UNITED STATES FOOD AND DRUG ADMINISTRATION

PUBLIC WORKSHOP

SCIENTIFIC EVIDENCE IN DEVELOPMENT OF HCT/Ps SUBJECT TO PREMARKET APPROVAL

Silver Spring, Maryland

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PARTICIPANTS:

Welcome/Opening Remarks:

CELIA WITTEN, PH.D., M.D.
Deputy Center Director of the Center for Biologics Evaluation and Research, FDA

Session 1: Keynote and Regulatory Scheme

IRVING WEISSMAN, M.D.
Stem Cell Biology and Regenerative Medicine

STEVEN BAUER, PH.D.
FDA Perspectives on Scientific Evidence and HCT/P Development

Session 2: Experiences in Product Development

JACQUES GALIPEAU, M.D.
How Mechanistic Studies on Mesenchymal Stromal Cells Inform Design of Human Clinical Trials for Autoimmune Ailments - The Fitness Paradigm

MICHAEL MATTHAY, M.D.
Mesenchymal Stem Cells for Treatment of ARDS Patients: Challenges and Lessons Learned in Pre-Clinical Testing, FDA Approval, and Ongoing Clinical Trial

GREGORY RUSSOTTI, PH.D.
Drivers and Methodologies for Making Cell Therapy Process Changes

DENNIS CLEGG, PH.D.
Development of ES-Derived Retinal Pigmented Epithelium on a Scaffold for Age-Related Macular Degeneration

CHRISTOPHER BREUER, M.D.
The Development and Translation of the Tissue Engineered Vascular Graft: From the Bench to the Bedside and Back Again

PARTICIPANTS (CONT'D):

Session 3: Views from Professional Societies

JONATHAN KIMMELMAN, PH.D.
Ethics, Evidence, and Regulatory Approval For Cell-Based Interventions

MASSIMO DOMINICI, M.D.
Dissecting Unproven Cellular Therapies: The International Society for Cellular Therapy (ISCT) Position

PETER RUBIN, M.D.
Clinical Adipose-Based Therapies

Session 4: Views from Other Government Agencies

KRISTY POTTOL
Delivering Mission Ready Medical Solutions to the Warfighter

MARTHA LUNDBERG, PH.D.
Enabling Development of Regenerative Medicine Technologies and Therapies at the NHLBI

Session 5: Patient and Society Experience and Expectations

JEFFREY KAHN, PH.D.
Societal Perspectives on Development and Oversight of Novel Cell-Based Therapies

BRIAN MANSFIELD, PH.D.
Perspectives of Stem Cell Therapy for Orphan Inherited Retinal Dystrophies

THOMAS ALBINI, M.D.
Severe Visual Loss After Intravitreal Injection Of Autologous Adipose Tissue-Derived Stem Cells For Age-Related Macular Degeneration

PARTICIPANTS (CONT'D):

MICHAEL MILLER, M.D., PH.D.
Glioproliferative Lesion of the Spinal Cord Arising from Exogenous Stem Cells

Closing Remarks:

IRVING WEISSMAN, M.D.

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P R O C E E D I N G S

(8:40 a.m.)

DR. WITTEN: Good morning. I think we will get started. My name is Celia Witten. I'm the Deputy Center Director of the Center for Biologics Evaluation and Research here at FDA. I'd like to welcome you to our public workshop on Scientific Evidence in Development of Human Cells, Tissues, and Cellular and Tissue-Based Products, known as HCT/Ps, that are subject to premarket approval.

The purpose of this workshop is to identify and discuss scientific considerations and challenges to help inform the development of HCT/Ps regulated as biologic products and subject to premarket approval, including stem cell-based products.

As you may be aware, next Monday and Tuesday, we are holding a Part 15 public hearing on the draft guidances related to the regulation of HCT/Ps. We have received some questions on how the Part 15 hearing relates to this workshop.

The purpose of the public hearing is to obtain comments on four draft guidance documents that were issued to provide clarity about FDA's existing regulatory framework for HCT/Ps. The purpose of this workshop today is to discuss scientific considerations in the development of HCT/Ps that based on the regulatory framework are regulated as biologic products and require an IND or BLA.

During today's workshop we are going to hear from a number of distinguished speakers. In Session 1, Dr. Irv Weissman will provide our keynote address covering stem cell biology and regenerative medicine.

Following his presentation, Dr. Steven Bauer will provide an overview of the regulatory framework for 351 HCT/Ps and FDA perspectives on scientific evidence and HCT/P development.

During Session 2, speakers will provide an overview of their experiences in product development and describe challenges, scientific questions, and lessons learned.

Following Session 2, the speakers will engage in a panel discussion, and the audience will have the opportunity to ask questions of the panel members.

In Session 3, we will hear views from professional societies. In Session 4, we will hear from representatives of government agencies on how their respective agencies advance product development, and finally in Session 5, we will hear from a bioethicist, a patient representative, and two physicians about patient and public expectations.

Following Session 5, there will be a panel discussion with the speakers for Sessions 3, 4, and 5.

To end the day, Dr. Weissman will provide some closing remarks.

Before I turn the floor over to Dr. Weissman for his keynote address, there are a couple of housekeeping items. Restrooms are located in the hallway outside of this conference room. Lunches, snacks, and beverages can be

purchased at the kiosk close to the registration desk.

With that, I'd like to welcome Dr. Irv Weissman. Just to provide a brief introduction, Dr. Weissman is the Director of the Stanford Institute for Stem Cell Biology and Regenerative Medicine, and Director of the Stanford Ludwig Center for Cancer and Stem Cell Research.

He is a member of the National Academy of Sciences, the Institute of Medicine at the National Academy, and the American Association of Arts and Sciences.

He will give the keynote presentation today. Thank you. *SESSION 1: KEYNOTE AND

DR. WEISSMAN: Thanks, Celia. Stem cell biology is actually very simple. The difference between a stem cell in the tissue and all other cells in the tissue is that when a stem cell gives rise to say on average two cells, one of the two on average is a stem cell. Still, that is stem cells are distinguished from any cell downstream from the stem cell because they are the only ones

that self-renew, and in the whole blood forming system, the hematopoietic stem cell is the one that self-renews.

Years ago, we wanted to be able to identify, isolate, and then transplant first in mice and then in humans, the hematopoietic stem cell. It turns out that is the only cell in the bone marrow that regenerates the blood. If you take away the stem cells and transplant everything else, it lasts for about eight weeks.

So, if you want a permanent survival of the cells and the biology and the medicine they bring, stem cells are the important ones. For us, the other cells are problematic in both autologous transplants and allogeneic transplants.

Years and years ago, Mike McCune and I, after we had isolated the mouse blood forming stem cell by saving lethally irradiated mice with as few as 100 of the cells versus 200,000 bone marrow cells, we didn't get volunteer medical students to say I want to be irradiated so you can find a stem cell.

So, we put into immune deficient mice human fetal bone, on the right, human fetal liver, human fetal thymus, and found those organs in that immune deficient mice would take, of course, and then you irradiate those mice and put into them the cell type that you think might be stem cells.

This is an old version. I'm sorry I put in that gray background. If anybody wants the slides afterwards, I'll get rid of the gray background.

They lack markers of the B cell myelomonocytic, T cell, and red cell lineage. You can combine all the antibodies to those lineages, make them green, with a green fluorescent protein or fluorescein, and then positively select, and no single marker on the surface will allow you to isolate stem cells, no matter what people tell you.

With a combination of markers, we could isolate the cells. That was 1988. Then in the humans, we found that very similar markers -- that is mouse, that is human -- no B cell

myelomonocytic, T cell, red cell markers -- again, very similar markers on the surface.

CD34 alone is not sufficient to purify blood forming stem cells. So, the idea for transplants is to take the blood forming tissue, which in the beginning was bone marrow, but now we know you can mobilize peripheral blood, many ways to do it, the clinicians taught the scientists that this could work, post-chemotherapy cells are mobilized. If you give Cytoxan by itself, now there is a whole panoply of things you can give to a healthy patient and you mobilize stem cells, and not just stem cells but many cells in the bone marrow into the blood. Also, marrow or mobilized peripheral blood or umbilical cord blood.

The one thing you should know is that hematopoietic stem cells make blood and only blood, no matter what anybody tells you. Every time we have tested with mouse or human, hematopoietic stem cells make blood and only blood. They don't make brain, they don't repair heart. None of those other things that have been

claimed over the years. They don't transdifferentiate under any circumstance to become a brain forming or liver forming or gut forming stem cell.

That means when you have a cancer patient like somebody with metastatic breast cancer, if you wanted to rescue them after high dose chemotherapy, you don't give that mobilized blood because over half the time you are giving back the cancer after you have ablated their immune system.

You want to get pure stem cells by a cell sorting method. The current standard cell sorter is a high speed cell sorter first developed by the Herzenberg's, commercialized by Becton-Dickinson, but there are a number of them out there and of course, they have to be qualified that they don't bring infections into the operation, because you don't want LPS, infectious agents and so on.

You can get pure hematopoietic stem cells and they work in transplant, but they have

to be free of cancer if you are, for example, a woman with metastatic breast cancer.

These are the results of the study. These are either using single marker or CD34 or two different commercial kinds of separators, and the only thing that gives you 250,000-fold depletion of breast cancer cells from the mobilized blood is multiple marker, high speed cell sorting, no solid device can get to that purity because you get non-specific adherence of the cells to part of the device, so it has to be flow sorting at least so far.

What would you do if you had breast cancer free or non-Hodgins lymphoma free or myeloma free stem cells you try to transplant? I formed a company called SyStemix, long gone, but we isolated the blood forming stem cell, we developed the sorters that were able to sort them, and we did clinical trials in those three diseases.

SyStemix was purchased by Sandoz and that merged into Novartis, and eight years after

the initial purchase, they shut it all down in the middle of a clinical trial.

Let me show you the results of at least one of the clinical trials. This is the Stanford clinical trial where we isolated cancer free hematopoietic stem cells from women with metastatic breast cancer, and they had to have evidence of metastasis in bone or liver or lung, the only exclusion was brain at the time this was done, between 1996 and 1998.

There were 15 patients that we treated in this way, and at that time, it was still popular to give back mobilized peripheral blood which by that time the bone marrow transplanters erroneously called it stem cell transplant.

So, don't you believe anybody who says they are doing a stem cell transplant unless they are doing purified stem cells. They are doing bone marrow or mobilized blood, and that inaccuracy in the language accepted by the journals, accepted by the bone marrow transplant community, leads people astray who have memorized

their way to their field rather than being able to understand the science and the questions that need to be asked, hard questions, before you treat patients.

So, it is now out 20 years for some, 18 for most. One-third of the women who were given cancer free stem cells still today, I still follow them, are cancer free. Mobilized peripheral blood at 11 years, seven percent were cancer free.

Now, if that was a pill or a protein, that would be a product, but the company that bought us in 2000, roughly at this part of the trial, made a business decision to move on to other products to emphasize what they were doing.

That brings up a second point. What we are trying to do is advance medicine for people. The function of a company is to make a profit. They are always going to be responsible to their shareholders and their money first.

I personally believe after lots of experience that we need a way to fund these kinds of projects in competition at academic or

not-for-profit institutions until the end of a phase 1 or phase 2 trial that tells you it is safe, it is worth going forward, because the amount of money isn't there, and the companies have other reasons to exist.

By the way, it is statistically significant both for progression free survival and overall survival at all time points, and the only place where this therapy now will be offered is at Stanford. I gave away all the stock I had. I negotiated on behalf of Stanford. We opened up actually the day before yesterday a cell sorting clinic, and we are beginning pure stem cell transplants. I will talk about that more in a minute.

Does the mouse predict the human in any meaningful way? This is the time to get either enough neutrophils or enough platelets to be safe in a mouse for reconstitution. You get a dose dependent survival, and at the dose that gives you 10 days to platelets or 10 days to neutrophils in the mouse, it is about 10,000 hematopoietic stem

cells or on a weight basis, four times 10 to the 5th per kilogram.

Here is the breast cancer study. This is the time to get to the 500 neutrophils per microliter or 20,000 platelets per microliter, and what you see is the break point is right about here between 5 and 10 times 10 to the 5th.

I won a bottle of wine with our Chief Medical Officer, Chris Yettner, who said mice do not teach you about humans. In this case, it did.

Now, allotransplants bring another issue, the T cells that are present contaminating the mobilized blood or the bone marrow cause graft versus host disease. The T cells have homing receptors that take them to the lymphoid organs, lymph nodes, spleen, and the activated cells go back to the thymus, function we don't know yet, but what means is the graft versus host disease starts in the lymphoid organs. It destroys the structure of the lymphoid organs. That is the primary reason at least in animal models for the immune deficiency that follows transplant, not

just the immunosuppression to get the transplant in but the graft versus host disease.

You can put in, as Judy Shizuru did, less than the number of T cells in a mouse model of a matched unrelated donor that then would cause systemic graft versus host disease, the one that could be diagnosed, and of all doses of T cells, the lowest dose she gave, there was a continuing graft versus host disease reaction in the lymph nodes and so on.

We wanted to get to pure stem cells to get rid of graft versus host disease. We showed in this mouse, no graft versus host disease with pure hematopoietic stem cells, lots of T cells are included. Mouse and pre-clinical for human, and we are now set up to do the first clinical transplants in human in allogeneic. It will be severe combined immune deficient patients.

Now, God I hate this. This is terrible. I put in gray so it would look good for you. So, listen to what I say, and hopefully it will show up on the graph.

We knew or we suspected that the genes that cause autoimmune diseases like Type I diabetes, multiple sclerosis, systemic Lupus, are genes that are expressed in either the cells that affect lymphocyte development, that is in the blood forming system, or intrinsic defects in the blood forming system, so that led to the idea that if you could allotransplant stem cells from a disease resistant donor, you might be able to change the course of that disease.

Again, Judy Shizuru and I took mice that had Type I diabetes, they get it four to five months of age, we give them a lethal dose of irradiation, we give them more antibodies to get rid of the residual autoimmune T cells in their bodies, then we transplant either whole bone marrow from them, it buys a month, that is as far as the transplant community has gone. That is the Richard Burt protocol.

Pure stem cells from the NOD mouse, the diabetic donor, buys another month, but stem cells from a third party even a matched unrelated donor

prevents progression of the disease for life, because stem cells self-renew.

Now they can deplete the T cells that would cause the autoimmune attack on the insulin producing islets because they have a system that can do it. We even co- transplanted mice that had been diabetic for six months with hematopoietic stem cells and islets.

The reason behind that, and this is probably the most important thing I can say, is that hematopoietic stem cell transplant, when successful, either as a partial chimera or a full chimera, induces transplant tolerance of any other organ, tissue, or tissue specific stem cell in many, many animal trials.

It does so because the thymus depletes autoreactive T cells, and it defines as autoreactive those that will react against the host, that is why you don't get GVH from the new T cells coming up, and those that react against the donor because the dendritic cells also deplete and induce regulatory T cells that give you permanent

transplant tolerance.

So, if you are planning to transplant organs like livers, kidneys, lungs, hearts, this will be the preferred way if you didn't have to go through the chemotherapy or the radiation that takes you next to death for a transplant of purified hematopoietic stem cells to work.

We want to replace genetically defective systems. We want to induce tolerance to organs. We want to treat autoimmune diseases. We also have success in the Lupus model. At any point, we can stop the progression.

If you want to gene modify, those are the cells that are most efficient to gene modify and the only cells that self-renew and therefore fixing a sickle cell gene, you would have to use it.

Now, we hope very soon to have the alternatives of either donors that don't have sickle or gene corrected self stem cells where there is no immunologic barrier. The chemotherapy is the problem.

To try to address that, we began looking at antibodies that might deplete hematopoietic stem cells, Agnieszka Czechowicz, Deepta Bhattacharya, and I.

If you take a mouse that is severe combined immunodeficient and you transplant 5,000 stem cells -- there it is right there -- that is 50 times the dose you need. You get one percent chimerism because those are the only open niches for stem cells. I will just say that's the problem.

If we give one dose of an antibody that depletes c-Kit positive cells and all hematopoietic stem cells are c- Kit positive, just one dose of the antibody in mice, we are up to 15 percent, and if we give three doses, we code so we know the first transplant is red genotype, second green and third blue, we got 80 to 90 percent chimerism.

Now, that is in a clinical trial for patients who have severe combined immunodeficiency at Stanford. Amgen has supplied the antibody, AMG

191, which depletes monkey stem cells that share the same c-Kit, and in our mouse, the human hematopoietic stem cells, and we will be testing first in human, this month it has been discussed with CBER, I know, not by me but by Judy Shizuru and Maria Grottsi, and Ron Parell, the first test, is this going to work.

It's not going to be exactly this protocol, but will it work. It's an important advance if we can get rid of radiation and chemo, but when we tried to do this -- this is the depletion of stem cells in immune deficient mice, the same antibody, only dropped one or two logs the number of stem cells in immune deficient mice.

Another part of my lab had been working with a "don't eat me marker" called CD47. It says "don't eat me" to macrophages, and we found that the antibody to that would allow macrophages to eat cancer cells, and that is now in clinical trials, phase 1 trials at Stanford in the U.K.

The antibody blocks the "don't eat me" signal and endogenous eat me signals, calreticulin

is the primary one, lets the macrophage deplete, but we found also that we could give a very strong eat me signal if we provided human IgG1, whether it's Rituxan or Herceptin, or Trastuzumab or Pinatuzumab, they synergize by blocking the don't eat me signal and providing a strong eat me signal for the high infinity FC receptor for human IgG1.

Students in the lab said well, couldn't we apply that in the mouse model to our conditioning. When we give the antibody condition where we have antibody to stem cell, antibody to T cells, because now they are immune sufficient, we're going to do a matched unrelated donor, plus anti-mouse CD47, and only that combination leads to full chimerism for life, and we are testing them for immunological tolerance now.

Antibodies exist that have been tested in humans to human CD47, they are well past the safety stage. They can be delivered safely. Antibodies to T cells exist. The Amgen 191 sees human hematopoietic stem cells and precursors, so you will lose some of that tan you got, but the

point here is that we are moving toward all antibody conditioning.

It will be a partnership with the investigators here at the FDA to bring that through safely because once you can treat patients with antibodies, it is likely to be in an outpatient setting eventually, you change whether you would put anybody through the risk, a newborn with sickle cell or Type I diabetes, to have the treatment. So, target removal, no radiation or chemotherapy, antibody conditioning.

Now, I want to just bring up the point, the reason that we have all argued so hard with the groups that don't want us to do embryonic or fetal stem cell research, is that embryonic stem cells, human, whether they are taken from pre-implantation embryos, or reprogrammed from adult cells, can make all tissue types in a dish, can they make all tissue stem cells?

Well, we have shown in fact that it can be done. In the distant future, we will do that. We needed to have - - yet another example, and I

want not to go over time.

I co-founded a company called StemCells, Inc. with Fred Gage, David Anderson, Ann Tsukamoto, and Nobuko Uchida. We isolated from human fetal brains a cell subtype that has markers I can tell you about, clears it away from all cells in the fetal brain, they can be expanded at least a million fold, they have been transplanted into the brains of immune deficient mice. We always want to go through a mouse model first to see the biology of the cells.

This give rise to human cells, you see the back one is mouse a year later, so you see neurons, astrocytes, even neurons in the cerebellum and the cerebral cortex when you do it in newborn mice.

This is a picture of a mouse brain 47 weeks after transplant of pure human brain stem cells. It is a ray gamma, that is a severe combined immunodeficient mouse.

This is the subventricular zone where stem cells reside. A year later, the human stem

cells are there, and like the mouse brain forming stem cells, are dividing and self-renewing.

This is the olfactory bulb, which in the mouse is critical for life, and those are human cells migrating from this zone to the olfactory bulb. That is an antibody to humans, and this is the olfactory bulb showing the perigiomerular cells in human in exactly the right place.

We showed that human brain stem cells in the context of a mouse brain in graft self-renew in the appropriate place, migrate to the appropriate places, and differentiate appropriately. It is a whole science that is going on there, and it led us to study lysosomal storage disease in mice and then for humans, Batten disease, and in Batten disease, they lose their hippocampal structure, CA1, CA2, CA3, and increasing doses of human cells into the mouse model, neural protected, did not neural replace, so the enzyme that was missing was made by the human cells, secreted with six phospho-manos, and retaken up into the disease cells, curing the

lysosomal storage disease.

It works in spinal cord injury, Aileen Anderson, Brian Cummings, so long as you don't severe the spinal cord, you have a contusion, you can transplant above and below, all the brown dots are human cells, transplanted 30 days after the spinal cord injury. They were paralyzed. The paralyzed mice, this is a large scale of their walking behavior, stay paralyzed if you put in mesenchymal stromal cells, which many people call mesenchymal stem cells, but they can't differentiate. Neural stem cells, they are walking perfectly normal.

Human cells are exquisitely sensitive to diphtheria toxin. Brian Cummings gave them diphtheria toxin. They were immediately paralyzed, and the re-myelinated axons in the area of the injury, because the cells in an ischemic injury like spinal cord injury, the cells that are in the ischemic region that have their cell bodies, not their cell processes, die.

They have naked axons going through the

region, the sensory neurons in the muscle, in the leg, go through, and they are re-myelinated by the human cells, but once you give diphtheria toxin, they are gone. This is the mesenchymal stromal cells or mesenchymal stem cells, they just sit in a pocket. They don't become neurons.

I am told, but I don't study it myself, they have anti-inflammatory properties. So, it is a cell therapy and one that I know you probably know more than I do, and that was done in clinical trials, and for thoracic spinal cord injury, half of the patients who has Asia A regained sensation.

However, this year, just a few months ago, the company closed down when it ran out of money in the middle of trials, and I'm willing to talk about that but I am going to finish this very briefly.

We know that a fertilized egg gives rise to a pre- implantation blastocyst. In the middle of that are the inner cell mass, and each of those cells have the potential to give rise to all cell types in the body, and all embryonic stem cell

research began by learning how to culture them, but they go through tissue stem cells to make liver, pancreas, brain, blood, and so on.

We published last month in Cell that we can make from human embryonic stem cells or human induced pluripotent stem cells, stem cells for the liver, that was two years ago, bone cartilage stem cells, verified, skeletal muscle stem cells, and cardiomyogenic stem cells in five to seven days.

You can massively expand the human ESCs, so cell number isn't a problem, cell sorting and cell differentiation becomes the issue. You don't want any of the pluripotent cells left behind because they cause teratomas.

This is a mouse that received the bone cell, and there is bone that comes from it.

So, what I have told you is that there are tissue specific stem cells. Every tissue we have looked for it, we found it. You can purify them from other cells. The purified cells are the only cells with the life-long regenerative potential. If you can combine hematopoietic stem

cells and one of these other tissue stem cells, say bone and cartilage or cardiomyogenic or skeletal muscle, the hematopoietic stem cell can induce tolerance from the same donor tissue.

Instead of waiting for somebody to die for a liver transplant, in the future, not now, in the future, we expect to be able to get liver stem cells and hematopoietic stem cells from the same donor, in this case, an embryonic or induced pluripotent stem cell line.

We expect to condition the patients with antibodies, not chemotherapy and radiotherapy, so we don't have a primary anti-cancer reason for putting people through a bone marrow or a mobilized blood transplant, we don't have graft versus host disease, so what I hope I have shown you is that the science of the past and the science that is going on now should change the future.

The future of not having to hospitalize everybody who gets a bone marrow transplant to treat their graft versus host disease, to keep

them away from infections, to give them life-long immunosuppression, should make the cost of medicine cheaper, so long as those who commercialize it try to remember that although their function is to make a profit, the most important thing we can do is to change medicine for people.

Thank you. (Applause)

DR. BAUER: Thank you, Irv. We have a few minutes for some questions if anybody has some for Irv. (No response) No questions. Thank you, Irv. That was very nice. We will move ahead.

My name is Steve Bauer. We just heard some very, very eloquent science, starting off with a perfect illustration of how important that can be in thinking of how to develop products, establishing proof of concept in animal studies, and helping facilitating bringing the important next generation medicines into the clinic.

I am part of the FDA Office that regulates a large number of the kinds of products that we will be concentrating on today, so I'm in

the Center for Biologics Evaluation and Research, and the Office of Cellular, Tissue, and Gene Therapies. I am going to be talking about FDA perspectives on scientific evidence and development of these HCT/P products.

I think in order to understand how important science really is in this endeavor, you need to understand a bit about the regulatory framework that is established through laws, regulations, and guidance, and how that important aspect of science interacts with the regulatory framework, and talk about regulatory science, so the second part of my talk will be that. I'll summarize with a few highlights, and at the end, leave a few slides up with some resources and contact information for people who want further information.

As I sort of just alluded to, in the U.S., we have a three-tiered system that is based on statutes or laws, and these are acts of Congress and they are signed by the President, so those are the underpinning legal authorities under

which the FDA acts, and two important ones are the Food, Drug, and Cosmetic Act and the Public Health Service Act.

Based on those, we have regulations that are actually written by the FDA but approved by the Executive Branch, and we see those in the CFR, and you will hear me quote from the CFR, Code of Federal Regulations, as we go through a little bit today. I'll try not to do that too much, but I do think it is important that people understand where these things come from.

Then there is guidance, and this is FDA's interpretation of the regulations, and those are written and approved within FDA. They are intended to facilitate product development and understanding by people who want to bring things before the FDA, and it's advice that is non-binding on the FDA or the sponsor.

HCT/Ps are biologics. I put up the USC Code that gives the definition. I won't go through that. Since the topic today is HCT/Ps, I put that up there. That is from 21 CFR Section

1271. That is the sort of scope of today's meeting. We are talking about products that include human cells, tissues, and cellular and tissue based products. You will hear this term "HCT/Ps."

These are articles containing or consisting of human cells or tissues intended for implantation, transplantation, infusion, or transfer into a human recipient.

Within the U.S., we have this paradigm for medical product regulation based on centralized authority, that is the FDA. We look at the entire life cycle, which I'll explain in a little more detail in a few minutes, from the investigational product stage to actually marketing and then post-marketing surveillance and study of products.

We look at it from the first clinical trials, and even beforehand, to post-marketing. This is all done within applicable laws, and FDA does have enforcement provisions, and these apply to clinical investigations and marketing

authorization.

The documents, the policies and guidelines are freely available to the public, and you can find them in the Federal Register or FDA guidance documents, and we intend there is as much transparency, and there are fora for public discussion. These include FDA advisory committees, public workshops, such as this one, and sometimes interactions with the NIH RAC.

The regulatory approach for HCT/Ps is a tiered and risk based framework, so tissues and cells that are highly processed and used for other than their normal function and sometimes combined with non-tissue components or used for metabolic purposes are considered to be higher risk, so they are regulated with more oversight, and they require demonstration of clinical safety and effectiveness, pre- market review, and the pertinent regulations are written down there. I won't read that.

More conventional tissue grafts that undergo little processing used for their normal

function, these are perceived as lower risk and can be regulated solely under Section 361 of the Public Health Service Act, and are meant to help prevent communicable disease transmission.

361 HCT/Ps, products can be regulated providing they meet all of these following criteria, not more than minimally manipulated, intended for homologous use only, and not combined with another article, and not have a systemic effect, and not dependent upon the metabolic activity of living cells, or have systemic effect or is dependent upon the metabolic activity of cells for its primary function, and is for autologous use, allogeneic use, and first degree or second degree blood relatives or reproductive use.

So, that is the world of 361 HCT/Ps. We are focusing on the ones -- I've given you a few examples of the 351 HCT/Ps that are in that higher risk category and regulated in the pathways that I'll be going through in a few seconds in my talk.

Examples include allogeneic unrelated

cord blood, allogeneic unrelated pancreatic islets, and these are just examples, autologous tumor vaccines, CAR-T cells, neuronal stem cells, and multipotent stromal cells. As Irv pointed out, some people call these mesenchymal stem cells.

In order to get those kinds of products from the idea in the lab and to the bedside, we're going to be talking about the premarket approval pathway a little bit, and discuss how science interacts with the different stages of that. I'm going to be putting in a little talk on some of the research that is actually done at FDA that is meant to facilitate development of these kinds of products.

We are doing this in real time, and I think you might have gotten a sense from Irv's talk and certainly just following the literature, we have to do this in real time with what is available now. We have to work within the constraints of these regulations, which I just pointed out. It is a complex system or set of

kinds of products that we are regulating, but it really is dependent on sound science. Again, available technology.

In our work, we try to identify knowledge gaps and help point those out, help people in the field, and sometimes address with our own research some of those gaps and come up with possible solutions.

That is our sort of focus for today. We are going to hear from a lot of people about some of the challenges in this arena, and some of the possible approaches to moving the field forward.

I do need to point out that our objective in review, the primary objective is all about safety in all phases of clinical investigation. We apply scientific principles to the study of the products, the pre-clinical knowledge, clinical outcomes early and late, to assure the safety and rights of subjects.

As we advance the products to later phases, we increasingly emphasize the importance of scientific evaluation to permit an evaluation

of drugs' effectiveness and safety.

There are lots of different mechanisms and resources available in translational development. There are FDA regulations, and there are ICH documents, FDA guidance documents, and standards that are being developed through a variety of standard setting organizations. Those interact with and help guide people who are interested in translational development as they do their basic research and discovery, product development, proof of concept studies, tox studies, and study things like cell fate and biodistribution.

There are opportunities to interact with FDA at multiple times during product development to discuss the scientific issues and to help you understand how they fit into the regulatory paradigm, and those can begin with pre- IND discussions that mostly focus on the very important pre- pre-clinical animal studies that are necessary to help us look at the safety profile of a new product, and then pre- IND

meetings where we have a chance without having a clock ticking in the background to interact with FDA and get our feedback, including the scientific issues that we will be talking about today and will come up during product development.

All of that culminates in an IND submission, the very first clinical trials with that product, proceeds through stages of clinical trials, and then the goals are to have a license application and lead to product licensure.

This is just a diagram of some of the opportunities and interactions with people along this clinical development pathway life cycle. I mentioned pre- pre-IND interactions during the developmental phase, pre-IND meetings, and then the IND has a 30 day review clock.

It is good to take advantage of these pre-meetings in order to help facilitate success at the IND phase, and then a few other times when meetings are sort of normal and expected. We are also available for interaction when other important questions come up throughout this

product life cycle.

I'm harping on the issue of how important science is. These are the terminologies that we apply to our different scientific review disciplines. There is product, looking at how the product is made, what the source of the materials are and so on. Pre-clinical, looking at how the in vitro and in vivo studies support the rationale and the safety of a product, and clinical and statistical.

We will be hearing people discuss each one of these topics today.

The goals of pre-clinical testing are to produce adequate information about the pharmacology and toxicology both in animals or in vitro, and that allows a sponsor to conclude that it is reasonably safe to conduct a proposed clinical investigation. There are a lot of details that go into this, and I'll talk a little bit more about that. Those are the goals of the pre-clinical phase.

In the pre-clinical program, one of the

challenges is picking a relevant animal species and model and relevant in vitro studies, and I think Irv gave some really nice examples of how one can go about that. It's not always so straightforward, but that is an important and challenging part of the science that supports product development.

In these animal studies, both the pharmacology and the safety are important. In the end, again, if we think at the end of this that it's reasonable to proceed to clinical trials, these pre-clinical studies, if they point to an acceptable risk/benefit profile, then we can go to clinical studies.

The bottom line for pre-clinical studies is follow the science, and what we really need to know is what is the scientific rationale that helps us look at this risk/benefit profile. We want through pre-clinical studies to identify a biologically effective cell dose range, and we want to collect safety data associated with the route of administration, the dose level range, and

the formulation.

It is also nice to know and important perhaps more later in product development to start understanding the mechanisms of action and look at things like real time and quantitative cell tracking, but the real issue is does that first submission for the first clinical trial, the IND phase one, contain sufficient information to assess the risks to the subjects.

These are important scientific endeavors. The stronger the science, the more likely things will proceed as planned.

Now I'm going to switch a little bit to product testing and development, what we often and those in the industry call "CMC" or chemistry, manufacturing, and controls. The goals of this are to ensure product safety and ensure consistency of process and product, and that can be a challenge.

Ideally, predicted in vitro activity, and this is a challenge, more of a challenge for some of our products than for others, and I'm

going to be talking about some research that we have done in that arena in just a few minutes.

Product testing really should be guided by a detailed understanding of the manufacturing process and the product, and this is what we term generally as "characterization." Again, strong science is what is needed here.

I mentioned this kind of life cycle approach in the different phases, and we need to know at the beginning what are you measuring to say the product is what you say it is as an identity, and then quality includes things like what characteristics must the product have or lack -- my timing is taking over my slides.

This includes microbiological safety and product specific safety, things like remnant undifferentiated cells that Irv mentioned in his talk, are important things to consider for stem cell based products. Purity, does the product contain appropriate cell populations.

