FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

ONCOLOGIC DRUG ADVISORY COMMITTEE (ODAC)

Thursday, August 13, 2020

8:06 a.m. to 11:41 a.m.

Morning Session

Virtual Meeting

Meeting Roster

ACTING DESIGNATED FEDERAL OFFICER (Non-Voting)

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Division of Advisory Committee and
Consultant Management
Office of Executive Programs, CDER, FDA

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C O N T E N T S

C O N T E N T S (continued)

P R O C E E D I N G S

(8:06 a.m.)

Call to Order

Introduction of Committee

DR. HOFFMAN: Good morning and welcome. I would first like to remind everyone to please mute your line when you are not speaking. For media and press, the FDA press contact is Kristin Jarrell. Her email address is kristin.jarrell@fda.hhs.gov, and her phone number is 301-796-0137.

My name is Philip Hoffman, and I will be chairing today's meeting. I will now call the morning session of today's Oncologic Drugs Advisory Committee to order. Dr. Joyce Yu is the acting designated federal officer for today's meeting, and we'll begin with introduction of this morning's meeting roster.

DR. YU: Good morning. My name is Joyce Yu, and I am the acting designated officer for today's meeting of the Oncologic Drugs Advisory Committee. When I call your name, please introduce yourself by stating your name and affiliation. I'll start. My

name is Joyce Yu, acting designated federal officer for the Oncologic Drugs Advisory Committee.

Dr. Hoffman?

DR. HOFFMAN: My name is Philip Hoffman. I'm a medical oncologist at University of Chicago.

DR. YU: Dr. Garcia?

DR. GARCIA: Jorge Garcia, chief medical oncology, University Hospitals, Seidman Cancer Center, Case Western Reserve University, Cleveland, Ohio.

DR. YU: Thank you.

Dr. Halabi?

(No response.)

DR. YU: Dr. Halabi, can you unmute your line, please?

DR. HALABI: Yes. Good morning, everyone. Susan Halabi, biostatistician, Duke University.

DR. YU: Thank you.

Dr. Hinrichs?

DR. HINRICHS: Christian Hinrichs, senior investigator, NCI.

DR. YU: Thank you.

Dr. Sung?

DR. SUNG: Anthony Sung, hematopoeitic stem cell transplant physician, Duke University.

DR. YU: Thank you.

Dr. Cheng?

DR. CHENG: Good morning. I'm Jon Cheng, medical oncologist. I'm the industry rep and I work for Merck Pharmaceuticals.

DR. YU: Thank you.

We'll be skipping Dr. Finestone. She's not yet on the line.

Dr. Morrison?

DR. MORRISON: Sean Morrison. I'm a stem cell biologist at the University of Texas, Southwestern Medical Center.

DR. YU: Thank you.

Ms. Pearl?

MS. PEARL: Good morning. My name is Diane Pearl. I am the mother of two Fanconi anemia post-bone marrow transplant patients, and I live in Park City, Utah.

DR. YU: Thank you.

Dr. Robey?

DR. ROBEY: Pam Robey, stem cell biologist and senior investigator at the National Institutes of Health, dental research.

DR. YU: Thank you.

Dr. Singec?

DR. SINGEC: Ilyas Singec. I'm a stem cell scientist at NIH.

DR. YU: Thank you.

We'll now introduce our FDA participants.

Dr. Bryan?

DR. BRYAN: Wilson Bryan. I'm director of the Office of Tissues and Advanced Therapies, in the FDA's Center for Biologics Evaluation, and Research.

DR. YU: Thank you.

Dr. Puri?

DR. PURI: Good morning. My name is Raj Puri. I'm the director of the Division of Cellular and Gene Therapies in the Office of Tissues and Advanced Therapies in the Center for Biologics Evaluation, and Research.

DR. YU: Thank you.

Dr. Oh?

DR. OH: I'm Steven Oh. I'm the deputy director at the Division of Cellular and Gene Therapies at the Office of Tissues and Advanced Therapies in CBER.

DR. YU: Thank you.

Dr. Bauer?

DR. BAUER: Good morning. Steve Bauer. I'm a branch chief in the Division of Cell and Gene Therapies.

DR. YU: Thank you.

And Dr. Klinker?

DR. KLINKER: Morning. I'm Matt Klinker. I am a product reviewer in the cell therapy branch in the Division of Cellular and Gene Therapies and the primary product reviewer for this application.

DR. YU: Thank you. That concludes our morning introductions.

DR. HOFFMAN: For topics such as those being discussed at today's meeting, there are often a variety of opinions, some of which are quite

strongly held. Our goal is that today's meeting will be a fair and open forum for discussion of these issues and that individuals can express their views without interruption.

Thus, as a gentle reminder, individuals will be allowed to speak into the record only if recognized by the chairperson. We look forward to a productive meeting.

In the spirit of the Federal Advisory Committee Act and the Government in the Sunshine Act, we ask that the advisory committee members take care that their conversations about the topic at hand take place in the open forum of the meeting.

We are aware that members of the media are anxious to speak with the FDA about these proceedings, however, FDA will refrain from discussing the details of this meeting with the media until its conclusion. Also, the committee is reminded to please refrain from discussing the meeting topic during breaks or lunch. Thank you.

Dr. Joyce Yu will read the Conflict of

Interest Statement for the meeting.

Conflict of Interest Statement

DR. YU: The Food and Drug Administration is convening today's meeting of the Oncologic Drugs Advisory Committee under the authority of the Federal Advisory Committee Act, FACA, of 1972. With the exception of the industry representative, all members and temporary voting members of the committee are special government employees, SGEs, or regular federal employees from other agencies and are subject to federal conflict of interest laws and regulations.

The following information on the status of this committee's compliance with federal ethics and conflict of interest laws, covered by but not limited to those found at 18 U.S.C. Section 208, is being provided to participants in today's meeting and to the public. FDA has determined that members and temporary voting members of this committee are in compliance with federal ethics and conflict of interest laws.

Under 18 U.S.C. Section 208, Congress has

authorized FDA to grant waivers to special government employees and regular federal employees who have potential financial conflicts when it is determined that the agency's need for a special government employee's services outweighs his or her potential financial conflict of interest or when the interest of a regular federal employee is not so substantial as to be deemed likely to affect the integrity of the services which the government may expect from the employee.

