catheter including controls on the catheter handles that deflect the tip and that extend and retract the needle.

The needle would normally be retracted into the catheter except when briefly extended for each injection that is made during a therapy session, and the syringe, potentially loaded with therapy suspension, is attached to an infusion port, also on the catheter handle.

The drawing on the right illustrates delivery of the catheter retrograde through the aortic arch, then, through the mitral valve, and into the left ventricular cavity. In this drawing, as you see, the catheter tip is deflected and the catheter is being used to make multiple injections from the endocardial surface of the ventricle.

There are also potential concerns that attach to the use of needle injection catheters for delivery of cell therapies for cardiac disease.

First, we believe this type of catheter may potentially be particularly prone to clogging by cell suspensions. Factors that might contribute to this include the following:

The potential desirability of using very small injection volumes plus highly concentrated

cell suspensions, the potential desirability of a small diameter injection needle that will also, thus, have a small diameter lumen, and the fact that injections may be made at more than 20 locations during a treatment session, thus, increasing the potential for clogging due to the repeat injections.

A second concern. As noted previously, it may be important to ask whether cell viability or functionality could be adversely affected by contact with catheter lumen materials. In this type of catheter particularly, we think it may also be important to ask whether shear forces produced during infusion of cells through a long, potentially a very small diameter injection lumen might also adversely affect the cells.

Third, it may be important to consider the safety, and this was brought up in one earlier session, of whether the safety of the cell suspension is accidentally delivered in the systemic circulation.

Of note, with this type of catheter, it may be very difficult to maintain continuous contact between the catheter tip and the moving endocardial surface of the beating heart, and when

contact is broken, therefore, you could have injection into the ventricular cavity and into the circulation.

There is a note, for folks in the audience, I don't know if you can see the reference, but if I understand correctly, we will have cells on the web site, is that correct? Okay. Otherwise, I was going to read the reference to you.

One note, if people want to look into this, this has been studied for cardiac ablation catheterization electrophysiology. I have noted one good study up here where they used intracardiac ultrasound imaging catheters to evaluate the difficulty of maintaining continuous contact when they thought contact was perfect.

A fourth consideration. Should we assume that it is important to control or limit the maximum needle extension? Factors to consider might include the following: Is it critical to avoid injection or laceration of the organs that surround the heart?

It may also be important to consider whether there may be safety concerns if the cell suspension is inadvertently injected into the

pericardial or thoracic spaces, or if it is drained from these spaces by the lymphatics and then delivered into the systemic circulation. Relating to needle injection, curves or bends in many catheter designs, including the 180-degree bend around the aortic arch that will normally be present, could affect the needle extension length of the catheter.

Finally, particularly in hearts that have minimal epicardial fat surrounding the left ventricle, it may be difficult to avoid occasional injection completely through the wall of the ventricle and into the pericardial space.

Factors that might contribute to this could include the following:

First, is locally thin regions in the ventricle, possibly related to myocardial infarction, possibly related to the normal indentations that separate the muscular trabeculae of the ventricle on the endocardial surface of the heart.

Second, is compression or stretching of the ventricular wall where the catheter tip is again pressed into contact with the wall, and, finally, the possibility that a forceful injection

could simply potentially separate both myocardial cells and epicardial cells, allowing cell suspension to flush completely through the ventricular wall.

A fifth and final question regarding needle injection catheters is the following: Are injection depth and the spread of injection of the injected cells potentially important therapy parameters?

For example, will injection of cells near the more ischemic endocardial surface of the heart provide therapy that is identical or equivalent to injection near the less ischemic epicardial surface, or is a minimally dispersed bolus of cells at each injection site equivalent to wider dispersion of cells at each injection site?

We currently suspect that catheter design, cell suspension characteristics, and injection speed can all affect injection depth and spread.

If a clinical study is performed using a specific injection catheter and a specific cell suspension, will the same therapy then be delivered if a different injection catheter is used to deliver that same cell therapy?

Of note, you can use animal studies, and

this is where the large animals become important, to characterize the depth and spread of the cell suspensions produced using a specific catheter.

Finally, and this is a question I think that unites all the questions listed above on this slide, when an investigational therapy is studied, how important is it that the therapy delivered be characterized? When an investigational therapy is poorly understood, is the characterization of therapy more or less important?

Today's meeting is focused on scientific discussion of cell therapies for cardiac disease, and because of both the focus of this meeting and the time constraints, this is not a good forum for discussion of regulatory concerns related to cardiac catheters, however, if you would like to discuss cardiac catheters intended for delivery of cell therapies, you may contact either myself or my branch chief, Mr. Elias Mallis.

I have listed our contact information on this slide. Again, it will be posted on the web site.

Finally, because this is one of the final presentations today, I have one final slide. My manager has repeatedly asked the following

question: Whether there are earlier or predicate devices that have been used to provide catheter delivery of biologicals.

He has also asked what we can learn from any earlier devices. Initially, I was unable to define a useful predicate for catheter delivery of biologicals. Then, I did find a useful example, and it was among photos from the Minnesota State Fair.

The obvious lesson from this photo, I would say is that intense concentration may be required during catheter manipulation.

[Laughter.]

DR. JENSEN: Thank you.

[Applause.]

DR. RAO: Thank you, Dr. Jensen.

I am going to suggest that we wait and hold off questions until we hear from Dr. Lederman, as well, since he may be perhaps answering some of those questions, and then direct questions to both people at the end of that talk.

Transcatheter Myocardial Cell Delivery: Questions and Considerations from the Trenches

DR. LEDERMAN: I am going to be quick. I am grateful for the opportunity to speak before

this audience and this committee. Thank you for your service.

I am speaking to you as a clinical cardiologist, so I like to think of myself as Joe six pack of clinicians. I will be talking about the considerations, the frustration that many investigators feel when we would like to talk with the agency to get some guidance about how to start bringing these interesting therapeutics to clinic assuming we have determined that the timing is right to bring therapeutics to clinic.

I am sorry there will be a bit of repetition. I will try to go quickly through repetitive slides.

We are dealing with integrated therapies and unfortunately, we are also dealing with a morass of regulatory purviews that don't necessarily intersect. You have seen already that we have considerations of delivery devices, as well as cellular agents, to combine in therapy, and we haven't discussed much in this room combinations of novel mobilization agents should we choose to use that route to drive our cells, and it becomes difficult when we have proof of concept in some animal models to find an appropriate proof of

concept to support our clinical trial and to support our safety considerations when some of our colleagues outside the U.S. have kind of moved ahead.

So, let's see if I can generate some interesting questions for the committee, and that is really what I hope to end on.

You have seen this slide from Strauer. I will review again just the different approaches to cell delivery, into coronary cell delivery is attractive because it is very easy, there is a wide dispersion into the target territory, and there are a lot of available devices to be used although they must be used off label.