Often times, one of the things we say and emphasize in our early interactions with

sponsors is we don't necessarily know that you need one cell type to get the kind of clinical effect that you want. That could be the case for some products. It could be different for others. This does say the appropriate cell populations.

Strength, how much will you dose. You want to know that sort of going in or propose those things. Then at the end, you really have to know about identity, microbiological safety, and potency becomes a challenging issue for cell based therapies and purity.

Presumably at the end, you should know what cells or cell types you want in your product to get the kind of effect that you want.

What gets you into the clinical trials at the beginning, if you don't continually try to understand your product, at least in some cases without an iterative understanding and approach to developing the science behind your product, you might not know how to characterize your product to lead to success. I am going to illustrate that a little later. Ideally, the testing should predict

the product performance in vivo.

Now I'm going to switch to multipotent stromal cells, MSCs, and talk about a regulatory science project that we have been doing here in FDA labs.

We first started with kind of a survey of what we actually see in regulatory applications that come into our office. This resulted in a publication, MSC-Based Product Characterization for Clinical Trials: An FDA Perspective. I won't go through that whole paper.

In the end, what people call MSCs is quite diverse in terms of what people characterize, how they manufacture them, what source they take them from. The question really is what are quality attributes that would lead you to the kind of product understanding I've been talking about, and what is the relationship to what people are measuring to performance in clinical trials, and those are open questions.

People often have a concept in mind when they talk about mesenchymal stem cells, that they

are anti- inflammatory, they can undergo tri-lineage differentiation. The question is when they really isolate these cells and characterize them the way they do, do they maintain those properties that they kind of start out with as their concept of how they are going to work.

So, the question is once you actually take these cells and manufacture them, and you can measure things about them, but does what you measure -- how does that relate to their biological properties and how does that relate potentially to outcomes in the clinic.

It is an important question. This is just a hypothetical but it's based on a lot of observation on our end in seeing the information in clinical trials, but it is a hypothetical clinical response. There is a Y axis, and comparing a control group to an active group, you might look at this data and say there is tantalizing evidence that there is clinical effect. Clearly, some response and some non-response.

The question is, is that due to heterogeneity of the product or is it due to heterogeneity of the patients, or a little bit of both. So, advancing our knowledge in those arenas might really help us to understand how you measure the effect of products, and if you can do that, you might figure out ways to manufacture differently, enrich for, or somehow identify the cells that lead to the clinical success and focus on those.

We have undertaken a regulatory science research project which we call our MSC Consortium, and we started with bone marrow derived MSCs as a proof of concept project. Our goal is to develop strategies to determine identity/potency assays that predict safety and effectiveness. That is a rather ambitious goal, but we thought the kind of studies that we are doing there could be broadly applicable to a good number of cell therapy type products.

What we did was purchase MSCs from commercial sources. These are the lines, variety

of ages from 22 to 47, male and female, and cryopreserved or analyze them at passages 3, 5, and 7. This was based on say five or six years ago, kind of common schemes of manufacturing for MSC type products.

We did this manufacturing in my lab. This was the overarching approach. We applied genomics, single cell PCR, and qRT-PCR in the Moos Lab, genomics in the Puri Lab, proteomics in the Alterman Lab, in the Hurst Lab, epigenetics and karyotypic analysis, high throughput.

We are looking to see if we can correlate any kind of product signals that might come out of these molecular analyses with in vitro and in vivo bioassay systems, and the McCright Lab is looking at in vivo and in vitro models of wound repair. My lab was looking at in vitro quantitative differentiation assays, and the Wei Lab was looking at in vitro and in vivo immunosuppression.

In my lab, we published several papers looking at things like low hanging fruit

adipogenesis and showing you could measure the progenitors, adipogenic progenitors in these MSCs, and very interestingly, you could see that if you took the MSCs from different donors, different capacities to make adipocytes out of MSCs, and then that capacity would diminish with passage. These were surprising based on other people's literature, but we did it for all eight of these lines in a very systematic way, and reproducibly quantitative.

We also observed the colony forming unit activity decreased for all of these during passage, and the size of the cells increased for all these cell line passage. I won't show you that data.

Clearly, when you take the cells out and under the conditions that we used and the sources of cells that we used, taking them out of the bone marrow environment, manufacturing them had clear effects on differentiation capacity, morphology, stemness, that is used in the field.

I'm going to talk now about a recent

publication. I mentioned a second ago that we saw increases in size in all these cells, so the morphology was definitely changing, and it changed differently for different cell lines, and we thought maybe we could take advantage of that by doing kind of a high throughput morphological assessment of these cells.

We chose Xylenol Orange staining and nuclear staining to kind of assess whether or not we were seeing what some people say is an important aspect of osteogenic activity. I know it's not necessarily 100 percent correlation, but this is a commonly used assay in the literature, but what we did was assess the morphology at day three after osteogenic stimulation and compared it to just growth at day three, and then did all these morphological signature collection, and then subjected that to a principal component analysis, and then compared that to the 35 day kind of standard osteogenic conduction assay with mineralization as the output.

We were able to show that two models --

you can take subsets of the measurements that you use and subject them to models, and we developed models from those, or even look at single parameters, but we were able to show that two models predict mineralization with 92 percent accuracy.

This was done automated, high throughput, three days rather than 35 days. It is sort of getting towards this goal of making predictive measurements about cell characterization that have some either in vitro or in vivo correlate. Of course, in vivo would be better. We are working towards that.

We did this with an original set of cells that we learned upon and then we applied it to other cell types manufactured with different serum concentrations, drugs, and so on, and were able to show that this model still had that predictive power.

One thing I wanted to point out also is that if you look at the cell surface markers that we saw in those INDs and that are kind of

community consensus markers for what people call MSCs, I talked about the biological heterogeneity of these cells.

This is an illustration, if you look at passage 3, 5, and 7 for all the cell lines with all of these different markers, you really don't see differences. Using these kinds of approaches as quality attributes might not give you the information that you want with some kind of correlation with biological activity.

This MSC Consortium, we have shown that the consensus MSC markers don't correlate with functional heterogeneity that we were able to assess quantitatively. They're not responsive to donor or tissue culture age differences.

We have had some success developing assays to identify and qualify predictive product characteristics, and we are publishing findings. I've been talking about what came out of my laboratory. I'll show you in a minute the publications that have come out of this group.

The potential applications are you might

be able to identify differences between MSC samples and work towards that goal I showed you earlier with the hypothetical clinical outcome thing.

Optimizing your manufacturing, figuring out ways to differentiate between cells that work and don't work, so you can evaluate the impact of tissue culture conditions in duration. You can correlate with other characteristics of MSCs. You might be able to guide purification techniques to help understand mechanisms controlling stem cell differentiation and function, and that is kind of an interesting biology behind this.

I'm not going to read all these papers. They will be available in the slides that you will get. There was a sector review. I mentioned three different quantitative assays. Four papers on proteomics, a paper on immunomodulation, some outcomes of the genomic studies, and seeing some predictive markers for proliferation and senescence, and then genetic and epigenetic stability studies that have come out of this

group. It's been the eight different labs I showed you at the beginning.

In summary, you heard in the first talk and in my talk about the importance of scientific evidence, crucial for development of these 351 HCT/Ps. That applies to product and pre-clinical. I think we will hear more about that, these other disciplines, later today.

The regulatory framework in current science allows development of these complex novel products. We have licensed products using this paradigm that I have been talking to you about and look forward to doing more of that.

The science continues to evolve. It is a challenge for all of us. It is important to keep our eye on those targets.

This is contact information for myself, and regulatory questions can be submitted. We have a wonderful Web based webinar series called "OCTGT Learn" for anybody who wants to learn more about regulatory framework, then there is a lot of information available on our Web site. You can

contact the Consumer Affairs Branch or Manufacturers Assistance and Technical Training Branch if you want. These are the places you can find those resources.

Thank you. (Applause)

DR. BAUER: We are going to move on to Session 2, and that session is about experiences in product development.

I didn't intend to take any questions at this time, but if you want to talk to me during the breaks about the research part of this, I'd be happy to do so.

I'd like to ask Dr. Galipeau to come up. We are going to start Session 2: Experiences in Product Development.

Jacques is a Professor of Hematology and Medical Oncology at the University of Wisconsin at Madison, relatively new, last week. As of September 1, he became the Inaugural Director of the University of Wisconsin Advanced Cell Therapy Program, and Assistant Dean for Therapeutics Discovery and Development.

His presentation today is entitled: How Mechanistic Studies on Mesenchymal Stromal Cells Inform Design of Human Clinical Trials for Autoimmune Ailments - The Fitness Paradigm. Thanks, Jacques.

DR. GALIPEAU: Steve, thanks for the invitation, the organizers, to speak today. I'm just going to jump straight into it.

Distinct from the hematopoietic stem cells that Irv spoke of, mesenchymal stromal cells are rare in bone marrow, maybe one out of a million of nucleated cells in the marrow, and probably the best reductionist marker that identifies the MSCs.

These cells in life and in all of you play an important role as nurse niche cells to allow Irv's stem cells to survive long term in the bone marrow space, but they also play another role. They are sort of cops for the immune system in the bone marrow. They play an important role in regulating how lymphocytes come in and out, and they can participate in tissue injury repair.

We now know a lot about how these cells tick at least in regards to modulatingthe immune response. When you tickle MSCs with Interferon gamma, that is how they sort of sense in their environment there is inflammation going on, the human cells will massively up regulate an enzyme in their cytoplasm called a IDO, it converts an amino acid, and this small molecule blocks T cells and also affects monocytes which are other cells that circulate in your body.

Now, when you add Interferon Gamma, you also have to regulate PD-L1, but also other genes that are expressed, COX2, this is something that is inhibited if you take Aleve, IL-6, HGF, and these talk a lot to the monocytes, and they secondarily, the monocytes, which are very abundant, the MSCs are quite rare, the monocytes in your blood are very abundant, start making buckets of IL-10, and IL-10 is a profound immune suppressant.

MSCs on their own, they are sort of the match that lights the gasoline that leads to the

systemic anti- inflammatory effect, and because of this, MSCs have been developed very robustly as a method to hose down over exuberant inflammatory disorders.

The reason MSCs are so popular as opposed to say skin fibroblasts or something else -- again, I'm a hematologist. You can take a little bit of bone marrow, local anesthesia, not a big deal, and as little as a couple of tablespoons of bone marrow, you can grow in the lab a gram of your tissue. You can grow in industry up to a kilogram of these MSCs because of their big proliferative potential.

Because of this property, everybody basically was saying so, here's a solution, what are the problems we can fix with it, because I can make a kilo of Irv's MSCs. That being said, you can grow them in the lab and you can harvest them. I'm going to dwell upon immune modulation, not talk about regenerative medicine.

In immune modulation, really the Eureka moment was an anecdotal case report made by

Katarina LeBlanc at the Karolinska Institute in Sweden, where a young boy had a bone marrow transplant. He had leukemia. He developed graft versus host disease. You get the diarrhea which is the white spots [graph on slide], you get liver dysfunction, that is a bilirubin [graph on slide], and the young boy was dying of graft versus host disease.

What Katarina did, she took marrow from the boy's mom and gave the boy mom's MSCs i.v.. Here is a dose here. You see the liver dysfunction and diarrhea went away, it came back, another dose of mom's cells, went away, stayed away.

Kids that have this die, it is as simple as that. Adults that have this die quicker. This was just an incredible Lazarus event that this was really like, you know, the face of Helen launched 1,000 ships, so there were a bunch of clinical trials done in academic centers in Europe that were seeing actually very exciting phase 2 -- the biggest one was the top one here, 55 patients.

Response rates, maybe 7 out of 10 patients were getting MSCs, not from their mom's but somebody else, were getting clinical response for their graft versus host disease, which was basically killing them. Multiple studies, virtually all of them in academic health centers in Europe.

Now, the logical next step after some development like this is deployment. A company based in the U.S., Osiris, started making MSCs, and this is from their Web site, from one donor -- I told you that you could generate a kilo, I was not kidding. They say they can manufacture 10,000 doses from a single donor.

Please note that the European studies are exciting, never manufactured more than 10 doses per donor. Remember that as we move forward.

They went ahead and they did a clinical trial, graft versus host disease, prospective randomized trial, placebo controlled, in the U.S., the Osiris product, it did not work in their hands, giving steroids only, which is the only

treatment that works, there is no FDA approved treatment for graft versus host disease other than steroids. It was no better in their hands.

This was presented as a press release and in a poster, the paper has not yet been published. This is in 2009.

What I haven't told you is there are literally thousands of papers demonstrating that mesenchymal stromal cells in mice will improve just about any inflammatory tissue injury anyone can think of, unimpeachable data published in top journals from Nature Medicine down. Yet, you go into people, and it don't work. Why is that so?

Do you just walk away and move on or do you try to figure it out? We are university based scholars, we tried to figure it out. Here are a couple of things that we felt, our group, would be playing an important role in why we could improve things.

I want to dwell upon my favorite, which is cryopreservation. If there is one thing you have to remember this morning, stem cells are like

sushi, fresh is best. Why is that so? Usually, I have a bunch of jokes at this point, since I have only 15 minutes, I won't. I can give you the jokes afterwards.

To come back to my point, the scientific data using mice or sheep or monkeys, if you can afford it, almost universally are positive, it always works. Nearly all the MSCs that are being used are syngeneic. That is a technical term meaning because all these mice are inbred, so they are like clones, if you take marrow from one mouse and give it to the other, it is like giving the marrow from the same mouse. They are all syngeneic.

Virtually all the studies used fresh cells, straight out of the culture. They don't put cells in their freezer and then thaw them in the vast majority of studies. The bulk, always, always fresh cells directly from culture.

As in human clinical trials, MSCs, nearly all the industrial studies, 100 percent of the industry studies use allogeneic MSCs, not

their own, somebody else's. Academic studies are about 50/50, your own or somebody else's.

Virtually the majority of studies utilized frozen cells, and the way the frozen cells are delivered is as follows: you take the frozen cells out of the freezer, you put them in a vat of warm water, you take that out, and sometimes the cells are washed or not, and it is given to a patient within four hours of thawing.

It's not that the cells are put back in culture and allowed to recover emotionally, like the astronauts in the movie Alien, none of that. They are given right after thawing. All those animal studies I spoke of don't do that. They put the cells back in culture.

The question is is thawed allogeneic the same as fresh. When you thaw cells, there's dead cells, and that's standard, maybe up to 30 percent are dead, and that's fine, versus live cells, a little bit of dead cells, Trypan Blue really doesn't detect that and Annexin PI does, and what we found was -- this is a T cell proliferation

assay. These T cells are growing in a petri dish after you tickle them. If you add live MSCs, you block that proliferation. That is a standard sort of assay, but if you take cells straight out of the freezer, you thaw them, you put them on top, don't work no more.

This is just different ratios of MSCs and T cells. It's not that the thawed cells don't work at all, they will, but their effect is markedly blunted. If your end point is to achieve statistical significance for effect, this is something that will let you down.

We looked at human MSCs from different subjects, and what we found was -- this is again T cell proliferation, you add cells that were in culture for a week, MSCs, you block T cell proliferation, cells straight out of the freezer, they don't work no more, no better than spit in our hands, but if you put those exact same cells in culture for at least a day, they fully recover all their potential to block suppression.

It's not that they are permanently

screwed up post-op, they just have to recover emotionally from the thaw.

Why is that so? When you take cells straight out of the freezer and you tickle them with Interferon Gamma, they cannot up regulate the enzyme I spoke of, whereas after a day of culture rescue, they can up regulate. Why is that so?

Interferon Gamma leads to phosphorylation of STAT-1, and leads to the Interferon Gamma effect. What we found was that cells straight out of the freezer cannot -- the protein is there, the receptor is there, the cells can't respond. Why is that so? When you thaw cells, this is three different volunteers, and we looked at the heat shock proteins. The cells undergo a heat shock response. Heat shock proteins are to metabolism what P53 is to DNA.

This was described actually a quarter of a century ago, a zillion years ago, when you take cells out of the freezer, they undergo a heat shock. It is called freezer burn effect. It is reversible. These heat shock proteins, their job

in life is to tell the cell stop everything, let's survive this insult, and then we'll move on.

What about homing of the freezer burnt cells? We took human cells from the same donor, froze/thaw them versus cells that were live, and injected them in the tail vein of a mouse, and when you inject in the tail vein, all the cells wound up in the lung.

We asked can we detect human cells in the lung of mice the day after we inject them, if you take live cells, we detect the cells, this is PCR signal, so it is counterintuitive, so yes, but the thawed cells from the same human donor injected in the mice, we could not detect any of the cells, zero, in their lungs after 24 hours. So, there is an accelerated clearance of thawed cells.

Why is that so? MSCs are different from hematopoietic cells because they have a skeleton, it is called a cytoskeleton, that is how they stick to things, and when you thaw cells, it completely melts down their skeleton. This

skeleton spontaneously refurbishes itself, but it takes 24 hours, and we have shown that for thawed cells their cytoskeleton is markedly impeded, so the structural integrity of the cells is messed up post-thaw, and you can replicate this by using a drug, Cytochalasin D, and although these cells are both live, those with the busted up skeleton will not biodistribute.

Again, showing this has nothing to do with function and phenotype, this is structure of the cell. You can only anticipate this by putting this in a mouse in vivo.

We are not the only ones that say thawing is not a good thing. Again, Katarina's group showed MSCs thawed, if you put whole blood on it, it generates blood clots, and they are susceptible to complement mediated - complement is the protein in your blood-- supposed to attack bacteria. If you put that on thawed cells, it lyses the cells.

What we found most recently, that we just published, is if you take the thawed cells

and you add T cells on top, which is a standard assay, the T cells start destroying the thawed cells as opposed to live cells, which are not destroyed. Even autologous cells can get destroyed.

If the T cells and the MSCs are mismatched, the T cells react to them and lyse them in vitro. If you put activated T cells, and there is a shield between them and the thawed cells, the thawed cells are protected. It is only if there is direct contact.

We went on to show T cells that have direct contact with thawed cells kill them. Thawed MSCs, they undergo a heat shock, they are susceptible to killing, they are susceptible to complement, increase coagulation, you have abnormal membranes, abnormal cytoskeleton, but otherwise, they are okay. (Laughter)

If you put them back in culture, all this gets fixed. Don't forget, this is what is done in mice, this is what is done in people.

I think fresh is best but fit is fine,

to come back to that.

How about those 10,000 doses? I told you all that exciting initial data that was done out of Europe. They were using MSCs generating no more than 5 to 10 doses per volunteer donor versus the industrial studies.

A massively expanded product, is it the same as a non-expanded product, which comes back to the presentation that Steve gave earlier. When you split cells over time, it is basically an experiment of an accelerated agent in a petri dish, and we saw these cells stop growing. They become big and fat, that is a characteristic of senescence, and these senescent cells have a phenotype that is identical to non-senescent cells, again to Steve's point that the phenotype is good for identity but not for functionality.

We found that senescent cells actually were unable to block T cell proliferation. So, there is a clear functional defect.

The last point that I want to raise in my closing minutes is clinical trial design.

Steve pointed out there may be things that are wrong with the cells, there could be, but there are also aspects of who are the patients you enroll in these studies because you want to give the cells to patients that you think have a good likelihood of responding and avoid giving it to patients that won't respond.

If you look at the negative Osiris trial for graft versus host disease, and Osiris was acquired by Mesoblast, which is a public company traded out of Australia that is now doing an ongoing phase 3 study of MSCs for pediatric steroid resistant GVHD in the U.S., and they have learned, although they haven't published their study, that their primary end point includes not only a CR day 28 but a partial response.

We also know that kids do better than adults. Restricting the enrollment to children and young adults as opposed to the Orisis study who went to older adults is a bias towards their being able to observe a positive effect, and they are also excluding people that had mild GVH

because GVH can affect the skin only, and those typically can do quite well on steroids on their own, so by excluding those, you are going to increase the likelihood of getting a delta with your control group, and they were also more demanding. You had to be sicker. You had to have at least bowel or liver involvement, and you had to be getting worse on steroids to be enrolled, and they were also doing it at a single site.

I think they designed the clinical trial, but they are using the same product, the frozen/thawed, but some don't work. I just think in the setting it is not optimal. They are always taking a chance they may not meet their primary point, but the way it is set up, who knows. Hopefully, it will move forward.

Compatibility is also a big deal. I could take Irv's MSCs and give them to everybody in the room and that is perfectly fine because they are magically immune privileged. I think that is not true.

In the setting of bone marrow

transplant, if you give -- MSCs are analogous to mice under MSC transplant, the engraftment of the donor hematopoietic cells goes up, but if you use MSCs derived from the hematopoietic stem cell donor, you make the outcome worse, so immunology matters, the source of the MSCs and the immune typing and compatibility with the recipient is a big deal.

Are human thawed heat shocked senescent allogeneic the same as mouse and genetically fit, I don't think so. Learning from this informs what we can do to improve the outcomes. If your Uncle Bill has a heart attack or a stroke, you don't have two weeks to grow his MSCs, because the outcome is going to play itself out in the first seven days. Allogeneic is the way to go but for many ailments, chronic inflammatory ailments, autologous makes sense, low passage meaning two weeks of culture, not three months of culture, fitness, and also clinical trial, rational selection of subjects based on biomarkers.

To Irv's point, academic health centers,

not for profit, historically played a role in development. I propose to this audience that these same centers need to play an active role in deployment, so we as academic health centers have made available bone marrow transplants for almost 40 years because it works, and it has never been developed by industry, industry is very, very good at developing certain types of platforms, and for reasons unrelated to effectiveness or outcomes may not be able to effectively deploy other platforms such as this like we can do in academic health centers.

A short and narrow pathway to make that feasible, I think, would be really useful. A lot of data was generated when I was at Emory, I was there for seven years, prior to my moving to Wisconsin just last week.

Thank you for your attention.

(Applause) Now, I wasn't planning to take any questions at this point because Steve just gave us 20 minutes to speak, and being very vocal speakers,

we figured the panel at the end would be when questions would come through, so I'm just going to do a segue right now, if you can put Dr. Matthay's presentation up, and introduce Michael Matthay, who is a Professor of Medicine and Anesthesia at UCSF, and will be talking to us about his use of MSCs in acute respiratory distress syndrome. Mike? *MESENCHYMAL STEM CELLS FOR TREATMENT OF ARDS PATIENTS: CHALLENGES AND LESSONS LEARNED IN PRE-CLINICAL TESTING, FDA APPROVAL, AND ONGOING CLINICAL TRIAL

DR. MATTHAY: Thank you, Jacques. Thank you very much for the invitation. I'm delighted. I'd like to endorse what Jacques just said.

I believe very much in NIH and related support for this field, just echo Jacques, what you just said, I was going to ask you, but in the interest of time won't, we don't know the details of the Prochymal product from Osiris, it's not published. FDA, of course, can't reveal that to us. We don't know how they were modified. We don't know how many passages they went through.

There are many issues there in all the private world of how MSCs are managed. I couldn't endorse the points you made more.

In terms of disclosures, I have no conflicts. Basic science and clinical grants from NIH and FDA, and research grants, two of them from industry that are not in conflict.

What I'd like to do briefly is talk about MSCs and the reason for our interest in their relevance for acute respiratory distress syndrome. The source of the MSCs, which I'll give a little more detail on particularly in view of Jacques' important remarks. The pre-clinical data for efficacy that we worked on, which as you will see advanced well beyond the mouse, and the issues related for testing safety, which I think are extremely important, and where we stand with the phase 1 and 2 clinical trials, and some conclusions.

Now, everyone knows that MSCs were discovered by Dr. Friedenstein when the Russian government compelled him to collect bone marrow

from volunteers in case of a Chernobyl or nuclear war, but he discovered/noticed that these cells that he thought were basically fibroblast contamination were really part of the stromal cells of the bone marrow, and subsequently they have been described in placenta, cord blood, adipose tissue, and other organs.

They do not engraft, almost for sure. They could enhance proliferation of stem cell niches. That is why the term "mesenchymal stem cells" is misleading. They are really mesenchymal stromal cells.

They do not normally express Class I or II antigens, although that can be modified by the presence of Gamma Interferon, so in general, allogeneic preparations are well tolerated in humans as far as we know.

This shows you our clinical focus for MSCs, which is ARDS, which is a syndrome of non-cardiogenic pulmonary edema where the patient is hypoxemic because of the edema fluid. This is not explained by heart failure, and the usual

causes are pneumonia, sepsis, aspiration, major trauma, effects over 200,000 patients a year in the United States, including the large pediatric population.

We have made progress in treating this syndrome with a lung protective ventilation strategy, but we have no other specific treatments.

Our experience with translating human MSCs for clinical trials began with mouse experiments, and as I am going to show you, we tried to advance beyond the limitations of mouse studies by using ex vivo perfused human lung studies, some rat studies and some sheep studies that FDA -- thank you -- recommended that we do, and I will show you how important they were in helping us and address some of the key points that Jacques made.

Then our IND preparation submission approval, funding from the NHLBI, and a phase 1 trial which is done, and our current phase 2-A trial, which I will update you on.

Here is a diagram just to very briefly tell you the problem in lung injury from ARDS. It relates to an increase in capillary permeability and epithelial permeability, and the influx of protein rich edema fluid into the airspace of the lung with several lines of inflammatory cells.

This slide shows you on this side the normal air filled alveolus with Surfactin that keeps the alveolus expanded.

The rationale for considering MSCs for treating ARDS are multiple. The anti-inflammatory effects, but perhaps more importantly their ability to restore endothelial and epithelial barrier integrity. As I will show you, they enhance the clearance of alveolar edema fluid. We discovered serendipitously that these cells have marked antimicrobial properties, which I think is a very interesting point and very clinically relevant. They do inhibit apoptosis, and there are both cell contact dependent and independent effects.

In our initial mouse studies, we found a

dramatic effect on reducing lung injury when we gave the syngeneic bone marrow mouse cells intratracheal to mice after a high dose endotoxin injury. You can see the marked improvement. This was reflected by better survival, less lung injury, and less edema.

In subsequent studies where we gave live bacteria, a more relevant model for human lung injury, we found the MSCs worked whether given intravenously or IT. They worked in comparison to appropriate controls with fibroblasts as well as PBS. We blinded the investigators doing the study so there couldn't be bias, and we actually gave, as other investigators have, human MSCs, as well as the mouse MSCs from Dr. Prokop's NIH repository.

I'm going to move on to tell you what we then did to try to really see if these cells might be effective for human lung injury. Our laboratory at the University of California, San Francisco, receives about 60 pairs of human lungs a year from the Northern California Transplant

Donor Network. These are lungs that are not used for transplant. Only 20 percent of lungs from brain dead donors are used for transplant. Most of these lungs are actually not very severely injured.

We studied these lungs, both one, isolating cells, and also doing a perfused human lung model, and for these studies, we actually add fresh blood to the perfusate to make it more clinically relevant, and the lungs are perfused at normal pressures, and the lungs are kept inflated with 95 percent oxygen, 5 percent CO2.

In the initial studies, we used high dose endotoxin as in the mice, to injure the lungs, then we used bacteria. What we found in the initial studies was the remarkable ability of the MSCs given intratracheal or intravenously one hour after the endotoxin injury to reduce endothelial permeability which had been markedly increased by endotoxin back to a normal level. Also, the pulmonary edema that was produced by endotoxin was returned to a normal level, and even

the conditioned media had a beneficial effect.

Now, the other property that I alluded to that is very important for the lung is the ability to remove edema fluid from airspaces. This is driven by active sodium transport, a process we described about 20 years ago, and in the presence of endotoxin, alveolar fluid clearance, this goes to zero. Fibroblast did nothing, but MSCs of the conditioned media restored it to near normal, which explains why the edema fluid was decreased.

Now, in these studies, we used initially cultured MSCs, as Jacques had said is often the case. Then we used cryopreserved MSCs, which had been thawed, which I will tell you about.

At this point we were thinking about translating the therapy, and we had various fora's and discussions with industry about a source of MSCs, but for a variety of reasons, I was not satisfied with their candor, so we linked up with Dave McKenna at the NIH Repository at the University of Minnesota, and began to use his

clinical grade MSCs.

In the human lung studies, we took the cryopreserved MSCs and thawed them, and we centrifuged them, and we removed all of the supernatant, which has the DMS cell, which I'm going to come back to in a minute, as well as the dead cells, and then re-suspended them.

At that point, we repeated all the prior experiments with the chemical grade MSCs, with endotoxin or live bacteria, and what you see here is with intrabronchial or intravenous MSCs, the alveolar fluid clearance with E.coli injury was restored to three-quarters of normal, and furthermore, the anti-inflammatory effect on neutrophils was achieved, again giving the cells in the perfused or intrabronchial one hour after the injury. We extended these studies so we had a lag time of two hours as well.

We had found in the mouse, as I alluded to in the beginning, that these cells had remarkable anti-bacterial effects. The MSC treated mice with no bacteria had a markedly lower

number of bacteria. We found this was due to the release of antimicrobial peptide LL-37, one that we all have, and by increased monocyte phagocytosis, but we wanted to see if this would be replicated in the human system, and indeed, we found again the MSCs had very strong anti-bacterial effects related to increased monocyte phagocytosis.

I think this is part of their evolutionary adaptation, probably in the history of evolution, the greatest threat to the bone marrow was infection, and these MSCs probably evolved as part of their properties to protect the hematopoietic elements with these antimicrobial properties.

Other people have confirmed this antimicrobial properties, and it is an area of quite a bit of scientific interest.

In the interest of time, I won't go through the detailed mechanisms that have been identified by our group and many others for how they work, but the release of paracrine

molecules, such as fibroblast growth factor 7 or KGF, and in our more recent work, the pro-resolving lipid, Lipoxin A4, shows that they can release factors that beneficially affect the injured epithelium or endothelium.

They also release mitochondria, which in a very nice Nature Medicine paper Dr. Bhattacharya's group showed can be transferred to the injured epithelium and restore the bioenergetics of the injured epithelium resulting in better function, and also they release microvesicles, which turns out can enter in macrophages in epithelial cells. It is quite interesting, the different pathways by which they work.

Finally, FDA said to me, Dr. Matthay, wouldn't you feel better if we had a large animal model before you introduce these MSCs into very ill, critically ill patients with ARDS. I said thank you, you're right. I appreciate the suggestion.

We were fortunate to in fact partner

with Dr. Dan Traber, the late Dr. Traber, and Dr. Enkhbaatar, and do some sheep studies at the University of Texas. These sheep studies were 24 hour studies where severe pneumonia and sepsis with pseudomonas aeruginosa, and we decided in concert with the FDA's excellent advice to give the MSCs exactly as we planned in the clinical setting.

We shipped the cryopreserved MSCs from the University of Minnesota. We thawed them. We centrifuged them. We again removed the DSMO and the cell debris and re- suspended them in PlasmaLyte, and gave them in that way.

I'll just show you very quickly. The first issue, of course, is always safety. We used two different doses, 5 and 10 million MSCs per kilogram. What I'm showing you here is for systemic blood pressure after the severe pneumonia and sepsis, the cells were given one hour later, there were no adverse effects on systemic blood pressure. In fact, at the end of the day, the higher dose was associated with a better blood

pressure than the control.

The biggest issue would be pulmonary arterial pressure. As an ICU physician my entire academic life, we are always worried about pulmonary hypertension. While we hoped for a therapeutic benefit here, the risk would be that if you give these cells into an injured pulmonary microcirculation, maybe there would be a transient rise in PA pressure and the right heart could fail. That was what FDA was concerned about, it is what we were concerned about.

We gave the cells. You can see here, the blue is the control, the red is the higher dose, and at the end of 24

hours, actually the PA pressure was lower, fortunately, in the sheep treated with either the lower or the higher dose of MSCs.

Briefly, from an efficacy standpoint, we measured oxygenation like we would in patients. Here the control sheep given PlasmaLyte, you see this big drop in oxygenation reflected by what we call the PaO2-FiO2 ratio, so this is severe

pulmonary edema, goes all the way down to 100, so this is very severe respiratory failure. The sheep are ventilated and managed like a severely ill patient in the ICU, and here are the two doses of MSCs.

I think you can get the impression that oxygenation is better statistically at the end of 24 hours. It was better. We gave 10 million cells, because FDA said look, you're planning on giving 5 million cells, Dr. Matthay, we want you to double the dose for safety.

Well, that turned out to be extremely helpful, again, thank you to the FDA. We found out when we measured the lung water, that it was the higher dose which had a more beneficial effect on the quantity of pulmonary edema. That is the dose we selected for our trial.

When you thaw these cells, as Jacques alluded to, about 65 to 70 percent of the cells are viable. That is what we found with Trypan Blue exclusion. You are certainly not giving 10 million cells per kilogram, you are probably

giving 60 to 65 percent of that in terms of live cells.