Related to discussions of today's meeting, members and temporary voting members of this committee have been screened for potential financial conflicts of interest of their own as well as those imputed to them, including those of their spouses or minor children and, for purposes of 18 U.S.C. Section 208, their employers. These interests may include investments; consulting; expert witness testimony; contracts, grants, CRADAs; teaching, speaking, writing; patents and royalties, and primary employment.

Today's agenda involves biologics license

application, BLA, 125706 for remestemcel-L, ex-vivo culture-expanded adult human mesenchymal stromal cells suspension for intravenous infusion, submitted by Mesoblast, Incorporated.

The proposed indication or use for this product is for the treatment of steroid refractory acute graft versus host disease in pediatric patients. This morning session will discuss issues related to the characterization and critical quality attributes of remestemcel-L as they relate to clinical effectiveness.

This is a particular matters meeting during which specific matters related to Mesoblast's BLA will be discussed. Based on the agenda for today's morning meeting and all financial interests reported by the committee members and temporary voting members, no conflict of interest waivers have been issued in connection with this meeting. To ensure transparency, we encourage all standing committee members and temporary voting members to disclose any public statements that they have made concerning the product that issue.

With respect to FDA's invited industry representative, we would like to disclose that. Dr. Jonathan Cheng is participating in this meeting as a non-voting industry representative acting on behalf of regulated industry. Dr. Cheng's role at this meeting is to represent industry in general and not any particular company. Dr. Cheng is employed by Merck & Company.

With regard to FDA's guest speaker, the agency has determined that the information to be provided by the speaker is essential. As a guest speaker, Dr. Sally Temple will not participate in committee deliberations nor will she vote.

We would like to remind members and temporary voting members that if the discussions involve any other products or firms not already on the agenda for which an FDA participant has a personal or imputed financial interest, the participants need to exclude themselves from such involvement and their exclusion will be noted for the record. FDA encourages all other participants to advise the committee of any financial

relationships that they may have with the firm at issue. Thank you.

DR. HOFFMAN: We will now proceed with FDA opening remarks from Dr. Wilson Bryan.

FDA Opening Remarks - Wilson Bryan

DR. BRYAN: Good morning. On behalf of the FDA, I want to thank the members of this advisory committee for taking the time to consider this biologics license application, or BLA, for remestemcel. This product is proposed to treat pediatric patients with steroid-refractory acute graft-versus-host disease.

Throughout our consideration of this BLA, it is critical that we remember that these are highly vulnerable patients. They are vulnerable because they have a life-threatening disease. They are vulnerable because as infants and children, they are not able to fully participate in or give informed consent for decisions regarding their medical care. And they're vulnerable because for the patients who are less than 12 years old, there is no FDA-approved therapy for steroid-refractory

acute GVHD, so there is a substantial unmet medical need. It is critical that we make our regulatory decisions with these patients and their vulnerability in mind.

The FDA is bringing this BLA for consideration by this advisory committee because this is a first-in-class product and because we have substantial concerns regarding this application. Remestemcel is a mesenchymal stromal cell or MSC product. There are a large number of ongoing clinical trials of MSC products, but no MSC product is FDA approved for the treatment of any disease or condition in the United States.

The FDA is concerned that a wide variety of MSC products are being marketed and sold illegally in the United States to treat diverse conditions, including but not limited to orthopedic, rheumatologic, cardiac, pulmonary, neurodegenerative, and oncologic disorders, and COVID-19. These products have not been shown to be safe and effective but are marketed to vulnerable and desperate patients who are often charged

thousands of dollars.

The FDA believes that the field of cell therapy has the potential to address many unmet medical needs, but that potential must be reached through rigorous science with regulatory oversight and not by exploiting vulnerable patients. For these reasons, we very much appreciate the efforts of Mesoblast to undertake clinical studies and develop their products to address an unmet need.

In today's discussions, we ask this committee to consider the rigor of the evidence, both the product's characterization data and the clinical trial data, in this first application for an MSC product. This afternoon, Drs. Bindu George and Kristin Baird will outline some of the FDA's concerns regarding the clinical trial data. For this morning's discussion, Dr. Steve Bauer will describe some of our concerns related to product characterization.

Because MSCs and all cell therapies are highly complex products, the FDA often finds that chemistry, manufacturing, and controls, or CMC

issues, can be particularly challenging, but it is our responsibility to address these challenges. If remestemcel receives marketing approval, it is critical that every pediatric patient receives a product that has the same safety and effectiveness as is seen in the clinical trial or trials that supported that marketing approval.

The discussion this morning focuses on product characterization issues that are at the core of providing this assurance to patients and their parents. I am very much looking forward to hearing the perspectives of this committee on these critical product characterizations and clinical issues.

I am also looking forward to the presentations from our guest speakers, to reviewing the public comments submitted to the docket, and the statements that we will hear today in the open public hearing. All of your deliberations and comments will assist the FDA in our consideration of this license application. I will stop there and turn it back over to Dr. Hoffman.

DR. HOFFMAN: We now have a guest speaker presentation by Dr. Sally Temple.

Presentation - Sally Temple

DR. TEMPLE: Thank you. I'd like to thank you for the opportunity to speak about some of the challenges surrounding assessment of therapeutic cell products. I'm going to use some of the literature on MSC use for graft-versus-host disease as examples, and the presentation will be in four sections: an introduction; discussion of the sources of variability in cell product and patient response; cell product characterization covering morphology, markers, and function; and then conclude with some suggestions regarding approaches to determine critical quality attributes, that is the --

DR. YU: Hi --

DR. TEMPLE: Hello?

DR. YU: Dr. Temple?

DR. TEMPLE: Yes?

DR. YU: Hi. I'm very, very sorry. Could I just pause for one moment and allow Dr. Hoffman to

inform the committee about the conflicts?

DR. TEMPLE: Okay.

DR. HOFFMAN: I'm sorry. I got out of order here.

DR. TEMPLE: No worries.

DR. HOFFMAN: Both the Food and Drug Administration and the public believe in a transparent process for information gathering and decision making. To ensure such transparency at the advisory committee meeting, FDA believes that it is important to understand the context of an individual's presentation.