The disadvantages have been mentioned that there is a potential coronary artery injury. One of the real clever innovations by Strauer's group and by Sawyer's group and the Hanover group is that they have chosen to deliver cells through an occlusive balloon deployed at the site of a recently deployed stent, so there is really almost no possibility of coronary injury from the delivery device, however, there is a possibility of a coronary microembolism, and when you test this is the setting of a recent acute myocardial

infarction, that coronary embolism may be difficult to detect, so clinicians have gotten away with it, or at least I should say their patients have gotten away with it.

Certainly, there is a great potential for direct washout of injected cells. Very few people have actually measured this or reported this, but there is some evidence that there is a low fractional retention of delivered cells, and really this intracoronary cell delivery is yet another way to expose the entire patient to the therapeutic agent.

This kind of approach is probably unsuitable to certain patient populations, especially those with chronic myocardial ischemia when the inflow arteries are occluded. Most investigators have taken advantage of transient coronary flow interruption ostensibly to improve local retention, but it is just not clear the value or importance of this transient coronary flow interruption. Certainly, a lot of patients can't tolerate prolonged coronary flow interruption without incremental myocardial injury.

There has been discussion about both surgical and transcatheter cell injection.

Certainly, most of us will recognize that catheter-based injection is less morbid than surgical epicardial injection. Primary surgery has been unattractive in investigational studies when the surgery is offered only for the sake of cell delivery.

The problem is in small studies, combining cell delivery with an effective therapy, I think has been mentioned by several people before, doesn't really generate meaningful safety or efficacy data, because the assessment of toxicity events is confounded by the concomitant surgical procedure, and the assessment of efficacy events is very easy to ascribe to the concomitant effect of therapy.

So, I think this kind of approach should probably be discouraged in small, single-center studies. I hope some investigators in the room have already overcome this in moving to larger studies.

Direct catheter injection of the myocardium is attractive because we can achieve a high local cell density and probably a high total dose. It is certainly very easy also, with the variety of catheters that I will describe, and the

entire myocardium is for the most part accessible irrespective of the patient's individual coronary anatomy.

But these devices are disadvantageous in the U.S. because there are no approved devices, although a few are available through investigational device exemption.

I will show you some data that there also is low retention of injected cells and that direct myocardial injection is yet another means of systemic exposure of the patient to the cellular agent. We are left even in the best situation with multifocal cell accumulation, meaning a heterogeneous dispersion of the cellular agent.

Whether or not that is important isn't clear, and there is the potential for damage to the myocardium or to the chordal structures or the valve structures, however, this potential has not been supported by experience with comparably aggressive or more aggressive intramyocardial catheters especially in the fairly large laser myocardial "revascularization" experience or angiogenic gene transfer.

There are a bunch of variants of myocardial injection catheters. There are

techniques to access the pericardium and bathe the epicardial surfaces of the heart with the cell preparation of interest.

Patients with chronic myocardial ischemia who have undergone coronary bypass surgery are, for the most part, not eligible, so this is a difficult approach in early clinical trials.

There are investigators who have demonstrated satisfactory delivery of genes and cells by retrograde coronary venous approach. I will show you a picture to show what that means. A company has commercialized tangential transvenous intramyocardial injection.

Then, there are a bunch of endocavitary catheters that go across the aortic valve retrograde and are pretty successful in delivering cellular agents. There are an array of what I call "dumb" catheters that we use just under x-ray guidance that are very attractive because they are quick and easy. Two examples are Boston Scientific Stilletto and Biocardia Device.

There a couple of smarter devices, it is not clear that they are better, but they employ a Static Roadmap like the Cordis Biosense electromagnetic guidance system with some

non-fluoroscopic guidance. There are also devices that have an integrated ultrasound, and the smartest devices, I will show you an example, in research mode only, of instantaneous imaging both of the tissue and the device.

We should all open our hearts to the possibility that surgical videothoracoscopic, a minimally invasive surgical procedure may accomplish cell delivery with very little morbidity even in a primary surgical procedure.

This is just a demonstration of one of the so-called "dumb" catheters. This is a Boston Scientific catheter going from a femoral artery of a pig, across the aortic valve, and can successfully guide whatever agent you want with centimeter, not millimeter, precision to any aspect of the endocardial surface.

Medtronic has recently bought Transvascular, which is an interesting device that has an integrated ultrasound to guide the deployment of a needle through a coronary vein and can access target myocardium through any of the coronary veins in a tangential fashion. This has been tested in clinic in a very small number of patients, and so far there haven't been any safety

disasters.

A retrograde transvenous approach has been demonstrated I believe only in animals. The Stanford group, Keith Marsh's group, have been interested in this for delivery of dyes or gene agents or even some cellular agents. I am sorry the pictures aren't very attractive. It is not clear to me how this can possibly work, but the proof of principle has been shown.

It is also attractive in that the inflow coronary artery anatomy is not a problem since coronary vein patency is maintained in virtually all patients. Dr. Perin is an expert in the use of the Cordis electromagnetic guidance system, the Biosense system that has its advantages in that it has been widely used and tested for a variety of investigational approaches.

It is disadvantageous in that it is a prior roadmap of the heart that may vary over time, and so it is not clear to me that you accomplish millimeter scale precision of your injections, but it is also not clear that that is very important.

I think Dr. Epstein's group also has great expertise in the use of the device for cell therapy.

Just to brag about some work in my lab for a moment, we have used real-time MRI to guide cell injection to very small targets with great precision and great ease. This is, as you see, a multi-slice real-time movie of the heart. You can see it in long axis and short axis, of a pig in which we have caused a tiny, little microinfarction, shown in white.

The catheter is shown in red and green, and we can see with great 3-dimensional sense where we are steering our catheter, and if we like, we can label cells, say, mesenchymal stromal cells, very easily.

Here, the mesenchymal stromal cells are showing up in black, so you can actually see interactively, as you deliver the cells, that at least some are attained in the target myocardium, and this is almost ready for clinical application.

I want to show a little bit of data. This is from my youth when I was just out of fellowship, a study that was funded by Boston Scientific while I was at still at University of Michigan.

We injected neutron-activated microspheres into the heart with an endocavitary catheter, direct surgical approach or postmortem, and what

was interesting is that we inject and then kill the animal within minutes, and most of what we inject, both surgically or transcatheter approach, is lost immediately.

There is some effect of volume. Smaller volumes were associated with slightly greater retention in tissue. This is 10 microliters. That is a tiny, tiny injection compared with 20 or 100.

A better study was published by Smits from the Thoraxcenter group in Rotterdam using scintigraphy and radiolabeled albumin, either plain radiolabeled albumin or a colloid, and they showed also loss of the majority of injectate after just a minute by scintigraphy.

Their colloidal preparation had great retention, which is interesting, and that there might, of course, be some interaction between biological agents and the myocardial interstitium, so conceivably, cells won't necessarily be lost. These are studies that can be done in the lab in healthy animals.