One other key point I have shown in these slides. When we did a few experiments in which we did it the way Osiris did, and almost every company in the field, where we just thawed the cells and gave the cells with DMSO, no effect, all the therapeutic effect was lost. We shared that with FDA, and we talked about the long history of how DMSO can be a problem, not in hematopoietic cell transplant but under acute conditions. We learned that, thank you again to the FDA's directions.

Finally, the clinical trial called START being run at Mass General in Boston, Pittsburgh, Stanford, Ohio State, and of course, the home site for us is UCSF. The phase 1 trial design was to test standard dose escalation, three patients at 1 million, three patients at 5 million, and three patients at 10 million cells, the target dose.

The enrollment criteria was moderate to severe ARDS, defined by a P/F less than 200 on a

PEEP of 8, within 96

hours of developing ARDS. We excluded patients with moderate to severe liver disease, treatment for cancer in the prior two years because there are still questions and concerns that somehow the growth factors are other features of the cells might enhance tumor formation, chronic lung disease, pulmonary hypertension, and children.

We very carefully identified with FDA's input what would be pre-specified adverse events, hemodynamic and respiratory. One of us, the physicians, is at the bedside always during the two hour baseline period and in the six hours afterwards. We don't leave the bedside. There is a coordinator, but one of the M.D. physicians, usually myself, is there.

We had secondary endpoints for respiratory, systemic, mortality, and biologic markers. Of course, we are underpowered for that in a phase 2-A design. The phase 1

results, fortunately, showed no safety

issues whatever, so we went on to the phase 2 trial.

We enrolled one last week, 51 of the 60 scheduled patients. The trial is 60 patients with two to one randomization, 40 patients to receive 10 million MSCs, and the other 20 PlasmaLyte.

The safety endpoints are still primary because in this field, we are very underpowered for efficacy with only 60

patients. In conclusion, I would say the pre-clinical

studies in several models, including the human lung and the sheep, support the rationale for testing MSCs in moderate to severe ARDS, in my opinion, and an NIH or equivalent source of MSCs is optimal. I'm very concerned about private sources of MSCs where it is not in the public domain what the passages were, how the MSCs were modified so the company could achieve intellectual property.

I'm far more comfortable with an open NIH like support, and I would like to see that

supported more. I think it would help the field.

There is no question that our consultation and input from the FDA has always been extremely helpful. I couldn't also agree more that studying the biology is critical. It is only in the last two years, for example, that we discovered these cells have the remarkable ability to generate the pro-resolving lipids like Lipoxin A4, resolving D1, a whole field developed by Charlie Serhan at Brigham, which I think is extremely important, and these lipid products, very important.

It is one of those areas where the pair of clinical trials and ongoing lab research is critical. We will see what we learn from testing safety and efficacy of the MSCs, and of course, all this work is being done by a group of investigators, both at UCSF, Dr. McKenna at the University of Minnesota, and the late Dan Traber, and I can't thank enough the investigators at Stanford, MGH, Pittsburgh, and Ohio State for their participation in the trial, the NHLBI, the

DSMB, and of course, most importantly perhaps, the patients who consented to be part of our clinical trials, and the ICU nursing and respiratory care staff.

Thank you. (Applause)

DR. MATTHAY: Do we have a break now?

DR. ANATOL: We are going to try to get back on time, so we will have our break and we will come back at 10:40.

(Recess)

DR. GALIPEAU: Okay. So again, the format is going to be 20-minute presentations. We're going to hold questions for the panel discussion that will follow at 11:40-ish.

It's a pleasure to introduce Greg Russotti. Dr. Russotti is vice president of Tech Operations at Celgene in Warren, New Jersey. Plays a role in CMC, and he'll be talking to us about drivers of methodology for making the cell process changes.

Greg?

MR. RUSSOTTI: Thanks, Jacques. And

thank you to the FDA for the invite. It's a pleasure to be here today.

So I'll talk about some of the approaches we've taken to making process changes within one of our cell therapy manufacturing platforms. And at Celgene, there are several different cell therapies we're interested in, and there's two that are currently in clinical trials. One is a mesenchymal-like cell product derived from placenta called PDA002. And it's given intramuscularly, and it's in trials of diabetic foot ulcers with peripheral arterial disease and diabetic peripheral neuropathy.

The other product currently in clinical trials is also placental-derived. These are natural killer cells. These are immune cells, and we have a trial currently ongoing in AML, acute myelogenous leukemia, and another trial for multiple myeloma about to start. But the examples I'll give today are really around the PDA002 product.

So let's just start by talking about why

you make process changes, and there's a variety of reasons. Some proactive, some reactive. Whenever you make process changes, the most important thing to remember is product quality, and safety comes first. Efficacy, if you have efficacy demonstrated, is important to maintain, of course. And process robustness. You want to make sure that you have a consistent product and a process that makes it consistently. Sometimes, and hopefully this can be proactive as you characterize your process as Dr. Bauer spoke about earlier, you want to characterize and get ahead of the curve. Sometimes it's reactive, solving a problem.

Invariably, capacity needs increase as you go through clinical trials, so if you have to make more product, you either do things like change a scale or change the way you make the product, changing the operations. And one of the examples I'll give today is about changing an operation.

Cost becomes a factor, and this is

really not just about making more money; this is about patient access. If you can't make a product at a reasonable cost, then you can't make a product.

So some of the things we focus on are reducing labor. A lot of the things you'll do initially to get into a trial involve processes that are very labor-intensive, and the one example I'll give today, the first example I'll give today is about reducing labor. Raw materials are often very expensive, so we can switch to less-expensive alternatives. That's always a good thing. And then you always look for opportunities to optimize your process. Again, getting something that's more consistent back to product quality.

So the challenge is that you understand more about your product as you march through the trial. So this is a timeline that shows starting trials, the initial pre- clinical studies, marching through ultimately towards commercial. And as you gather knowledge on analytics, knowing ways to better measure your product, as you

understand the product better, what makes it work, what keeps it safe, as you understand your process better, these things all increase as you go through the trials. Tracking and trending refers to the data you get as you make more and more product for that trial in a very consistent fashion. But the ability to make changes will certainly decrease because your process evolution should decrease as you march through the trials.

If you think about safety risks and risks to your efficacy of your product, both the regulatory concerns and the business concerns of changing a product increase as you march through the trial. So this is the challenge we face. We know more as we go along, but we're less apt to make changes as we go along.

So as we make changes, just to kind of -- this is really a summary of my whole talk really about how you go about doing it, and I'll give a couple of examples of how we've made a couple of changes in our placental-derived mesenchymal cell. So most importantly, you need

to understand your process. To do prude process characterization, the first thing you need to do is be able to define the output. What are the key product attributes they're trying to maintain? And then from there you want to be able to define acceptable ranges for your critical inputs, knowing that if I stay within these ranges I'll hit my outputs. That ultimately leads to process validation.

We have a group of bioengineers and biologists that comprise our process development group and they complement our analytical development and our production and QC groups, but the engineers really take this methodical approach to understand how the changes we make affect the product, and thinking about things like effective scale and other parameters, but ultimately, we want to come up with an acceptable design space that we know if we stay within this space, the product should look the same. And I say "should," and then we'll prove it next with our comparability tools. And there are a number of

tools listed here. I won't read these. These are things people typically look at and measure. And ultimately, you do want to look at some in vivo performance when making a big change before you go into clinical studies.

So at the end of the day, you want to be thinking about all the effects of the change in taking a risk-based approach. So what are the physical effects on the cells, on your product? What are the chemical effects on the cells? Thinking about the micro environment. And then other incidental things, like processing times. And people don't often think about these because you think about the most important things that affect a cell and the changes you make, but you don't realize that sometimes when you make a change, whole times may increase, transfer times of a product from one container to the next may increase. You need to be thinking about all these things.

So the analytical tools are very critical, and I'm not going to go over all the

different tools. This is just an illustrative slide that gives you an example of the things you might consider, but it's really important to think about what you want to measure. What change are you making, what might be affected, and what tools do you want to use? And you're not going to necessarily use every tool for every change, but you want to use the right tools. And how to measure those things is important, too. And there's a lot of choices there as well.

So let's dive into a couple of examples. And this is just a schematic of the process we use to make this PDA002 product, the placental-derived mesenchymal cell. We start with placental tissue. Do a primary culture after initial isolation. You'll see a two-tiered banking system with a master stock and a working stock. For those of you who are not that familiar, this is really just to allow the proliferative potential of the cells to be taken advantage of so you can get a large number of cells which you could never process in one batch, so you freeze down at these

intermediate points and have a consistent starting material for every batch you make.

As we march through the cell expansion, we do start in cell factories. We've moved towards this bioreactor approach growing cells on microcarriers, which can be suspended in a bioreactor, centrifuge the cells down to remove residuals and other wastes, add our DMSO at the latest possible moment, and then cryopreserve.

So the two areas that I'll focus on are a change to the bioreactor and a change to the final container circled on this slide.

So the first example is making this change from growing cells on these static cell factories. These are very much like T-flask only scaled up in greater capacity. A good way to start your trial. You don't want to spend a lot of time making the perfect process when you start a trial, but as you go forward and you think about cost of goods and capacity, this is a very hard thing to sustain. And we made a decision to examine better platforms pretty early on once we

had a sense that this was something we were going to go forward with. So the concept here is the cells need a surface upon which to attach. They can't be grown in suspension like a CHO cell like you might use for monoclonal antibodies but they can stick to a surface like a microcarrier which you can suspend.

So the platform switch included a couple of things. It included going from these cell factories to the bioreactors, and also the cell concentration step we had done in BAT centrifuge mode. We switched it as kSEP system, which is a continuous centrifuge mode. So today I'll really just talk about the cell culture, the cell expansion change, not the centrifugation change.

So as we did this we, of course, had many reasons to make the change. The benefits listed on the left, as I mentioned, scalability, cost of goods. We think better robustness because now you have one container making all your product rather than a bunch of parallel containers which, and if you think about cell factories, there's

going to be natural heterogeneities of the cells within the cell factory. Within a bioreactor, you're aiming more towards homogeneity. And then the operational steps and controls are just better and sterility risks should be less because again, one container versus many.

But there are many risks. It's a more complicated process. It's not like your t-flask that you started in when you first discovered your cell product and wanted to develop it. So you have to understand your process better and put the proper controls in place.

Comparability is key here. So we wanted to define which parameters we wanted to look at as input parameters because ultimately, we need to minimize those differences in the final cell that we make. And we have a variety of things that we measure, and I'll talk a lot about those.

The other thing which I won't really talk about today but I just want to highlight is bringing microcarriers in added a whole new facet to the drug product purity, and that's the fact

that microcarriers could bring impurities in. So anything the product comes in contact with could bring impurities in, but these microcarriers are a new material. They do break apart. They don't have a perfect uniformity when you buy them. There's different sizes. So we had to make sure we minimized the small particulates before we cultured. We have process controls in place to minimize those particulates once we purified our cells and we characterize those particulates extensively to ensure that the amount left -- and there's always going to be something left. Hopefully it's very little, but there will always be something. The amount left was reasonable and the type was reasonable for safety.

So again, it's a risk-based approach, data driven. As Dr. Bauer said, it's all about data. The science should be driving where you go. So to do this comparison, of course we used our -- I put current release assets because this is what was in our release panel at the time, looking at how the cells grow, their viability and the

phenotypic purity. We had functional assays, which were not part of our release panel yet, but potency is the most important thing. And we had a bead T-cell reaction, which is like a mixed lymphocyte reaction except we use beads to present the antigen as opposed to dendritic cells. And some other potential potency candidate assays which I won't talk about today.

We did further characterization as well. Of course, we looked at the effect on stability, both long-term in the freezer and short-term after you thaw the cells before infusion. And then we did some in vivo studies as well that were relevant to the diseases we were going to be studying in the clinic.

So just going through some of this and how the data looked, the first is the functional test, the bead T- cell reactions. So we look at both CD4 T-cells and CD8 T- cells like you would in the mixed lymphocyte reaction. And you can see on the far left is the CD4 and the far right is the CD8. And within each one you have your 50

liter bioreactor results versus your 10 tray cell factor results. And you're looking at T-cell suppression, and there really is no difference at all here. So we've got great confidence that the most important thing that is that the cells are functional when we make this change.

Trypan blue viability is just a simple measure of viability. No difference, but we think there's much more sophisticated measures of viability, and we did look at some of those. So one in particular -- and I'm not going to talk about all the data that we did, it's just a couple of snapshots of the key things we looked at. We did something called a cell health assay. This is a flow cytometry assay that looks at both annexin which is a marker of the apoptotic state of the cells and TO-PRO 3, which is a much more sensitive marker of membrane integrity than trypan blue. And again, good news here. Things look very similar across the two platforms.

And another thing is morphology. You know, it's funny. I always the scientists and

engineers in my group, as you move towards these more automated things, like using a Vicell counter for cell counting, which is what we use, which also measures the trypan blue viability, don't forget to look and see what the cells look like.

And here's just a simple picture of morphology. I don't know how well you can see it but the cells do look similar in terms of their shape, in terms of their density. The cells had a bit of a growth lag in the microcarriers but then caught up. And that's, we think, because of the initial attachment time that's required in a dynamic system like the bioreactor as opposed to a static system like the cell factory.

Now, cell size is where we saw one difference. And you can see the average cell diameter in the top left graph a bit lower for the cells growing in microcarriers as compared to cell factories. And we looked at this a lot, and one of the things we wanted to understand was were the cells really inherently changed or was it just the dynamics of the system, the sheer in the system

that was causing them to get a bit smaller.

And if you look at the graph on the right, you can see the red line is cells growing only in cell factories, so they maintain their average size pretty well. The blue line of cells grown in cell factories of passage five switched to bioreactors in passage six, which is representative of what we do. But you see if you put them back into cell factories they recover their size, so we think this is a transient effect and not an inherent change to the cells, more just of what they're seeing in culture.

The other most important thing in my mind is if you look at size distribution, yeah, the average changed but the spectrum of sizes that these cells are no different in the cell factory than in the bioreactor. So when you think about the fact that we did clinical studies already in a cell factory, the body had already seen cells of all these sizes and we're just shifting the spectrum a little bit. We also did some animal studies which I won't get into today to give us a

better understanding of what cell size might do when you infuse the cell. And at the time we were thinking -- this change was made both for our infused product or IV administered product, as well as an IM product. But we were more concerned about the IV and whether the cell size would make a difference. And again, I won't get into that today but we did some animal studies there.

So overall, just in conclusion, there were a variety of studies done. I didn't show you all of them today but in all, it looked like the cells were not changed as far as their key product attributes, and these other things like cell size. We had a pretty good understanding of why they changed and why it wouldn't make a difference.

I didn't talk about stability or post-op preparation, but those were comparable as well and the animal models also showed comparable results. Just in the interest of time, I'm not going to show those today.

So we have one other example. So the first example is a nice example that showed you a

very comprehensive comparability strategy with really a lot of good analytical tools that look at the most important things and give us confidence we're making the same product. This example is really one about using proper engineering to understand the effect of the change on the cells. So this is switching our final product container from blood bags, which is what they initially use for a long time, to cryopreservative vials. And this is a vial made by Aseptic Technologies. They make them in a variety of sizes. In this study we looked at both the 2 ml size and the 20 ml size. And what you can see here is the differences in geometry are quite difference. And the surface area to volume area is quite different from a bag to the vial.

So this is just some background on the vial. If you want to know more, we can talk later. But the beauty of these vials which came out a number of years ago is that they can be cryopreserved. They do come already sterile, gamma radiated, and you can get your product in by

going through the septum which reseals, and you can reseal it with a laser. It just actually naturally reseals on its own after you puncture it with a needle.

Many challenges with this. And you can imagine it's a different product now that the cells are coming in contact with the time of filling, how you visually inspect, which is a whole talk in itself which I won't really get into today. Leachables, extractables are important. What we're going to talk about today is the geometry and its effect on freeze and thaw.

So when we first did our quick and dirty study and said how will the cells do if we use the same freezing program? And what's interesting, if you look on the left and you look at trypan blue viability and you compare viability of cells frozen in the bag on the X-axis to viability in the bag frozen in a vial on the Y-axis of course from the same batch of cells made. You see really no difference. It's all within a five percent difference. So if you're just using trypan blue

you're going to fool yourself is the point of this.

We had a potency measure at the time which we thought was indicative of the efficacy of our cells and the functionality of our cells, and what we saw is that the potency of cells in the vial was considerably less than that made in the bag, as much as 30 to 50 percent less.

So back to Dr. Galipeau's point about freezing and potentially damaging your cells. It's very important you understand how you freeze and what you're trying to have your cell do.

So we looked into this a bit, quite a bit, and the graph on the left shows in the dash line the freezing chamber temperature. This is a control rate freezer. And because there's a lower surface area to volume ratio in the vials, what you can see on the red graph is a much longer time to get through the freezing, and then what ends up happening -- I don't think this pointer works. Oh, it does work. There we go. What ends up happening is then the rate of freezing here in the

vials is much greater than the rate of freezing in the bags. And we did some separate studies, independent studies which showed that the freezing rate affects potency directly.

So once we saw this and looked at differences, we started pulling apart what differences were the ones that affected the cell potency? And sure enough, it's freezing rate. So then we went ahead and changed our freezing protocol, changed the freezing chamber profile so that we can match this freeze rate that we previously had in the bags which gave us what we thought was an acceptable potency. And when doing that, our trypan blue viability still stayed the same but now our potency numbers are much better. Everything was within 10 percent in a vial as compared to a bag.

So again, trying to understand, using the right comparability matrix and then trying to understand the physical effects of the system on your cells, and then making the right engineering changes so that those effects become minimized and

what the cell is seeing is now very much like what it would have seen before.

So in summary, when you make these changes, it's really important you understand your process. Process characterization is something you should be doing from day one as much as you can. I always like to tell my peers and my management, whatever resources we have that's excess, we're going to just do more characterization. We have some minimum we want to do, but we're going to be opportunistic and do more so that we can solve problems proactively rather than reactively.

Engineering approach, you obviously have to understand what's affecting your cells and design your systems and your parameters so that you can make the same cell. Understanding the science. Back to understanding how the product works. Measuring the right thing. How the product is affected in your system. And that analytical tools are the key. You have to have the right ones and enough of them.

So when you consider making changes, I mentioned earlier it's easier to make them earlier in your clinical process than later, but it's not impossible to make them at any time. And of course, as you go later, you're going to have to scale up, hopefully. So it's really important to consider the benefit of the change to the risk it's presenting and then the timing of that change within the clinical development spectrum. And all along it's important that you must maintain product quality, whether it's safety or you understand efficacy, but you have to make sure that at the end of the day you're making a product that's going to be safe for patients, and then if it's efficacious, it continues to be efficacious.

So just to acknowledge in the groups that all contributed to the study, we have the Bioprocess Development, Analytical Development groups. Our production group, we have a GMP facility at our site in Warren, New Jersey, and the quality operation groups that go with that. And I just want to note a lot of these ideas and

approaches are published in an online journal, which you can take a look at. It was published earlier this year. This journal, Bio Insights is doing a four-part series. This was in the first part. A four-part series on the cell and gene therapy manufacturing pathway, and I think the third part is about to come out. Didn't have as much -- the same examples, exactly, but a lot of the methodologies are there if you want to know more.

So thanks very much. I guess we'll take questions on the panel. Okay, thank you.

(Applause)

DR. GALIPEAU: I think we have to do here a PC to Mac swap, so while Dennis is setting himself up, I'd just like to introduce Dr. Clegg, who is the founding co-director of the University of California-Santa Barbara, for the Center for Stem Cell Biology and Engineering. He's the co-principal investigator of the California Project to Cure Blindness and multi-disciplinary effort to develop stem cell therapy for age-related macular

degeneration.

Dennis?

DR. CLEGG: All right. Thank you. And I'd like to thank the organizers for the invitation to speak today.

So I'm going to tell you about development of an embryonic stem cell-derived product, retinal pigmented epithelium that we're growing on a scaffold for the treatment of the dry form of age-related macular degeneration.

And one disclosure, I'm cofounder of a startup company called Regenerative Patch Technologies with Mark Humayun and David Hinton at USC.

So if you go to the Internet and search for cell therapy, the first thing you might find is a shampoo, but I'm not talking about that today. Instead, we're talking about what some have called perhaps the next pillar of medicine, a third pillar of medicine to go along with small molecule drugs and biologics. And of course, when you're dealing with cells, it's a completely

different situation than a small molecule or a biologic.

And according to the Alliance for Regenerative Medicine, there are over 600 clinical trials underway using cells and stem cells, and 74 approved products already. So it's keeping the FDA very busy as I'm sure they will tell you.

I'm going to talk about blindness, and the September issue of the National Geographic had a beautiful story about sort of a global perspective of blindness. Roughly one in every 200 people on earth, 39 million can't see, and another 246 million have reduced vision. And in the case of age-related macular degeneration, it's actually a small percent worldwide that in developed countries in the elderly population it's one of the leading causes of blindness.

If you go into the optometrist or ophthalmologist and they give you that bright flash, they're taking a picture of your retina. It's called a fundus photo, and it looks like this in a normal eye. If you have the early form of

macular degeneration, you see these drusen yellow spots. I don't know how well that shows up. And that can progress to two forms of the disease, the so-called wet form where you get improper angiogenesis and bleeding and the dry form where you get something called geographic atrophy.

About 10 percent of the wet form and 90 percent have the dry form. Now, there's some pretty good treatments already using inhibitors of VEGF for the wet form, but for the dry form there's really no good therapy and that's what we're targeting.

Now, in both cases, most people believe -- and there's good evidence for the theory that the disease is caused by the death of RPE cells, retinal pigmented epithelial cells. And this is a monolayer of epithelial cells, pigmented that lie right behind the retina. And you can see in this diagram the RPE here are at the top. And so as these disappear during age-related macular degeneration, they are important support cells for the photo receptors, the rods and cones here, so

pretty soon the rods and cones die and you lose vision.

So how can we engineer an RPE replacement for the dry form of AMD? Well, I'm going to talk about two general challenges that we've faced. One is the cells. How do you make the cells? How do you differentiate an undifferentiated stem cell and expand it to make enough cells for therapy? And then, two, how do you deliver it? And there are two general approaches that people are taking. One is to just inject a suspension and the other is to implant a graft that's grown on a monolayer. And of course, there are considerations if you're going to use a scaffold, should it be biodegradable or biostable?

Well, I'm going to tell you about work that was carried out, funded by the California Institute for Regenerative Medicine so-called disease team project that we called the California Project to Cure Blindness. It's led by Mark Humayun at Keck School of Medicine. And myself and David Hinton are co-principal investigators.

And it involves in addition to USC, UC-Santa Barbara; University College of London; Caltech-City of Hope; and the startup company, Regenerative Patch Technologies. And we've benefited greatly from Jane Lebkowski, who is part-time consultant for Regenerative Patch Technologies.

Now, I'll tell you about the different contributions as we go through this.

Well, the first question we wrestled with is what stem cell should we use to make RPE? And you can make RPE from both IPE and ES cells. You can't make RPE from adult stem cells. We chose ES cells when we started this project back in 2010, and I'm going to focus on the studies that we've done with the H9 cell line from Wisconsin.

Now, the first person to report that RPE could be derived from HSC was a group at ACT and Irina Klimanskaya, et al., showed this figure in a paper in 2004. And what they did was to grow undifferentiated cells in a dish and then just

simply remove the FGF. If you remove the FGF, you start to see differentiation into all kinds of different cell types. And after about six to eight weeks, they saw little pigmented patches that they could pull out and expand, and they went on to show that it had a MRNA profile very similar but not identical to fetal human RPE.

So we looked at this and collaborated with ACT early on, and then went on to do studies on our own asking can we increase the frequency of differentiation toward RPE? And then can we speed up the process to make RPE?

And to make a long story short, we tried many different conditions with this so-called spontaneous method where you just remove the FGF and wait. And using the right substrate and cell line and media, you can get conditions where about 40 percent of the cells start to make pigmented colonies and then by selective enrichment and selective culturing methods, you can get nice homogeneous cultures that are 99 percent positive for RPE markers. As shown on the right here, you

can see staining for PMEL, a premalenazone marker in green and DAPI in blue where most of the cells are PMEL positive.

And very importantly, we didn't see any undifferentiated HESC markers in these experiments, and we've devised several different assays to look at potential contaminating cell types. The one percent we think is probably neural cells. We can see some that have a neurophenotype. Those are not dividing as far as we can tell and we're not too worried about them. And they may be RPE precursors because RPE is derived from the interior neural plate.

I'm not going to go over all the characterization, but we've looked using a variety of methods at mRNAs, proteins, and functions, both in vitro and in vivo, and these cells are similar but not identical to fetal RPE, which we can obtain and use as sort of a gold standard to compare our cells to.

Now, one problem with this method is the current method that we're using to make cells for

the clinic takes about six months. It's quite a lengthy process. It works, but one of the things we've been looking at more recently is can we speed this up. And one idea is to mimic what goes on during development by forcing the cells to become RPE by adding growth factors to bring them through these stages that normally occur in the embryo. And we just published recently a study by Lindsay Leach and collaborators where after 14 days we can get cultures that are about 90 percent, are positive for early RPE markers by using a late WNT pathway activation and manipulating FGF and WNT pathways and TGF beta pathways in a progression of times that are similar to what goes on in vivo.

But I have to say at 14 days they're still not mature and we need to grow them for another 60 days before they start to express mature RPE markers.

Okay. So now on to delivery. How do we install these cells for therapy, suspension versus monolayer? And we had done some studies early on

with Pete Coffey's group at University College of London and Pete's now at UC-Santa Barbara. And what we're trying to do is deliver these cells into what's called the subretinal space, right here between the RPE and the photoreceptors. And when we tried suspensions, what happens mostly is that the cells clump together and don't integrate into the endogenous monolayer. And these are IPS RPE stain for human-specific antigen in green. And you can see these clumps. And one important function of the RPE is to carry out phagocytosis of the outer segments. You can tell if they're doing that by staining for rhodopsin and looking for the red fluorescents inside the green-labeled cells. And occasionally we can see that, but we don't see a lot of phagocytosis. And they're not oriented properly to carry that out. The apical side should be right next to the photo receptors and it's not in most cases.

So our approach has been to grow the cells on a biostable scaffold made of a substance called parylene. Parylene is a xylene polymer

that's already approved for use in the eye. We collaborated with engineers at Caltech, especially Y.C. Ty, to develop a scaffold that has ultrathin regions so that the permeability mimics the normal Brooks membrane that the RP cells lie on. And we coat it with vitronectin, which allows these cells to grow and polarize, make tight junctions so they have apical micro villi that can interact with the photoreceptors and a normal basal apical polarity.

So why polarized RPE on a scaffold? We grow these for 30 days before implanting, and studies from David Hinton's lab have shown that the polarized RPE behave much more like real RPE than suspension RPE. They're more resistant to stress. And if you look at growth factors they secrete, for example, PEDF, here is staining from a polarized monolayer and here's staining from a suspension. And you can see much better behavior of the cells in a polarized monolayer.

And the parylene, we've done a number of studies using this. This is used in coating stents and electrodes already in the clinic, and

the studies that I said, show the permeability is similar to Bruch's membrane.

We've done side-by-side comparisons in the nude rat of subretinal implantation of the monolayer versus the suspension, and we see improved survival when the cells are implanted as a monolayer. And we've shown efficacy, at least proof of concept, in the RCS rat model.

Now, one issue with studying macular degeneration is there's no perfect animal model for macular degeneration. Rats don't have a macula, which is the center of the retina. Different structure, slightly. They're nocturnal; we're diurnal. But the RCS rat has a defect in the RPE. There's a mutation in the MERTK receptor, and the cells can't phagocytose those outer segments that I told you about. So the rats are born with vision but the RPE don't work so the photoreceptors die over time after about 12 weeks. And so the experiment is to put in the RPE and see if you can rescue those photoreceptors.

And a lot of studies have injected cells

at day 21. We had to wait till day 28 because we're putting in a patch. And we made a much smaller version and it's actually quite a challenging surgery, but we were able to measure phagocytosis, photoreceptor survival, and visual function over time in this model.

And so first thing, can they rescue photoreceptors, and this is work from Biju Thomas and David Hinton, and you can see in the area of the implant -- here's the implant in this section. You can see the nuclei of the photoreceptor layer here that are rescued where as if you look away from where the implant is, you don't see that nuclear layer. And this is after two months after transplantation we can see rescue of those cells.

Are the RPE working? Well, we can look at phagocytosis using the assay I showed you earlier, staining for rhodopsin, and in this case now the RPE is oriented properly and you can see phagosomes containing rhodopsin immunoreactivity in the transplant itself compared to the native RCS retina where you don't see any phagocytosis.

And that's 60 days after transplant.

And we wanted to look further to see are these rescued rods and cones still connected to the brain? And one thing you can do is to actually put an electrode in the superior colliculus and measure responses to light. And what we found was the area in the superior colliculus that responds to light is the same area that corresponds to where the implant is in the retina. And these luminescence threshold mapping experiments were just published in the Journal IOVS by Thomas, et al.

Okay. So that's our efficacy. And then it was a real challenge. We just heard about process development and making cells large scale. You can imagine there are some specific challenges using a scaffold with a monolayer. And we worked with City of Hope and a manufacturer spin-off company from Caltech called California Memstek to make a scaffold suitable for humans that's 3x6 millimeters, a little smaller than a penny, that has a frame where it can be excised off the frame,

has a handle that the surgeons can grasp, and we built a tool to deliver it to the eye which I'll show you in a minute.

We worked with City of Hope. We transferred our protocol to the GMP manufacturing facility at City of Hope, and we introduced a step where we freeze down cells as an intermediate cell bank in vials and then thaw those out, grow those 30 days on the parylene, and they're delivered in a 37 degree incubator to the point of care and implanted. And we were able to complete our preclinical studies and submit our IND which was 3,300 pages long. And I'm wondering if anyone in the FDA read it from cover to cover, but I know they have large teams that do this.

We showed efficacy in the RCS rat, as I mentioned. Very important, we looked at tumorigenicity in the nude rat. We wanted to show we could deliver this reliably, and for that we used a pig study and human postmortem eyes. And the IND was cleared.

And I'm going to show you an animation

of what this surgery looks like. What is done is to go in and remove the vitreous first, which is the gooey stuff in the middle of the eye, and then inject some fluid behind the retina. And you can see it's creating a retinal detachment here. And that's where we're going to put our implant. So you have to cut a little hole in the retina. We're going to come in intravitreally. And here's the implant. The surgeon trims the handle, and using this special tool that we've designed, pulls the implant into a cannula, and it folds up kind of like a taco which protects the cells. And then that's inserted through a hole in the side of the eye, through the hole in the retina, and the implant is extruded where it unfolds, such that the apical side of these cells is directly opposed to the photo receptors. The retinal detachment is then closed by adding oil to the center of the eye to push the retina back down and the surgery is complete. There's also a laser to seal the blood vessels that might have been damaged in the retinotomy and then the oil is applied.

Okay. So we've shown in the pig that we can deliver this and we've used OCT to look at the implant after delivery and then cut sections and looked at the photoreceptors. We can see pigs with good placement show good preservation of photoreceptors. And included these studies in the IND.

So we've begun now our clinical trial at USC Tech School of Medicine. It's underway. The startup company is the sponsor. And I don't have much time to go over the details. We're looking at 20 patients in the first phase 1/2A. The first cohort of 10 is 20/200 or worse. We started at 20/400 and now we've gone to 20/200, which is legally blind. And then the second cohort will be 20/80 or worse. And the idea is to intervene early. We're not adding photo receptors. And some of these patients that are pretty far gone have already lost their photoreceptors. So we have to add the RPE at an early stage to rescue the photoreceptors.

Okay. So just to wrap up, I've showed

you that we can make the cells and showed you how we're delivering them on a scaffold and told you about this clinical trial that we're starting.

But looking forward, I wanted to mention that we're not the only ones in this space. A lot of folks think that this might be a good idea to use stem cells to treat the dry form of AMD and there are a lot of different approaches -- people using suspensions, people using different kinds of cells, and IPS cells that you may have heard about as well, and people using different kinds of scaffolds. So we'll just have to see how these work, and it's a very exciting time as phase 1 trials are underway.

So I'll just wrap up by thanking the wonderful team and the California Project to Cure Blindness at all those universities I mentioned. The folks in my lab, including my dog, whose name is Iris, and our funding sources, especially the California Institute for Regenerative Medicine.

Thank you for your attention.

(Applause)

DR. GALIPEAU: So thanks, Dennis. While Chris sets himself up. So Dr. Breuer is a professor of surgery at the

Ohio State University. He serves as the director of the tissue engineering program and also director of the surgical research at the Nationwide Children's Hospital, and he'll talk today about the development of translation tissue-engineered vascular graft.

DR. BREUER: Well, good morning, and thank you very much for this opportunity to share some of my work.

My name is Chris Breuer. I'm a pediatric surgeon and a researcher at Nationwide Children's Hospital, and I'm the director of the Tissue Engineering Program at the Ohio State University.