For this reason, FDA encourages all participants, including the applicant's non-employee presenters, to advise the committee of any financial relationships that they may have with the applicant such as consulting fees, travel expenses, honoraria, and interests in the applicant, including equity interests and those based upon the outcome of the meeting.

Likewise, FDA encourages you at the beginning of your presentation to advise the

committee if you do not have any such financial relationships. If you choose not to address this issue of financial relationships at the beginning of your presentation, it will not preclude you from speaking.

We will now proceed with presentations from the guest speaker, immediately followed by presentations from Mesoblast, Incorporated and FDA.

DR. YU: Thank you so much.

Dr. Temple, please proceed.

DR. TEMPLE: Thank you.

I'm going to use, as I said, some of the literature on MSC use for graft-versus-host disease as examples; and the presentations in four sections covering an introduction, discussion of the sources of variability in the cell product, and the patient response; and then cell product characterization covering morphology, markers, and function; and then conclude with some suggestions regarding approaches to determine critical quality attributes, which are attributes of the cell product that indicate efficacy and that can be used

to assess differences in cell product preparation.

Allogeneic stem-cell transplant of hematopoietic stem cells is the treatment of choice for patients with several high-risk malignancies and other life-threatening, non-malignant disorders.

Acute graft-versus-host disease is a leading cause of mortality and morbidity. While steroids remain the first-line treatment, about half the patients don't respond. So second- and third-line treatments are needed cryopreserved unmatched. Our generic mesenchymal stromal cells, or MSCs, are currently used in several European countries to treat graft-versus-host disease.

So why MSCs? MSCs can be derived from different sources, including from bone marrow, and they have been shown to exhibit plasticity, taking on the features of cells such as fat, bone cartilage, and muscle. They've been shown to secrete numerous factors that can be beneficial in an injured environment, and some of these factors have immunomodulatory properties.

For example, they can suppress the proliferation and activity of T cells, B cells, natural killer cells, and they can positively regulate activated regulatory T cells.

In this review, acute graft-versus-host disease mechanism is laid out as shown in this figure, and they describe it in three phases. First, there's initial tissue damage and antigen presenting cell activation. Then the donor T cells from the graft are primed, differentiate, and migrate. Then there's a third stage. When the activated immune cells destroy the host tissue, especially in the gut, liver, and skin, after infusion, the MSCs become activated and are thought to inhibit processes at each of these three phases.

However, there are several sources of variability that can affect outcome. There's donor variability that's shown here on the left of the slide. That includes genetics, age, health, medications, et cetera. Then once the MSCs are isolated, they enter a multistep manufacturing process with several opportunities for variations

such as cell plating density, the vessel type used for culture; the culture components that are used; the length of time in culture; and the number of passages.

All of these factors can affect the specific cells and the final cell product, their characteristics and properties, and how they interact with the disease environment. The recipient patient is also a source of variability, having different genetics depending on the stage of disease, the specific tissue locale, and prior treatment they have received, all potentially affecting outcome.

Now, I'd like to delve a bit more into these sources of variability and product effectiveness because understanding these factors is critical to understanding therapeutic efficacy. I have to point out that the data that I'm going to show were collected from several different studies using MSCs and they do not reflect a single product or a single manufacturing process.

There's good evidence that the MSC donor

impacts outcome, and in this experiment using a T-cell proliferation assay, two of the donors, indicated by 303 and 308, had significantly lower inhibition of T-cell proliferation at the 1to 9 ratio of MSCs to peripheral blood mononuclear cells. Dr. Bauer will go into more detail about donor variability in his presentation, but it's clear from studies in the literature that we need to have a better understanding of the impact of donor on cell product characteristics.

This figure describes the basic steps in MSC manufacture from tissue collection, cell isolation, expansion, harvest, and then release testing prior to patient administration. In addition, the cells may be frozen and cryopreserved during this process. So a significant challenge is knowing how to optimize this multistep complex process and how to make sure it's standardized and reproducible.

As agents, reagents, supplies, and donor are changed, how do we determine that the product is sufficiently similar to one that previously demonstrated efficacy and safety?

In this study from 2012, the impact of passage number was assessed, and it was demonstrated that if MSCs are used at early passages, they were more effective in patients than late passage cells. So again, we need to understand how MSC properties change with time and culture, with passage, and different culture conditions.

There are numerous different culture modes for MSC production whether you're using a particular type of multi-layered spec system or bioreactor, and as I mentioned different culture components. The impact of these on the cell types produced and the cell therapy success is important to determine.

I mentioned previously that MSCs are used in Europe for graft-versus-host disease, and in this review from 2018, data from 17 European centers were analyzed to assess differences in product manufacturing, including the tissue source, how the MSCs were isolated, the growth factors that were used to expand the product, and the methods used

were different at different centers, as you can see. Further details were taken, including the markers that are used for acceptance criteria, which also varied substantially.

Given we have different manufacturing protocols, different criteria to characterize the product, and there is information on how the patients responded, it may be worth while analyzing these data and potentially studying the cell product and any retained locks, and relate this to patient outcome.

I also talked about variation that comes from the particular recipient. The recipient varies in genetics and stage of disease, and the same product can elicit different responses in patients. These are typically recorded as complete, partial, or non-response.

Clearly, we need to understand how differences in the recipient patient impact the cell therapy outcome. An important question is whether we can identify responders versus non-responders for a particular treatment by using,

for example, genomic sequencing of patient or assessing parameters related to stage of disease.

Going on, building further into impact of recipient, we know that after the MSCs are infused, the allogeneic MSCs live for a short while in the host. This occurs in mouse models, and in this study, it was shown that MSCs infused into mouse models of graft-versus-host disease underwent cell death, as shown in B. Further, in these graft-versus-host disease models, the MSCs successfully reduced the graft-versus-host disease effector cells in both the spleen as shown here and in the lung and another one.

They also then went on to ask whether the MSC death was important. They compared the outcome and second mouse model, which was mutant for perforin, in which the MSCs did not die. What they found now is that, as shown here, these graft-versus-host disease effector cells were not reduced in the spleen or in the lung. So in this model, then, it appears that the MSC death is critical for beneficial response.