But I think it is easy to say that local myocardial injection is at best an exaggeration, that most injectate is lost rapidly and exits either by backflow directly into the myocardial

cavity, which we can see directly, or with intracardiac ultrasound or even with high-resolution x-ray, that there is also a clear, what I call "intravasation" or return to the coronary circulation or coronary lymphatics.

When we inject too deeply and directly into the pericardium, that is another mode of exit, but clearly, the interstitial myocardial target retains only a fraction of what we intend to deliver there.

So, where does this material go and is that really important? I think it is important at least that we assume that what we think we are injecting by any route goes everywhere, and I think that means that conventional toxicology or biodistribution experiments can be conducted in uninfarcted animals without the needle of interest just by modeling it as a left atrial or left ventricular cavitary injection that is not device specific.

I think also it is interesting to talk about open- label autologous unfractionated bone marrow data, what is the incremental value of animal safety or tox experiments in light of the fact that provisional safety has been shown in

open-label studies if we are convinced that the safety reporting has been complete, and I am pretty comfortable that it has been.

For autologous leukapheresis products, say we apherese CD34 cells for a direct myocardial injection, those cells are circulating already. It is not clear to me what is the value of incremental biodistribution experiments regarding systemic exposure.

I will qualify that by saying that allogeneic material perhaps should be treated differently, but for autologous material, it is not clear to me that these animal data are valuable, and to require it of investigators before going to clinic sounds dubious in my opinion.

Also, it is worth noting that the importance of targeting is just not established. While I am very interested in precise anatomic targeting, it is not clear why we need it.

Delivery targets certainly vary by application. We may want to target infarct borders. Doris Taylor had some data today that infarct borders may be unattractive for certain therapies.

Do we want to target ischemic zones, or do

we want to avoid ischemic zones, do we want to avoid thin myocardium? Is roadmap data worse than blind data or worse than instantaneous real-time MRI data? It is just not clear.

Certainly, good targeting is attractive in that it may reduce overlapping injections and waste of injections, and overlapping injections may increase systemic loss. It is hard to imagine that we might be exceeding some therapeutic index.

So, in other words, the value of targeting is just not clear to me, and if we are able to show some efficacy, it is not clear how much we must ask of investigators to establish these catheter-based information before going to clinic.

A point that has not been mentioned, but that I have encountered in animal studies is that operators need feedback regarding delivery of their therapeutic agent, and I would like to encourage the committee and the regulatory agency to consider contrast labeling at the time of cell delivery. It is certainly clearly more important than needle stability measures.

There are lots of ways to label injection mixtures. You can admix contrast into your injection cocktail. Iodinated radiocontrast is

clearly tolerated in myocardium. We inject high-dose, full-strength intracoronary radiocontrast, replacing blood inflow for many seconds in patients in all settings, acute MI, acute and chronic ischemia, and healthy myocardium.

It is certainly very well tolerated. It is hard to believe that iodinated radiocontrast injected into the myocardial interstitium is not tolerable. It is certainly well tolerated in animal experiments, but this kind of feedback under x-ray guidance, for example, is critical in knowing that we are delivering the cells into the target that we think we are.

If we are doing injections under MRI, then, certainly we can admix gadolinium-based MRI contrast agents in very dilute form just like the agent that reaches the myocardium after systemic exposure, and these are very easy to be tested biocompatible in vitro.

Certainly, we can do test injections of contrast to test the purchase of our needle in the myocardium before injecting the cell of interest, but the problem with these test injections of radiocontrast is that the catheter and hub dead space often exceeds the volume of the desired cell

injection, so that is a problem that is difficult to overcome.

Alternatively, we can label our cells. That is certainly easy if we want to deliver our cells under MR or under echo, but it is not clear what options we have under x-ray, so to the members of this committee, please facilitate solutions to this clinical need.

Some engineering concerns have been mentioned in the very excellent guidance materials supplied to members of the committee. I just want to speak to some of them.

The issues of biocompatibility of lumens and potential clogging of lumens, this is easy to test on benchtop and doesn't require animal experiments.

The issues of balloon injury of target coronary arteries is an important one. You have seen creative solutions by investigators in Europe by protecting the target coronary artery, inflating their occlusion balloon inside a recently deployed stent.

Certainly, there are noninjurious compliant occlusion balloons that are clinically approved for a variety of peripheral artery

applications, that are used widely in the cerebral circulation, that are also used for coronary protection and substantial equivalence data are already widely available.

We have also heard discussion of considerations of the pressure capacity of balloon wire lumens. The European investigators, for example, are administering their cells via the wire lumen of an inflated coronary balloon.

As a practicing interventional cardiologist, I don't consider that an important concern because every balloon, every over-the-wire design balloon that I put into a patient, I expect to use for intracoronary angiography, and I use for intracoronary angiography with a fairly high pressure system.

There are many times I need to know that my balloon is in the right place, so I pull out the wire and I inject contrast at a fairly high pressure directly through the balloon lumen.

That is not, of course, an indicated use, but it is a wide use by all operators of coronary artery balloons, and I think the test of time has already been past, but if you like simple benchtop pressure data, they are easy to acquire.

Regarding endomyocardial injection catheter engineering concerns, the same biocompatibility and clogging concerns are easy to answer on benchtop tests. This issue of variable needle extension is probably an important one if injection depth proves to be important, and it is not clear that it is, but this can be addressed in benchtop testing.

It has been mentioned that purchase stability is important to assure injectate reaching the target tissue. My assertion about marking or labeling injection cocktails with contrast might address that concern.

The report from UC/SF from Jonathan Coleman using an old intracardiac echo device reporting instability is actually a spurious observation because of through-plane motion of the target that the UC/SF group was inspecting with a fixed vena caval or a right atrial intracardiac echo device. In other words, I don't think it has been shown that the contact of EP catheters or myocardial injection catheters cannot be maintained, in fact, just the opposite, especially from the Biosense device which has a local cardiac electrogram capability to assure contact stability.

So, I think this is not really an important problem for us to worry about.

The issue of potential myocardial perforation is often raised when we discuss the possibility of delivering cells directly into patients after a recent large myocardial infarction, and I think that is an important one. It is interesting that you refer to myocardial biopsy devices as predicate devices, because as I view the biotomes as some of the most dangerous devices we ever laid hands on, they are so incredibly stiff and indeed perforations do sometimes occur.

Fortunately, there is an animal experience from my lab. I guess we should probably get it out there, of a large number of injections directly into freshly infarcted myocardium, and I think this kind of data is easy to obtain.

But the bigger issue is not that freshly infarcted myocardium can be safety injected, it is that the device companies can't really control the operators. I have seen this so many times. An engineer walks into a lab and cringes as the interventional cardiologist effectively abuses the device. How do you model operator misbehavior? It

is kind of difficult.