Tissue engineering is a multidisciplinary science that attempts to leverage the cells and the ability to replicate and to self-organize into functional units that are called tissue.

One method of tissue engineering uses a biodegradable scaffold onto which the cells can be seeded. The scaffold provides sites for cell attachment and space for tissue formation and can actually serve as a template that can control the formation of the tissue. As the scaffold degrades, neotissue forms and the resulting neotissue that can then be used by a surgeon to either repair or replace tissues that have either been damaged, diseased, or congenitally absent.

For the last 20-plus years, I've been working with my collaborator, Dr. Toshi Shinoka, trying to apply tissue- engineering principles to develop devices for use in congenital heart surgery. Taken together, congenital cardiac anomalies represent the most common birth defect, affecting nearly one percent of all live births. And despite significant advances in the surgical/medical management of these patients, it remains the leading cause of death in the newborn period. And one significant source of complications are the fact that most major

reconstructive operations require the use of synthetic manmade materials in the form of either vascular grafts, vascular patches, or replacement heart valves. And complications associated with the use of these materials are a significant source of post- operative morbidity and mortality.

We developed the first tissue-engineered heart valve, and then subsequently the first tissue-engineered blood vessel designed specifically for use in children. Our scaffold is fashioned from polyglycolic acid fibers, which are coated with a copolymer of polycaprolactone and polylactic acid, and they degrade by hydrolysis once implanted in the body. It takes about six to eight weeks for the scaffolds to lose their biomechanical integrity and about six months for the fibers to disappear completely.

My colleague Dr. Shinoka performed the first implantation of the tissue-engineered vascular graft in a child at his home institution, the Tokyo Women's Hospital. In this particular case, he used our original method where he

harvested a biopsy of a small blood vessel from this child. He then isolated the cells by explanting the tissue and then expanded the cells by serially passing them in culture. Once he achieved a significant number of cells, he seeded the scaffold and then incubated the scaffold outside of the body for a period of time before implantation. And he used this to replace a portion of the pulmonary artery in a child undergoing congenital heart surgery and the clinical results were excellent.

He used this technique on several more children and it worked well, but ultimately, he abandoned this technology for several reasons. One is its utility was quite limited due to the large amount of time required to make one of these blood vessels that took a couple months. Two, because the cells needed to be cultured for such long periods of time, there were insignificant risks of potential contamination or even malignant dedifferentiation. But the real nail in the coffin was the fact that sick people had sick

cells and he had multiple children that came in for this procedure but he couldn't passage adequate number of cells in order to create the tissue-engineered vascular graft.

So we began to explore alternative cell sources and came upon the fact that you could actually use bone marrow, or more specifically, bone marrow-derived mononuclear cells to perform the same procedure. You could seed these cells onto the scaffold and use them to form neovessels. And the bone marrow-derived mononuclear cells had the distinct advantage that they were available in such abundance that they didn't need to be expanded in culture. So from a single bone marrow aspirate, we could obtain enough cells to actually seed our scaffold and implant it immediately, and this dramatically reduced the amount of time needed to make one of these grafts.

Using this technique, he initiated the first clinical trial evaluating the issue of the tissue-engineered vascular graft in an operation called the Fontan operation. And this particular

clinical target was chosen for two reasons. One, it's one of the more commonly performed operations in congenital heart surgery; and two, it had an excellent safety profile. You see, in the Fontan circulation, the graft is used to connect the inferior vena cava to the pulmonary artery, so it's a large blood vessel with a high flow, so there's a low chance of catastrophic graft occlusion due to thrombosis. And it's also implanted in a low pressure system so the chance of aneurism formation or graft rupture is significantly lowered.

Clinical results, the one year follow up on the first 25 patients implanted with these grafts were quite good. There were no graft-related deaths or graft failures. The one graft-related complication as demonstrated in the CT angiogram, a patient developed a partial neurothrombosis, which was successfully treated with anticoagulation and went away. Long-term results were interesting. We demonstrated that the tissue-engineered vascular graft did possess

growth capacity, making it uniquely suited for these particular applications and for use in congenital heart surgery.

Long-term follow up also demonstrated that the primary graft-related complication was stenosis, and now some of these patients are up to 14 years out and our incidence of stenosis is up to about a third of these patients. Fortunately patients that develop stenosis can be adequately treated with angioplasty, but this is an invasive technique, and as we move forward, if we really want this to be widely used, we need to develop tissue-engineered vascular grafts that possess growth capacity but don't stenose.

In 2007, I recruited Toshi back to the United States to join me and together we went to the FDA. And not surprisingly, they had some issues with our technique for making the grafts. The grafts proceeded using a manual technique, and the graft actually had to be palpated with your hands in order to get the cells to go into the hydrophobic scaffold. The FDA suggested that this

might not be the best method moving forward. So we went back to the drawing board and developed an operator independent method using vacuum seeding to seed the scaffolds and developed GMP compliant methods for making our tissue-engineered vascular grafts. And in the summer of 2007, implanted the first tissue-engineered vascular graft in the United States in a little three-year-old girl who had a single ventricle cardiac anomaly and was undergoing Fontan surgery. And six months after surgery, the patient was doing quite well, and she's actually completed enrollment in the study and is now five years out. And this is an MRI demonstrating a widely patent graft that has increased in length as this child has grown in size.

As we would have predicted, some additional patients in our trial have developed stenosis, similar to the Japanese trial. You can see an angiogram of one of these patients. Fortunately, these patients have all been able to be successfully managed with angioplasty and

continue to do well.

This is a post-angiogram picture demonstrating that the stenosis can be cured with the angioplasty. And this is that little patient, Anderson, six months after his angioplasty.

About 12 years ago, with the help of the NIH, we began a series of investigations that tried to get at the cellular molecular mechanisms underlying neotissue formation, and specifically, the cellular molecular mechanisms underlying stenosis to see if we could stop being so empiric and actually enact rational design. And based on a mechanistic understanding, develop methods for making tissue-engineered vascular grafts that might not stenose.

And to this end, we needed to develop mouse models to take advantage of the vast number of molecularly agents that are readily available in mice that aren't available in other species that can enable things like cell tracking.

We needed to develop methods for fabricating our scaffold on a much smaller scale,

and we needed to develop murine models and microsurgical techniques so we could, in fact, study these. And we were ultimately able to develop an inferior vena cava interposition graph model that worked quite well for this purpose. Results of implantations of the grafts in the mouse models seemed to be fairly similar to our human experiences and that some of the graph stenosed. And we were finally armed with the tools that would enable us to ask and answer some of the basic questions that we had wondered about for quite a long time.

Our first question was what was the fate of the cells that were seeded onto the tissue engineered vascular grafts? According to classic tissue-engineering paradigm, the cells seeded onto the grafts are supposed to be the source of the tissue, the building blocks upon which the tissues are made. To test this question, we created a chimera by making our tissue-engineered vascular grafts with human bone marrow-derived mononuclear cells but implanting them in an immunocompromised

SCID beige mouse model that readily accepted the xeno transplant. And then using human- specific markers to track the fate of the cells. And what we discovered to our surprise is that the cells actually disappeared and disappeared quite rapidly. Nearly 99 percent of the cells were gone within the first day. We wondered if this might be an artifact of this complex model, so we repeated the study using several different techniques, including labeling the cells with superparamagnetic iron oxide particles, and certainly monitoring the grafts with MRI over time. And each experiment showed the same thing. The seeded cells disappeared very rapidly.

If the seeded cells weren't the source of the vascular neo tissue, what was? We had multiple hypotheses, but one was that they might be arising from the native vessel necks into which the vascular graft was implanted. To test this hypothesis, we took a cuff of labeled blood vessel and sewed it to our tissue-engineered vascular graft and then implanted this composite graft into

a nonlabeled host. And what we discovered is that the cells within the tissue- engineered vascular graft, the neo tissue, did possess label and co-localized for markers for endothelial cells and smooth muscle cells.

So ultimately, the vascular neo tissue is arising from the neighboring blood vessel. So this really resulted in a paradigm shift. Originally, we had assumed that the cells that we were seeding onto the grafts were very important for the tissue formation, but instead what the seeded scaffold was doing was enabling tissue regeneration.

The next obvious question was did you even need to see the cells at all? And in our initial pilot studies with large animals we would always include an unseeded graft, and frequently these grafts would fail. But when we did a larger study using our (inaudible) models, what we discovered was that the seeded cells, in fact, are not needed for vascular neo tissue formation. But very importantly, they inhibited the formation of

tissue- engineered vascular graft stenosis. So there might be something there in our efforts to try and improve the design of this graft.

We also observed at this time that most of the cells during the early period of tissue formation were actually immune cells, specifically monocytes and macrophages, and the degree of monocyte and macrophage infiltration actually correlated with a degree of stenosis. And furthermore, the seeded grafts had less cellular infiltration and better patency, suggesting that perhaps this was an immune-mediated phenomenon.

To determine whether this was correlative or causative, we went back to our mouse model. And we implanted our tissue-engineered vascular grafts and monitored tissue formation. Then we used a type of drug called quadrinate. Quadrinate liposomes, which are a selected macrophage poison. We did this with the hope that we could knock down the macrophage infiltration and decrease the incidence of stenosis. But to our surprise, this had such a

powerful effect that it actually stopped tissue formation. There was no tissue formation in the animals that were treated with the quadrinate, suggesting that not only were the macrophages important in the formation of tissue-engineered vascular graft stenosis, but they were essential for vascular neo tissue formation.

To confirm these findings and validate this discovery, we used an inducible conditional monocyte knockout mouse and implanted our tissue-engineered vascular grafts and observed exactly the same phenomenon.

So identification that the macrophages are really the critical cells in this process prove really to be a very important discovery and have enabled us to start to perform rational design.

From an engineering perspective, there are only three ways that we can try and affect the host macrophages. We can do it with our cell seeding. We can do it with our scaffold design. Or we can try to manipulate the host through

pharmacologic methods to alter the macrophage response and control the formation of tissue.

Our studies into cell seeding have been quite interesting. It's embarrassing that this is a publication from this year, but we finally went back and did the basic study of looking at cell dose. And what we discovered is that the cell dose, the more cells we see on the graft, the lower the incidence of stenosis. So there is a cell dose response. So this provides one viable strategy for inhibiting tissue-engineered vascular graft stenosis.

Altering scaffold designs, another viable strategy. When the tissue-engineered vascular graft is implanted in the host, it induces a foreign body reaction, and it's well known in the literature that the chemical composition, in addition to the morphometric characterization of the scaffolds are ways of altering the macrophage response to the scaffolds. So simple things like the fiber diameter of the scaffold, the porosity of the scaffold, or even

the fiber alignment of the scaffold are effective strategies for changing the degree of macrophage infiltration and altering tissue formation.

But when you think about this, there are so many parameters that if you try and take an empiric approach and attack these one at a time, there would be an infinite number of experiments. So we've actually turned to computational modeling and have now developed a mathematical model that can actually accurately describe tissue formation in our tissue-engineered vascular grafts, and we are currently using this model to optimize the design of our scaffold, and we think this holds great promise for moving forward.

Finally, we've probably had our best results by altering the host pharmacologically. Initially, as I mentioned, we discovered that the macrophages are critical to tissue formation. We tried to modify or inhibit macrophage infiltration using the quadrinate liposomes, which was an effective strategy for inhibiting stenosis but it was too heavy a hammer. So we began to look at

what other signaling pathways were involved within these macrophages and discovered that the TGF data pathways were very, very important. And subsequently started looking at different inhibitors of the TGF beta receptors. And we discovered that a small molecule that inhibits the TGF beta one receptor was actually quite effective inhibiting stenosis but didn't adversely affect neotissue formation, providing another strategy for moving forward.

And interestingly, when we characterize the macrophages from these cells, not only did it alter the degree of macrophage infiltration but there was a certain phenotype that was very, very important and associated with the development of stenosis versus nonstenosis.

Most recently, we've been able to do the same thing using losartan. We think this is an off-target TGF beta effect of the losartan, but this is an important discovery because the use of losartan is already FDA approved and has an excellent safety profile in our patient

population.

We've also continued to try and do process improvement, to modify the way we make our grafts. One of the unintended side effects, developing our GMP methods for making the tissue-engineered vascular grafts is that it takes a lot longer to make the grafts than in the original Japanese studies. In the Japanese studies, the grafts could be -- the cells could be harvested, isolated, seeded, and the grafts were ready for implantation within a little over two hours. Currently using our technique takes us about six hours, which is feasible but does present some problems and some additional potential complications for these patients.

One of the reasons why this technique takes so long is because we use density centrifugation and Ficoll and this is a labor-intensive process that adds time and complexity to the procedure. So we began looking at alternative methods. There is a filtration elution method, a filter that can be used to trap

the mononuclear cells from the bone marrow and then these cells can be eluted off the filter and collected in a seeding chamber. And we demonstrated that while there are some differences in the cell populations obtained using these two different methods, that biologically they're the same. They form excellent vascular grafts in both small and large animal models, and that they both function by altering the macrophage response. But they have the added advantage that it's a much simpler technique and can save substantial amounts of time.

So how do we roll out these findings from the bench to the bedside? Well, I think it's already been mentioned that one of the primary problems with any animal study is the question of relevancy. You know, mice aren't small humans, and sometimes discoveries made using muurine models do hold true for humans but other times they don't. How can you actually validate these discoveries?

Well, one method is to use human tissue.

And this year for the first time one of our patients died from a non-graft-related complication and consented to an autopsy. So for the first time we actually have human neotissue that can be examined. And the resulting vascular graft is beautiful. The neovessel looks like a real blood vessel, both grossly and histologically. It's got a monolayer of endothelial cells surrounded by concentric layers of smooth muscle cells. It's got a similar extracellular matrix. But being able to examine this tissue and look at some of these characteristics has been very helpful in trying to validate some of our work.

A more traditional method in the United States is to do validation work with large animal models. For our tissue-engineered vascular graft, we've developed a lamb model which seems to be a very relevant and important model. And we've used this to validate some of our discoveries or some of the changes that we're trying to implement like the use of a filter.

And finally, in some studies where the risks are able to be calculated and are fairly minimal, it can be appropriate to go directly into man.

So how do we suggest putting all these pieces of the puzzle together and moving forward? Well, we feel we've reached a point in our study where we know that stenosis is a critical issue. And we're interested in carefully rolling out these different strategies that we've developed in the clinic. And we thought it'd be best to start simple. We think some of our process improvement measures, such as changing to the filtration method, seeding more cells, and eliminating the incubation period are logical and safe ways of potentially bringing these discoveries into the clinic. And then if, and as needed, adding additional therapeutics, such as the use of losartan in our patient population in an attempt to improve the design and develop our second-generation tissue-engineered vascular grafts.

So what lessons have we learned? Well,

I think one, this is a high risk, high reward game, and that anything you do, or anything you don't do, has very, very significant consequences. It's important that we try and push forward but do it in a safe and careful way.

Two, you always require a leap of faith. No matter how robust your data, no matter how long you've been doing this, at some point you just have to move forward. And you know, this is the sort of thing that causes handwringing and keeps you up in the middle of the night but it's part of the game.

Three, it's very interesting doing this work with congenital heart surgeons. They're about as polar opposite as you could be from somebody trying to do a well-controlled study. You know, it's in their DNA. Every single congenital heart defect is different, and so when they get in there they have to be able to change on the fly. And you know, to come in with this protocol or trying to do things the same way, sometimes I feel like the guy in this video.

(Video shown.)

DR. BREUER: But the single most important lesson that I've learned, and I think if I had to say one thing today it's this -- it's that translational research has to be a two-way street. You need to be able to go from the bench to the bedside and back again on a very routine basis and that no matter how strong your data, really at some level the experiments don't even start until you get into humans. And at that point you need to be able to identify where your problems are and you need to be able to go back to the lab and develop rational solutions for overcoming your problems. And that's how we're going to safely help our patient populations. Thank you.

(Applause)

DR. GALIPEAU: So thank you, Chris. I'd invite the speakers that spoke this morning to come up front, please. So for people in the audience, the idea of the panel that follows now was to sort of

have an open-ended discussion with panel members. And we felt that maybe a structured approach to this would be productive to get a lot of ideas out. At first we had some set questions that I'm going to be posing to the audience, the panel, and afterwards we can open it up to the audience if there are some pressing questions from individuals.

So let me kick it up here. So one of the questions that we wanted aired was -

- and I'm asking everybody that's sitting here. Based on your experiences, what advice do you have for product developers, whether it be academic or industry, promising manufacturing changes? The idea of course is to get a better mousetrap moving forward. So to the panelists, what's your advice?

The guy from industry first. There you go.

DR. RUSSOTTI: Thank you. So just one point, I guess, I already made in my talk that I think is first and foremost is I would not try to

make the perfect process to get into the clinic. I think an important strategy is to get answers quickly. So don't worry about costs and capacity when you first start out. I think it's more important to learn. Of course, you have to make good quality product, and of course you care about safety, but I think it's important, and I think this leads to another question we're going to discuss later about iteration. I think it's important to get answers as quickly as possible because as we heard this morning, animal data is great but it's not always predictive. The best data is in humans. So get in early, learn, iterate in the background, then work on better processes and plan for success when the time is right.

DR. GALIPEAU: So Chris, actually, you had to do like a full like 180. You started off with a cellularized graft and then you realized well, we don't need the cells because it's a host immune response to the cellularized graft. How do you manage that moving forward trying to get the

ball rolling because you're working on IND and you want to treat people? So how was that interaction and dealing with the FDA?

DR. BREUER: The FDA has been a wonderful partner. You know, I think we're both on exactly the same page. We want to help patients. And I think the road to perdition is paved with good intentions. I think everybody goes into this and is driven by their enthusiasm. They want to do the right thing. But it doesn't always work out that way. And I think having somebody that can second guess you and look at your work critically and then, you know, provide additional controls is very, very important for moving forward and making good progress. I also think it's incredibly important if at all possible to understand mechanism. If you can understand mechanisms of action, not only does it help you in developing your process, but if things don't work, a lot of times you can potentially throw out a very valuable technology. And if you understand how the mechanism of action works, then you can

figure out why something doesn't work and then improve your design.

SPEAKER: Could I ask a quick follow-up to that?

(Inaudible) by the way. I'm just kind of curious. You mentioned that the

FDA -- said that the two-hour procedure was maybe not the best method and they recommended the GMP method. The thing that I didn't understand from that was I didn't hear you say that there was a practical problem of doing it

(inaudible) complications. I'm curious. It wound up being disadvantageous to patients in the sense it was six hours instead of two as we've said. Was there some reason that that alteration was made other than the theoretical?

DR. BREUER: Fortunately, there were no graft complications using the non-GMP method that was performed in Japan, but I think it's only a

matter of time. And I think the added benefit of doing things in a GMP-compliant method speak for themselves. I mean, it was absolutely the right thing to do and absolutely the right way to go forward.

I think now the onus is on me to figure out how to do it faster and better, and we're in the process of doing that. But especially in the early phases, you know, following and tracking all the data and making sure we have all the quality control and quality assurance measures put in place and having good release and post-process monitoring testing in place is the only way it can make sense.

You know, the Japanese trial was very intriguing, but it was very poorly performed. There's excellent patient follow-up. All 25 patients have been followed up through now 14 years, but again, it's congenital heart surgery. So you know, everybody was followed up in a different way. There weren't great release criteria, so the data's flawed. And I think doing

it the way that I developed it in collaboration with the FDA is the right away of moving forward.

DR. GALIPEAU: So a follow-on question to that was are there ways the FDA and academic and industrial stakeholders can foster open access for novel development of tools, the CMC, toxicology, clinical? Now, open access is actually big deal. And you know, Dr. Matthay spoke to that point this morning that academics are compulsive showboats and published and dock publicly and posters and do everything else while industry for, you know, obvious IP protection reasons sometimes are going to be more discreet. So maybe Mike, I'll let you just kick off. What are your thoughts here?

DR. MATTHAY: Well, I think it's not we're just showboats. We have to show for our peer review publications the details of our methods. And that's good. And we need industry very, very much. We can't go without industry. But I do think once the IP is established there should be a mechanism in cell therapy for the

pathways that the particular industry or company has used to be more in the public domain. I don't think that would interfere with the patent, and I think it would really help both the companies involved and the academics. I'm not quite sure how to do that. I'd like to know what FDA thinks and what others think.

DR. GALIPEAU: So panelists, anybody else who would like to -- Irv, you had some strong feelings about those things.

DR. WEISSMAN: Well, I think I said at least for the cell types that I've been dealing with, the stem cells, they organize themselves if you get them in the right place. So for the cell types we've looked at, making scaffolds is not necessary. You put blood-forming stem cells into the blood. They have homing receptors that make it to the bone marrow. We didn't know if you put neural stem cells into the brain if they could find the right place, and mainly we located them where they should be. But in both of those cases, blood forming and brain forming, academic success,

discovery, preclinical discovery, even early successful clinical trials have not led to a single therapy. It's not the FDA that's the problem; it's our culture that we try to get our discoveries out early.

Second, and this is really important, NIH, although it wishes to support clinical translation, hardly supports at the level that's necessary. That's why we began proposition 71 in California, to try to get funding in via the state so that you could carry it in a not-for-profit setting until you got into mid- to late-phase, phase 1 clinical trials when any fool would notice that you had a success. When I say "any fool," I mean any fool. The most likely problem you have when you try to build something that will be therapeutic in humans is that you have your leaders, your business leaders, even your CMOs, that come from an industry that was small molecule-based, not cell-based. And so they always live by the culture they grew up with and that made them successes in the recent past. But

it doesn't work.

So just as an example, not one of the small molecule pharmaceutical companies developed a protein therapeutic. There were new names -- Amgen, Genentech, Biogen. You know, all of those, because the culture wasn't there. And none of the protein therapeutics have even ventured companies, have ventured into a cellular therapeutic. But the cellular therapeutics as you just said, requires a whole team, a herd of cats, people who are MDs, who will try to take the lessons they know about the pathology of the disease and advise you how to go next to try to treat the disease while you're trying to bring in your cellular therapy.

Now, we do have cultures that create silos. Companies are absolutely great at making a silo so that research hardly talks to development, hardly talks to operations. And hardly any of them get to talk to a CEO who wants to funnel everything and keep the process to him or herself. I know I'm being very negative here but the stakes

are incredible. That is that we have developed scientifically -- we, the community, not just me -- things that should be making their way. And the surprise was the FDA was our partner in every one of them, not the barrier. The barrier is how we finance clinical translation. How we train people to be translators.

I went to medical school a long time ago, but even the medical students at Stanford now don't learn clinical trials. They don't learn everything that starts with, I have an idea. I've got to now set up a clinical trial. I have to go talk in my pre- pre-IND. I have to worry about pharmacology, PK/PD, viability. All of the issues we don't train. That may be boring training if it's didactic, so maybe it needs to have like business or law, case-by-case approach so it's exciting. But unless we move those fundamental problems, I don't see how we're going to move this fast enough to save the people while we're still alive to watch it happen.

DR. GALIPEAU: So, Dennis, go ahead.

DR. CLEGG: Yeah. I just wanted to follow up on something that Irv said, you know, in terms of teamwork. That's been super important in our project, and it's a concept that CIRM -- really embraced with their disease team grants and provided the funding. But coming from an academic perspective, that's a challenge. I mean, academics are raised in a competitive environment where they have to write grants and get their papers out before their competitor. And we were fortunate to actually approach our competitor and collaborate with them, which turned out to be great collaboration. But I know that's not always easy. And I also want to second what Irv said with regard to the FDA coming into it, I didn't know what to expect from the FDA and they've been super helpful and helped us all along the way.

DR. GALIPEAU: So actually, go ahead.

DR. RUSSOTTI: So just to comment on some of Irv's thoughts about doing things in a nontraditional way. I think he's absolutely right. You can take a small molecule or even a

biologic mindset when developing cell therapies. Obviously, you can take your learnings and apply them. So I was very fortunate. I worked at Merck for a number of years before I went to Celgene to lead a process development group in cell therapy and at Merck I worked on highly complex biologics, live virus vaccines. So a lot of the principles applied on the CMC side.

But I think when you look at the industry players that have gotten into this, and if anybody from large pharmacy that's been in and out of it, I'd be curious to hear your point of view because I think Celgene is becoming large pharma, but when I started at Celgene, Celgene was less than a thousand people. Now it's over 7,000. But what Celgene did that was very smart was we made a very independent vision of the company to focus on cell therapies. And we were everything from discovery to clinical, regulatory, commercial strategy, and the CMC that I run. And I think by doing that we've allowed ourselves to grow and think out of the box and develop things in a way

that draws upon experiences from traditional, but writes a new story. And I think that's why these smaller companies, and I think of our subsidiary like a smaller company. These smaller companies, and I won't name names. You know who the players are that are well funded and are thinking differently, are the ones in the best position for success.

And I also just want to comment that it's really - - really should be commended at the FDA is really thinking out of the box, and I think our interactions with the FDA on this have been nothing but outstanding because you take guidances that were written in many cases for other types of therapies. They've been adapted for cell therapies but they've really drawn upon experiences from traditional types of biologics and small molecules. And the FDA has thought in an evolutionary kind of way to adapt these towards cell therapies. And we've thought with them. And it's been a great partnership. And I think other companies would probably say the same thing.

We've heard our panel members from academia saying the same thing. So that's not the problem.

I think the problem is you have to have -- the solution is you have to have all players thinking outside the box and this is where big pharma, smaller biotech, like Celgene, have to be able to put the right kinds of groups together that can think creatively. And I think the field is going.

One other account I'll make -- sorry for taking so long -- is I think on the CMC side, there are efforts by academic players that are trying to pull consortia together which will include academic partners -- there's one being led by Georgia Tech, another by Wake Forest -- to really focus on the CMC questions. And I hope that these consortia are being built across the board, not just for manufacturing, because this is where we'll get many of the key academic, government, industry players to work together and solve these problems. It's a new field. It's going to take a long time to figure it out, and

hopefully we've stated to turn the corner now but it's going to take people working together to figure it out.

DR. GALIPEAU: Chris?

DR. BREUER: Yeah, I'm not so against big pharma or big device companies. I think they've made a lot of great products and saved a lot of lives.

I think there's a special place though for academics, and I think one of the things that I've found very enabling is the Office of Orphan Products, and that it provides a more streamlined, less expensive way of bringing your technologies to the clinic. And it enables you to really target in on the population that might not be a big commercial market but could potentially benefit from your specific product. And I think that's one of the things that I think has been done that is just incredibly enabling. It's a great thing and I hope more and more people can take advantage of that, especially within academia.

DR. GALIPEAU: So actually, I'll pose this question to Michael because he spoke about that. So Mike, what's the relevancy of preclinical animal models of predictive value for human cell therapy studies?

DR. MATTHAY: Well, it's a good question that I think applies to all of us. And there's been discussion in different models. It does depend very much on the questions you're asking. I really like Chris's last slide here about the two-way street. I think we all experienced Dr. Clegg talked about this, and of course, so did Irv. How we venture into the clinical setting, we learn from it, and we go back to do problem-solving. But I think all of us have used mouse work to establish some general efficacy model in most cases, or to test the mechanisms as Chris described in some detail. But then it's clear that in some cases going to a larger animal model really is helpful. And in our case, I think it was really excellent. I actually began some of my research career with sheep work but then long

ago left it, but then was lucky to partner with a large animal facility. It's not easy for industry or for academics to find really effective large animal facilities to work with, and that's again where NIH could help us more really with funding large animal work. So the sheep work, I think, was very helpful to us. Other investigators use pigs. It's not always necessary, but you learn usually not just about safety but additional mechanisms.

DR. GALIPEAU: So I'll pitch in at this point. Maybe I'll maybe give it a more skeptical -- because I'm looking at it from an immunology perspective. If you think about tissue injury and bone repair and vascular repair, I guess there's a lot of uniformity among vertebrate species, but immunology, the difference between the immunology of a mouse and a human, even other vertebrate species that are used is very, very, very substantial and often cannot be truly predictive. So I'd make the case that in vivo or

(inaudible) so that maybe the only

true testing of certain technology, especially for immune modulation, is to go fairly promptly to first in human clinical trials. Speaking rhetorically here, the case report that Katarina Le Blanc did in that trial of Graft-versus-Host disease, they went in with in vitro data because there were no good animal models at that time. So they went from in vitro data to first in human. Not to belittle the utility of animals to attempt to predict toxicity, but in their utility of (inaudible) models, mice and rats in particular, it should be predictive of immunology, and in particular it is extraordinary challenging. And you have to be cognizant of the limitations. I don't know what's the experience with others. Limitations, maybe,

of animal models, or how much or how little should we be put to the test before promptly moving forward to the people.

Irv, you had a thought?

DR. WEISSMAN: So I'll differ with you a bit, or a lot.

There's been conservation of gene cells and cell functions that you can follow, and you get pretty accurate results. And then there are species differences where you can't follow them. And so I think you have to be aware of that as you try to apply it.

The reason back in 1986 to 1988 that Mike McCune and I made the SCIDHU mouse was we knew that the hormones or the cytokines or the proteins in a mouse didn't always act on the receptors for those hormones or cytokines or chemokines in humans. So we put in the whole human fetal organ.

I think I've just been subpoenaed recently for the use of human fetal tissues in

experiments. And the reason I say that is that society also brings in its own cultural background, and doesn't always try to look at the issue in a balanced way. Meaning, how can we go from an understanding of the biology to medicine rather than a political or religious or a moral background to allow or not allow these things to go forward. You may think it's minor, but I don't think it's minor at all.

So I think if people had picked up on making SCIDHU, as we did, you would have been able to work with the subsets of human immune cells as Mike McCune and I did for years. We were lucky enough in that model that HIV, which didn't infect any animal in a productive way, infected the SCIDHU mouse's thymus, lymph node, and so on, so that we could follow HIV and eventually know what therapies didn't work. We couldn't find the therapy that would work.

But the important point is to be able to look at what animal model you need to use to study what's going on. The fundamental principles I

believe are the same. The exact interactions of defined subsets of CD4 T-cells may not be exactly the same, but conserved behaviors will be conserved and will predict.

DR. GALIPEAU: So we have precisely 10 minutes left, so I'd open it up to the audience. Invite people to walk up to the microphones. Madam, please identify yourself before a question.

DR. ELGENDY: Hoda Elgendy, LifeNet Health. Actually, since we are witnessing a stem therapy

avenues and more than 70 clinical trials ongoing, I think it's time to hear and brainstorm and know your opinion about cell dose, especially you mentioned that cell dose affects your system. And in addition to cell purity index from a surgeon's standpoint. And also the systemic side of toxicity, knowing that those cells are baby cells. They live inside the body. They may go into the blood stream. Even with a scaffold or without scaffold. In suspension or on a monolayer. We know that. They can go especially

in the eyes, you know. You put it in the eyes fiber blast and it could migrate on that surface no matter what. If it's God-made the fiber blast will come and then it will lead to blind. I understand that the patient is either to be terminally ill or blind as some of you mentioned, but at the same time we are doing something to prevent that in nonblind or semi-blind people in terms of the eye.

So I would like in a nutshell to hear your opinion about cell does and purity index and cytotoxicity. Short and long-term. Thank you.

DR. GALIPEAU: Do you want to grab this one?

DR. WEISSMAN: I think purity is important. Or you'll never understand what you're doing. I think you can work out the dose. You get an approximation from the animal study but then the actual first-in-human trial in a dose escalation rather than a dose de-escalation. I think you can get at it.

Toxicity, hopefully you'll see,

depending on the cell type you put in. Toxicity hasn't been the problem for us with pure cells. Toxicity is a problem of impure heterogeneous populations of cells.

DR. GALIPEAU: Dennis? C: Yeah. In our particular case, using a

scaffold and growing the cells for 30 days, they form a mature polarized epithelium monolayer where they're held together by tight junctures and adherent to the scaffold. And we see very little cell division. And for us that was a desired outcome. We don't want them to be dividing. We don't want them to be migrating. It's know that if RPE cells get on the surface of the retina they can become myelofibrosis and contract and cause a retinal detachment. That's a really important problem. And if you're injecting them into the subretinal space, you can get reflux out and cells can come out. So we approached it that way.

And in terms of dose for us, it's "one size fits all." We made the scaffold big enough

to cover the geographic atrophy lesion in patients, and so we didn't have to worry about dosing. And then peers, as I mentioned, you know, we developed assays to look at the different potential contaminating cell types that might be there. And one nice thing about growing these on a 3x6 millimeter patch, we can look at every cell on the patch using automated microscopy. And we can't detect any undifferentiated antigens, and of course, we've done the tumorigenicity studies to make sure that the cells are not tumorigenic.

DR. GALIPEAU: Thank you. You've got a question? Microphone number two.

MR. WEISS: Hi, my name is Dan Weiss. And I'm a physician scientist from the University of Vermont, interest in long-regenerative medicine.