Importantly, they also showed that the peripheral blood mononuclear cells, the PBMCs, from the patients with graft-versus-host disease could kill MSCs in vitro, while those from healthy controls did not. Moreover, as shown in B, the cell death in vitro correlated with patient response as shown here. In this case, then, there's an in vitro assay that appears to protect clinical outcome in different patients, which is very valuable information.

So how do we better define the cell product characteristics that are related to clinical benefit? We typically characterize cell products with three main areas of assessment: cell morphology, markers, and functional tasks. Assessment of morphology is a fundamental aspect of cell culture method.

Experienced researchers can look down the microscope at growing cells and rapidly tell whether a particular culture is on track. So integrating this fundamental method of assessment into the manufacturing process is very important.

Cell morphology depends on many different factors on the cell type, the substrate, the culture medium, the growth factor, and the passage number. Morphology is a powerful indicator of cell type, cell health, and state, and therefore it can inform about cell identity, purity, and potency, but we need methods to assess morphology that ideally are operator independent.

Computer-based image analysis has advanced to very sophisticated levels, and in this study led by Dr. Bauer, MSC images were collected, segmented, and captured by computer software and then analyzed in depth. This digitized information about the cell features can then be used to predict MSC properties relevant to patient outcome such as degree of mineralization or immunosuppressive activity.

Recent analytical methods use deep learning with large sets of training images to assess cell properties, as shown in this study led by Dr. Kapil Bharti at the National Eye Institute.

Here, induced pluripotent stem cells were

used to generate a monolayer of retinal pigment epithelial cells. Image analysis and deep learning methods then enabled prediction of important features of this cell, including the tightness of the monolayer and the polarized secretion of vascular endothelial growth factor.

Analyzing images by non-invasive methods combined with customized software programs can be very valuable tools to assess the cell product during the manufacturing process.

Stem and progenitor cells divide and they produce progeny of different types, and dynamic features such as division mode, cell migration, and process outgrowth, these can help indicate cell health and properties.

In this paper from 2010, computer-based analysis of time-lapse movies was used to predict the retinal progenitor cell fate from the movies, and in fact the successful prediction rate, as shown here, was very high. Static images or movies can be captured by non-invasive methods during manufacturing and provide critical information that

improves the efficiency and the reproducibility of the manufacturing process.

The marker expression is another key characteristic used to assess cell products, and numerous markers have been identified on MSCs, including cell-surface markers that are valuable to identify and also select cells.

However, despite extensive study, no markers have yet been identified that accurately predict clinical outcomes, so how do we know whether we have correctly identified the key markers to follow? We need a comprehensive understanding of cell markers, including cell-surface markers, that can be used as a reference to correlate with product performance.

An understanding of the molecules expressed on the cell surface is especially useful, as these are the molecules that will interact with the host environment. For example, the surfaceome encompasses specific receptors, surface ligands, and adhesion molecules, which could impact product performance after transplantation. The surface

proteome is a small percentage of the total genes expressed in a cell.

This figure from Dr. Rebecca Gundry shows the method she uses, that the surface glycoproteins are captured, and then mass spec is used to sequence the associated peptides. Dr. Gundry has also developed sophisticated bioinformatic tools to analyze the surfaceome and identify which molecules are unique to a particular cell type.

Just as an example, she's used this system to identify a cell-surface transporter, which one, that is expressed on the surface of pluripotent stem cells and can be used to remove residual stem cells during cell culture to improve product purity and safety.

Any cell product has a degree of heterogeneity due to different numbers of cell subtypes or two different cells being different states. How many different subtypes of cells are present? We need to know also whether the heterogeneity is actually important for product success. Are some populations beneficial and

others harmful?

Selecting subpopulations could improve product performance. Are some subpopulations inert? This could impact dosing. So for many reasons, we need to have a good understanding of the heterogeneity of cell products.

Single-cell analysis has greatly advanced our understanding of cell population heterogeneity. For example, single-cell transcriptomics has become routine to define the diverse cell types in a mixture and provide information on gene expression in individual cells that can inform about cell state and cell health.

In this study, adipose cells were isolated and cultured, and then thousands of the cultured cells were analyzed with RNA sequencing. In this case they used a 10x platform, then a bioinformatics analysis was performed as shown in B. After correcting for cell proliferation, several subpopulations of cells were revealed.

Currently there are a number of different platforms to perform single-cell sequencing and

there are several bioinformatic tools. Both of these impact outcome. So in order to use this technology to provide information on the heterogeneity of a cell product, it's crucially important to standardize the platform and the bioinformatic analysis pipeline that is used.

It's also valuable to use the single-cell technology to understand more about the original cell isolate. I already mentioned that the cell product can vary with different donors, and in this recent paper, bone marrow mesenchymal cells were studied using single-cell transcriptomics, and this revealed several different cell subpopulations with distinct gene expression pattern.

Hence, defining the composition of the cell isolate, which we can consider the starting material in this cell manufacturing protocol, can be informative and potentially valuable to predict the performance of the final cell product.

I previously mentioned that MSC preparations can vary in its efficacy with passage. In this study, bone marrow stromal cells were sequenced at

single-cell level and found to change gene expression with passage, and some of these markers are selected in seq. This information could potentially explain how passage impacts clinical outcome.

Note that in addition to the single-cell sequencing that I have mentioned, other methods are available and are being developed to analyze features of cell population. These include, for example, single-cell ATAC-seq, which gives information about chromatin state. New technologies that are coming online have great potential to improve our understanding of cell products.

Overall then, it would be useful to gain deeper information about cells at several stages of the manufacturing process, at the beginning in the original cell isolate; during manufacture to track changes that occur with time and culture and passage; to assess the impact of critical steps such as cryopreservation; and then to more fully characterize the final formulated product. A

challenge is determining what tests should be done, and when, and how that information will be used to assess the product.

In addition to morphology and markers, functional testing plays an important role in product characterization. MSCs are known to exhibit plasticity and acquire features as adipo-, osteo-, and chondrogenic lineage cells. This plasticity is induced, for example, by using specialized inductive culture media that push the cells down these different pathways.