In reality, proof of principle has been established.

The issue of inadvertent pericardial injection probably has little or no clinical importance especially when compared with the loss of injectate via other routes, and its only value is that you are not delivering what you think directly into target tissue again, the value of instantaneous visualization of injections.

The issue of distribution of injected material within the target myocardium, I think it may be reasonable to assume that this distribution is different in normal myocardium versus fresh infarct versus chronic scar, but the value of these data are just not clear compared with the efficacy data in support of preclinical or early clinical experiments, so having this information of how many cubic centimeters of myocardium are exposed to target cell based on a given volume or dose of cells, it is just not clear why we need that information. This kind of information ultimately can only be valuable in patients.

Are endomyocardial injection catheters generic? In my opinion, assuming benchtop

biocompatibility has been determined, and assuming that mechanical performances are satisfactory compared with predicate devices, I think that a myocardial injection catheter is pretty much the same from one to another.

One needle device should be translatable to another, and the scientific and regulatory value of additional data from large mammals, healthy ischemic infarcted, is really pretty small and hard to justify, in my opinion. So, I keep giving this message, nihilistic message about the large animal models investigators have been asked to provide.

To summarize my opinions, I think engineering and biocompatibility concerns can be addressed with benchtop data. I think that animal model safety experiments matching a given catheter device with a given putative therapeutic agent don't meaningfully contribute to patient safety and are, in fact, potentially misleading.

I wish that there were a way to get a screening IDE or IND capability to support testing new cell preparations without repeating unnecessary preclinical experiments as we switch from device to device, and ultimately, careful human experimentation is what is most important.

Let me just make a few more points before I turn to some questions and step off the podium, so people can go home.

I want to reiterate some points made by other speakers today, and I want to reiterate it especially to the regulatory officers here. I think that blinded placebo groups are mandatory even in first experiments.

Why would you conduct an experiment without a suitable matched control in the name of safety? That is just bad science. Interestingly, there has not been a single open-label or "Phase I" safety trial that fails to make an efficacy claim without a suitable matched control.

Unfortunately, the agency is inadvertently discouraging blinded controls, for example, when they ask for a delay in between exposing a given subject within a given group of patients, asking for seven days or four-week delay between patients to look for safety of individual patients. This often frightens investigators away and makes them drop placebo groups.

I think that in cardiology, we rarely conduct classic Phase I studies in end-stage subjects in spite of the conversation in Doris

Taylor's speech.

So, I would like to encourage people in the committee and encourage regulatory agencies to facilitate inclusion of blinded placebos in early, first in man even, experiments.

I was also asked to talk a little bit about the safety of direct myocardial injection, and unfortunately, there are no large series of direct myocardial injection of cells or any other agents, however, a related catheter has been tested in a few hundred patients.

Cordis had a myocardial--they called it a DMR, direct myocardial revascularization procedure, but it was a way to burn the myocardium from a transcatheter approach. In this Cordis-sponsored study presented by Martin Leon and Ron Kornowski a few years ago, a Cordis Biosense derivative, a device much like the myocardial injection catheter shown today, was used to steer into the myocardium of 300 patients with refractory ischemia, mild or moderate left ventricular dysfunction, and preserve wall thickness.

One hundred patients underwent sham procedures, placebo burns of the myocardium, and 200 more received laser in two different doses.

This is just the clinical complications. I will point you to left ventricular perforation. There were none in the 100 placebo patients, there was 1 out of 100 in the highest laser dose, which is comparable to some other laser trials.

I think this establishes a relative safety base from a perforation perspective. These other events unfortunately weren't very well described, and this study unfortunately has not been published, and I am not sure it ever will be submitted for publication, but the acute safety of this device, I think is relatively self-evident.

So, is placebo and the myocardial injection safe in principle? I think yes, and it is not a reason to discourage these placebo groups in early first clinical studies.

So, again, we are trying to bring therapies to clinical testing, and we are trapped between delivery devices and cellular agents that we would like to use together.

I want to just ask a few hypothetical case questions to the committee before I step down. I certainly don't have answers, but I hope you find it provocative.

Let us say that an investigator identifies

a novel marker, an HL321, let's call it, that identifies some kind of progenitor cell for which there is no clear animal homolog.

There are some limited preclinical efficacy that when an enriched human HL321 population is injected to intrinsically immunocompromised rat infarct, it causes functional recovery compared with a population of known cells that are relatively depleted, so this is not the most robust type of experiment, but based on experiments like this, and based on patients who are clamoring for therapy of their massive myocardial infarction, investigators may want to bring some test of this therapy to clinic even now.

How could we test local autologous HL321 cells assuming we had a feasible way to mobilize and recover these cells? How could we test that for safety and efficacy?

In the example I want to give you, there already is a commercial cell system available, marked with a CE in Europe, and in Europe, hundreds of patients have successfully undergone bone marrow transplantation with a population positive selection for this marker, and also in Europe, dozens of patient underwent local cardiac delivery

trials in a variety of applications, and Phase II trials have been offered.

Is it unreasonable to permit U.S. investigators to conduct similar experiments now that provisional safety has been tested in Europe, if incompletely reported in Europe?

For this kind of clinical experiment, are additional animal data really necessary when human studies have already been conducted, and when there is no animal homolog of that positive selection marker? What animal model is really adequate?

Can we use the experience of investigators like Dr. Epstein, investigators like Dr. Perin, using undifferentiated bone marrow to support the local delivery of other autologous cells, and are individual cell preps, autologous cell preps substantially equivalent in this case when they have just been derived directly from the patient irrespective of sources? Is bone marrow that much different from apheresis product, from a mobilization product?

So, how can we apply non-U.S.A. human safety data to support U.S. clinical trial proposals, or should we continue the way we are now and just sit and wait for others to do their

experiments without us?

Thank you very much for your attention, and I hope you found these questions provocative.

[Applause.]

DR. RAO: We have time for a few questions.

Q&A

DR. NOGUCHI: I think that was a terrific representation of the tension between belief and what is published or not published, and what we might call the paradigm for FDA, which is absence of evidence is not evidence of absence.

I will just challenge you a little bit. When we see safety data whether it is from another country or here, one of the critical questions for an adverse event is, well, is that really showing that if you give a product that you have a lack of an adverse event, or could it be that, in fact, you didn't give a product, which by your own arguments you would say most of the time, you lose most of the product all over the place.

So, some of it falls into the category of our experience is that adverse events actually other than from the actual injections of others for biological products don't occur unless you actually

also have bioactivity, and sometimes if you don't have any cells, you may not have any bioactivity.

So, I think that you have a number of very genuine points very well worth arguing, but I would just caution that it is very simple to say something is safe. We rarely say look at all the published data and it's safe, because we don't really know if a product was actually being used there, and perhaps that is one of the points you might want to just think about.