One of the reassuring things that I'm hearing from this panel, and I think reflects this particular audience, is the underpinning of careful science as a primary guide in terms of how the cell therapies are developed and brought to

the clinics. We all believe in the incremental progress and the two-way street and like, too, but it's a real world out there. And so I'm curious about the position of this panel on movements afoot out there currently. We may hear a little bit more about it this afternoon, but to almost fast- track some of the cell therapies. Things like have happened in Japan and that have been proposed now here in the United States with something called the Regrow Act, and a simple consolidation of that is to take a product that made it through phase one and phase two and then skip the phase three. Go directly to marketing, if you will, and bring that to a needy public. So I'm curious as to thoughts on this. Should we be doing this? Should we put a break on this and say absolutely no way; it's the wrong thing to do?

DR. GALIPEAU: Okay, so everybody gets to put their hand in a bucket of crabs here? You know, let's give Chris at the end here a chance to --

DR. BREUER: I think it's very

product-specific. If you've got a disease and there's nothing you can do for it and the patients are going to die then, you know, have at it.

DR. WEISS: But that's a compassionate use. That's almost a separate equal issue.

DR. BREUER: But I think you can do that with Orphan Pathway. So in the Orphan Pathway, you know, you prove safety and then you can have an HDE and continue to use your product and sell your product but you study it at the same time. So I think it's possible to do right now. And I think for the right applications it's a wonderful way of doing things.

DR. RUSSOTTI: So I'd sure love to hear the FDA's response to this. I'm just going to tee it up though. I'm not a clinician. I'm a CMC guy and I have been at Celgene

years. There's one story at Celgene I'd like to share. On one of the earliest therapies that Celgene was

approved on from one of their first IMIDs, which is very effective in the field of

multi myeloma and related types of hematological cancers, and one of the first indications Celgene got approved on was on less than 30 patients worth of data because it was a very clear understanding of a genetic disposition of a patient population that was going to respond to this therapy. So you can talk about Japan all you want and the Regrow Act. I think the Regrow Act is very dangerous in a lot of ways but I'm not going to comment any more on that. I think what's important, back to science. If the science is there and the data is there, the FDA will fast-track. The FDA will find creative ways to get things to patients quickly. So again, if the FDA wants to comment, great, because they probably have more tangible stories than I do. I have one that I know of very well but I'm not going to comment anymore on that but I think it's all about the data and we can do it. We just have to have the right data.

DR. GALIPEAU: So it's about the science. We're not going to put the FDA on the spot because this afternoon they're going to be on

the spot with all this stuff.

So microphone number one.

DR. BERTRAM: Yes, sir. Real quick question. Following up on the previous one which relates to

dose, I think dose selection, if you look at that --

SPEAKER: Closer to the mic.

DR. BERTRAM: Closer to the mic. All right. One of the key --

THE REPORTER: Please say who you are.

DR. BERTRAM: Ken Bertram, RegenMed. Quick question for you, really relates back to

dose, which is a key issue. If you look at dose in clinical trial failures, whether it's small molecule, large molecule, or cell-based therapy, dose selection is the key reason why a clinical trial fails, and so we're unable to bring a particular product onto market.

Two-part question for you. All of you looked at dose. In the cell therapies itself, did any of you see any evidence of any toxicity as you

escalated your dose? And if you did, if you could give some examples of general toxicity effects that you did see.

DR. BREUER: No toxicity other than that in my case it's autologous, so if you're taking more bone marrow, your chances of a transfusion are higher but there's ways around that. You could reinfuse the red cells, but no other toxicity.

DR. WEISSMAN: There was no toxicity in animals or in humans with purified hematopoietic stem cells. You couldn't reach a dose, give an IV that would lead to toxicity if you used stem cells. If you used an impure product, it's completely different. When you put the cells in a defined region, as we did in the spinal cord or the sub-retinal, or even into the brain of children, we suspect there is a cell dose issue even with pure cells, but we only suspected. We never got to the dose with the amount of money that was there to follow. So I think it's an important issue. I think you can approach it for

toxicity in animal models if you have the right animal models.

DR. GALIPEAU: So our experience using autologous fresh cells in Crohn's Disease, we went 2/5/10 million cells per kilo without a problem. But then if you use autologous cell therapies there's a practical limit of how much you can manufacture. The limit is not so much the dose but the time it takes because if you've got a big fellow like me, 10 million cells per kilo is a lot of cells. So to go through 20/50 -- in mice we go up to 50, 50 million cells per kilo. If you go above that the mice start getting sick. The only other cell type that you could go off scale would be universal allogenic, and I guess the only company that went to high dose was what, I guess Athersys went up, what, 10 million cells per kilo repeatedly with their product. And that's probably the higher dose of the MSC-like cells. I'm not aware of anybody going a higher dose IV, IV, unless somebody else has some experience. And they haven't disclosed any toxicities.

MR. BERTRAM: Maybe rather than putting forward the second question which may take time and others would like to ask, one question or one comment, I guess I would give not only the FDA but to us as scientists trying to bring something forward and actually develop it and that is thinking about our dosing paradigms different. I mean, Irv, as you said, we use dose escalations in clinical trials. We're all faced with randomized control trials. I can't help but wonder if in partnership with the science we bring the understandings of science forward into our clinical trials so that we look at different dosing paradigms, possibly dosing metrics. I don't know what it is, but when I look at all across all different cell types, when we get into the millions and hundreds of millions, that's about where we start seeing a fax, and that's about where most of us considered to be probably the maximum to manufacture. It may be in partnering with FDA one of the things to think about is what different, unique dose metrics or

clinical trial designs might we use in order to take this very safe product generally and start and advance our protocols in the humans at a higher dose and look at dosing regimens in a way that will allow us to actually, rather than look at unsafe methods which I agree with Greg on, I think Regrow has got some safe issues, but to actually allow us to be able to accelerate the development of these products more quickly.

DR. GALIPEAU: I guess the point you raise has to do with the open access we spoke with. Academics will publish, but often their industry studies or small startups are under no obligation to publish. And that data typically is, for obvious reasons, kept confidential.

So it comes back to microphone number two.

DR. GROSSO: Yes, Rob Grosso, Bethany, Connecticut.

I just had a question for the panel. There seems to be a great interplay between the FDA and small developers, but one thing that sort

of came out was structure and function of cell therapies. And during the process of process improvement, how do you guys feel, and ladies feel, about FDA guidance on process improvement with structure and function in cell therapies? Is there a clear guidance for you all?

DR. RUSSOTTI: I am not sure if I fully understand the question but I think it's just about back to making changes that make sense. So I don't know if you want to come back and help elaborate because I really don't fully understand the question. I think it's back to the FDA being willing to listen to science and just doing things that make sense. Can you just maybe rephrase?

DR. GROSSO: Sure. With Irv's discussion about structure and function of cells, maybe as you change from one process to the other in development from early bench science through clinical procedure you're trying to increase production. How do you guys handle guidance on process improvement versus totally different product? What's the --

DR. RUSSOTTI: Well, I don't think you can do a totally different product, unless you want to start from square one. I think that's the whole point.

DR. GROSSO: Right.

DR. RUSSOTTI: In my presentation I talked about the fact that we were trying to make the same product. You have to understand what your product is, number one; how your process affects the product, number two; and then do things that won't change the key attributes. There may be some attributes that are not unimportant, right, but it's all about function, really. I mean, structure is nice, too, but it's all about what your cells are trying to do. So I think it's operating within that paradigm that you have to understand your product and your process. And the more you understand, the better your chance will be that you'll make a change that will result in the same structure and function.

DR. GALIPEAU: So I apologize to the rest of the people lined up at microphone two

because we're going to take the last question from microphone one.

DR. JONES: Thank you. Alice Jones, LifeNet Health.

I was curious. You were talking about your graft growing. What sort of objective measurements did you take to discern that it actually grew and perhaps did not stretch?

DR. BREUER: Yeah, that's a really fantastic question. You know, people always say, well, how do you know it's not just dilation? And then it's really, really complex because the inferior vena cava is like a (inaudible) so it changes size and diameter all the time. So I think one thing is to look at length, and that's really key. Marking your grafts when you implant them with something. A radiographic marker is also very key. But we've got some interesting data now 14 years out where we, not on purpose, but did an experiment that I've always wanted to do. In my mind, if I wanted to do the experiment to prove growth, I'd have to take the same thing

and put it in an organism that's supposed to grow and see that it increases in size, and put it in an organism that's done growing and show that it doesn't increase in size. And when we compared the rate of growth in our younger patients versus our older patients there was a much steeper rather of growth in the children that were implanted in the two- and three-year-age range compared to young adults and older adolescents who had had the grafts in place.

DR. JONES: Interesting. Did you also find any commonality in the stenosis formation in the patients that did become stenotic? Was there some common underlying clinical feature or something with the dose of the cells that was delivered? And also, do you see elastin in that explant?

DR. BREUER: We see some elastin. Whether it's functional or not is always the big question. And we're doing some biomechanical work trying to get at that right now. The cohort is too small at this point to really know who

stenoses or doesn't stenose, but I can't imagine it doesn't have something to do with the immune system, that there aren't going to be patients that have a higher rate of stenosis and other groups of patients that have a lower grade.

DR. GALIPEAU: I'm just going to have to stop you at this point here. Miss, I'm just going to have to stop you because we're getting a bit late.

I know there's a lot of terrific questions coming from the audience. Please, our folks are going to be here for the rest of the day, so walk up to our panel members and ask them questions.

I'd like to thank all the panel members for their excellent contribution.

(Applause)

DR. GALIPEAU: I apologize we're breaking late for lunch, but I'm sure that our FDA sponsors would like us to start on time at 1:20.

This closes Session 2. Thank you.

(Recess)

DR. WITTEN: Could people come in and take their seats, please. We'll start in another two minutes. Okay, if people could come in and take their seats we're going to start with our first after-lunch session which is the "Views from Professional Societies," and to start off the session is Dr. Jonathan Kimmelman. He's an associate professor at McGill University with a specialty in biomedical ethics. His research focuses on ethics policy in scientific dimensions of drug and diagnostic development. He chaired the International Society of Stem Cell Research Guidelines and his talk today is on the regulation of unproven stem cell-based interventions, the ISSCR, and he will also serve as the moderator for the rest of this session.

DR. KIMMELMAN: So now for some ethics to help you digest your lunch. So first I want to thank the FDA for the tremendous honor to address you.

So over the last 10 years or so there have been numerous reports about clinics that are

offering unproven cell-based interventions to patients outside the context of clinical trials. Now, most of the time when we've heard about this it's been in the context of far-flung locations; places like Italy, Moldava, Dominican Republic, Russia, et cetera., but as people like Leigh Turner and Paul Knoppfler have meticulously researched and demonstrated most recently in a publication in cell stem cell, there are an abundance of clinics within the jurisdiction of the United States that are offering unproven cell-based interventions to large numbers of patients outside the context of clinical trials.

Now, there are many who would see this as a good thing and there are many advocates out there who would like to see the reins on providing cell-based interventions to patients loosen, and indeed as we've heard earlier today there have been some jurisdictions that have done exactly that, so for example we know in Japan that there is a policy that allows cell-based interventions to patients for a fee after demonstration of

safety in very small clinical trials, and we also know that in the United States there have been policies that have been floated -- not yet successfully taken root but nevertheless floated -- in Congress namely through the REGROW Act that would also authorize in the context of regenerative medicine unproven cell-based interventions to patients outside the context of rigorous clinical trials.

Now, regardless of where you stand on whether these are good developments or bad developments, it really invites a question, an ethical question about how regulatory authorities ought to establish the benchmark or threshold for making cell-based interventions available to patients, again, outside the context of trials, and so what I want to talk about in my 20 minutes is first, what is morally at stake in establishing a threshold for approval of drugs or cell-based interventions, and then in the second part I want to explain how that moral understanding of what's at stake articulates with the guidelines written

by the International Society of Stem Cell Research.

Okay, so let me start with part one. Typically when people talk about what is morally at stake with the demonstration of safety and efficacy with whatever the regulatory standard is for allowing a drug or cell-based intervention to be marketed, there are sort of four kind of narratives or typical arguments people tend to hear that kind of organize the moral debates.

The first one is that there's a tradeoff between private interest and public interest. On the one hand you have the interest of patients who have very serious life- threatening disorders. You have them wanting to exercise their autonomy to get access to potentially life-saving interventions traded off against the interest in the public in being protected from unsafe and unproven interventions, and in this way of understanding what's morally at stake you can think of the drug innovation as kind of a zero-sum game. Whatever you do to increase the private

autonomy, the personal autonomy of patients is at the expense of the public interest.

Now, the second common narrative one hears is that there's a tradeoff between caution and innovation. You can have a really cautious regulatory environment, one that keeps us really safe but that doesn't really allow the flourishing of research and innovation, or you can have a highly-innovative environment, but if we're going to do that we're going to have to make peace with the fact that people may be actually harmed in that process.

A third really common narrative one hears and that context often, again, it's kind of zero-sum game; whatever you increase in caution you do at the expense of innovation. Now a third common argument one hears is that it's really a matter of patients versus bureaucracy, of patients with felt medical needs, caregivers who want to advocate for those patients versus governments that had a logic that may be disconnected in some way with the very intense needs and yearnings of

patients and other caregivers. And the third argument one often hears is that it's really a matter of demonstrating safety versus requiring efficacy; that one option is to allow cell-based interventions or drugs to be approved on the basis of safety, leave it to the marketplace to sort out whether they're efficacious, or we can have regulatory standards that really require demonstration both of safety and efficacy.

Now, what I want to suggest is that each of these different ways of trying to frame what is at stake in regulatory standards is really deficient on understanding the problem. So first let's take the issue of private versus public trade-off. The fact of the matter is that the vast majority of interventions that are put into clinical development in areas where the clinical need is the greatest fail to vindicate their clinical promise in rigorous clinical trials. So, for example in cancer, for every 20 new drugs that are put into clinical testing only about 5 percent will actually demonstrate safety and efficacy in

trials.

Now, once we move into the realm of things like neurodegenerative disorders which is a thriving market for unproven cell-based interventions, the numbers have to drop to less than 1 percent. There's very little we can do for neurodegenerative disorders that at least have a neurological basis of their cause.

And in fact where we have been able to be successful with translating complex interventions, it's taken an enormous amount of time. So for example, the very first gene therapy clinical trial was conducted in 1989. That was the year that song "Love Shack" was at the top of the charts, so you can imagine at this point to my knowledge there are no FDA-approved gene therapies. Is this correct? Okay, so although there are some very, very promising and exciting gene transfer techniques, some of which actually show tremendous promise for very niche disorders, it gives you some sense of the scale, the timeline that it takes to unlock the clinical utility of

very complex interventions, so it's not clear to me that patients -- the (inaudible) of patients have access to unproven cell-based interventions or drugs necessarily deprives them of medical opportunities. It may deprive them of a choice, but it doesn't deprive them of access to a life-saving intervention.

The second issue about caution versus innovation; this assumption is that regulation in some way stymies innovation, but the fact of the matter is, at least in my analysis, there are many ways that oversight actually enables the very conditions under which innovation occurs, and there are a number of different arguments by which I can cash this out. One that I'm going to make right here is just the simple notion that if you are a for-profit company and you know that you're going to get data exclusivity, exclusivity on your product, if you demonstrate safety and efficacy of your product you have an enormous financial incentive to generate high-quality evidence about how to unlock the clinical utility of your

intervention. So, maintaining stringent regulatory standards creates incredibly strong financial incentives for companies to generate evidence, and this is just one way that stringent regulatory standards actually drive innovation as opposed to impeding it.

Another critical way, just to mention really quickly, is that it ensures that by maintaining high standards requiring demonstration of efficacy it ensures that you maximize the information you get for exposing each patient to unproven cell-based interventions; that is if you only allow patients access in the context of clinical trials you're gathering important information about safety and efficacy. If you allow that efficacy information to be collected outside in the marketplace, you're diminishing the efficiency with which we are actually gathering important information about the properties of these interventions.

Finally, I think critics who would see that there -- as an argument -- there's a tradeoff

between innovation and caution should address the fact that the USA enjoys one of the strictest regulatory regimes for drugs and cell-based interventions and yet has one of the most vigorous innovation enterprises, so it's not clear to me that one can argue that stringent regulatory standards by themselves stifle innovation although of course there are some regulations that are better than others.

Okay, what about the issue about patients and bureaucracy? Certainly there are many patients that organize patient groups who argue vociferously that we ought to diminish the standards or requirements for making cell- based interventions available to patients, and I think these are voices that we need to take very seriously and we need to listen to, but it's not clear to me that patients with these organizations necessarily are democratically elected to represent the opinion of all patient groups. Certainly I know many patients in my personal experience, in my family who have had serious

life-threating disorders, who I think value knowing that there is an agency making sure that the drugs that they take are proven to be safe and effective.

And finally the issue about safety and efficacy -- what's always puzzled me about this argument is this notion that you can have something that's safe but not efficacious. Let's just take the example of X-ray radiation, something that's pretty safe in the context of a patient who's being treated for a tumor but not a particularly safe modality for a patient that's being treated for a wart or a skin tag.

Safety is by its very concept context-dependent. It depends on the value or the utility of the activity that you are evaluating, and moreover it's not clear to me that cell-based interventions by themselves can generically be called safe. There are all sorts of different kinds of risks that we have to worry about; risks of contamination, risks of cells integrating inappropriately and disrupting organ activity, et

cetera., and there's certainly a number of cases out there of patients who have been harmed from receiving unproven cell-based interventions.

So, in a sense you can think of regulatory standards as a pivot point that determines how we as a society want to distribute the burdens of medical uncertainty. Do we want to put the burdens on health care systems and patients, or do we want to put the burdens of uncertainty on the backs of companies that are expressly privileged to profit from marketing those products? That's one question.

Another way in which you can think of regulatory standards as a pivot point is in a question of patient autonomy. If patients receive cell-based interventions in the context of clinical trials they're typically undergoing a very rigorous informed consent process. They understand what risks and benefits they're receiving.

If they're receiving unproven cell-based interventions in a care context, typically the

informed consent process is less stringent and less demanding, and in that way I think there's a very strong argument about why we would want to restrict access to unproved cell-based interventions to patients within clinical trials.

Let me explain how this kind of moral understanding of what's at stake in drug regulation fits with the ISSCR guidelines. Now, just a few words about the ISSCR. The ISSCR is probably the largest and most influential society of stem cell scientists; about 4,000 members from around the world. In 2008 the ISSCR issued a set of guidelines on clinical translation. In 2016 the revised guidelines were released addressing clinical translation as well as basic research, and I chaired the effort to revise the clinical translation guidelines.

Just a few words about the process. The process involved a very large and diverse group of individuals who represented different constituencies, different expertise, different countries, and also we strove to have as much

diversity, both gender balance as well as cultural diversity within our working groups, and the process started in 2014.

We issued the first draft of the guidelines in the summer of 2015, received about 26 comments from various experts who we solicited for a peer review, made some revisions, sent out a draft for public comment and there we received about 80 different comments. We addressed those comments, incorporated these into revisions of the guidelines, and then released the guidelines in 2016, and there are about 6 different core sections of the ISSCR Guidelines that directly speak to the question of how regulatory authorities ought to oversee the process of evaluating cell-based interventions.

The very first call out of regulatory authorities is actually in the first section; the section on principles. Two principles in particular speak to the issue of regulatory standards. The primacy of patient welfare; there's a passage there that says it's a breach of

professional medical ethics to market and provide stem cell- based interventions to a large patient population prior to rigorous and independent expert review of safety and efficacy.

In the section on social justice there's a statement that says it's a matter of social justice that the cost of proving the safety and efficacy of a medical intervention be borne by entities that are expressly privileged to profit when such interventions are marketed. Again, it gets to this question of how we want to distribute the costs and burdens of medical uncertainty.

The second section that deals with regulatory standards actually directly speaks to the question of regulatory approval, and that section states the introduction of novel products into routine clinical use should be dependent on the demonstration of an acceptable balance of risk and clinical benefit appropriate to the medical condition and patient population to which new treatments are designed. Now, within the expository language underneath that there's also a

statement that says that national governments and regulatory authorities should maintain rigorous review pathways to ensure that stem cell- based products conform to the highest standards of evidence- based medicine.

The third section is actually a warning within the guidelines about the provision of unproven cell-based interventions outside the context of clinical trials and innovative care. Basically it says this should not happen unless -- you should not be delivering unproven cell-based interventions unless it's in a trial context or a special innovative-care pathway.

What is an innovative care pathway? Well, that's the fourth section that deals with this. The ISSCR lays out conditions that need to be met for a legitimate care pathway; small numbers of patients. There needs to be a protocol. The protocol has to undergo peer review. Patients should be ineligible for clinical trials. There needs to be a mechanism to systematically collect information on outcomes,

and these outcomes need to be reported in the peer review literature.

The fifth section deals with the question of patient-sponsored clinical trials or pay-to-participate clinical trials. Many of the clinics that are offering unproven cell-based interventions to patients are doing so in the context of trials where patients pay to participate in them, and the ISSCR guidelines take a very skeptical and restrictive view of pay-to-participate clinical trials on a number of grounds. One ground is that it's hard to imagine how pay-to-participate clinical trials incentivize the kinds of rigorous data collection activities that you would need to actually rigorously evaluate the stem cell based product. After all, it's hard to imagine patients paying to be allocated to the Sham treatment arm, for example, so the ISSCR take a very restrictive view of the pay-to-participate clinical trials.

And the sixth part of the guidelines that deal with regulatory standards is actually in

the section -- the penultimate section of the guidelines that deals with communications, and they are the guidelines that state regulatory and law enforcement authorities are encouraged to investigate and where appropriate restrict unsupported marketing claims made by commercial actors to the extent that these violate relevant consumer protection truth-in- advertising securities and commerce laws within a given jurisdiction.

Now, these guidelines, I encourage anyone who's interested in them to go online, to download a copy of them. They're very easily accessible or you can read very brief synopses. We've published a series of these in major medical journals. This is one that we published in Lancet. There's another longer description of the guidelines published in The Stem Cell Reports as well.

Let me just close with a last thought. You might be sitting there wondering why would a professional society that's primarily devoted to

scientific research take such strong views on regulatory standards for cell-based interventions, and the answer is that the process of innovation is an area that is as cutting edge as cell-based interventions is very long and it's very arduous, and it depends on sustained collaboration of many, many different kinds of actors all of whom are putting a lot of their interests at stake in that collaboration and all of whom come to that collaboration entertaining a different set of goals.

So, for example, patients participate in clinical trials not necessarily to make big discoveries but to get access to cutting edge treatments. Companies fund clinical trials not so much to make big discoveries. They might like that, but because of a prospect of earning revenues from products that get regulatory approval. Scientists conduct clinical trials partly to burnish their CVs, to get publications in medicine, so all these different actors are coming to the collaboration entertaining a mix of

different kinds of motivations, some of the altruistic and others self-interested, and in that environment you really want to create conditions where actors can trust that when they collaborate with other actors who are pursuing other kinds of interests, they know their personal interests are not going to be undermined, that patients when they participate in clinical trials when they receive cell-based interventions that they're not going to be putting their health and welfare at stake.

And I would suggest to you that regulators play a key function in establishing those conditions where these different disparate actors can trust that their interests are going to be protected when they collaborate with people who are utterly strangers to them. So, in that respect I would encourage people to think of regulatory authorities like the FDA not so much as agencies that are there to protect the public from unsafe and ineffective drugs, but to think of FDA as probably one of the most important regulatory

agencies that is driving the innovative process, that creates incredibly strong incentives to generate the kind of information we need to practice a high standard of evidence-based medicine. Thanks. (Applause)

Okay, so we now move on to the next part of the program. I assume there's no question now. I think I'll take as a panel. Does that sound right? Okay, good.

So I'm going to introduce the next speakers. The next speaker is Massimo Dominici. He's a clinical scientist at The University of Modena and Reggio Emilia, Italy, developing cell-based and gene therapy approaches for cancer patients. Dr. Dominici served as President of the International Society of Cellular Therapy from 2014 to 2016. He's currently Chair of the ISCT Advisory Board and Chair of the ISCT Presidential Task Force on Unproven Cellular Therapies. Thank you.

DR. DOMINICI: Thanks for the introduction and thanks to the FDA for hosting

myself coming from Italy. I've been working around cell therapy for quite a while, and I'm going to share with you some of the reason why I start to work also on these improvements in cell therapy as part of the ICT.

ICT's a global society dealing with translational research in cell therapy including immunotherapy, stem cell therapy, and since 2010 we felt the need I think, together with other societies has been already explained, to take some action regarding what in 2010 was called medical tourism. That time the President Kurt Gunter felt a need to act and to propose a plan that involved the society to try and face the issue of medical tourism, and since that time the society established a task force regarding what we now call proven cell therapy and why is that?

I think we should start with numbers. It's a massive issue. Some estimates are indicating there are -- the market regarding the unproven cell therapy is very wide, and there are 2.4 billion of U.S. dollars that are involving

patients that are paying to be treated with a single treatment can be paid up to $40,000 U.S. dollars. And that's, I think, is quite significant. But this I would say is just a part of the issue.

At some very recent publication from a friend, John Ruskin, in Australia where they were dissecting the numbers of stem-cell based clinic that were available on the web, so they did some web research and the intensity of the redness has indicated the numbers of clinic for each country. So you see that these three global challenge, and the global challenge as I was saying, is related to the type of diseases and to the cost of the diseases. I was mentioning the cost of each of these treatments. This 2009 publication of stem cells, these authors who were essentially picking up a series of clinics where there was a doubt of proven cell therapy approach. They were calling them asking some questions. One of the questions; how much is the payment for the treatments, and in some cases there was no answers, but the answer

that they got were between $10,000 and $40,000 as I was explaining.

How about the clinical indication of the 2009 publication? As was said, the majority of the cases are related to neurological disorders because these are frontier for medicine, is a frontier for regenerative medicine, and so we move from multiple scleroses, Parkinson, stroke and if we compared those data we'd -- a more recent publication in 2016 realized the situation is likely changed. This once again the stem cell publication from John Ruskin, you see there's a big difference.

There's something else that's been incredibly increasing; that these treatment with stem cells for aging. The majority of the website were advertising treatment with stem cells just for aging, considering aging as a disease. This, I think, was to me at least -- it's very surprising how the landscape of clinical indication has been changing the last seven years. Would aging (inaudible) the rest of disease

versus, you know, the neurological disorders that are still appearing in the list here.

Neurological disorders are certainly a big issue and we know all that, and the reason why I personally have been involved in this unproven cell therapy field is for a reason that is related to my own country. I think the previous speaker was showing one of the scripts that were present in Rome at that time. This was 2012.

In 2012 I received a call from the Minister of Health asking my lab to analyze a cell preparation that was under investigation in a public hospital in Italy, and for clinical indications which were mostly related to neurological disorders. This group of individuals who were essentially performing the following procedure as I show you here in the slides, so they were starting from bone marrow aspirate. They were isolating the cells in a regular lab, forgetting about the CGMP or the CGLP regulatory frameworks. They were amplifying the cells. There were some testing. They were freezing down

the cells. They were keeping the cells for quite some time in a nitrogen tank. They were defrosting the cells and they were treating the cells with retinoic acid for a couple of hours, and then they were infusing the cells and (inaudible) almost to size. One end is fine and one (inaudible). The second (inaudible) intravenously. The numbers of cells which was used about 200,000, and we have been talking about millions of cells this morning, so I (inaudible) of cells in this indication. And this was -- I mean I'm not certain here to question the way in which the cells has been delivered. If you have data that (inaudible) reporting the approach, this is very fine. If you have data that are supporting that you can get some amelioration of your diseases just using this type of delivery, then that's very fine. These authors did not have any type of preclinical findings regarding this type of infusion.

While I was more and more involved in this story I realized that the protocol that we're

applying was very, very weird; not done in the way in the cell types but in the way in which the cells were delivered, so approximately or currently about 40 patients have been treated according to this protocol and the treatments were very different from one to the other, so one patient in this case, patient AB was receiving patient cells in a sort of (inaudible) setting so the bone marrow aspirates and then transplant. This was the first infusion. The same patient, patient AB was receiving cells from an (inaudible) donor in an allergen A setting for the second infusion, and then for the third infusion the patient was receiving cells from another patient. In a very weird manner the authors were calling that multiple (inaudible) transplantation since we forgetting like 60 years of immunology and more. So this is essentially what was shocking me, and this was why I start to work in this field as scientist involved in cell therapy development.

And the cost of this treatment has been estimated between 40,000 and 80,000 euros and the

patient had to make loans with a bank to pay the treatment which was another shocking surprise to be seen.

So we talking about unproven cell therapy and why this protocol can be considered unproven cell therapy. I would say there were no scientific basis on mechanism of action. There were no scientific basis for the proposed delivery schedule. There was no evidence -- even preliminary evidence of safety. One of these patients actually died during the infusion. There was a lack of primary data and expectation of efficacy, and the ratio between risk and benefit was not even taken into consideration and informed consent was an option for these guys here.

And then the last part which certainly one of the

> (inaudible) is terrible is the pay to be treated model, so this is why I'm proving -- this why (inaudible) protocols can be considered unproven cell therapy. So this is

just a case and I leave myself with my lab and with my country I would say with the patients that were starting to look for this type of treatment, but if we trying to dissect which are the (inaudible) of the cell-based intervention I think we can try to simplify some -- apologize for oversimplification, but certainly we can have this type of example unproven, unregulated medical procedure which are marketed as beneficial therapy without waiting for (inaudible). Then we have a novelty medical care, non-approved but possibly proven approach provided by legitimate care givers which, you know, they take their path of investigating the efficacy and the safety, and I would say this is innovative medical care. Of course then we have the clinical trials

development as we all know.

The problem that is emerging (inaudible) cell therapy is that sometimes the first line and second line a bit confused. So there's some confusion of unproven cell therapy in the novelty medical care, so we don't want to take this cue of innovate to propose unproven approach. That's something that should be clear.

So when we start work around unproven cell therapy inside the ICT and also with other colleagues involved in this field we started to ask ourselves which are the key question on unproven cell therapy because that was very challenging. So the first question was how to define unproven cell therapy. The second which are the minimum level of biological evidence that we can apply to a cell-based product to advance this product into a clinical scenario, and then which are the minimum requirement for cell manufacturing and those things which seem to be obvious for us but they are not, and we should talk about that in this context and outside these

rooms.

And then which are the interaction between the improvements in therapy in the global regulatory frameworks which are very different from one region to the other. And then what is the role of pharmaceutical and biomedical industry in this context, and once again, you know, what is the role of scientific community in this field.

So we start with the definition of unproven cell therapy. I think presenting this (inaudible) protocol I went through all these points and it certainly unclear scientific rationales (phonetic). I'm not going to repeat this data, and what I think should be outlined here is just uncontrolled (inaudible) procedures that are taking place in human being.

Then I wanted to pick something else that was shared and discussed inside the group. This is dealing with the basic core practice for

cell therapy production. This is work that was done by Paul Eldridge in collaboration with other colleagues in the Society. There is certainly some common sense guidelines on manufacturing, but it's very relevant to be respected to generate a product that is essentially standardized and ready to be injected and reproduced in a variety of patients for a precise indication.

Of course there are some risks related to this direct to consumer marketing of cell-based therapy. Patient arm, there's lot of psychological impact in patients and in their family. There is financial loss for sure that has been also subject of justice-related issues. Damage to the integrity of the entire healthcare and of course this is generating unrealistic expectations.

The way in which these clinics are providing and approaching providing therapies and approaching the community it's changing rapidly. The way of communicating is changing, so they generally use, you know, testimonials on websites.

They use social networks and they link stem- cell based intervention to some sort of scientific journals that they support their approaches. Essentially they are identifying some sort of even fake scientific societies or fake regulatory body that can certify their structure. So it's very diversified the way in which they can justify this approach. What is generally happening is those guys are outlining the benefits and hiding the risk.

This once again is a 2009 publication that I was mentioning to you at the beginning. Essentially the authors were calling about 25 clinics asking questions regarding the benefits of the approach and the risk of the approach. As you can see here in the majority of the cases those clinics were outlining vague positive claims of efficacy and in the majority of cases they were hiding the risk of those treatments. That's part of 7 years ago publication, but it's still going on in a more subtle way.

So which are the future steps and why I

like to share this work with the scientific community. Of course we cannot stay closed in our lab or stay closed in our scientific community. We want to embrace patient organizations, other professional societies, and regulatory agency to outline the action to ensure the patients are protected.

We'd like to -- it's something that might not be easy but to implement a long-term program to promote a global regulatory organization which might not be easy to do in a short time-frame, but is something that should be a focus of different regulatory agency. We would like to promote (inaudible) scientific development in the field and cooperate with other society so that we can try to speak with one single voice because I think the success of this strategy will come if there will be a global collaboration and a unified voice regarding this relevant issue.