However, MSCs are not the only cell type with this property. In a paper we published in 2012, we demonstrated that retinal pigment epithelial cells, that were derived either from adults themselves or from pluripotent stem cells, were able to acquire features with these lineages after exposure to the same inductive media that was used for MSCs. Even single identified RPE cells could take on these different phenotypes. Retinal pigment epithelial cells are central nervous system cells and not an MSC, so this feature is not unique

to MSCs. It may be an important and necessary to define them, but it's not sufficient.

An important property of MSCs is cytokine secretion, and typically one or two factors are used to assess a particular cell production process. But we can now take advantage of larger scale methods, such as using cytokine arrays to determine the secretome more completely and be able to define differences between one production process or another production run -- one process or a different production run and another.

Functional tests that predict outcome in patients would be ideal. In this study, a typical in vitro test was performed to assess whether MSCs could inhibit patient PBMCs. The outcome of this in vitro test was then compared to clinical outcome with the patients classified as responders or non-responders.

It's notable that this test did not predict the patient response. So we need to identify functional tests that can be used to assess the cell product that ideally are demonstrated to be

predictive.

Just as a reminder, I talked earlier about this cytotoxic in vitro assay that was found to be predictive of patient response. So such tests will be useful to help identify cell populations that would benefit specific patient populations.

In conclusion, defining the critical quality attributes of a cell product is an iterative process that demands knowledge about the cells and how they perform in patients. It would be best to gather wide knowledge about the cell product. It's very important to consider which data to gather, which methodologies to use, and at which time points in the production process.

Similarly, it's important to consider what information to gather about the patients and their clinical responses, and correlating the two in an iterative manner may be used to identify the critical quality attributes and the patient population that the product is best suited for.

Finally to summarize, the field of cell therapy is growing and has great potential to

produce new medicines and that we acknowledge that it is a relatively new field and that cells are complex, and dynamic, and it is challenging to define identity, purity, and potency assays. I mentioned the MSCs are being used for graft-versus-host disease in Europe with a variety of manufacturing processes and markers used to identify and release the product, so it may be worthwhile to delve further into existing data.

The MSC mechanism of action is likely multifactorial, so there is value in gathering wide information on the product to correlate with patient outcomes in order to define critical quality attributes.

Finally, we're at an exciting time when multiple technologies are maturing that enable us to characterize cells in depth and the key stages of the manufacturing process to better understand how these novel therapeutic agents work and may benefit patients. Thank you.

DR. HOFFMAN: Thank you, Dr. Temple. We'll now move on to the applicant's presentation,

Dr. Storton.

Applicant Presentation - Geraldine Storton

MS. STORTON: Yes, I'm here.

Good morning, Mr. Chairman, members of the advisory committee, and the FDA. I'm Geraldine Storton, the head of regulatory affairs and quality management at Mesoblast. We're pleased to be here today to discuss remestemcel-L, which I'll refer to as remestemcel throughout the presentation.

Here is the agenda for this morning's presentation. I will introduce remestemcel and elaborate on the manufacturing process and quality controls in place.

Dr. Silviu Itescu will then provide background on the pathophysiology of acute GVHD, a disease caused by cytokine storm and T-cell activation. He will also speak to the mechanism of action and the ability of remestemcel to reduce the cytokine release and inhibit T-cell activation. He will conclude by explaining how our clinical trial outcomes have been able to validate and demonstrate the selection of appropriate potency assays.

Remestemcel is an allogeneic cell product that comprises culture-expanded mesenchymal stromal cells isolated from bone marrow of healthy adult donors. Since the mesenchymal stromal cells are hypo-immunogenic, cells from a single donor can be used in recipients without tissue matching. Remestemcel is an off-the-shelf product that can be readily available to treat patients when needed.

Remestemcel is manufactured in Mesoblast's contracted GMP manufacturing facilities over a three-stage GMP compliant process. In the first stage, the bone marrow is obtained from healthy donors. The bone marrow then goes through the process to isolate and purify the cells, which then proceed through the initial steps of expansion into a cell bank. At this point, the quality is confirmed and the banks can be stored for some time.

In the second stage of the process, cells are further expanded and formulated into the final drug product and undergo cryopreservation. In the third stage, the product is packaged, stored, and

distributed under strict quality control. As the product is cryopreserved, it can be stored in distribution centers ready to be sent to hospitals when a patient needs treatment.

Let me elaborate on the donor program, and then I'll walk you through each of the manufacturing stages in more detail. The donor program is well established with potential donors evaluated for eligibility and safety. The donors must be prescreened and blood testing undertaken up to a week prior to donation to check for a full infectious disease profile.

In addition to meeting all the requirements for good tissue practice, we have added additional criteria to donors such as body mass index, age, and bone marrow cell count. The screening process works to prevent introduction of possible communicable diseases, and the additional measures are included to reduce the variability in the attributes of the cells such as their proliferative capacity, leading to more consistency in the yields of the manufacturing process.

The first stage of manufacturing is the production of the donor cell bank from the bone marrow aspirate. The nucleated bone marrow cells are isolated from the bone marrow aspirate. They are then culture expanded in a process using two passages of expansion.

Following passage 2, the cells are harvested and cryopreserved as the donor cell bank. Each donor cell bank lot is derived from a single donor, which following culture expansion generates multiple containers of donor cell banks per lot. Several donor cell bank lots are currently available for continued manufacture of drug products.

The second stage involves the continued production of drug substance and formulation and fill of the final drug product. One vial from a donor cell bank is stored and further culture expanded for three more passages. The cells are harvested following passage 5.

These cells are the active drug substance, and they are then formulated in a cryoprotectant

solution and filled to create the final drug product. The drug product is cryopreserved and stored below negative 135 degrees C in liquid nitrogen vapor phase. For each manufacturing campaign of drug product, a single container of donor cell bank is used. Overall, one donation of bone marrow can manufacture enough drug product to treat more than 400 patients.

Throughout manufacturing, there are a number of in-process controls to monitor the quality of the cell and the manufacturing environment to ensure that the process is being executed consistently. Cell count and viability are routinely monitored to assess consistency of cell growth throughout the process. Attributes such as sterility are also periodically monitored to ensure the process maintains an aseptic environment throughout.