DR. LEDERMAN: I, of course, can't even answer your question, and I want to point out I want to thank the regulatory officers and the FDA for trying to protect our patients, and to try to protect the American public. You are in a very difficult position that is often a thankless position, but I just hope you are open to this kind of conversation.

DR. NOGUCHI: Absolutely. That is why we would like to have you end it here, because I am sure it will provide the focus of discussion for tomorrow.

DR. RAO: Any other questions?

DR. EPSTEIN: Bob, I really enjoyed that presentation, it was really great. I would just

like to raise one point. For example, to my knowledge, no one has really tested in depth the safety of the transvascular administration of an angiogenic agent or its cells.

The reason I feel that might be different, for example, than a transendocardial or a transepicardial injection is because you are injecting it right around a large artery. It is conceivable that there can be pro-atherosclerotic or pro-restenotic effects.

DR. LEDERMAN: I am sorry. Let me interrupt your question. Do you mean intracoronary approach or the Medtronic transvascular approach?

DR. EPSTEIN: The Medtronic approach, so where you are injecting it, not downstream at the small vessel level, but at the large vessel level, so if you are injecting it, for example, through the venous system, it is contiguous with the arteries that have atherosclerosis in it.

So, I would think that for that special case, the FDA would require that you have to show that there is no deleterious effect in terms of a pro-atherosclerotic effect. I would be interested in your thoughts about that.

DR. LEDERMAN: I think that point is well

taken and every such safety request or demand is interesting and valuable, but how do we answer that kind of question satisfactorily.

Let's take the question you just asked, a tangential myocardial needle or perhaps a retrograde venous administration of agent X, and the problem of an unrecognized atherogenic effect, how on earth do we test that? Are apoE knockout mouse experiments satisfactory?

DR. EPSTEIN: For this particular, I would injure a vessel in a pig, and then inject whatever agent you are interested in transvenously in the area of that injured vessel and just see whether there is an increase in the neointimal response.

I don't know how you carry--I mean your question would be so what, whatever you see, and that would be a good question.

DR. LEDERMAN: But that is exactly right, that might reassure us, but that is also not atherosclerosis.

DR. EPSTEIN: That's right, but the AMI studies, you know, you are doing angioplasty, so if you were to increase the incidence of restenosis in the pig, you know, quite predictively, it would certain add a major cautionary note to approval of

such a protocol.

DR. TAYLOR: I would ask two questions. One, very short, but one is you said that you would argue that exogenous delivery of any given cell population is equivalent essentially, but I would think that the GCSF, or if you have given one bone marrow cell population, is it right to go ahead with all the others without necessarily more safety data?

I would argue that the GCSF data that just came out would actually argue the converse, that the only difference there was mobilization of cells that would otherwise be endogenous to that same patient, and yet an increased number of those cells clearly caused some negative effect.

DR. LEDERMAN: I wasn't actually making that assertion. I was making the assertion that needle injection catheters are ultimately very similar.

DR. KURTZBERG: But the difference, there have been, I don't know how many tens of thousands of patients have had GCSF, we have gotten bone marrow in their right atrium, so I mean the dissemination of those cells is not the issue, it is combining that with a local technical injection

and trauma to that site that is different.

I mean there is experience with these cells disseminated through the human body for two decades, so that is not the issue. The issue is what do the cells do in the setting of a local technical injection into an artery or other part of the heart that is sick.

DR. TAYLOR: I think that is sort of the point I am trying to make, that exogenous delivery is not necessarily the same thing as mobilization of cells, and that having more cells there, that we don't understand, we can't just interpolate from other data.

I want to ask a very short question. What do you think about clinicians moving forward who don't have experience with preclinical studies? I mean one of the things that probably enabled Philippe to do the studies he did is he had that six years of preclinical experience making mistakes or whatever.

What do you think about any clinician moving forward in a trial without having previous preclinical experience?

DR. LEDERMAN: My short answer is who cares what I think, and we all operate as parts of

teams with expertise in our respective areas. In cell therapy, for example, it would be outrageous for me to do an early clinical study without the close collaboration of cell therapy experts like some that are fortunately in the room.

And would we need to have our local on-site preclinical experiments? It is not clear to me how important that is. It is more important to me that our agents be well characterized, that the studies be well conducted, and that they be designed in a way that the data can be interpreted rather than open-label, early clinical experiments that are very difficult to interpret.

DR. RAO: I really agree with on the emphasis you made about the fact that you should have a placebo-controlled trial, but then on the same token, you know, you also said that one catheter is much like the other, but I don't think that we can extrapolate that from saying one is much like the other, you know.

I mean we worry about drugs when we say whether it is a generic formulation or whether it is a formulation which contains the same active ingredient, and to me, when you are looking at the device, you are making it with cells, you have to

worry about how clearly or how similar the device is to any other device.

We can't simply say, well, you know, the benchtop pressure was the same. You know, I can take syringes and I can show you that the benchtop pressure on that cell agent injection is exactly the same because of how I do it, but, you know, I can put cells through it, and I can guarantee you that there would be a difference.

I mean Dr. Menasché showed in his data you use a 27-gauge needle, and it is very different from using a 29-gauge needle. It doesn't matter whether you have got the same pressure or not.

So, I think that it would be hard put at least for me to be convinced that most catheters, even if they are giving delivery externally in much the same way, that one can logically extend it and say that it will probably be the same.

DR. LEDERMAN: So you are telling me--and I don't mean to belabor the point--but if you have two catheters by two different vendors, that have satisfactory benchtop testing for biocompatibility, have satisfactory hydraulic characteristics, that what you inject at one end comes out the other end, and one such catheter has satisfactory efficacy

data in some kind of preclinical model, that you would require a repetition of that preclinical model for another catheter that is virtually the same?

DR. RAO: I am saying that right now we can't make the assumption that it will be the same, and the reason I say that is that we know that when we make minor manipulations to cells which we are delivering, for example, if we take CD34 cells, which have been kept in culture for 48 hours as opposed to 12 hours, we have a very different endpoint result. We know that.

We don't know what the interaction will be with the catheter, and we can't make the assumption that because we know five parameters, that those will be adequate in making a reasonable prediction, so until I have a lot more data, I will be very surprised that one could make that statement or anybody would agree that that is okey.

DR. LEDERMAN: The end result is that we have an unmanageable number of permutations, an unmanageable number of permutations that makes it hard to make progress.

DR. RAO: But again I think this is to reemphasize what Phil said, it is not that we can't

do it, so that means it shouldn't be done. It is to try and identify what is critical, so that you make sure that the critical points are done, so that is the critical issue to me.

DR. RIEVES: Dr. Lederman's presentation was excellent, and I think it raised some excellent points. I think the important part will be to discuss them tomorrow, and can give one example, because for every point that was raised in that discussion, there is always the other hand. It is like the two-armed economist. I will give you one example right now.