So, I'm ending here thanking once again FDA for the opportunity and thanking all the people which have been participating in the

process of establishing this debate inside this society and am looking for the final debate to listen to questions you might have regarding the work that we have been doing, and we hope to continue in collaboration with other society, so thanks very much. (Applause)

DR. KIMMELMAN: Thanks so much.

DR. MANSFIELD: Okay, our next speaker is J. Peter Rubin who's the Chair of the Department of Plastic Surgery, the UPMC Endowed Professor of Plastic Surgery and Professor of Bio-engineering at University of Pittsburgh. In addition to his active clinical program, Dr. Rubin directs a basic science research program in biology of adipose-derived stem cells and serves as the Co-Director of the Adipose Stem Cell Center at Pitt. Dr. Rubin is Co-Chair of the American Society of Plastic Surgeons Task Force on Regenerative Medicine and a regulatory chair of that same society. Thanks.

DR. RUBIN: Thank you. Thank you very much to the FDA for the opportunity and honor of

being here today. Talk about adipose therapies, we're going to shift gears a little bit and talk about the world of adipose-based therapies; where we are now and where we're going with these therapies.

The American Society of Plastic Surgeons is the largest surgery-specialty organization in the world for plastic surgery and represents 94 percent of all board certified plastic surgeons in this country, and many of the adipose therapies that are being conducted are being done within the field of plastic surgery, and no financial disclosures for this talk.

So at the University of Pittsburgh I chair the Department of Plastic Surgery. I'm a clinical plastic surgeon and I also do basic science and clinical research. I sort of work in all the different spheres of translation, and this is really focused around adipose tissue where we have the Adipose Stem Cell Center at Pitt, and in this talk I'm going to cover two main themes that are distinct but really interrelated, so we have

first the theme of autologous fat transfer, and this is a surgical tissue grafting procedure where we use particles of adipose tissue that are generally two to five millimeters in size, and in current plastic surgery practice these particles of adipose tissue are implanted, and these small particles are composed of all the components of adipose tissue; adipocytes, stromal cells, connective tissue and blood vessels.

And then the other part of this talk is about adipose stem cell therapies, again which is interrelated to autologous fat transfer because we derive these isolated cell products from these extractive fat particles. In these adipose stem cell therapies adipocytes are excluded and we have a population of very bioactive cells, and these cells are really the biologic engine that are responsible for a lot of the tissue remodeling that we see in fat transfer procedures.

So, again, the first part of this is going to be about fat transfer which actually has a really long history. We talk about many cell

and tissue therapies and this is one that actually goes back to the 19th Century with the first publication on this topic coming out of Germany in 1893 where a surgeon used small grafts to treat scars of the forearm, and in modern fat transfer this has really become a very common clinical practice using simple methodology and this has broad-reaching applications in aesthetic and reconstructive plastic surgery. Moreover, this is something that's undergone many refinements over the last two decades.

How do we get these particles out of the patients? Well, this is a minimally invasive harvest, and we use hollow-bore cannulas either under hand-held suction or with machine-driven suction, and you can see in this picture the tip of the cannula which is about the same size as the particle and those apertures really determine the particle size. We often will use infiltrative solutions containing epinephrine in the subcutaneous tissues so that we can minimize blood loss, and once we have this tissue extracted very

simple centrifugation at about 1,200 g and then we can use blunt-instrumentation specially designed to inject this material after we separate the aqueous and oil layers. So here we have a photo from my operating room showing many of these small tubes of fat processed in the O.R. and ready to go, and we can do this in fairly large volumes, and when we do this with large volumes we'll simply use larger instrumentation to extract and deliver the tissue.

So there's a lot of variation in how this is practiced, and that's given rise to a veritable supermarket of devices that are out there, and the cannula world from the harvesting and injection steps up through the fat- processing steps, and this is an example of a commercially available filter canister that we can keep online sterile in the operating room, and there are other devices that will irrigate the collected tissue with saline solutions, and there are very expensive devices that we can bring into the operating room as well but in its base form I've

really outlined these straight-forward steps.

And this was survey data that we published from our lab a few years ago showing that in the plastic surgery community there is a fair amount of diversity in how surgeons will use these different processing steps: centrifugation, simple filtering, washing with saline, other methods that remain undisclosed by the participants in the survey and to no preparation at all or just simple decanting of the material.

So why is this technology important in reconstructive surgery? That's because fat is actually our best tissue for reconstructive surgery. It's our best soft tissue replacement. It's a natural component of soft tissue that defines form and shape throughout the body, so now we have a method of doing this with minimally invasive technique, less donor site morbidity, and very importantly a tissue-remodeling affect, so the current clinical applications will span aesthetic facial volume augmentation where this really got its start as sort of a not really

mainstream procedure many decades ago, and it's now widely practiced in breast reconstruction, buttock augmentation, scar treatment, cranial facial reconstruction, limb reconstruction, just to mention a few, and it has very important applications in the treatment of radiated tissues and reconstructive surgery as well.

So I want to highlight some of the data that we've collected in our clinical trials or clinical studies that are really geared toward the devastating limb and cranial- facial injuries that are seen in military trauma from IED blasts, and this is work that is funded by the Department of Defense through different programs including the Armed Forces Institute of Regenerative Medicine and the Biomedical Translational Initiative, the Congressionally determined medical research programs as well, so it's a very important area for our wounded warriors.

And we did some very detailed analysis in a cohort of 20 subjects that we treated with cranial-facial deformities. Five of these

subjects underwent repeat treatments. There were no serious adverse events, and one of the things I want to really highlight with these adipose grafting therapies is that the safety record is overall very, very good, and the recovery for these patients tends to be much easier than the traditional open procedures.

All of the procedures were performed on an outpatient basis is this cohort and we followed the patients out through 9 months with a battery of quality-of-life measures, high-resolution CT scans, and a number of other assessment instruments.

So this is a young woman, a trauma victim who has a left temporal deformity that you can see on this photograph, and we followed her out through 9 months. This is with a single outpatient treatment where we were able to restore her facial form without having to use alloplastic implants or a more complex procedure.

This is a patient with a more severe mid-face injury where she had significant volume

loss in her mid-face that we were able to restore, and this is about 9 months after the initial treatment with these injectable therapies. Moreover, on patients with severe scarring, depressed scars such as this military veteran we were able to attain a pretty significant remodeling of the scar out through 9 months.

And in a more extreme example, this is a young trauma victim who had a very profound cranial deformity that would have required a significant procedure using autologous rib grafts, a free tissue transfer, very major surgery that would have kept him in the hospital for probably a couple of weeks and about a 10-hour surgery. So, he was one of our patients that we enrolled for a second treatment, and you can see with two outpatient procedures that we were able to make quite a difference for him. This is 24 months after the initial treatment and 9 months after the second treatment, so these are really evolving as very important reconstructive tools for our patients, and quality of life measures as expected

are greatly improved in all of these patients going out over time.

When we look at the tissue healing with high- resolution CT scanning where we can do very detailed volumetric analysis we see that this is characteristic of these adipose-grafting therapies. We see that there is a loss of volume of the tissue out to about 3 months and then a stabilization of the volume from 3 months out to 9 months where things really level off, and the volume and the form at 3 months is very predictive of what we're going to see at(inaudible) months. There's also a clustering of these patients at around 65 percent retention of the material, healing of the grafted tissue based on what we inject measured out over time.

Interestingly the stromal vascular cell concentration in the native tissue correlates with graft retention really suggesting that these stromal cells are playing an active role in the healing of the tissues, and this is very consistent with what we've seen in our animal

models that we published where we've been able to do much more precise measures, and there's a very strong correlation between the presence -- concentration of endogenous CD 34 positive, 90 positive, 31 negative, 45 negative cells and the healing of the tissue over time. More on that shortly as we talk about the adipose-cell therapies.

So very importantly while volume and form can be restored, what's really striking about these therapies is the tissue remodeling that we see, and above and beyond volume there are very significant applications in treatment of radiation injuries.

So this is a case that was published by one of our colleagues, Gino Rigotti in Verona in Italy where he had a patient with osteoradionecrosis of the chest wall following therapeutic radiation. This was a refractory wound and with multiple injections of lipo-aspirate fat grafting he was able to get these tissues to granulate enough that he could

put a skin graft on the tissues and get them to effectively heal. And these are principles that we've carried over into treating our patients.

This is a patient of mine, a 54-year-old woman with a very aggressive T4 squamous cell carcinoma who had a full thickness cheek resection, partial mandibular resection, and you can see that bulge right off of her lip is a free flap. It's from her forearm and this is how she came to me. Very tight, constricted, contracted tissues and by all standards her reconstruction was over, and if we were going to do anything more we would have to do another free flap, another free tissue transfer in a patient who's not really in the best shape. So, with three outpatient procedures we were able to soften the tissues sequentially and get her to this point which is a pretty dramatic improvement in quality of life for this patient.

This is another one of our patients, one of our wounded warriors who was also a bilateral amputee, and you're seeing at the base of his

thumb a skin graft that is adhering to the bone. He needed that surface to push his wheelchair and he had a lot of breakdown, so we were able to provide some good, soft tissue there but most importantly, actually get suppleness and new elasticity to the skin on his hand, and that really stopped the breakdown from pushing his wheelchair.

I want to talk a little bit more about breast applications, and again this has become very widespread for use in breast reconstruction. This is more survey data published from our lab, and when we asked in 2013 the plastic surgery committee how many of you are doing fat grafting for the breast, 70 percent said that they were using this practice and 88 percent of those were using it for reconstructive applications.

So, here's one of my patients with a lumpectomy deformity -- single treatment with autologous fat grafting. This is a year out. Another patient with very severe capsular contractures. Some of these patients get severe

scar contractures around their implants. It's very painful. The implants are in place in the picture on the left. On the right a year later they had been removed and replaced with her own fat tissue, so this was a year after the procedures.

And this is a patient who in any practice 10 years ago would have had implants or a free tissue transfer. This was a case done by one of my colleagues who was able to affect a complete breast reconstruction using injectable autologous fat, and to put this in perspective this is the treatment that every other patient would ordinarily be having tissue removed in block from the abdomen transferred by microsurgical technique to the chest to get this result, and that case was achieved with autologous fat injection.

What about oncologic issues in breast fat grafting? There are some questions about growth factor release, cancer surveillance, malignant transformation. In some of the in vitro studies, and this is from our lab, show indeed if

we culture malignant pleural effusion cells with a feeder layer of adipose stem cells we're going to get tumor nests. We're not sure what this means because clinically none of the recurrence rates in the large published series are any different, even a good case controlled series, but we're looking more closely at that under NCI funding. We're looking at new experimental models to better represent the scenario of residual microscopic disease treated subsequently with fat grafting for reconstruction.

What's our Society doing? One of the really key things that the American Society of Plastic Surgeons is doing is data collection. We have a large prospective registry for fat grafting, and all of our members are expected to introduce data into this registry to really verify our results over the long time.

So, I want to spend the last part of this talk focusing on adipose cell therapies which again are derived from these particles of fat, only it's obviously a higher level of processing

and isolation because we're going to get the non-lipid-laden cells separated from these tissues, and if you look within the structure of fat tissue you see that most of our cells of interest, these 34 positive cells are Perivascular and really within the stroma, so we're going to use enzymes to digest these cells out, and here we have our initial isolate we refer to as the stromal vascular fraction. That's our fresh isolate, our sushi as our friend from Wisconsin would call it, and once we plate these cells we get the adipose stem cell fraction that can be expanded through many passages, and we can get very significant cell numbers.

We've worked out GMP methodology through PAC funding to do this by manual techniques, but there are also automated machines that will do this, and for our clinical trials currently we are actually using one of the automated machines under an IDE so we can bring this down to the point of care and do rapid isolation, and we know that that will be more transposable to other environments.

What do we get in our cell isolate? Well, this was data or summary from a Joint Position Statement from ISCT and the International Federation for Adipose Therapeutics, IFAT, so it was published in 2013. Dr. Dominici was one of the co-authors on this paper, and we really get a heterogeneous mixed population with parasites, endothelial cells, stromal cells, a few hematopoietic lineage passenger cells as well -- tissue monocytes and macrophages and those will not adhere to plastic, so those tend to wash out when we culture the cells.

So a lot of people in this room know ISCT. IFATS is not as well known. It's really a fat stem cell and regenerative medicine society that's actually going into its 14th year right now, but this is an event where we really focus on the best science in adipose therapeutics.

Some of the very, very attractive properties of these cells are the release of veg-F under hypoxic conditions and other angiogenic growth factors. This is data where we sorted out

the cells and looked at veg-F expression under low-oxygen tension, and we're applying these therapies in the pre-clinical phase for wound healing, in porcine models, and also because of the immunomodulatory properties and the ability of these cells to be suppressive in MLR we've been looking at them in rodent transplant models. So, this is a hind limb transplant that is out past 100 days where we have just 30 days of FK506 and a large cell load of adipose stem cells as well as bone marrow stem cells and this is adapted from some protocols that we've been doing in Pittsburgh.

And our strategy right now for applying these clinically is to really capitalize on the bio-active properties of these cells, isolate autologous adipose stem cells and mix them back in with this fat graft to get a more cellularly enhanced fat graft, so we're doing this under an IDE.

This is some of our pre-clinical data that we developed in our rodent models, and this

is applied toward treatment of traumatic amputation sites where we have about 1,500 people in our military population who have lost limbs from IED blasts, so many of them have poor soft tissue coverage and pain at their amputation site.

So, we're conducting a randomized clinical trial with 30 subjects, randomized to receive the cell therapy or the fat grafting alone and following them out actually over

years, so this is an example of how we're able to build up that tissue. This is a patient who's now back on his limbs rehabbing. We're able to get really good remodeling of the tissue, and importantly 8 out of 9 of the patients that we've treated so far have had resolution or at least a great improvement of their phantom pain, and that's something that's been very difficult to treat.

So in summary, adipose tissue grafting is a very powerful reconstructive paradigm in current surgical practice and adipose stromal therapy has really held a lot of promise for

tissue repair and we certainly want to move forward with the responsible evidence-based approach and deal with all of the challenges of cost, dose, and potency, et cetera. Thank you very much for your attention.

(Applause)

DR. MC FARLAND: So, I'm Richard McFarland from the Office of Cellular, Tissue, and Gene Therapies and we're going to switch before the afternoon break one more time, and as the title suggests get some views from government agencies, and as my first speaker works her way up here I'm going to tell you that these are just two government agencies, the DoD and the NIH, from a multi-agency group that coordinates our efforts across regenerative medicine and tissue engineering.

So the first speaker is Kristy Pottol who's the Project Manager and Director of Tissue Injury and Regenerative Medicine, Project Management Office of the U.S. Army's Medical Material Development Activity at Fort Detrick.

MS. POTTOL: Thank you, Richard. Thank you so very much for inviting me to be here. It's really my honor to be here to speak to you all today. I first must disclose that these views are my views alone, and I can't represent the Department of Defense, so there you go. There you have it.

About 25 years ago I was trying to pay my way to go to school and it was really hard. I didn't have any money. I had to do it all on my own, and I got kind of hungry after a while. I wasn't really certain if when school loans ran out in the summer if I was going to actually have a roof over my head. I started selling everything that I owned just to have food the next day, and it occurred to me that at least if I joined the military I could have food and there would be a paycheck and there would be a roof over my head. That was pretty much my main objectives as a young kid, and I went into the military.

What does that really mean when you go into the military? You have no idea. It was

really simple little hierarchy stuff, but a couple years ago I was standing next to General Caravallo and he was -- when we launched AFIRM, the Armed Forces Institute of Regenerative Medicine, he said when these young men and women choose to join the service they write a check to the United States of America payable up to and including their life, and they sign their name and that's co-signed by their family.

As a little kid that was not on my mind. I just really wanted to eat, but really listening to the two-star say that now 25 years later it really just impressed on me the importance and the responsibility the Department of Defense takes for these young men and women that are joining the service, and then we want to return them back to their homes in whatever community they live whether it's Iowa, Michigan, or Texas. And so that's part of the responsibility that we have in medical research for the military is to make good on that promise.

And so how do we think about medical

research and any technology space inside the Department of Defense? What we really think about in terms of -- I just titled this and try to make it in English instead of in Army-speak, so we have kind of occupational-driven research. Our occupation just happens to be really different than most people's occupation, so we try to really think about what is it going to take to help these young men and women be able to function and survive and live when they're on flight decks or they're out at sea or they have a blast happen or a gunfire happen or they're jumping out of airplanes or walking into an area where suddenly there's Dengue or something like that. So, we really think about what is the occupation that we have and how do we develop and drive medical solutions to help keep them healthy.

As a project manager for tissue injury and regenerative medicine what we think about is we need to get that to them right now. We need to hurry up and be really focused. We think about it in a very military way. How do we push forward?

How do we get there fast? We owe it to these folks to help them be ready to go protect our country. If they get injured while they're protecting our country, can we save them at their point of injury and help bring them back home and give them -- restore form, function, and appearance back to their life, and then can we help them enter back into their own community to be rehabilitated and restored?

It's a very powerful mission for us, and it's something that we drive very hard when we think about how to move forward. As we do very well, we've saved more people in these last wars than we've ever saved before, but we also have more significant injuries than we've ever seen before, so how do we continue to be innovative to help restore form, function, and appearance to help put these young men and women back together?

Well, we think about it in a couple different ways. One is are there medical solutions already ready today? That's really important for us. The military's not a commercial

manufacturer, so whether there are already solutions that are available today -- anywhere in the world today -- that will help us achieve this goal to keep these guys ready to do their jobs.

Are there ways that we can partner with non-government entities, and that's where we often see us working or the Department of Defense working is in some partnership where we can't go it alone, but we have a need so we need to partner with somebody. And then there are cases where we lead the military or lead the research and development for very unique military needs, and often you might see this in the chemical and biological side.

So this our focus. Obvious the top is a little bit cheaper for us than the bottom, but we have to go through that order. Behind every one of these is a mission is how do we drive forward. But going back to that responsibility that the General talked about is we really need to think about how we do that responsibly for any one of these folks that are anywhere in the world helping

to protect and defend our country, and so in that responsibility we have the Food and Drug Administration thankfully looking out for some of the elements of safety and efficacy and all that's involved with that, but the DoD also has a requirement of regulations because if we buy something it's got to be incorporated into our whole system, and so what does that mean? And that means that there's a ton of regulations out there for sure.

We also bring a bit of a systems engineering approach, so that framework that you see up on the top is the same framework that we would use to build new tanks, to build a new F35. It would be how we'd make new radios. That's also how we think about developing new medical products, so that systems engineering approach is very structured. It's definitely cumbersome, but inside there are elements of how to make your thinking more robust to ask more questions and in that we want to fail fast. We need to get those solutions out now and so we need to fail fast, so

we think about a lot of questions and probably the biggest power that we have if you're working with the Department of Defense is the power of the question; trying to figure out where the gaps are and how can we solve the problem in a different way because we're very mission focused. We have to solve the mission, so let's just figure out how we're going to do it successfully.

We need to translate the research into products. This slide is really just meant to say that's something that we cannot do alone. We have to work with academic institutions. We have to work with patients. We have to work with regulators. We have to work with other researchers and scientists, other government agencies, and it has to be an ongoing conversation. It's not easy. It's, you know, we always laugh at ourselves inside the DoD -- I think the FDA, too -- when you ask us a question we always say, "Well, it depends." Right? Because it always depends. There's so many rules and regulations specific on your product and

that's really for us all these gears and all these people you have to talk to is how do you get to the answer of "it depends?" You have to talk to everybody to figure out what it depends on, and then try to get to your next bit of information, so we work very hard to try to figure out how we work together and ultimately navigate the pathway through to get to the final side.

And really where's the risk that's involved? We need to work on that question together. We really, truly need to be partners together in what we're doing, and as we look forward into the future in front of us, particularly in these new product technologies that we're talking about here today, what is it going to take to get us to move forward? How will we be able to get to the right side of this image and help deliver products to our service members so that they can continue to live happy lives and also help us protect our country if that's what we ask them to do?

And so in my shop what we've tried to

perfect is the power of the question. This work, what we're doing and what you're doing is too important to leave to chance. We want everyone that's working in this field to be successful because we need answers to these questions. We need to be able to restore us back to full form and function and appearance after you've given us so much for our country.

And so how we think about not leaving this to chance is taking a look at where can we influence, where can we find partners? And what are the questions? We talked a lot about science here but it does not end at the science. It's not just the regulatory questions that are important. Is there a way to reduce costs of goods sold? Is there -- you know, what's your profit margin? What's your long-term sustainability plan? If you don't have a sustainability plan I'm not going to be able to promise these men and women five, ten years from now, and that's important to us. How are you going to get funding to continue on? How will you be able to continue to innovate? Can you

scale up to match your marketplace that you intend to do? Do you -- in that scale of how big of a scale does that really need to be and do you have sufficient facilities to do that, and what if it gets better? Do you have a way to scale up from that even still? How are you going to get paid eventually for selling your product? If you don't know the answers to that question now, at what time do you think you're going to get the answer to that question?

So we want to start asking those questions now. It's really important to start asking all those really hard questions, and it's like beating your head against a wall because you just don't know. It always seems to depend, but at some point you've got to figure out the answer to that question, and that's what we really try to do.

One area in here is because it's so hard and it's so complicated. The DoD did a request for information and asked the United States in some way where are you stuck in regenerative

medicine manufacturing? Where are your real problems? Where are your real gaps?

And there's a pretty good response from that and as a Presidential Initiative for the National Manufacturing Institute they released a funding opportunity announcement for the Advanced Tissue Bio-fabrication Manufacturing Institute, so the Department of Defense on behalf of the President put $80 million out to find answers to those questions that we heard were important in advancing tissue bio-fabrication.

What is it really going to take to have standardize cells and material processes to create platforms to find appropriate lot release testing, inline testing, non- invasive testing? What is it going to do to really create stability so that we can continue to have products, so we can answer these questions of scale up, so that we can reduce cost of goods sold to be able to make a company viable down the road? So that's (inaudible). It's in open competition right now, but it's -- these are the questions that are important to us

to try to help to answer because of this commitment that we're making back to our country, and how do we restore form, function, and appearance to our wounded war fights that helped us defend our country today.

And so with that I'd just like to say that there are a lot of ways that we think about solving the problem, but for us there's always one goal. How do we take that young man or woman who was at the point end of the spear and give them back to their home, take them back home so that they can be with their family again when they're done. Thank you. (Applause)

DR. MCFARLAND: Thanks, Kristy. So the DoD the last 10 years have been really transformative in many of these fields of regenerative medicine and tissue engineering. Our next speaker is from -- someone who we all know -- National Institute of Health, one of the other institutes that funds this. Martha Lundberg is the Program Director of Advanced Technologies in Surgery Branch in the basic and early

Translational Research Program in the Division of Cardiovascular Sciences of the National Heart Lung and Blood Institute, also known as NHLBI.

DR. LUNDBERG: Well, good afternoon and thank you to the FDA and Richard and the MATES group for inviting me here. I think it's an important and timely topic. There's a lot of things going on in the field, and it's great to see you all here giving us your advice and opinions on where we are.

Today -- let's see if I can work this thing -- I'm going to talk about enabling development of regenerative medicine and technologies at the NHLBI. As a disclosure I am a full-time employee of NHLBI as mentioned, and this is delivered as part of my regular duties so there's no disclosure.

I'm going to talk a little bit about the resources we have at NHLBI as well as some of our programs in the context of preclinical and clinical studies, and just in terms of resources, we do support a number of programs that are

available at no cost to investigators. But really what we see is that the path to clinical application is long and it's expensive. There's funding gaps, lack of non-technical expertise, a lot of knowledge gaps, and decreased risk tolerance among investors at this point in time.

So what is NHLBI doing in terms of addressing those problems? Well, we've developed a number of in-kind resources. We put together panels of advisory experts to help people think about, you know, are we going to bring this novel discovery to commercialization? Almost all of these resource programs include a training component and educational component, so we're trying to train the new scientists in the field, the innovators, with education and training so that they can think about regenerative medicine in a commercializable way.

And finally through our SBIR program we participate in the investor forums to try and facilitate and develop partnerships with those that would be interested in carrying on the

preclinical studies that are ready for commercialization or clinical application.

So, some of the resources -- these resources can provide a great deal of value to developers even though they're not direct grants or funding. The resources are available to not only academic investigators but companies, non-profits, and other researchers that are in the field of innovation.

One resource that is important to talk about from our point of view is the Biological Specimen and Data Repository Information Coordinating Center which is BioLINCC. It's a long mouthful for me, and it really provides access to sample sets that are linked to clinical data which can be useful to support an IDE or marketing application to the FDA.

We also have IND-enabling programs. You may be familiar with the Gene Therapy Resource program, the SMART program, as well as the PACT program which is the Production Assistance for Cellular Therapies.

So these IND enabling in-kind services provide GMP manufacturing, GLP foreign talks, testing for gene therapy for small molecules, biologics, and other therapeutic modalities. So this can increase the value of the technology really at no cost to the developer, and there are some other institutes. NCATS is a new institute at the NIH that also provide these kind of in-kind resources, so folks would want to be able to check with that particular institute at the NIH to see what's available to them.

So PACT -- PACT is a long-standing NHLBI funded initiative. I'd like to mention my colleague, Liz Welniak is here in the audience, so if there's particular programmatic questions that you have she's here to help me answer those questions.

It began in 2003. It renewed in 2010. It closed for a brief period of time, but now it's back up and running, and it currently has five cell-processing facilities and a coordinating center. And the primary goal is to provide

assistance with cellular therapy, translational research, and manufacture of cell-therapy products for IND-enabling preclinical studies.

This is a little bit different than the first two iterations of PACT. Basically this program wants to help investigators scale products requiring more complex manufacturing than they normally have at their institution. It's really not designed to provide products for administration to human subject in clinical trials. That's the new change for the PACT-3 program.

So as I mentioned, PACT-1 and 2 was really designed for preclinical through the phase 1, first in human studies, and looking at dose escalation, safety and toxicity studies. These were really, you know, safety studies that needed manufacturing and a scale of validation release criteria for the cells being used.

In PACT-3 which has just been renewed, we're focusing on the preclinical phase which provides all of the GMP manufacturing and support

needed for proper preclinical (inaudible) studies that is required by the FDA. Another -- I need to bring to your attention our SBIR Advisory Group and the Office of Translational Alliances. These folks really have expertise in the regulatory affairs that this development aspects (phonetic), regulatory strategies, and they're available pretty much to anyone. You don't have to necessarily be an SBIR/STTR applicant, and they provide answers to questions that you may have in taking your innovation all the way up through clinical application and commercialization.

And there's really more than -- more people than we can talk to one-on-one, so NHLBI is also providing Small Biz Hangouts. These are all archived on YouTube, and they're really focused on the common issues that innovators face regardless of whether it's a technology in our mission space, so advisor experts here, Chris and others, Gary, really are hanging -- holding these hangouts that

encourage everyone who has a need to figure out how to find the information you need through FDA. They're there to help you navigate the FDA website and provide answers to questions. Again, these are archived on YouTube.

Next I want to just briefly touch on the fact that NHLBI sends their staff to a lot of the investor forums, so these forums allow -- and they're listed here, across the U.S., some of them. They really -- our staff are there to help bring together those investors with innovators in the field, and we go to these events throughout the year.

So switching gears a little bit, some of the preclinical programs that we have ongoing is an example -- is NHLBI Progenitor Cell Biology Consortium, and this program was started in 2009. It had a 7-year $170 million commitment and was just recently renewed for 5 years. It has now -- has a translational focus and renamed the Progenitor Cell Translational Consortium. This is headed up by Denny Buxton, and we have a team at

NHLBI that are working with the new center, but the original goals of the consortium was to bring together multi-disciplinary teams from the heart, lung, blood, stem cell technology research areas to develop the regenerative medicine field. It has an administrative coordinating center, and throughout the program it brought in additional ancillary projects and pilot studies as well as education and training to help develop the regenerative medicine field.

A complimentary program is the Lung Repair and Regeneration Consortium which was funded in 2012, and basically this was to further help the lung community to -- mechanisms that control lung repair and regeneration. This did also have a strong educational and training component to help scientists in the field figure out and navigate this very complex field.

The renewal of the PCBC, now the PCTC, is really combining the Lung Repair Consortium and the Progenitor Cell Consortium into one but focusing on translation. The applications were

laid out, so-called road map, about how they plan to address disease in our mission space and barriers to progress, how they plan to overcome these barriers, and when they expect to reach key milestones.

The focus areas are patient-specific disease models using genome editing to understand disease pathology and to design therapeutics. They expect to further the field of cell therapy and tissue engineering using progenitor cells including gene-modified cells and differentiated progeny. An additional area of focus is to direct reprogramming of cells in vivo to treat disease.

These are some of the resources related to the PCTC, and I'm going to try to catch up on our time and jump right into the clinical programs that we have ongoing. These are just a few of them and I'm not covering everything obviously. We have a very large investment in this space. The Cardiovascular Cell Therapy Research Network or CCTRN, funded in 2007. Its aim was to provide infrastructure to develop, coordinate, and conduct

multiple clinical protocols to facilitate bench-to-bedside application in this area. It performed three trials, time, late-time, focus, which all demonstrated safety and can be found online -- those results. These were all three trials looking at autologous bone marrow mononuclear cells. The renewal occurred in 2012. There's seven centers, and the scope has expanded to include peripheral arterial disease and to focus on more innovative cell types that have the potential for enhanced therapeutic efficacy.

One study, the Concert Study, is looking at C-positive cardiac cells versus MSCs in patients with ischemic cardiomyopathy. A second study, the Seneca Study, is looking at allogeneic MSCs in cancer survivors with anthracite induced cardiomyopathy.

Another area that we're trying to focus on is the cardio-thoracic surgical trial network which just completed a phase 1 safety trial which demonstrated safety of mesenchymal precursor cells as adjunctive therapy in recipients with LVADs.

The network renewed in 2012 and they're now currently enrolling a phase 2 trial of MPCs in patients with end-stage heart failure, ischemic or non-ischemic that are being evaluated for LVADs for which to transplant or destination therapy.

And what's interesting to note here and suggests a notion of a strong public interest is the easy enrollment. We're ahead of -- in our accrual and it really demonstrates or suggests the notion that folks are eager to see the use of these cells in improving their heart disease.

I'd just like to summarize that we are using a number of mechanisms to promote this whole area and move towards clinical translation including our in-kind resources, educational support, facilitating partnerships, and clinical networks. And we also have independent, robust portfolio which Chris Breuer is a nice example of an independent investigator that has really capitalized on the NIH system to promote this area and really looking at science-based advances in the field.

And finally I'd just like to thank the folks at NHLBI that helped me put together this presentation to be at the meeting. Thank you.

(Applause)

SPEAKER: We're going to take a short break. I mean short, and reconvene at five after three.

(Recess)

DR. WEISSMAN: Okay let's get started. The moderator for our next session is Dr. Jeffrey Kahn who is the Andreas C Dracopolous Director of the Johns Hopkins Institute of Bioethics. He is also Robert Henry Levy and Rider Hex Levy professor of bioethics in public policy and professor in the Department of Health, Policy and Management in the Bloomberg School of Public Health. So he's professor of bioethics, public policy and public health and Dr. Kahn is going to talk to us initially about societal perspectives on development in oversight of novel cell based therapies.

DR. KAHN: Thank you. I'm going to kick

off this part of the program and then I'll be moderating the rest of the session this afternoon. I don't have a disclosure slide because I really have nothing to disclose except to say Dr. Bryan and Dr. Witten know that I chaired a committee for the Institute of Medicine, now National Academy of Medicine that was sponsored by the FDA on the first human uses of mitochondrial replacement techniques. I disclose that really mostly as a matter of information and also to say some of the lessons that came out of that report and the recommendation from that report are quite relevant to the discussion here today. One more thing to say about that my colleague Jonathan Kimmelman who spoke before the break was a member of that committee as well.

So I'm just going to make some quite general comments about how first society and then individuals who are involved in early trials of cell based therapies might be thinking about the issues and what we want in place as we move forward.

Dr. Pottol's comments about being hungry and a student made me recall that when I was a graduate student I was also hungry and I didn't enlist in the military I rather enlisted as a research subject and made a fair amount of money over a fair number of months doing things in retrospect were probably not so wise. Most of them early phase research trials. They were not for cell based therapies of course given my age but they were sort of novel approaches and part of what it reminds me of is that we have to be thoughtful about how we proceed. So let me dig in here. You see I only have a few slides so really you'll hear me more talk than you will see slides for me.

So as we, the royal we, the society thinks about moving forward in enrolling subjects who are likely to be patients and then eventually as we move to approval and introduction of these new cell based therapies we want to make sure certain things are in place. And society not only ought to expect this but ought to demand that

these things be in place.

