version of that. I'm just showing you what it looks like.

And if we did exactly that-- if we created models of myocardial infarction where we either inject saline, this collagen biomatrix, or this optimized matrix, what we saw was that we were able to dramatically reduce the amount of scar size whether we use the proteins with just collagen, or if we used these proteins with an optimized matrix where we really got sustained release over three days. And we could really in this model drive down scar size to a negligible amount compared to what our typical model gets. This was associated with a functional benefit. In other words, the mice that received that injury with saline, continued to do poorly. Whereas, the ones that received this protein had an acute injury but then the injury stopped right there and didn't continue to proceed. There was a leveling off and even an improvement in their ejection fraction associated again with this reverse remodeling.

And a similar effort has now been made in the of our porcine model. This effort combines these discoveries in our patient populations but also looks at what's secreted by our stem cell population. And so this is a more complex protein delivery model that we're providing to our porcine model of heart failure. And this is essentially a similar model. We put a balloon in the left anterior descending. Similar to that angiogram I showed where you had the blood clot. This is just a balloon blocking that blood flow for about two hours. We get a scar that's formed that you can see on the MRI. And you get diminishment in function. And if we do give these proteins after re-perfusion, in other words, after we restore blood flow, we see that we can protect these hearts against catastrophic injury.

This is correlated with a reduction in scar size histologically as well as on MRI. And is in part reproducible. Although, the data is actually pretty new, we do see that at least over our experience, we can reproduce this beneficial effect on the heart. So something that we see as beneficial as part of our stem cell technology or something unique to patients that are protected against heart attack, can now be harnessed and delivered back into small and large animal models of the same disease to show how you can develop this biological effect. So you can see that we're sort of again, transitioning towards man. And really now, trying to identify these vulnerable patients at the time of heart attack. And providing them with a new technology to protect them against heart failure and potentially death.

So with that, I'll just summarize here in saying that the Center for Regenerative Medicine's main mission is to really take bench based discovery, utilize the big clinical power that we have here at Mayo Clinic to translate these fundamental discoveries into large animal models, and ultimately, with help of the FDA, translate them really into to applied science to help our patients. And really this new type of technology transforms patient lives when applied correctly. And the patient's experience-- these patients suffer from chronic disease and their families that suffer with them-- if we are able to bring these discoveries at the level of the bench to the patient population, it really is a transformative entity.

So with that, I'd like to just thank everybody who has participated in this partnership. It's been a huge and long project spanning over a decade. And several of the people in this room actually are integral to this project. And I'd be happy to answer any questions.