In addition, quality control release testing is performed on the donor cell banks and the final drug product. As the drug substance is immediately processed, the tests on the drug substance are

focused on in-process sterility and mycoplasma testing to ensure the aseptic environment has been maintained. Our critical quality attributes, which are attributable to safety, efficacy, and yield, have been established, and we have consistently carried them through the development process, including multiple manufacturing site and raw material changes.

The characteristics and attributes of mesenchymal stromal cells are well understood, and robust quality assurance processes ensure final product with batch-to-batch consistency and reproducibility. Once manufactured and packaged, the third stage of the process involves shipping the final drug product to distributors, where remestemcel is packaged into cartons containing either 1 or 4 vials. The product is held at distribution centers until it is requested by a treating hospital.

The product quantities are prepared and shipped under strict temperature conditions to the treating hospital to ensure the quality of the

product at the time of treatment. Products can be stored for up to four years under cryostorage conditions. This gives hospitals the ability to have off-the-shelf product available when needed to treat a patient.

To prepare for administration, the required number of vials are thawed based on the patient's body weight, resuspended, and transferred into an infusion bank with 40 mls of Plasma-Lyte A or an equivalent solution, and infused. The infusion typically takes no longer than 30 minutes. Mesoblast works with the hospitals to ensure the staff are trained in the handling, thawing, and administration procedures for the drug product.

To understand the quality attributes that we use to ensure a quality product is distributed to these patients, you need to understand a bit of the pathophysiology of GVHD and the mechanism of remestemcel in this disease. To describe this, I will now pass to Dr. Silviu Itescu to explain the mechanism by which remestemcel functions in the treatment of patients with graft-versus-host

disease.

Applicant Presentation - Silviu Itescu

DR. ITESCU: Thank you. My name is Dr. Silviu Itescu. I'm the chief executive officer of Mesoblast. Remestemcel is a novel cellular therapy for the multimodal mechanism of action. It modulates in terms of excessive immune response to foreign tissues, autoantigens, or infections, allowing resolution and recovery of healthy tissues.

Due to these characteristics, we have developed remestemcel for the treatment of acute graft-versus-host disease in pediatric patients when they have failed to respond to treatment with systemic corticosteroids.

Acute graft-versus-host disease is a serious and life-threatening complication of allogeneic hematopoietic stem cell transplantation that occurs when alloreactive donor T cells within the hematopoietic stem cell graft recognize the recipient's tissues as foreign and trigger an immunological response.

The pathophysiology of acute GVHD disease is complex and is characterized by three phases: tissue damage from conditioning treatment; immune cell activation and cytokine storm; and end-organ damage, primarily involving the skin, the gut, and the liver.

In phase 1, the bone marrow transplant conditioning regimen causes profound damage to host issue, which leads to the release of inflammatory stimuli. This activates antigen-presenting cells. In phase 2, following the bone marrow transplant, there is substantial immune activation of donor macrophages and T cells, which results in a cytokine storm that mediates tissue damage. Phase 3 is the end-organ damage involving the gut and the liver that results from the macrophage and T-cell cytokine storm and is frequently fatal.

This slide demonstrates two major characteristics of remestemcel's mechanism of action. Firstly, the cells use surface receptors such as tumor necrosis factor receptor type 1, or TNFR1, to sense the presence of high levels of

inflammatory cytokines such as TNF-alpha produced by the inflammatory macrophages and T cells within the micro environment.

TNF signaling through TNFR1 activates cytoplasmic NF-kappaB, which moves into the nucleus and is the master regulator of multiple anti-inflammatory factors, which ultimately result in polarization of inflammatory M1 macrophages to M2, anti-inflammatory macrophages, switching off TNF-alpha production and inducing production of the anti-inflammatory cytokine, interleukin 10.

Measuring surface levels of TNFR1 is a sensitive predictor of the ability of the cell to respond to the inciting inflammatory stimulus, in this case TNF-alpha, and orchestrate an anti-inflammatory response, and is a good upstream assay to quantify the ability of the cell to evoke a downstream, anti-inflammatory matrix response.

The measure of the cell's bioactivity is its ability to inhibit CD4 T cell activation and proliferation, the end result of multiple anti-inflammatory factors produced either in

response to signaling through TNFR1 or via other surface cytokine receptors, including interferon gamma. Measuring the cell's ability to inhibit CD4 T cells provide the qualitative bioactivity that reflect the combined effects of multiple anti-inflammatory factors and pathways.

The next slide shows the ability of remestemcel to inhibit production of high levels of TNF alpha produced during an active cytokine storm. Shown in the second panel, significant induction of TNF alpha, lymphotoxin, and interferon gamma is seen following activation of peripheral blood mononuclear cells with anti-CD3 and anti-CD28 monoclonal antibodies.

Co-culture with remestemcel from two distinct product lots result in potent inhibition by over 90 percent of both TNF alpha and lymphotoxin production, both ligands for TNFR1 but not interferon gamma, indicating a specific and selective pattern of proinflammatory cytokine depression within both T cells and macrophages.

This suggests a feedback loop whereby high

levels of TNF alpha activate remestemcel via its surface receptor to secrete paracrine factors responsible for specific shut down of the inciting TNF alpha produced by inflammatory M1 macrophages and T cells.

To evaluate whether specific levels of surface TNFR1 are indeed related to the intracellular bioactivity following TNF alpha signaling of remestemcel, we used siRNA technology to establish remestemcel lots expressing reduced TNFR1 expression levels, as seen here. The right-side panel shows the effect of TNFR1 knockdown on phosphorylation of NF-kappaB following TNF alpha stimulation.

When remestemcel is activated with TNF alpha, there is significant phosphorylation of NF-kappaB as shown in black. This response is dependent on the level of TNFR1 expressed by remestemcel, providing a direct link between TNFR1 levels on the surface of the cells and intracellular bioactivity as measured by NF-kappaB activation.

This process ultimately results in nuclear translocation of the activated NF-kappaB, where the complex is able to initiate transcription and use expression of multiple target genes. This translocation is inhibited when TNFR1 is downregulated, as shown in the middle panel, but not when TNFR2, the second receptor for TNF, is downregulated, as shown in the right-hand panel. This demonstrates the critical requirement for signaling via TNFR1 in NF-kappaB translocation and its bioactivity.