First, that there is a pretty clear and established set based on evidence that safety has been established in an initial way and that we minimize to the extent that we can tolerate the risk to the parties involved. So we want to make sure and I think we don't get many chances to get this wrong before we get it right. This is a really important point in the development of a new technology. Getting things wrong at the early stage will lead to continued problems of trust and willingness of the public not only to participate but also to allow their tax monies to be invested in new areas of research.

The second thing we want to make sure after safety is that there is some established evidence for the likelihood of efficacy based on pre-clinical that is non-human based research models and as the investigations go forward that they are limited to the appropriate populations and we don't see proliferation beyond the kind of narrow research population certainly at the early

stages and probably even more as we move into initial adoption as a matter of approved therapies. So just sort of a signal about one of the things I want to leave you with at the end of my remarks and I hope we can talk about during the panel is how to make sure that we don't have this sort of proliferation of technologies out into the public once something is approved in ways that are not as responsible as we might hope. So what effectively look like off label uses are not the most responsible or in the best interest of individuals or society.

The second thing I think needs to be really focused on is that we move forward in what is presumed to be a cautious way so sort of safety first is a really important principle I think and one that is an important one to honor as a matter of societal interests. So go slow and do these initial kinds of research introductions with as few individuals as is necessary. So we don't want to expose more people to risk than we need to. So careful and small numbers to the extent it makes

sense to get the data that we need. If we're talking about studies that are performed in more than one place by more than one entity maybe even funded by more than one company that the designs are standardized in a way and to the extent possible that we can compare and pull the data that comes from them. So make the most use of information that comes from what would be a relatively few numbers of people in early stages. So that is not typically done. You may have heard this from others. This was among the recommendations that came out of the mitochondrial replacement techniques report. Because we are talking about such small numbers that it is hard to get good and useful data unless you're able to combine data from multiple sources somehow.

The expanded version of that point is the next bullet which is about data from places that are outside of typical FDA jurisdiction or FDA analysis that might be outside of this country for existence and so we want to pull information from as many places as feasible to support coming

to conclusions about both safety and efficacy as we move forward into the research trial and into eventual, potential approval.

Maybe most difficult if not controversial is how to collect long term longitudinal information from the people who are involved in both research and maybe even in early applications of approved therapies. How are we going to learn over a long period of time what the effects of these new kinds of therapies are going to be? That requires somebody to pay for that work. It is really hard to collect longitudinal data. One you have to get people to agree but two someone has to pay for it. But I think that is something that society needs to be made confident about. I'm not giving you ways to do it but rather raising it as an issue that needs to be addressed.

Switching from societal interests to individual interests to what individuals need to be thinking about and need to be informed about as they are offered the opportunity to participate in

what would be early trials. There needs to be special attention paid to communicating what are the novel aspects of whatever the initial trials are for a particular cell based therapy. So you would want to know and be told whether this has ever been done in humans before. So if you're among the first human subjects to be participating in a novel cell based therapy trial that is important for you to understand and the implications of that. So what information has been learned from non-human models but of course understanding that this is the first time for a human and there will need to be some human trials and we've heard a lot about that today.

A clear explanation and communication of the risks involved that the potential benefits are highly prospective if any in particular in these early stage trials and a longstanding issue in the ethics of research involving human subjects is the misunderstanding and I don't mean that people don't get it but rather the improper balancing in people's minds about how much risk versus how much

potential benefit. So part of that is because people want to perceive potential benefit in research participation but part of it is because the communication of that information is often skewed at least historically it has been. Empirical research probably now 10 or so years old shows that there is a pretty consistent skewing of information shared with potential research participants overemphasizing potential benefits and underemphasizing potential harms.

In the context of cell based therapies we need to make clear the difficulty of what it means to withdraw from research. You may be able to no longer be in a trial but it is hard to get the thing that you got as part of that trial to be taken out of your body. It is not like a drug that washes out it is not like a device that can be removed and that is something that needs to be communicated and made clear. For some people the source of materials if they are controversial in nature would be important and should be disclosed just as a matter of information and people may

decide that that's not something they want to participate in as a matter of their own conscience.

Stepping back and talking both from a societal perspective and individual perspective the kinds of principals that ought to be part of an oversight scheme for moving forward with novel first in human early phase development of novel cell based therapies.

First to the extent that we can figure out how to do this and do it with transparency. Share with the public in a timely way the information coming out of these early phase trials. It would be great if sponsors were willing to commit to depositing the protocols and of course the identified results in publically accessible places so that it can be seen by people who would like to see it. An exploration of the views of stakeholders involved in the kinds of research that we've been talking about and hearing about today and that can be done in a variety of ways. This country we're not terribly good at

public engagement around what are socially, societally and sometimes individually controversial areas of science and biomedical research. European countries in particular have had much better examples and experience and built up approaches to doing this but this is an area where I think we could do better and need to do better. There needs to be partnership among the regulatory authorities responsible for oversight of this kind of science and I mean not only in the United States but across borders. Just to reiterate something that I said earlier enabling sharing of data, pulling of data, cross referencing of data, so maximization of data quality to make the most use of the data that comes from asking people to put themselves in uncertainty if not in harm's way and take advantage of that to the greatest extent possible. So it is a privilege to do research on humans and we should take the most advantage of it as we can. I don't mean exploit the people but I mean take advantage of the data.

That is the end of my remarks. I wanted to just to a very general overview as I just did and there will be lots of opportunity to talk with as you see a number of people at the panel stage. I want to move to the next part of the program. I now have the privilege of introducing the remaining speakers in this part of today's program. The next of whom will by Dr. Brian Mansfield. As you get the slides ready let me introduce Brian. Brian is the Deputy Chief Research Officer for the Foundation Fighting Blindness a position he assumed in 2011. In that role, he assists the Chief Research Officer of the Foundation in directing the early translational research investment program and ensures implementation of the Foundation's research strategic plan and manages day to day operations of its science department.

DR. MANSFIELD: Thank you for the invitation to talk today. What I'd like to do is to outline a little bit about what the foundation does, the patient perspective of their disease and

where we stand in our guidance not only in the research that we support but also in the guidance we give to our constituents when they approach us about these therapies. So the Foundation Fighting Blindness is a non-profit organization. Our mission is to provide preventions, treatments and cures for people who are affected by this group of often retinal degenerative diseases. So these are all awful diseases and these are for a small group of people who feel they are not well represented by the major efforts that are going on in the large pharma companies. Our research budget runs around $28 million a year. We have a scientific advisory board of about 100 experts in this field who give us very good input and guidance in the things we do and in our decision making. We fund a lot of preclinical research and more and more now we are starting to step into fund the proof of concept studies in humans which we generally do in a cofounding manner with another partner and we are also looked upon to provide education and support to our constituents who are affected with

these devastating blinding diseases.

So I just want to remind you that the inherited dystrophies the IRD these are diseases that affect the back of the eye. The eye is full of fluid and there is only that small part of tissue outlined partly by that white box there where the retina sits. But the retina is a very complex tissue. There are at least 10 cell layers in there we'll come back to in a moment. Different diseases affect different cells in that complex and different parts of the retina are reflected by different types of diseases. So you can see if the disease affects the middle of the eye there. The are macular diseases which are distinct from diseases that affect the edge which are the peripheral diseases.

Now these diseases are literally in your face all the time every day. They are progressive diseases so you can't avoid the point that they are progressing and you are very well aware of their progression each and every day. In the peripheral diseases your vision just gradually

disappears from the periphery until you end up as if you're looking down a very narrow tube ultimately you lose vision. The macular diseases are the opposite. They start in the middle, the high central acuity part of your eye where you really depend on a lot of color vision, a lot of ability to read and see detail and then that area of blindness expands out until you lose all vision.

So one of the things that we're very focused on is the importance of vision to our constituents and recently we held a patient reported outcomes meeting with them where we were asking about quality of life, what they want out of therapeutics and what is most important to them. I'm going to summarize on the next few slides a couple of these findings.

The first thing that comes out broadly is nearly everyone with this disease tries to hide the fact that they have it because of a number of societal things such as fear of loss of employment. If you depend on computer use or

reading and your employer senses you may not be able to do that efficiently that creates a great fear. And particularly if you lose your job will you get reemployed. There are concerns about health insurance. What is the insurance consequence of discovering that I have a disease like this? By the way there are no treatments for these diseases. There is one prosthetic which can treat a small minority of diseases but there are really no therapeutic choices for these people at all. And then there is discrimination. These people look different. They will often wear tinted glasses, fat glasses, they will use a cane, they will be less confident in their movement, many different things make them stand out and they are particularly aware of that difference.

Vision is also important to social interactions. Think about how someone can say something but depending on whether they smile or frown it has a completely different meaning to you. When you don't have good vision you lack that context that it comes with. It is obviously

important for our interaction with others. For instance, if we don't have peripheral vision and someone walks past us and we don't acknowledge them that can be considered perception of being rude to them. That has horrible consequences downstream. There is also the perception of disability. The number of people who say when they're out with their partner the person talking to them is either talking to their partner or is shouting at them. As they say it is a vision problem not a hearing problem. Vision loss also affects daily life. The biggest thing here is they can't drive. In our society being able to drive is independence and particularly if you have a young family where you have to pick up the kids and take them to their activities this is a major consequence when you get to a stage where you can't drive. And then there are other consequences which are harder for us to appreciate maybe such as July Fourth fireworks photoxia which should go on whether your eyes are open or closed it just keeps going.

So what do constituents want? Well it is graded. Of course they would love to regain lost vision but most realize that is not possible at the moment. They would like to stop that loss of vision at least stop it where it is now or possibly slow it down. Most people are looking to finish a productive life. They would like to get through to retirement or they would like to see their daughter married or something like that. There are always these things that they look forward to so any of these outcomes would be valued to them.

When we ask them what functional outcome they want the majority of them say they want to drive but when you talk more about this they really want independence. The ability not to have to ask someone to do everything for them all the time. A number of them or 40 percent of them also reflect the need to improve a social interaction and to be able to remain active in their careers.

When we ask them about therapies safety is really a prime one that they all bring up not

only for the eye but they also are worried about what more generic effects this may have on my body. I don't want to suffer a heart attack because of something I've taken for my eye. That is going to be an adverse consequence for me. The risk tolerance to what they will do varies. If they have very little vision loss but they know they're going to lose it, they are often open to being a guinea pig because they feel they can afford to lose a little vision if it will benefit someone else. That is great for pharmacological treatments probably not for the self-therapies. If they have a large vision loss, there is often a reluctance to do anything because they really hang on to just seeing the sunrise and knowing it is daytime again.

So the stem cells for the IRD that we support fall into three categories. There is the neurotrophic support where cells are generally injected as a bolus. They are not intended to migrate or differentiate or proliferate but they produce neurotrophic factors that we hope will

keep the retina healthier and maintain vision, slow or stop that degeneration. An example of work that we funded led to the neurotech device which is the small encapsulated device that contained cells genetically engineered to release a neurotrophic factor and that is placed just inside the eye in that watery compartment and diffuses proteins across to the back of the eye.

The second sort of stem cell therapy that we support is cell replacement therapy where we are looking for migration, integration, differentiation and the creation of productive connections in the eye. And as you've heard from Dr. Clegg and others formulation may involve bolus injections, a single sheet of cells, 3D layers where you provide a matrix to support some of these spindly cells and help them be more secure and you may look at a single cell type or you may be looking at trying to put in several of those different layers together as an architecturally structured component.

Again, just to give you a flavor of sort

of the work that we have cofounded most of it is preclinical. We support autologous and allogeneic work induced pluripotent stem cells, embryonic stem cell work, fetal retinal precursor stem cell work and you'll notice that second to bottom we did support autologous non-homologous use for CD34+ stem cells. We'll come back to that in a moment.

We support a number of primary indications. The technologies you can see range across the different matrices protocols trying to get optimization and better differentiation protocols. Some of the work that we have supported has ended up in the clinic.

The third stem cell we support is in situ differentiation where you're trying to encourage native cells in the retina to differentiate into a different type of cell to replace the damaged cell. So for instance we can induce the

(inaudible) expression or we can use small molecules to manipulate

permanent expression for transcription factors.

Now as I said the retina is a complex tissue. There are six primary cell types but actually 59 distinct subtypes so when people start going and putting cells in there be aware this is a very complex environment you're putting things into. Placement of the cells and migration differentiation integration are critical. This is a highly organized structure. There are very, very precise connections going on in there and when those synaptic connections go wrong we don't know what is going to happen to the perception of vision. Certainly in the diseases as they progress further and further those synaptic connections change and that is obviously a challenge in that you would like to see those rectified in a therapy that is addressing that stage of disease.

The other thing to realize is that this structure is actually essentially a computer as well. It is already starting to synthesize and

interpret shades, contrast, edges in your vision before it is even communicated to the brain. We must be aware that some cells if inappropriately activated can have negative consequences. For instance, glia, activated glia can lead to scarring so this is not something to go into lightly when you start playing with cells.

So what are the key challenges facing our therapy? I think we've heard other speakers talk about these. Key to us is how do you get stem cell differentiation to a particular cell type you wish to replace, where do you place it, how do you promote the functional synapse connection to make sure the signal gets back to the brain in a sensible way. What is the function of creating a synapsis which is not native? Where do cells go I think we all have that and the ability to trace them.

Now we are particularly concerned about non-homologous therapies that are appearing on the market where people are starting to take bone marrow and inject back into the eye for a fee.

There are many studies which are poorly characterized. The cells not well characterized by type, consistency, purity, quality. Route of administration is often poorly defined. In fact, often some of these protocols will suggest there are five or six different things they may do depending on the clinician. There is no disease focus. Most of these therapies that we are aware of are offering to treat nearly any retinal disease. That doesn't seem realistic as this is a complex tissue. There is a cost to the patient and generally there are few or no peer review publications showing anything about preclinical evidence with safety, efficacy, dosing, or administration route.

So we are very worried about the safety and oversight concerns. Some of these studies for instance are listed on clinicaltrials.gov and I'm particularly concerned that they can give the impression that they are federally mandated. You find the health authority being defined by a variety of different terminologies. There is in

fact no way when you go on clinicaltrials.gov of finding whether there is an IND for this procedure and whether it is FDA authorized. And clinical trials can tell you you can sort of get there but you can never be certain. You actually have to ask the person doing the procedure.

So what is our perception on this. Well there are very few as I say published outcomes and it worries me when we start seeing a lot of promotion in social media, in the mainstream newspapers where they're talking about the publication of a single case study but notice that the PI says he's treated 278 patients and 60 percent respond. Well why that one? Why did he publish on his 278? This really bothers us. We have to be careful though. There are some studies which do have regulatory oversight which do use a similar source of material maybe slightly more enriched and refined so we have to be very careful that we don't use a broad stroke to wipe out all these sorts of studies.

So what is our perspective on these sort

of procedures? Again, very much what other people have said. We very much support the regulated or careful approach that the FDA has understanding mechanism action if you can, defining your cells, defining your procedures, understanding toxicity distribution, efficacy and preferably having that peer reviewed and open.

So what do we tell our constituents because I often read an article in the paper and say you should be jumping for this this is the treatment you want and we tell them our studies. Is it on clinicaltrials.gov but be aware of what that really means. Is it authorized by the FDA when you know there is going to be an emphasis on safety. Is it focused on the world defined condition or is it a fix all. Are the clinicians experienced preferably knowing the complexity of the retina and therefore specializing in the IRD and talk to your own specialists before you go jumping into another study or clinical. Be wary of personal testimonies. They are often taken immediately after treatment where this is a strong

incentive to say it is working, it is good, it is beneficial. You don't know what happened later on and you don't know what their perception of it is. And we feel very strongly that participants should not be paying for any of these out of pocket if it is said to be a clinical trial study.

Finally, think of your risk tolerance. This is a trial, it is not approved and don't forget once you've been in a cell therapy trial you are going to be excluded from any of the other trials that are coming down the road probably for 10 years. So if you really think that there is something else on the horizon think very carefully about your risk tolerance as you enter into these trials.

So in conclusion, the Foundation for Fighting Blindness seeks to make our constituents scientifically aware of the issues. We believe in the potential of stem cell therapy, absolutely. But we believe it will come through well controlled peer reviewed studies on cell therapy. We have supported some of these autologous bone

marrow cell transplant experiments but they have been under the oversight of the FDA. We are aware that some of the procedures that have been out there have had complications and I believe you may hear about them soon and we advocate that all of the therapy should have (inaudible) data and have that regulatory oversight. Again, we do not advocate the use of pay policy for these sort of studies.

DR. KAHN: Thank you Dr. Mansfield. Our next speaker is Thomas Albini. Dr. Albini is an Associate Professor of Clinical Ophthalmology at the Bask and Palmer Eye Institute in Miami.

DR. ALBINI: Thank you very much for the invitation to speak here. Thanks to the FDA and Dr. Kahn and it is also a pleasure to be following after Dr. Mansfield and my colleagues at Baskin Palmer Eye Institute and myself have been familiar with Foundation for Fighting Blindness. I've worked with them for years and I have to say I have very little of substance of what he said from the patient's perspective. I think it is a great

organization in really helping patients who have these blinding conditions. But what I'm going to share with you is what I think is a very rare instance of a dreadful outcome from a "health provider" in South Florida where I think there was really demonstrated neglect for both patient safety and for any sort of scientific integrity from what was being done. Just to discuss a real case scenario with three patients who were treated, came from elsewhere in the country, were treated in South Florida and then were seen at my facility within two or three days after the treatment because of complications and of note this happened three times not just once. It was really a very horrifying situation. I have no relevant disclosures.

We've heard already about macular degeneration this morning and a little bit in the last talk and its important to say that for most patients with macular degeneration they preserve their vision for quite a long time. Dry macular degeneration especially in its early

manifestations is really consistent with retained vision for decades for the vast majority of patients. But for about 10 percent of the patients they will go on to develop one of the severe forms. There is the wet form of macular degeneration. This is the form where patients are routinely getting monthly injections of various biologic agents to control the fluid that accumulates underneath the retina within the retina and causes vision loss. Another 10 percent of the macular degeneration patients will develop severe dry form macular degeneration where there is atrophy of the retinal pigment epithelium as we discussed earlier this morning.

Now these patients will go on to severe vision loss for the vast majority of them if left untreated. We have very good treatment now for the wet form. For the dry form we still don't have a very good treatment. So the story is the patient moves to Florida and is getting excited about taking up golf as their main retirement activity and then loses the ability to drive, read

and recognize faces. These patients maintain good peripheral vision so they can get around a room but they lose their central vision.

So the wet macular degeneration outcomes these are outcomes from pivotal trials for a drug called Ramibizumab that was introduced by Genentech back in 2006 and really have shown marked improvement of visual acuity from baseline whereas in the control groups the patients lost visual acuity. This is maintained out at least through the first three or four years, we've got data through seven years. You lose a little bit of vision but if you continue with these monthly injections of biologics you can really maintain that visual acuity for a long time.

In dry macular degeneration the number of different strategies that are out there trying to find a treatment including biologics, small molecules, gene therapies and cell therapies as we're talking about here today. Perhaps these embryonic stem cell derived retinal pigment epithelial cells much like the cells that were

discussed earlier today. But provided from another source a company called Ocata Therapeutics that published their results in The Lancet, this is for dry macular degeneration and really showed some modest but apparently real visual acuity gains and some other biologic findings on various imaging studies demonstrating that these cells actually took residence and where the retinal pigment epithelium should be in the sub-retinal space and had some demonstrated function as well in that space. So we are having some victories in this field. They are coming slowly but I think this is very welcome news that there is some real data that stem cells are working for dry macular degeneration.

Now one of the delivery methods that we haven't talked about today is just intravitreal delivery which is perhaps the simplest form of delivery. It is the form of delivery that we use for intravitreal injections of biologics for wet macular degeneration like that drug Ramibizumab that I just mentioned. In this case these four

different trials that are listed on clinicaltrials.gov are looking at taking stem cells and delivering them straight into the vitreous cavity. One of these studies was using adipose derived stem cells where the adipose tissue was harvested on the same day that the intravitreal injection was done and was then processed in a very quick fashion under an hour. In the same procedure injected them bilaterally into both eyes. It is the outcome of this procedure that I'm going to be talking about. This company that was doing this unbelievably without an ophthalmologist directly involved in what was happening without and M.D. injecting the cells. They were injected by a nurse practitioner without M.D. oversight. This has resulted in really bad outcomes and as I said again demonstrated a complete lack of regard to the patient safety and to any sort of scientific integrity. They did have a trial that was posted on clinicaltrials.gov. The patients when I saw them and they came to the hospital a day or two

after this happened, by the way this was a very painful procedure for them which is not typical for injections. They are usually somewhat painful but not the type of profound paint that these patients were describing. They were under the impression that they had participated in a clinical trial. When we finally were able to see the informed consents that the patients had signed the informed consents actually weren't for any clinical trial they were just for a basically fee for service procedure which was explained to the patient to have the possibility of resulting in blindness and that it was outside the standard of care of treatment. That's how this particular set of injections was performed.

So we're reporting here three cases of bilateral vision loss after bilateral intravitreal injection of stem cells in a stem cell clinic. All patients underwent intravitreal injections of the usual dose of volume that is injected with autologous adipose tissue derived stem cells. All three of the patients paid $5000 for the

procedures. One of the patients also had the same stem cell preparation injected into both of her knees on the same day for an extra $1200. They signed a consent form for the procedure but not a study consent and all three of the patients here had seen the clinicaltrials.gov website. One of the patients, the first patient I'll describe was a statistician who had been involved in clinical research and these patients were under the impression that the clinicaltrials.gov website lended some credibility to the study.

The first patient is a 72-year-old female with a history of dry macular degeneration and vision of 20/60 and 20/40 which is pretty good vision. 20/40 is good enough to drive if that were your only eye at least in the State of Florida and it is good enough to read standard newspaper print. This is not perfect vision but it is very functional vision. She came in with three days of decreased vision, pain and vomiting and nausea. Visual acuity on presentation was hand motions only, she couldn't see the large E on

the eye chart out of either of her eyes and she had extremely high intraocular pressures. What we found in this particular patient these are ultrasounds showing anterior displacement of the crystalline lens which the zonules that hold the lens in place apparently became loose somehow. This is probably not because of the needle or a direct pushing or ripping of the lens but we think it is an enzymatic digestion that something that was injected into the eye. Trypsin for example is known to digest the zonules and used to be part of standard intracapsular cataract surgery. It is a way to remove the lens. But some protein that was injected in the eye probably dislodged these lenses that pushed forward that causes the obstruction of outflow of fluid from the eye, increases the pressures in the eye which of course is bad for the optic nerve that causes an acute glaucoma. She was found to have a vitreous hemorrhage in both eyes there was no view to the back. We had to remove the lens emergently, remove the vitreous that was in the back of the

eye and remove the blood that was there. She was found to have a retinal detachment in the right eye and over one week her vision dropped down to no light perception in either eye. She ultimately had retinal detachments in both eyes that needed to be fixed. Here you see a lot of intraretinal hemorrhage all throughout the fundus, retinal detachment, displaced lenses, really a disastrous outcome that you never see after routine injection of biologics for wet macular degeneration.

The second patient is a 78-year-old female. She had wet macular degeneration which was well controlled. She hadn't required an anti veg-F injection for two years prior to undergoing this therapy. Her visual acuity in the right eye had just dropped to a point where she was losing her driver's license and therefore sought out this treatment as a potential remedy for herself that her daughter found on the web. Again, from the web was referenced to the clinicaltrials.gov website and mentioned that when we spoke to her. She was also under the impression that she was

participating in a clinical trial. Similar diffuse hemorrhage in both eyes worse on the left than right. She presented without retinal detachments. Also very bad vision, counting fingers again not formed vision in either eye. She was initially observed and eventually developed retinal detachments in both eyes requiring treatment and now at least has one eye with visual acuity of 20/200 and the other one doesn't have any formed vision.

This is the third patient 88-year-old female, dry macular degeneration. Had a visual acuity of 20/40 again relatively good vision in the right, 20/200 in the left eye prior to injection. She came in seven days after the procedure with light perception vision only in the right eye, 20/200 in the left eye. A very mature looking retinal detachment with what we call PVR, Proliferative Vitreal Retinopathy which one of the reasons the retinal detachment surgery fails is that you get a growth of scar tissue on the surface of the retina fibroblast that contract and

pull the retina back off of the wall of the eye. It is possible that the stem cells which in some of the imaging that we have seen to take residence on the anterior surface of the retina and they may be actually pulling the retina off and being the reason why all these patients eventually developed retinal detachments in both eyes as this patient did in her left eye about a month later.

So these were the initial vision of the patients ranging from 20/40 to 20/200. Their presenting visual acuity when they came to the clinic ranging from 20/200 to light perception. Five of the eyes had lens subluxation, some were along their course. Four eyes had severe intraretinal hemorrhages. All of the eyes eventually developed retinal detachment and the ultimate visual acuity was legal blindness in all three of these ladies. We had a one year follow up and unfortunately none of the patients have gotten any better.

So what are the potential causes for these findings? Well they include contamination

of stem cell with toxic substances during preparation. Use of trypsin or collagenase during stem cell isolation which we're looking at because of that zonular weakness that we've seen and maybe it is not appropriately washed out. And there may be some genuine affect from growth factors in cytokines and the vitreous and blood derived undifferentiated stem cells to myofibroblast cells resulting in detachment of the surface of the retina. There is some biologic effect of these cells and some of the phase one studies have also been stopped because of retinal detachment where there has been inadvertent seepage of cells that were injected into the subretinal space coming back into the vitreous cavity and then retinal detachment was seen in a lot of those. So there may be some danger from this mode of delivery. There is real science being done on intravitreal injection of stem cells so I certainly don't want to put any hindrance to that. There may be a good way to deliver intravitreally but we hypothesize that intravitreal delivery at least in these cases

may have caused some of the problems.

The patients were referred to clinicaltrials.gov which listed an IRB approved study however the patients were not enrolled in the study. Injections were being performed without FDA oversight. There was no IND obtained by this clinic. These were patient funded research procedures and we've talked about the dangers of that. Unbelievably, an American licensed physician was not involved in the care and the injections were performed by a nurse practitioner.

Sorry to share this with you but I think it is an important thing for patients to know and as I've learned today the extent of the industry that is around these unregulated stem cell clinics I hope we don't see more of this with intraocular delivery. Thank you.

DR. KAHN: Thank you. Our last speaker for this session before the panel is Dr. Michael Miller. Dr. Miller is a Senior Clinical Fellow in neuropathology at the Brigham and Women's Hospital

in Harvard Medical School in Boston.

DR. MILLER: Good afternoon. I'd like to thank the organizers for the opportunity to be here today and to present on this very interesting and unique case that we saw recently at our institution. I don't have any conflicts of interest to declare.

I'd like to start with I think the reason we were contacted to present here is because when our case was reported a couple of months ago in the New England Journal it received a fair amount of press coverage about stem cell tourism and such and so I'd like to take the opportunity today to go into some detail about this case. What were the things that we learned from this patient and what types of conclusions might we be able to draw about stem cell research and the potential risks of tumors and other such outcomes?

So let's take a step back and look at the history of this patient. So this is a highly educated man who had worked as an attorney and at

the age of 60 he suffered an MCA ischemic stroke which resulted in hemiplegia on the left side of his body. Over the next bit of time he underwent physical therapy, a standard treatment after a stroke and saw some improvement in the strength of his left leg and he was able to then walk with some assistance but his left arm remained paralyzed. He also underwent a number of other procedures not necessarily done in the conventional setting including acupuncture as well as other physical therapy and off label use of anti-TNF therapy. These did not produce any improvement in his weakness. So then he traveled around the world to receive a series of stem cell treatments and I should note that all of this is reported by the patient. Unfortunately, we don't have any official records of what therapies he received and the patient was not willing to furnish literature such as dosages or other information nor was he willing to report the names or locations of the clinics. According to what the patient did tell us he went to China and

received embryonic stem cells and also in China subsequently received and allo mesechymal stem cell preparation that was injected intrathecal to the area around the spinal cord inside the dural sac. He then subsequently over the years traveled to Argentina and received autologous mesenchymal stem cells as they were labeled and then traveled to Mexico and also received mesenchymal stem cells and neural stem cells. A few things to note, one is the autologous stem cells that he received in Argentina, these were actually injected into his carotid artery I guess with hopes that they may reach the MCA distribution in the area that was affected and reconstitute some of the dead neurons. Then the treatments in Mexico he received two sets of them. After the first set he thought that he had some improvement which lasted about a month and then regressed but it is not clear whether there any physicians involved in the evaluation in these therapies. One of the real tragedies of this is the expense that he underwent himself as well as the risk that he took and the

fact that the community as well as the patient has not really gained a lot from this. This underlies the point of a lot of speakers that the investigation of these therapies really benefits from being in a controlled setting where we're surveying and gathering information that can then be offered to the broader community.

So subsequent to this he presented to our hospital with progressive lower back pain, lower extremity weakness that was not bilateral as well as sensory loss and urinary incontinence all of which raised concern for a lesion involving the spinal cord. So an MRI was performed. Here you can see that there is a lesion that extends from T12 to L5 and the superior and inferior extent of the lesion are marked there with arrows. It is contrast enhancing and in this axial image you can get a sense that the legion is encasing the spinal cord within the dural sac.

The patient was taken for neurosurgical exploration and the image at the left shows the epidural exposure so the dura mater has not yet

been opened here but they noted tightness and appearance that suggested it was bulging. Then when they sectioned and sliced through the dura the image at the right shows the exposure beneath the dura. They noted that the arachnoid mater was abnormal. I see the tortuous blood vessels here and the spinal nerve roots where the arrow points appeared to be caked together by some sort of substance. So they took a series of biopsies and this is from a specimen that they indicated was part of this intra-dural mass and here we see a large number of cells. This is very hyper cellular for an adult spinal cord. The cells appear as these purple structures, the nuclei of the cells and they have a primitive morphology that is they have a very high nuclear to cytoplasmic ratio and they resemble cells that you might find in a developing fetus as well as stem cells.

In another specimen that was nerve root you can see part of peripheral nerve there we also saw tumorous cells that were encasing the nerve

root. And then in a portion of another specimen there was an area of the tumor that was differentiated. So rather than these primitive cells with a high nuclear to cytoplasmic ratio and minimal cytoplasm in this particular area we saw tumor cells with a glial morphology here resembling astrocytes as well as this structure here which is known to pathologists as vascular proliferation and that is a hallmark of glioblastoma.

So next we used immunohistochemistry to better characterize the lesion and here in each of these images the pale blue is background staining of the nuclei of cells and the brown is a positive result where the antibody has detected the protein that we're interested in. So GFAP indicated that it was expressed positive and indicated that the tumor cells were forming astrocyte like cells. oligo-2 was also expressed in some cells suggesting a glial differentiation. A number of the cells expressed sox-2 which is found in (inaudible) as well as neuro stem cells. The

cells were also highly proliferative expressing the proliferation marker NIB-1. This is an index found in only very aggressive cancers and in the normal brain or spinal cord it is less than one percent.

The cells were negative for neuronal markers such as NEUN and synaptophysim and also negative for CD-34 stem cell marker and negative for CD-45 a marker of leukocytes because a tumor like this could be a lymphoma if you just look at the way the cells look but it doesn't look like that according to the immunized chemistry.

So based on this information just looking at the histology there is a very broad differential diagnosis for a tumor like that. It includes a lymphoma, glioblastoma, perhaps a primitive neuroectodermal tumor which we're now referring to as primitive neuronal or embryonal tumor. So the data that we have indicates quite clearly this is a malignancy and that is comprised of rapidly proliferating cells and they seem to have some glial differentiation. However, it

doesn't clearly fit the phenotype of any known tumor entity that is currently accepted in the WHO book of tumors. So we gave it the name of glioproliferative lesion to try to give our clinicians some sense of what this was to assist them in making decisions on how to treat the patient. So there is a series of questions we then proceeded to try to ask. One is are the lesional cells from this patient or are they exogenous? So to address this we used short tandem repeat genotyping this is the same type of test that is used in paternity testing as well as in forensic testing and it uses loci across the genome on multiple chromosomes that are known to be polymorphic and they have a different number of repeats in different individuals. So here is one locus, the Penta E locus and in the peripheral blood of this patient we noted that there were alleles with 13 repeats and

these two peaks here. And in the tumor we saw these same peaks indicating that some of his cells were there but we also saw 10 repeats

and 16 repeats indicating that the DNA of another person with those alleles was present there. Then when we put all of the alleles together we found that in the peripheral blood of this patient there was no non-patient DNA so his peripheral blood contained all his own DNA. However, in the tumor 62 percent of the cells were foreign. So in our specimen the majority of the cells there were from another person presumably one of these stem cell injections.