It is true that one study with what is a laser TMR system, there were very few perforations. There was another blinded, randomized study, completed in the U.S., published in JACC, 140 patients. Only the treated patients, 70 treated patients actually were catheterized.

Now, in that study, 5 of them had perforations, so it is often difficult to--just as one example, there is always another side to this, and the important thing is I think you have raised some excellent points. We have left the tough questions for our committee members to address.

DR. SIMONS: To come back to the point

that catheters are different, not only are they different from each other, they are different from the cells depending on the cell type used. I absolutely do not think there could be universal device.

DR. LEDERMAN: And these questions were not answered in benchtop testing?

DR. SIMONS: No.

DR. RAO: I think your point is well made, though, that I think the way devices need to be regulated is somewhat different from cells, because of the number of variables one might have to consider are somewhat different, and I think that is a very valid point.

If there is no more questions, we will go to the open part of the question and answer session.

Open Public Hearing

Before we can have the open public hearing, by law, I am required to read a statement. I will do that right now.

Both the Food and Drug Administration and the public believe in a transparent process for information gathering and decisionmaking. To ensure such transparency at the open public hearing

session of the advisory committee meeting, FDA believes that it is important to understand the context of an individual's presentation.

For this reason, FDA encourages you, the open public hearing speaker, at the beginning of your written or oral statement, to advise the committee of any financial relationship that you may have with any company or any group that is likely to be impacted by the topic of this meeting.

For example, the financial information may include the company's or a group's payment of your travel, lodging, or other expenses in connection with your attendance at the meeting. Likewise, FDA encourages you at the beginning of your statement to advise the committee if you do not have any such financial relationship.

If you choose not to address this issue of financial relationships at the beginning of your statement, it will not preclude you from speaking.

There were two people who had asked to be recognized before the meeting started. The first person is Dr. Vulliet.

DR. VULLIET: Thank you for the opportunity to come and present some data to you. Am I supposed to make a statement I have no

financial interest in this?

DR. RAO: Yes.

DR. VULLIET: Okay. I have no financial interest in this.

This is an example of some studies that were done very recently where we have been investigating, using my research team, which is myself, a cell biologist/pharmacologist, Dr. Greeley is a pathologist, Mitch Halloran is a cell biologist, Kristin McDonald and Mark Kittelson are both board-certified cardiologists, so we have a very interdisciplinary team.

We are at a vet school, which is probably novel for this group, and we are very specifically interested in animal models. I was very pleased to hear quite the discussion of animal models. I disagree with almost everything every one of the speakers complained about, not being suitable animal models.

I guarantee we see animal models that definitely are real patients, that have real disease. It is not induced, it is a real disease. It is there, it needs to be treated. For that reason, we have decided to investigate the possibility of using cytotherapeutics to see if we

can produce a beneficial effect in animals.

A good example of our animal model--and this slide is probably Peter's example of what they think we do with animals--but this is Oscar, and I guarantee you he will grow up to have somewhere later in life, lumbar disease, lumbar disk disease, either at the L2/3 or the L3/4, and he is a great model if you are into disk disease, but that is a different committee we talk to about that.

Steps for successful cytotherapeutics. This is my perception, not the committee's guideline, the first step is safety studies. This is about where we are at. In fact, most of the stuff we are squabbling about right now is whether these things can be done safely or not, if you think about it.

Very little good data on dose response, nothing on time course that I am aware of. Nothing or very little on clinical endpoint. What do I mean by clinical endpoint? When I am giving an antibiotic, I can tell you I need to hit serum concentrations of 1 microgram per ml.

Okay. I can design a pharmacokinetic regimen, I can hit 1 microgram per ml. If I am treating dilated cardiomyopathy, what is my

endpoint? If I see something four to six weeks later, I will be lucky.

So, when I am administering cells, I really don't have a defined clinical endpoint at this point other than the lack of adverse reaction. Think about it. It is a very interesting point of view.

Anyway, because we started off with safety studies, that is what we did. We asked a very simple hypothesis, and we started with can a half a million cells--we are using mesenchymal stromal cells, we call them stromal cells rather than stem cells because we are not convinced that primordial germ layers have been demonstrated coming from MSC, so we refer to them as stromal cells, you can call them stem cells if you like.

We also use a terminology I don't think anybody else has used in this room yet, is we are using MICs [ph]. That is a million cells per kilogram. We are very interested in a dose-response relationship. Doses are a key in any therapeutics.

So, we are giving half a million MSCs per kilogram of body weight, and can they be safely injected into the coronary arteries of the

anesthetized dog?

Simple experimental design, they are autologous. We collect bone marrow somewhere about a month later, we inject 10 million cells. These are 20 kilogram dogs, half-million cells per kilogram.

Seven days later, the dogs were anesthetized, physical exam, CV exam, necropsy and histo and immunocytochemistry.

I should also point out everywhere in this study, all of these dogs, after recovering from anesthesia, passed the cold nose test. They were perfectly normal, you would not be able to tell. They jumped, their tails wagged, they licked your hand. They were nice dogs.

So, in that regard, on physical exam, they looked good.

Abbreviated methods. Autologous, four sites, collect them. Freshly dispersed, and this will be a key point at the end. These are autologous cells, freshly dispersed MSCs into the circumflex artery. Catheter placement verified before injection, after injection, with fluoroscopy, physical exam. All dogs basically appeared to be normal once the effects of

anesthesia had worn away.

This is the first dog we did. This is the highest dose we did, and we did 1 million cells per kilogram, injected in the coronary. At 2 hours, took a section, you can see the catheter placement there, took a section of ventricular myocardium. It's a lightly stained hematoxylin section. These 2 cells, basophilic cells here, translate to the CMFDA-labeled cells or fluorescent cells there.

This is one of the things we are looking for. Our research team, probably different than many of the people in this room, feel that the only way we will get effective therapeutics is to have intimate contact between these cells and the dead and dying cardiomyocytes that they are going to replace. We don't believe in direct injection, and we can talk about that later.

So, that was our first dog, 1 million cells per kilogram. The reason he went--it was at two hours--was he went into V fib and died. Two hours, so it is fairly easy to do your necropsy in that. So, we then bacted those off to a half-million cells per kilogram and injected them, and the injections are 5 injections of 2 ml each and 1 million cells per ml, and this was the

control, the anesthetized dog, this is the ECG.

Dogs normally have inverted T waves, I don't know how many people know that, and it has to do with chest dimensions and chest geometry more than anything else. This is a normal ECG for a dog.

After the fifth injection, the T wave has converted to a normal position, but more importantly, what you see here is ST elevation, very profound ST elevation. This increase with each dose and at the fifth injection it was the most severe.

Twenty-four later, post-injection, you can see you have got back to an inverted T wave, but you have got bizarre complexes here. One-week post-injection you have got normal ECG, and this was published recently in Lancet, so I am not going to go through a lot of this published thing two weeks ago.