We next sought to directly show the effect of TNFR1 signaling on secretion by remestemcel stem cell of NF-kappaB regulated immunomodulatory factors. In the left-hand panel is shown the dose-dependent induction by TNF alpha of CCL2 secretion by remestemcel, an immunomodulatory factor which is regulated by NF-kappaB. Maximal induction is seen when using 10 nanograms per ml of TNF alpha.

In the right-hand panel, we show that progressively increasing concentrations of siRNA

targeting TNFR1 abrogated the effect of TNF alpha at 10 nanogram per ml to induce CCL2 secretion. This shows that there is direct relationship between the level of TNFR1 expression and CCL2 secretion by remestemcel.

A similar response is seen for secretion by remestemcel of another immunomodulatory factor regulated by NF-kappaB, in this case, M-CSF. On the left is shown dose-dependent induction by TNF alpha of M-CSF secretion by remestemcel, while in the right panel there is again a progressive reduction in the secretion of M-CSF with increasing knockdown of TNFR1.

Collectively, these data demonstrate that TNFR1-dependent induction of at least two factors regulated by NF-kappaB, CCL2 and M-CSF, both of which play the key role in polarizing macrophages to an anti-inflammatory M2 state.

In summary, these data very clearly demonstrate that the absolute level of TNFR1 expressed by remestemcel determines the response to TNF alpha, and in turn NF-kappaB activation and the

secretion of immunomodulatory molecules.

We next sought to directly show whether CCL2 production by remestemcel, a TNFR1-dependent and NF-kappaB regulated factor, result in M1 to M2 macrophage polarization. Purified CD14 positive monocytes co-cultured with remestemcel in the presence of TNF alpha showed significant increase in IL-10 secretion. This IL-10 production was inhibited in the presence of a blocking antibody to CCL2. These results were seen consistently when using distinct remestemcel product lots.

So these data show TNFR1-dependent signaling of remestemcel leads to production of CCL2, which plays a key role in the polarization of macrophages to an IL-10 producing immunomodulatory state.

In addition to the level of surface TNFR1 expression being an upstream measure of the integrity of remestemcel's ability to regulate NF-kappaB dependent immunomodulatory factors, it is important to measure the cell's downstream bioactivity to inhibit CD4 T cell activation and proliferation since these are important in the

clinical outcome of GVHD.

Shown in the right-hand panel is a reproducible inhibition of multiple remestemcel lots of the ability of CD4 T cells to proliferate following activation with anti-CD3 and anti-CD28 monoclonal antibodies. Measuring the cell's ability to inhibit CD4 T cells provides a qualitative bioactivity that reflects the combined effects of multiple anti-inflammatory factors and pathways, either in response to signaling through TNFR1 or other surface cytokine receptors such as the interferon gamma receptor.

Given our understanding of the multimodal mechanisms of action by which remestemcel inhibits T cell proliferation and inhibits macrophage polarization and retains the M1 phenotype, we utilized the matrix-based approach to develop our potency assays.

This approach is consistent with FDA guidance, which recommends the use of one quantitative bioassay and one qualitative bioassay, which together are sufficiently robust in terms of

reproducibility as indicators of product quality and stability.

In summary, our in vitro understanding of the multimodal mechanisms of action of remestemcel has informed the selection of our potency critical quality attributes. TNFR1 is upstream of NF-kappaB signaling and secretion of immunomodulatory cytokines, and can be determined quantitatively.

IL-2 receptor alpha inhibition on activated peripheral blood mononuclear cells is an early marker of T cell activation and was selected as a qualitative bioassay based on our knowledge that inhibition of T cell activation is critical to the immunomodulatory activity of remestemcel, both directly and via macrophage polarization.

As I'll show you, data from the broad and long clinical development program has further informed on the absolute levels of these potency measures and the correlation with clinically meaningful outcomes in patients with steroid-refractory acute graft-versus-host disease.

Our clinical program has included three

studies in patients with steroid-refractory acute graft-versus-host disease: Protocol 280, a randomized-controlled phase 3 trial; Protocol 275, an expanded access program in children; and Study 001, the pivotal, single-arm phase 3 trial in children.

During the yellow-shaded period in 2009, several manufacturing enhancements were made to optimize and streamline the overall manufacturing process of remestemcel, which Mesoblast has consistently carried forward. The most important change was to set a limitation on maximal trypsinization time, a process modification that has been shown to significantly impact the surface expression of TNFR1.

In this figure, pale blue indicates product made using the original manufacturing process resulting in a lower level of TNFR1 potency. The product made using the original process was used in clinical studies 280 and about three-quarters of the Expanded Access Protocol 275. The dark blue shows the product made using the optimized

manufacturing process, which has a much higher level of TNFR1 potency. This product was used in about a quarter of the Expanded Access Protocol 275 and in the pivotal trial Study 001.

Shown in this slide are the changes in the two key potency critical quality attributes between critical product lots made with the original process in light blue above and with the optimized process in dark blue below. The product made with the optimized process demonstrated a significantly higher mean TNFR1 expression level and a shift to the right in the overall distribution of TNFR1 expression levels. Additionally, the product made with the optimized process demonstrated a significantly reduced variability in mean IL-2 receptor inhibition than product made with the old process.

An assessment of the measured potency attributes on product used in the three steroid-refractory acute GVHD trials showed that patients treated with remestemcel in trials after 2009 received product with higher critical quality

attributes as a result of the optimized manufacturing process.

This table shows that mean TNFR1 levels and percent inhibition of IL-2 receptor expression will both increase in product used to treat all patients in Study 001. Cell viability was consistently high throughout this period.

We next examined whether these observed differences in potency attributes in products used in each of the study protocols had an impact on day 28 overall responses and on day 100 survival. As you can see, our pivotal Study 001, where all patients received optimized product, showed the highest day 28 overall response and the highest overall survival. Study 271 only measured survival through day 100. That's why we're showing here day 100 survival only.

Stratifying patients across all steroid-refractory graft-versus-host disease trials on the basis of having received only product made with the original or the optimized process demonstrated that patients who received only

product made with the optimized process had significantly higher mean levels of TNFR1 and IL-2Ra inhibition, IL-2Ra percent inhibition, and significantly higher day 100 survival.