Next we asked which genetic aberrations are present in the tumor cells. So the field of tumor pathology has evolved quite a lot over the last 10 years and now a large number of tumors can be understood in terms of the genetic mutations that they have. For example, most glioblastomas have an extra copy of chromosome 7 and often have amplification of the EGFR locus. So we were looking for characteristic genetic changes that might help us to understand this tumor. So we did targeted exome sequencing using a panel of 309 tumor associated genes. At our institution we

call this Onco Panel and we found no mutations or copy number changes of known clinical significance in these genes. We did find some variance but none of them were a classic variance of any clear clinical significance and some of them may have just been polymorphisms. So we weren't able to fit this into any known tumor entity based on this data.

So again putting this all together we call this a glioproliferative lesion. We were able to conclude that it was derived from non-patient cells. So this is a schematic that shows how a pluripotent stem cell can differentiate into mature forms as has been discussed in various aspects today so you can have pluripotent stem cells that can differentiate into neural stem cells which can then differentiate into forms like neurons, astrocytes, and oligodendrocytes. And so I'm going to take a look at the markers that I showed you earlier and we'll try to get a sense of what line of differentiation these tumor cells were showing. So they were

Sox-2 positive but negative for Oct-3/4 suggesting that they were not induced pluripotent stem cells or embryonic stem cells. The did express OLIG-2 found in neural stem cells. They were negative for neuron markers and positive for astrocyte markers and for oligodendrocyte markers. So based on this data at the time the patient presented to us the lesion appeared to be acting like neural stem cells. That being said we can't say exactly whether that was the state of the cells when they were transplanted into the patient. It is possible that less differentiated cells were transplanted and then subsequently differentiated.

For a clinical follow up the patient was seen by our colleagues in neuro-oncology and radiation oncology and they made the decision to treat the tumor with a three phase technique of radiation from cervical level C to the thecal sac. The patient did show some improvement of their spinal cord symptoms and on MRI three months later there was a modest decrease in the tumor bulkiness although subsequently it has grown in size. But

the patient is still alive at this point.

So I'd like to take a few minutes after sharing this case to talk about some other reports of stem cell derived tumors. One that received a fair amount of attention a few years ago now was a 9-year-boy with a genetic disease ataxia telangiectasia. He was given fetal neural stem cells. He traveled from his home in Israel to Russia and four years later presented with severe headaches. He was found to have two separate lesions. One in the posterior fossa adjacent to the cerebellum and the second one in the lower spinal cord. Examination of this lesion showed there were cells with neuron like differentiation with the marker new N as well as cells with astrocyte like differentiation expressing GFAP as well as areas that resembled the cells that line the ventricles within the brain. So this group described this as an extra axial multifocal glioneuronal tumor hinting at those dual lines, multiple lines of differentiation. They did note that it did have a low proliferation rate and that

it was well differentiated. So this overall is a better behaving tumor than in our patient where we saw very aggressive characteristics both in the clinical realm as well as pathologically.

Another case that has been reported was a 50-year- male with Parkinson's disease. He was transplanted with fetal neural stem cells, a procedure that has been done in a variety of places and has produced some very interesting science. He presented two years later with progressive lethargy, breathing difficulty and then suddenly passed away. I apologize, these figures were from 20 years ago so they're in black and white. In the occipital lobe and in the fourth ventricle we see that there is material that is filling the ventricles, filling these ordinarily empty spaces with cerebrospinal fluid. When these areas were examined microscopically they noted that some areas looked like cartilage and other areas looked like hair shafts. So we have two different lineages mesenchymal and ectodermal lineages. Interestingly, they didn't

find any neural tissue even though supposedly the patient received fetal neural stem cells. So they described this as differentiated intraventricular tissue of unclear origin and offered a number of explanations perhaps contamination by other cells during the procedure or perhaps the fetal neural cells de-differentiated and matured but it wasn't clear exactly how they got there but a fatal effect of this therapy.

We should not be surprised that stem cells can form tumors. It has been well known in animal experiments that when embryonic stem cells are injected into mice that they have the capacity to form teratomas in which a diverse range of differentiated cell types can be formed such as gut, neural, bone and so on.

I'd like to also step out of the stem cell area for a moment just to note that neoplasms can be transmitted between humans under certain circumstances. There are reports of transmission during solid organ donation. A preexisting neoplasm in the donor was transmitted to the

recipient generally requiring immunosuppression in the recipient and it has also been reported that in umbilical cord blood transplantation that leukemia has been reported. There is also a report of transplacental transmission from the mother to the fetus of a lymphoma.

So in summary, I presented a case of a 66-year-old male with a glioproliferative lesion of the intradural space and we found that this appeared to be derived from donor stem cells those it is not exactly clear which type of stem cell it came from. There are multiple previous reports of other tumors coming from stem cells and other exogenous sources that have grown in humans although none showed the same high grade characteristics that the case we saw demonstrated.

So as a closing point it is clear that stem cell therapy does hold great promise but must be balanced against the potential adverse effects and for this reason the clinical use and investigation of these therapies really needs to be evaluated in a rigorous setting and follow up

surveillance and standardized assessment by physicians and other professionals is really a critical part of this if we're going to be taking risks with patients' lives.

I'd just like to thank the others at Brigham and Women's Hospital as well as at the Dana Farber Cancer Institute who have been involved in various aspects of working up this case.

DR. KAHN: So I think now we're going to move to the panel. Everybody who spoke after lunch please make your way to your seat where there's a tent card. There is going to be more people at the table then the audience but we'll manage that. So we have about a half hour is that correct? So I think we'll also obviously have some time for questions from the audience so be thinking as we get settled here. I guess we'll do what has been done through the course of the day and have people line it up and we'll manage it that way.

So I've been thinking as we've been

listening to the afternoon presentation and we've had sort of how to be responsible from the professional society prospective, how to manage both moving forward responsibly and making sure we do so for the people who need new therapies from a government perspective. Then we just heard two very cautionary tales which makes me want to ask the following question and I hope you all can opine about this. In the history of research on human subjects the emphasis in the past going back to the 1970's when the regulations were first promulgated the emphasis was on protecting people from being taken advantage of and making sure that they weren't exposed to too much risk for the benefit of other people and not to themselves. Over the course of this decade there has really been a shift to talking about people as participants and partners in research. But the stories we're hearing sound a whole lot more like we need to protect people then we need to make sure they are partners that have access to the benefits that research offers. So let me ask you

whether you think we just haven't got it right for this particular category of research or whether we've got it right but there just needs to be an assertion of control, establishment of parameter, something or some third option that I haven't thought of.

DR. KIMMELMAN: So it is true that historically research ethics has been built on a premise that the fundamental function of research ethics is to protect the welfare of the human subject. But I think in a contemporary era where so much hinges on the quality of evidence our healthcare system solvency depends on it, our ability to deliver appropriate healthcare to patients hinges on having reliable evidence. I think it is time for research ethics to be thinking a lot about the downstream consumers of evidence. At the end of the day when you conduct clinical research it is not strictly a private transaction it is a public transaction. There are stakes that exceed or transcend merely the dyad of the patient and caregiver and those ought to

factor in to how we make decisions to risk, benefit and access. So I think Jeff really eloquently in his remarks noted that. For many novel scientific endeavors, you only really get one at bat. You conduct a clinical trial. If you don't have your conditions optimized, if you don't have good preclinical evidence behind it you go into a trial prematurely, you get a negative result and potentially you have withdrawn funding, public support, et cetera and that potentially can really set back what might otherwise be a very promising scientific endeavor and I think it is those kinds of considerations of public interests that ought to be driving in part our decisions about how we set regulatory standards rather than merely the question of how we protect patients from risk.

DR. ALBINI: I think it is difficult to be transparent about what level of regulatory standards you have and I think what the South Florida story shows, one of the things is that the patients assumed that there was a certain level of

regulation and a certain level of quality and science that they were buying into. They weren't aware of the tell-tale signs that it really wasn't there like the fact that they were paying for it, that there was no doctor involved. I mean some things in retrospect you say how on earth could you let this have happened to you. But they'll go back to well it was on clinicaltrials.gov and it turns out they weren't even in a research study. There was no outcomes data that was planned to be obtained. They didn't even follow up with the patients. It was just mind boggling. It was clearly just fee for service procedure without a doctor and I think that there just needs to be more transparency or more awareness in the public to what they're getting involved in. If you're going to deregulate it has to be very clear somehow to the participants in the trials that this is not the same as the type of clinical trials that they've been used to.

DR. KAHN: So only because I can't see all the way down to where Mike is but why don't we

just move serially from Dr. Dominici.

DR. DOMINICI: So I think you comparing the current situation with the seventies. Specifically, in this field there was a revolution in the way in which we can essentially isolate and amplify the cells. It is relatively easy and it is relatively cheap to do that if we compare with what was done in the past. You can take bone marrow, you can take fat and it is cheap and it is easy. That one side is a good thing because we can work in the lab and do things that were very difficult to do in the past. On the other side this is allowing those types of treatments in an environment that are easy and once again cheap to be approached. That is one of the problems I would say. The second problem which is part of the (inaudible) that we are being see on information on the web. In the seventies we didn't have that. These people have access to clinicaltrial.gov and they look at that as we learn. Those types of web based information can be misused and the patients may essentially be

captured by the fancy images that are related and are included in the website. They can't dissect the good and bad information. So I think the scientific society should be collaborating with patient associations and with the other stake holders in trying to clarify and work and starting from the source of information that the patient can get and provide resources. I think we should move along with the technological improvement to provide services for patients.

DR. RUBIN: I think the standards are not elusive. In fact, Jeff, you laid them out really eloquently in your talk and a lot of the issues of transparency and informed consent they're just so intuitive and all the things that we as a doc would want to do when we have to look a patient in the eye and say whether it is experimental or not, these are the risks, these are the benefits and lay it out and really have that transparent decision making process is really important. But there are mechanisms obviously to circumvent the standards that we've heard about in

the last few talks that therapeutic misperception of a patient funded trial or pay to participate trial combined with the desire for hope with patients who don't have a lot of hope. In fact, when patients call me and they want regenerative therapies that can't be done and I say it can't be done they get angry with me. And the no discussion is longer than the yes I can help you discussion because they get angry and they want to know why can't you help me, when can you help me, what do you mean you can't help me. So it is very easy, I could see how easy it is for them to show up in someone's office and be treated with standards that are less than what we would desire because of what they want from it. Those mechanisms and the people who are out there not adhering to the standards is a problem.

MS. POTTOL: I can appreciate the point where the patient is a little bit more difficult than it is for us. At least at my office I sit a couple of steps behind the scenes in an office and I don't see the patients. In the DOD we do have

one additional hurdle there to try to really making sure the clinical trials are in the best interest of the patient and that is with an additional layer of our human resource protection office on top of the standard IRB's and the FDA. So I think that it certainly takes a little bit longer to get a trial started but it helps us ask additional questions on what is right for the patient and are we doing all those things to care for them. The other challenge is are you challenging yourself to make sure that you're staying responsible, you're close to your research, are you having enough different voices involved to challenge you to make sure that the decisions that you're making are the choices that you're putting in there for your patient that you bring maybe opposing views so you can help place your own self and your own thoughts being too close to the work that you're doing.

DR. LUNDBERG: So I agree with a lot of what has been said already. I think another challenge which we haven't really touched on is

the fact that again with the internet being so available is that the problem is larger than just the U.S. As the example of the spinal cord tumor people are going outside of the U.S. and the access is just out there so that's a challenge that I think we as a group should be thinking about in ways how can best protect U.S. citizens from this kind of impact.

DR. MANSFIELD: I think engagement of the patients is very important. I think that the foundations and other organizations that fund some of this research and that support the patients have really got to have a strong educational role. They've got to help these people to understand what is involved, what the risks are. As a person who interacts with people with a disease we're very much aware of this real urgency that they have to get a treatment. Understanding the scientific process is very hard. We all know that you don't go from a concept to a drug in a year or two years it is a very long process with a defined set of steps and it needs to be done carefully.

And when there is an urgency for treatment it is very hard to understand why is it taking so long. One of the questions that we had when we were supporting gene therapy was once one gene therapy was successful they wanted it to be plug and play. Why can't you just cut it out and put it into the next, next one, next one and solve it all for us now. So there is this urgency, there is a need to educate to understand the time involved in these developments but I also think there is a need to control the development of the non-homologous use of autologous cells. I think that is a particular area that needs particular attention. That would be my thought. I think the existing regulatory framework that we have in general is good.

DR. KAHN: Yes I agree with a large amount of what has been said and I think one of the critical things that the speakers have done today is to lay out an ethical framework for how to address these questions. I think in one particular case there were two things that were very difficult. One is that there is great

variation in patients in terms of the degree with which they're willing to accept risk and the way that they see benefit. I think the second thing is to really be realistic about expectations to not be over promising. In the case of our patient he actually was highly motivated to seek treatments to the point that he traveled all around the world and spent potentially hundreds of thousands of dollars on these treatments. I think one thing that is very interesting even after all of this and even after the tumor he doesn't regret having taken this action. As some of you may have seen he volunteered to be interviewed by the press after our paper came out and on some level I think he was happy that he took this risk and it was a risk that he felt was worth I which I think it is important to respect the patient autonomy in that way but it also may still reflect over promising. Even if no individual physician has promised him something unrealistic from advertising from the way that scientific papers come out clearly he was expecting the potential benefit that made it worth

it for him and the amount of money and risk to himself.

Jeffrey Kahn: So I see we've got a few people at the microphone. I just want to make one observation and then we'll turn to the question which is at a time in our history when it seems like pressing government is not at its all-time high that one of the explanations for why people thought these cases we heard about were worth pursuing was because it was on a government website that seemed to endorse it as safe or something that would not harm them. So I think it really comes back to this notion of trust and we've said this over and over in the course of the day. It is very easy to lose trust and it is very difficult rebuild it. This particular area of biomedicine is kind of at a tipping point for moving forward and this notion of trust really cannot be squandered. Let's go to the second microphone because you're been waiting.

DR. RODRIGUEZ: Yes my name is Ricardo Rodriguez and I'm from Baltimore. I got approval

for a grant for a human study using SVF three years ago and we have gone through the process of INDs and IRBs which I believe is right and has made us think about our process very carefully. As I have been going through this process what strikes me as how many people are out there sort of doing it on whatever basis they can. So for me the problem as I see it at large is not so much a regulatory schema as it is, rather how those other people are flouting the existing regulatory scheme and that a quicker way of tackling this problem is identifying and bringing to light those people and publishing the information rather than complicated regulatory overhauls.

DR. KAHN: Okay we will go to the first microphone.

MR. CLEGG: His Dennis Clegg, UC Santa Barbara. I had a question for Dr. Miller and maybe you mentioned this and I missed it. Did you determine that the transplanted cells or the cells with the different DNA were human? Could they have been animal cells?

DR. MILLER: They were human. From the STR we were able to confirm they were human as well with the deep sequencing even though we didn't find pathogenic mutations.

MR. CLEGG: Okay. And then a general question for the panel. What can be done about clinicaltrials.gov? That really surprises me that they're listing these kinds of trials.

DR. KAHN: I'm not an expert about clinicaltrials.gov but maybe Jonathan you can opine.

DR. ALBINI: Well I think that one thing that could be done is you could just say on there this doesn't condone any government approval. I think the supposition is that somehow this is government approved. The purpose of the site is really just to make sure that everybody is aware of any clinical trial that is being done so anything can be put on there and it is not verified. So I think if there were a disclaimer that was written in English easy to understand by most patients on there than that would be a great

way to start. The other recommendations that we've had are to list whether or not an IND was obtained. For example, this group that I dealt with while they had IRB approval they didn't go through getting and IND they thought they didn't need it. But to the point of enforcing the regulation that we already have I mean the fact that this clinic was able to do this three times was very frustrating to me because as we saw the patients coming in we notified the Florida Department of Health and we notified the FDA and they all have mechanisms in place to investigate. But they are all slow going investigations and they didn't stop. The company is still around that is doing these things. They stopped doing eye research but they are doing other treatments. But really besides call the police there is no other way to regulate or do anything about it.

DR. MANSFIELD: I'd like to add that clinicaltrials.gov if you do have an IND it is actually captured on there it is just not visible to the public. I don't know why that can't just

be declared. You don't have to reveal more than it has been applied for.

DR. WEISS: If I may interject something on that. Is there not legislation pending that is meant to upgrade clinicaltrials.gov for all these reasons that are being articulated. If I'm not mistaken there is a motion afoot to number one tighten the requirements for entry of anything onto clincialtrials.gov. Number two to tighten the reporting requirements because that is the flip side of course you need to know what happened with these trials. So all of these sentiments are good but what disturbs me a little bit is that we as the experts in the field in this room don't all know what each other are doing number one. Number two, we're not communicating and presenting as best we could a unified front. The ISSCR, the ISCT et cetera should be hand in hand with the NIH, the FDA, loud and clear, consistent broadly circulated to have a loud message to patients and caregivers about the dangers of unproven cell therapies and where to go for the information.

This is where transparency, communication, visibility can go a long way. So the fact that nobody up here seems to know about pending legislation in the clinicaltrials.gov reporting and entry system is a little disturbing. So how can we all best learn from this. The other thing I'm curious about is the point you made. The FDA we spent a lot of time tracking down snake oil if you will that is on the web. We reported it to the FDA and they go through their diligent processes but there are no teeth in the FDA. They can't necessarily enforce anything it has got to go to the justice department to go out and take the action to throw these people in jail. It happens rarely and not frequently enough and so one of the things that we as a group can hopefully send a strong message is to the people that would have the power to shut these places down. There is a range of things out there and none of us can put ourselves in the shoes of a patient who is desperate or a family. We're not saying that. No one is trying to play God. What we're trying to

do is to identify the more egregious snake oil people out there and just shut them down as best as possible. Now one thing I'm curious about is the ISSCR experience a few years ago. You all gave a solid go at trying to do this and then you got threatened somehow and you had to back off. How can we as a community again learn from that. What happened to you? Why did you have to back off? How can we move around that to try to again wipe some of this garbage off the map?

DR. KAHN: That's a bunch of things. Why don't we start with ISSCR and maybe we'll move in reverse? Go ahead Jonathan.

DR. KIMMELMAN: I'm guessing Irv can probably better answer this question better than I can. This actually preceded my time here but Irv was president during this period.

DR. WEISSMAN: So in 2009 when I was about to retire as president of ISSCR I wrote an article about unproven clinics and that it should be the ISSCR as the agency that looks at it. So we had a group of people who looked at unproven

therapies. And we decided at the end to warn the patients and warn the caregivers because we couldn't be another FDA for the world. We couldn't take on the expertise to judge which one was good or bad so we said you as a caregiver or you as a patient should ask two questions of the people who are about to give you a therapy or charge you for a therapy. The first is the name of the head of the institution review board that oversaw the in house first in human clinical testing. And as you know IRB is responsible prospectively and during the early part of the trial for the safety does not judge the efficacy. The second question you should ask is in your country or in this country you find out the documentation from their FDA or their FDA equivalence that was responsible for overseeing the three phases of safety and efficacy. That's all we asked. We didn't take on anything. And we set up a website called A Closer Look and had many, many hits and we said we will even help you if you don't know who to ask the question. We'll

ask the question for you. So a few months later I was still head of ISSCR. I had the most distinguished board of scientists, stem cell scientists around the world with me and at a board meeting the executive director showed us a letter from an attorney in Chicago that said by what authority are you asking my client these two questions. And all of that board of distinguished physicians and scientists except two of us and the administrative head of the society said we're closing the site. They were afraid of litigation. We are afraid of litigation. So it takes courage, it takes the resources of the FDA maybe to step in and say these are the questions you should ask or have us ask because this is a most serious defect and for me it was an alarming revelation of my colleagues who were the leaders of the field.

DR. KAHN: Anyone want to comment? I will say in response to the previous comment about clinicaltrials.gov and reporting of results the NIH is moving towards a policy to require result reporting of all clinical trials that they fund.

So we've moved towards greater and greater transparency and of course the idea behind clincialtrials.gov in the first place was to give people greater opportunity to understand what trials were available. To the point that people see it and they think therefore it is endorsed. That wasn't ever the point. So somebody is taking advantage. It isn't so much the site that's the problem it is the people that are taking advantage of what the site does as a way of exploiting desperate patients. I don't know how more bluntly to say it than that. Anymore comments or questions?

DR. BERTRAM: Tim Bertram, Region Medical. I was musing on the last statement because it is kind of interesting as we've debated here today it has been interesting to see as we've looked at the different things and the expectations of the agencies and what I was really struck by is the ocular changes that we saw as a result of that implantation. A couple of points was it was done by a nurse practitioner. It

reminded me as I was listing to the story of the Maturini case that has been carried on, a fair amount of notoriety that took place in Europe where in fact is was also a medically trained professional. What I'm wondering about if there was no conversation here about how we are training our professionals to actually communicate with the patients. So maybe the papers and there is always going to be a nefarious aspect and I'm not saying no regulations here, I'm just saying there is an element that we will not be able to control. But in one part what we haven't discussed here today is how are our physicians being trained in order to go forward. We've got some very illustrious physicians that have presented but is it a general training that is going on because as this therapy comes forward there is obviously going to be the use, ultimately the misuse but more significantly the inappropriate use and I'm just curious what the board thinks.

DR. ROSS: Duncan Ross from Chimera Labs. As far as the training is concerned I was

wondering if we saw that result with the collagenase. In the case where we saw that result with collagenase have we seen that collagenase in other labs and when we talk about the training I know that that group has a training operation. I've come in and found various discrepancies. So when I think about taking collagenase away from the field I think that there needs to be better training. Maybe we could form some kind of training system.

DR. ALBINI: I can't speak to the enzymes but what I can speak to is that as a retina surgeon I don't think that my colleagues were aware this was going on. This was sort of off the map. So in terms of what we're training retina specialists I think the answer is nothing because I think there just wasn't awareness of this. When I've presented these cases at retina meetings everybody has been in shock and is unaware of other problems or that clinics like this even exist or what the market place is for stem cell clinics and so forth. So that's not out

there and the only thing I can say is that I do think probably a minority of retina specialists would recommend to their patients to try something like this. I can imagine I don't know how small the minority is but I can imagine and I think I've heard of some physicians who have recommended to patients where there are no other options why don't you try this -- not the particular one that I discussed but why don't you try some sort of stem cell therapy. I think that just comes out of the fact because we as retina specialists don't really talk about any of this. So I've learned a lot today and I think there probably should be a mechanism that some of what I've seen here today be communicated back to my colleagues.

DR. KAHN: Okay really, really, last, do you want to say something?

DR. KIMMELMAN: I just want to say two things about this. So first of all, I'm not a physician, I don't dispense medical advice but it seems to me that physicians dread having to tell patients that their options are incredibly thin

and I think when we talk about training it is not merely in terms of helping physicians to convey to patients the dubiousness of some of the medical options that they may be hearing about but also maybe training physicians to have those kinds of uncomfortable conversations with patients. That is my first comment and the second comment is one thing that we haven't talked about here is journalism and media coverage because of course patients get a lot of their information not from physicians but from news stories. Just the other day there was a wonderful story in USA Today about Bart Starr going to a clinic in Florida or somewhere else. Not exactly a hard hitting good scientific coverage and I think we could probably be doing more to be training our journalists on how to cover cutting edge science as well.

DR. WEISS: One last thought to add to that. So the training point is an excellent one, the journalism point is an excellent one. It is hard of course to implement training in an effective way across entire disciplines. It is a

good goal and a worthy goal that will take some time. One parallel that could be and hopefully is being effective is to utilize the professional societies. Parallel with the ISSCR and the ISCT have your American College of Retinal Surgeons, the American College of Cardiology, plastic surgeons take a visible and uniform stance such that on their respective websites at their annual conventions there is prominent visibility. Just by hammering away at it that you're going to eventually get the message across. This isn't something that is going to permeate the entire medical profession quickly but it needs to be a consistent concerted effort. So to give an example --

DR. KAHN: I think we need to give Irv the last word here.

DR. WEISS: Ten seconds. So as an example in the lung world we've just published an editorial against stem cell medical tourism in the flagship journal of the American Thoracic Society that is on the website but most importantly it is

a joint statement that is now shared on the website of probably 50 different respiratory disease societies ranging from private, non-profit foundations to patient advocacy groups to the actual foundations. It is an example of at least a small step in the education process against stem cell medical tourism.

DR. KAHN: Thank you. Thank you guys all eight of you. I'm going to turn it back over to Irv.

DR. WEISSMAN: So first I want to thank the FDA for organizing this session. When you hear so many negative stories about stem cells and tumors that are derived from not proven stem cells you may start to get disappointed about the field. It is an anomaly that right now this field is busting wide open and it is moving better and better towards rational scientific based animal model studied clinical therapies and clinical trials. But we must have funding for the basic science to do this. And you may have thought well don't you have enough stem cells? So far we have

blood forming stem cells. We can prospectively isolate transplant not in clinical trials. We have brain forming stem cells. Some people think we have skin forming stem cells I'm not sure. And in humans that's all we know. But we know by other kinds of developmental biology or what we call lineage tracing experiments that stem cells exist for every tissue. So obviously if we're going to move the field we have to fund the field. That's NIH. And NIH has to do it and the best method of scientific inquiry that is Fund R01, investigator, initiator, research and make sure there is enough funds in this area to do it.

So that is tissue stem cells and I've told you that we've now had since the 1990's the understanding that pluripotent stem cells can turn into all tissues and go through tissue stem cells to get there. So that is our opportunity to fast track the identification of the tissue stem cells. Embryonic stem cells cause teratomas. They are not therapeutic objects. When you purify a differentiated stem cell say bone and cartilage

stem cell you have to be able to differentiate it and then you have to purify it away because even one in a million cells could make a teratoma. So it is the responsibility of the regulatory agencies to look carefully at that proof of principle. Because the neuro tumor you described of the child coming back from Russia almost certainly came from a very undifferentiated cell or a poorly regulated cell that had been grown and was abnormal.

The next step I already told you is for this field to move forward we have to find ways like we are trying to where you remove life threatening chemo therapy or radio therapy to prepare the patient for a stem cell transplant. Hematopoietic tissue transplants were invented by the oncologists so they could give super lethal doses of chemo therapy. But now we have this other side to it, regenerative medicine. And we and I hope others are going to do that and again every single antibody we use will have to vetted through the FDA as being safe. One of the

difficult issues for any stem cell therapy is if you make a cell line and it goes bad and you have to make another cell line how can you guarantee it similar, biologically similar and biologically effective. That is really important.

The major focus of this meeting, oddly but I can understand it, is around mesenchymal stromal cells. And as Jacques said at the beginning why were people trying to put these cells in all the tissues. Well I know at the very beginning with Arnie Kaplan at Case or Cleveland trying to make the bone healing cell. One of the amazing things about getting that cell out in culture is it is easy. It grows like mad. So when the commercial outfits came into it they went where the light was. The easy cells and then kept trying them and many different things. I think it is a miracle that they've turned out to be great or at least interesting anti-inflammatory cell types. But that doesn't substitute for the hard research to find the other tissue stem cells. What we saw was a lot of the nitty gritty about

what will be important to the patients, what will be important to the FDA is how can we make these cells better, how can we manufacture them safely and because money is always involved how can it be done with a margin that will keep funding going for the company. And delivery of the cells of course as well.

Now I will say going back over the mishaps that we've seen on cells that cause tumors, cells that cause problems is you need to purify the cells. We should no longer be in an era that we accept all of the bone marrow or a homogenate of liver being the cell type that you use in a therapy. You should learn what the stem cell is, learn how to purify it and if it tests out right in the right models then carry that forward. Because most of the whole field of bone marrow transplant since the late seventies when Don Thomas and others invented it has not advanced very much at all. It is a little bit of this combination, a little bit of that but it is still mixtures of cells. Twenty-five years after it is

possible to isolate the cells. I don't want to make this all self-referencing.

By the way I forgot to put up a conflict side. I was the founder of Systemics bought by Novartis I have no financial interest in Novartis. I started at least two other companies that are dead or dying. Stem Cells Inc. that did brain stem cells and just recently ran out of money before it ran out of trials to do, closing down. So I guess for those I don't have a conflict of interest but I just formed another company called Forty Seven and I mentioned it in one of my talks that antibody to CD47 currently in clinical trials for cancer could also go into clinical trials for stem cell transplants.

I want to go back to terminology. I can't imagine why we're sloppy that we allowed the whole bone marrow transplant community to say they were stem cell therapists in which led of course to the cord blood community saying yes and the stem cells we have can probably give rise to all cell types in the body but they don't. They only

have cells that give rise to blood and if a few of them have enough mesenchymal precursors to mesenchyme. They do not give rise to brain, they do not give rise to heart and so on. We allow our clinicians, our journals and our journalists to get away with calling stem cell therapies when they're not stem cell therapies they are bone marrow or some other cell type. I'm an M.D. and like most M.D. I had to compete with the pre-meds to get into medical school. So I remember those people. They were the great memorizers. And all the time during medical school and after they advanced because they could memorize way better than I could. Those are the people who are asked to judge whether the therapy will work or go forward. Those are the people who added the word stem cell to all of these therapies. So somebody is going to have to insist if you advertise that you just had a stem cell therapy for breast cancer that you really did a stem cell therapy. Because the other memorizers will read the title and say well stem cell therapy doesn't work for breast

cancer.

We've had the answer since 2000 that one-third of our patients in a tiny trial were cured. But we can't get anybody to spread it because they read a paper by Statenauer and they read a New York Times article by Gina Colada that says this was all a fraud and a sham and it cost a lot of people a lot of trouble and they missed the boat. They threw the baby out with the bath water. So we should insist the same standard of terminology that we would insist for any chemical or any drug.

So you heard a little bit about the unproven, a lot about it. It is a very serious issue. It is not going away. I gave you the example of the question that I think still should be asked. We just the ISSCR wasn't the group who knew how to ask it or had the courage to back it up because we didn't have the resources to cover litigation, at least that's what they told me.

So maybe one way to do this is to have more of a global interaction between three bodies

that are really relevant to these kinds of advancement of medicine. First and foremost is the FDA. I am absolutely against the Regrow Act that takes away from the FDA the possibility that they oversee whether it is effective not just safe. If the FDA had an alliance with all of the FDA like bodies and I know you talk but I don't know how strong the alliance is then maybe you could get a handle on it.

I was called about eight years ago by the pulmonary acute care physicians that an 18-year-old girl was in the Stanford Hospital with pulmonary emboli. A day before she had been in the Dominican Republic where a South Florida doctor injected into her mesenchymal cells grown either from her or another source. So I called my Dean and I called the hospital administrator and I said what can we do about this. They said it was done in the Dominican Republic? Well there's nothing we can do about it. We can't do anything in Florida because we're in California and Florida covers the medical licensure. So medical

licensure should be dependent on non-participation in unproven therapies no matter where it happens in the world. Whether you send the patient to that place or you go there to help the trial or you collect money for the trial. Licensure should be at risk.

The final part of it which surprisingly I heard first in Thailand when I gave a talk and the head of the FDA and also the head of licensure which was under the emperor of Thailand. He said well we've added something. We've added no false advertising. I'm not sure if that is under FDA purview. I don't think so but you guys can tell me afterwards. So the triumvirate of a strong FDA that demands not only IRB but phase three trial with efficacy and comparative efficacy the risk of losing medical licensure and a penalty perhaps a very severe penalty for false advertising in the stem cell field. Maybe then when you Google stem cell therapy the first 100 won't be phony stem cell clinics. I think that that's most of the important part but we've heard over and over again

that there appears to be a small defect in clinicaltrials.gov, perceived or real so that that needs to be fixed. So that people can look at it and know whether an IND supported type clinical trial.

Then I will say the final one and it is mainly by Steve Bauer's early discussion on how they were characterizing the cell lines at the single cell level and then talking with a number of people at the break the FDA has to be funded to continue to do research in the relevant area. So it is not only a regulatory and oversight body and I know it is funded, I don't know the extent to which it is, but it is critical to have people who are also confronting the difficulties of digging secrets out of nature. How hard it is and how important it is to be part of the discovery apparatus if you're going to regulate it. So those may be more opinions then summaries but I want to thank you very much.

DR. WITTEN: I'd like to thank all our speakers and I'd like to thank the audience for

attending, thank you.

(Whereby, at 5:05 p.m. the PROCEEDINGS were adjourned)

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CERTIFICATE OF NOTARY PUBLIC

COMMONWEALTH OF VIRGINIA

I, Carleton J. Anderson, III, notary public in and for the Commonwealth of Virginia, do hereby certify that the forgoing PROCEEDING was duly recorded and thereafter reduced to print under my direction; that the witnesses were sworn to tell the truth under penalty of perjury; that said transcript is a true record of the testimony given by witnesses; that I am neither counsel for, related to, nor employed by any of the parties to the action in which this proceeding was called; and, furthermore, that I am not a relative or employee of any attorney or counsel employed by the parties hereto, nor financially or otherwise interested in the outcome of this action.

(Signature and Seal on File)

Notary Public, in and for the Commonwealth of Virginia

My Commission Expires: November 30, 2016

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