ST elevation made us think of troponin. We measured troponin at various times after injection. You can see it goes up, increases to about 45 nanograms per ml. If you ligate an LAD in a dog, you normally see ranges in the order of 150 to 200, so we had subnormal, if you will, levels of

troponin, but the time course it would be consistent with some sort of myocardial ischemia.

One of the things you also don't see in many of these preparations that you are seeing, which is my personal--I will give you guys my personal things that sort of irk me a little bit about science--is we are using H&E histology.

This is the gold standard of pathology. I don't care how many immunocytochemistry studies you see, H&E is what medical pathologists use to evaluate an outcome of a case. It is essential, it should be included, I don't know why people don't use it, but it is a great technique, I like it although I didn't do very well in pathology.

Here is a good example of a section of the ventricle of one of the injected dogs. What you see is three areas here of hypercellularity. This is the normal myocardium here as you zoom in. This is at 4X or 40X, this is 100X and 400X, and we are zooming in on this area right in here.

You see it is more hypercellular. What you see in here is you see mononuclear cells, rounded nucleus. You see some elongated nucleus. You see fibrosis. You see some lytic lesions. I looked at that as a cell biologist, and I said,

great, that's where my stem cells are, right? Wrong. My pathologist looked at it and said, "Rick, you have got a problem. Those are macrophages. You have just produced a heart attack in this dog." And I said, "Oh."

So, to verify that, these were CM dye/I labeled cells zooming in. You can see that the CM dye/I label is in the vicinity of this hypercellularity, so both the MSCs and these macrophages are here, but how do we know they are macrophages?

Again, using canine-specific antibodies that we have available in the vet teaching hospital, this is an H&E section, this is a CD18 monoclonal antibody specifically raised against canine macrophages. You can see you have punctate lesions, it is very characteristic, and, indeed, to confirm this, 7 days later, what we also see characteristic of myocardial infarctions is increased fibrosis and collagen deposition.

As you can see here, this is normal myocardium, it stains very red in Masson's, and what you see here is you see the blue here is collagen fiber deposition. This is very characteristic of myocardial infarction.

Five cardinal signs of MI are ECG changes, we showed that; proteins released from damaged myocardium, we showed that; decreased wall motion, we did not see that. We did ultrasound, but I don't know that we could. We have the sophistication to measure wall motion. Myocardial infarction is just not a common veterinary disease that we see. Characteristic cellular infiltrates, we saw that. Collagen deposition.

Basically, our conclusion is that at 0.5 million cells per MSC, will produce myocardial ischemia, microinfarctions in these dogs.

Our original interpretation of this was that this was a dose/rate of delivery problem. So, our feeling was, because there really is not much, is you critically look at the clinical studies out there in humans, they have got doses all over the place. It is very hard to extrapolate what the dose is. That is why use milligrams per kilogram. I would encourage anybody doing these studies to use some sort of a normalization like that.

I would encourage the committee to require it, so that you can start comparing, but more importantly, what we did, just to give you an in idea, is possibly post-injection cell clumping. We

didn't consider this as a possibility. We have taken the holding media the cells were in, and the injection media, to inject them into the cells.

At the end of 2 hours of holding them in the holding media, I personally inspected most of the dogs. We inspected the cells. None of the cells are clumped, so we assumed clumping was not a problem.

Just to give you guys a little bit more gray hair in terms of your job as far as making decisions, what we didn't do is we didn't do the right control, and the right control was to put these cells in 100 percent serum, because that is what you are doing, you are injecting them into the artery. From the time they leave that artery, they are going into 100 percent serum.

We did that and the cells started clumping. I don't know why. They didn't clump when we pulled them out of the bone marrow because they would have been clumped when we put them in tissue culture dish.

They didn't clump then, but during the process to 2 weeks of preparing them, sticking to plastic, their adhesive properties had changed. One dog, and I have heard several people talk about

fetal bovine serum, I don't know if it is known to this group or not, Darlen Procoff [ph] last year had an abstract, I don't think he has published it yet, had an abstract where he looked at fetal bovine serum in cells. It carries over, I believe it was about a milligram per million cells if you grow cells in FBS, at least with the MSCs, will pinocytose and hold about a milligram per million cells of fetal bovine serum.

It is not released by washing. These cells were rinsed three times in Hanks' balanced salt before they were injected. If you want to remove the fetal bovine serum, you have to grow them for at least 48 hours to get rid of it.

This may explain some of the early dieoff of the myoblasts that you are seeing, if you have got FBS in there, because you will get a reaction from it.

Basically, this research team does not feel that we are in a position to perform these studies on client-owned patients at this time, although that is our long-term goal.

What do we think is happening? What we think is happening is, we think the cells are coming in, we think they are clogging, producing

microinfarctions by clogging either a second or third order arterial, and causing areas of ischemia and microinfarctions.

Are there clinical techniques to detect this? That is one of those things. Would you see that, or does all the old imaging, does the modern world ultrasound pick up something like that? I don't think so. So, that is why I am saying histology is pretty important.

How do our cell preparations compare?

DR. RAO: Dr. Vulliet, we would like try and make sure that we stay on time.

DR. VULLIET: Sure. Let me finish this because that is actually what I think we are more interested in.

This is bone marrow from canine bone marrow. As you can see, you have got metas and milas and bands, and that kind of stuff. This is canine MSCs. Our cell size is about 19 microns, 20 microns. Mean cell size on this population, because they are much smaller, is 10 to 12 microns.

More importantly, look at this. The range on these, again, these are characteristic things. These are done in dog, they don't publish them in humans. Range is 7 to 50 microns, okay, and it

could go even go higher, go as high as 80.

Plasticity in this population of cells, this is probably being generous, saying it is about 0.01 percent. If you take the canine population of cells, and you go through a CFU selection process, you can get plasticity on the order of 40 to 60 percent.

If you are starting to compare potency, potential potency between these two preparations of cells, this preparation of cells, after it went through a CFU selection process, would be about 4,000 to 40,000 times more potent than just crude bone marrow in terms of plasticity potential, if you will.

Intrinsic properties of these cells, I don't know. These cells have a tendency to lay down collagen if you just leave them sitting in a tissue culture dish.

Successes for safety studies, therapeutics, efficacy studies need to be done in well characterized model diseases and patient diseases.

What I would like to do is leave you with a couple of philosophies. We believe that bone marrow stem cells have potential to treat many cell

loss diseases, especially the myocardium, and we will continue using these even in spite of the negative report. We believe this is a technical problem. We believe we will solve it by adjusting the dose and a few other things.

However, strict scientific disciplines are necessary to avoid a train wreck. As Doris Taylor said, we do not want to repeat the gene therapy trial.

The other thing I can give you is those of us who used to have gray in our hair, and now I don't have hair, is the comment, when clinicians read their own press clippings, patients are going to suffer.

Thank you for your attention.

[Applause.]

DR. RAO: Thank you, Dr. Vulliet.

Our next speaker is from Genzyme. Let's try and restrict the time to about 10 minutes.

MR. DU MOULIN: It is very tough being the last speaker of the day, but it is absolutely worse being the last speaker of the day and following a vet who talks about puppies.

[Slide.]

Good afternoon. My name is Gary C. du

Moulin. I am vice president of Quality Systems for the cell therapy operations at Genzyme Corporation in Cambridge, Massachusetts.

Genzyme Corporation is collaborating with Professor Philippe Menasché of Paris, France, in a multicenter, Phase II/III clinical study autologous skeletal myoblast implantation in Europe.

[Slide.]

Ensuring the therapeutic success of cardiac cell therapy is predicated on a rigorous scalable autologous cell culture program based upon the principles and practices of good manufacturing.

Our long experience with the scale-up and delivery of cartilage and keratinocyte-based cell therapy products and services to thousands of patients has confirmed that each element of good manufacturing practices contributes an essential part of an overall program that optimizes chances of providing cell therapy products expressing the attributes of safety and consistent quality for patients.

Controls required for the manufacturing process begin at the collection site of the muscle biopsy and ends approximately three weeks later as the suspension of cells exhibiting quality and

safety characteristics that once implanted can consistent initiate a robust repair process.

Maintaining the sterility of the cell culture system and ensuring lot segregation are critical attributes of success.

[Slide.]

All the elements of the GMP-based manufacturing program are essential in order to control the inherent variability representative of autologous cell culture. Briefly, these controls must be established based upon the following aspects of GMP.

These include, and they are listed here, a process that is validated, personnel who are trained and certified to manipulate cell safely, an appropriate facility expressing stringent environmental controls, records and documentation of all processes conducted, equipment that is calibrated and validated, raw materials that have been tested for their quality, accepted formally, and released into the manufacturing stream. Unique to an autologous cell process is to maintain stringent patient lot segregation.

[Slide.]

This is a photograph of our sole

manufacturing facility. It is approximately 10,000 square feet, contains about 70 biosafety cabinets in which individual patient's tissues are manipulated, but other organizational requirements necessary beyond the GMPs include these elements here, beyond the manufacturing, a purchasing element, materials handling element, logistics for the shipment of cells, customer care for communicating with the surgeon and the patient, engineering and facilities to maintain your facility, manufacturing technical services responsible for the training of personnel, process development, and clinical manufacturing, a formalized quality assurance, quality control, and validation services program.

[Slide.]

In order to scale up manufacturing activities for a clinical development paradigm, an organization must be created to effectively manage a myriad of direct and ancillary responsibilities, but here, quality controls including environmental monitoring for the manufacturing facility are critical components of the operational elements of cell therapy productions.

Robust testing programs ensures--and I

have listed them here--that cell therapy products meet the highest standards of safety, effectiveness, and reliability as a therapeutic modality, that transmission of communicable diseases is prevented, that one ensure that all manufacturing and processing controls are in place and consistently followed, that there is compliance with existing and anticipated regulatory requirements, that validated assays are performed which support lot release and performance monitoring of materials and components, and, finally, encouraging the development of new assays which enhance product safety, and, finally, generating and analyzing that data, the quantitative data to support continuous improvements to your process.

[Slide.]

Putting these concepts together, a manufacturing process whose key manufacturing events from biopsy receipt through final product fill finish are well understood, can optimize the ex vivo cell culture process, and supported by validated quality controls can ensure safety and product consistency.

[Slide.]

We believe that call product characterization is possible based upon validatable measures of viability, purity, identity, and yield, with safety indicators of sterility, endotoxin in the absence of mycoplasma.

Here are shown the percent viability and percent CD56 expression of three cell therapy products prepared during process validation studies from cadaveric skeletal muscle tissues.

Included in these data is evidence of cell product stability over a 72-hour time frame, one reasonably to be expected if transportation of the cells over a long distance to the patient is required. Note that the lot release parameters remain stable over 72 hours.

Viability and CD56 expression, both flow cytometric and validatable measures of cell product identity can be consistently maintained above the 90 percent range.

[Slide.]

Measures of sterility and potency are shown in this slide. It is crucial to ensure that sterility is maintained throughout the manufacturing process. In the case of short shelf life cell therapy products utilizing automated

microbial detection systems can provide benefits to improve time to detection should microbial contaminants be present.

Finally, in order to demonstrate the potential for a therapeutic effect, the presence of myotubule formation can be used as a measure of identification and perhaps potency. Myoblasts are undifferentiated muscle precursor cells which, when fused, become the differentiated myotubules. The presence of multinucleated muscle cells is a strong visual indicator that muscle differentiation has occurred and may be an important predictor of cell function.

In these photographs, one can readily see multinucleated myotubules indicated by the yellow arrows from freshly prepared samples and in cells after a simulated 72-hour shipment period.

[Slide.]

In conclusion, quality, safety, and effectiveness must be designed into cell therapy products. Quality cannot be inspected in or tested into cell therapy products. Despite the fact that we may, at this time, not know every aspect of cell product characterization, institution and maintenance of stringent manufacturing controls

through rigorous observance of GMPs can contribute to the safety and consistency necessary for the production of cell therapy products intended for cardiac cell therapy.

Developers of cell therapy products must consider, understand, and incorporate quality requirements at the earliest possible stages of the clinical development program, and in so doing, can optimize the therapeutic potential of these promising technologies.

Thank you.

[Applause.]

DR. RAO: Thank you.

If there are no questions, I would like to ask if anybody else from the audience wishes to make any comment at this time. I am going to ask that they limit their comments and be brief.

[No response.]

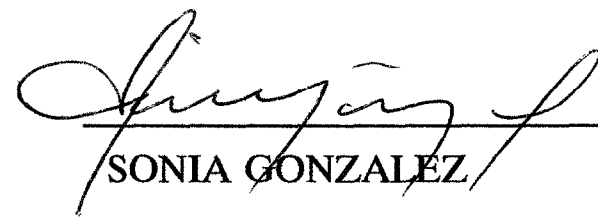
DR. RAO: If there are no more comments, then, I can declare the meeting adjourned.

[Whereupon, at 5:46 p.m., the proceedings were recessed, to reconvene at 8:00 a.m., Friday, March 19, 2004.]

- - -

CERTIFICATE

I, **SONIA GONZALEZ**, the Official Court Reporter for Miller Reporting Company, Inc., hereby certify that I recorded the foregoing proceedings; that the proceedings have been reduced to typewriting by me, or under my direction and that the foregoing transcript is a correct and accurate record of the proceedings to the best of my knowledge, ability and belief.



SONIA GONZALEZ