In pediatric study Expanded Access 275, children who received a single-donor lot of product made with the optimized process had a significantly better survival than those who received the product made for the original process. This demonstrates the relationship between optimization of critical attributes on a single-product lot and survival benefit within one single trial and consistency of patient demographics.

In pivotal study phase 3 001, where only product made with the optimized process was used, there was an almost identical survival outcome at day 100 of 74 percent, demonstrating a second trial, which confirmed the survival benefit associated with the optimized manufacturing process seen in the 275 study.

An analysis of donor lots from single donor used in patients treated in the phase 3 Trial 001

showed, in fact, that all of these lots have consistently high TNFR1 levels, on the left, and high T cell inhibitory function, as shown on the right.

Shown in the next slide is the relationship between in vitro inhibition of IL-2 receptor expression for lot and reduction in activated CD4 T cells in vivo after 28 days of treatment with remestemcel in the phase 3 Trial 001 in patients who received a single product lot. Activated CD4 T cells were defined as expressing IL-2 receptors in HLA-DR. The inverse relationship is significant using linear regression. Change in activated CD4 T-cell levels was seen in all patients for 28 days of treatment, indicating that this is a consistent in vivo measurement of the product's bioactivity.

Taking into consideration our understanding of the manufacturing process and controls and being further informed through the clinical outcomes, we've revised our specifications to ensure the commercial product lots will consistently reflect the potency and activity of the product used in the

001 phase 3 study.

In conclusion, remestemcel has a well-considered consistent and robust manufacturing process that uses well-defined release criteria. We've identified two important product attributes, TNFR1 expression and IL-2 receptor inhibition, that have demonstrated a relationship to the clinical performance of specific drug product lots. The survival outcomes in our clinical development program further inform determination of TNFR1 specification and IL-2 receptor inhibition in vitro associated with in vivo reduction of immune activation.

The optimization of product manufacturing for remestemcel has resulted in greater potency of these assays and improved clinical outcomes over time. Data from our clinical development program support that TNFR1 and IL-2 receptor inhibition correlate with clinical outcomes and highlight the importance of these clinical quality attributes to ensuring the manufacturing process consistently produces remestemcel lots of acceptable quality.

This concludes our presentation. Thank you.

DR. BAUER: Good morning. This is Steve Bauer. Shall I proceed?

DR. HOFFMAN: Please.

DR. BAUER: Thank you.

FDA Presentation - Steve Bauer

DR. BAUER: As I said, I'm Steve Bauer. I'm the chief of the Cellular and Tissue Therapies Branch in the Division of Cell and Gene Therapies in the Office of Tissues and Advanced Therapies, and I'll be giving the FDA presentation on product characterization for remestemcel-L. In FDA terminology, product characterization is also referred to as chemistry manufacturing and controls or CMC for short.

(Pause.)

DR. BAUER: Before going further, I would like to acknowledge my fellow CMC experts, our office leadership, and our colleagues in OCE and ODAC for their assistance with this presentation. I also want to thank our special government employees and advisory committee panel for their

participation today. We look forward to the discussion and input from you folks.

The purpose of my talk is to discuss the quality attributes of remestemcel-L and their relationship to product quality and effectiveness. In plain language, this product can be described as an off-the-shelf product, and the assumption is that each batch of product will have the same quality.

So we can ask this question. How do you know that the next batch has the same activity as the batches used in the clinical trial? What we rely on to answer this question is overall control of the manufacturing and testing for quality attributes as explained in the next two slides.

We rely on three key types of control as part of our strategy to ensure product quality and consistency. For each type of control, we determine characteristics to assess or measure and specifications for those measurements. One key is source control, meaning that we control the quality of the starting materials used in manufacturing.

Another key is process control of manufacturing, but the focus for today is control of product testing. At the end of manufacturing, we want to test the product to make sure it has the same characteristics from batch to batch or lot to lot. Product testing focuses on properties of the product that we call quality attributes.

DR. YU: Hi, Dr. Bauer. This is Joyce Yu.

DR. BAUER: Yes?

DR. YU: I'm sorry to interrupt you. Do you see the left-right arrows at the bottom left of your screen on your slides?

DR. BAUER: I did. Yes. I was pressing those and nothing seemed to be happening.

DR. YU: Okay. If it's not working for you, then I can move them for you as well.

DR. BAUER: Yes. I will try next time, but if it doesn't work, I'll ask you to do it. Thanks.

DR. YU: Sure. Please proceed.

DR. BAUER: So what are quality attributes? A quality attribute is a molecular or other characteristic of the product that is selected for

its ability to help indicate the quality of the product, and we also identify critical quality attributes, or CQAs, which consist of a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. Collectively, quality attributes define the safety, purity, potency, identity, and stability of the product.

In contrast to small molecule inhibitors, or monoclonal antibodies that target specific molecules, remestemcel-L is a cellular product. And as we heard earlier, cellular products and MSCs are inherently complex since cells express thousands of proteins, thousands of genes, and respond to their environment, both during manufacturing and in patients.

So how do we determine what are useful quality attributes and critical quality attributes for a cellular product? Quality attributes can be developed and established through understanding the characteristics and biological properties of the

product. The applicant has defined many characteristics of remestemcel-L as shown in this slide. This chart is from the applicant briefing document.

These characteristics overlap considerably with characteristics the scientific literature and stakeholder community have developed as consensus markers and properties that define mesenchymal stromal cells or MSCs.

These characteristics include morphology, the cell surface markers that are present and absent; the ability of MSCs to undergo trilineage differentiation; their ability to proliferate; their ability to evade host immunity and to be immunomodulatory, which is the key functional attribute of remestemcel-L that we focus on today; and finally their karyotype, which should remain stable through culture expansion as an indication that they will not likely be tumorigenic.

Among the characteristics the applicant chooses the characteristics they identify as being essential for product quality, this slide shows a

list of these critical quality attributes. Each product is tested for these CQAs, and each lot must meet acceptance criteria or values for these tests.

These tests of the CQAs are called release tests. This means that lots that pass the release test and meet predefined specifications are accepted, while lots that don't meet these values are rejected. The CQAs include tests for identity, purity, potency, activity, and safety.

I've highlighted the CQAs for identity, potency, and activity because they are the focus of our discussion this morning. The identity tests define what is in the product. CD105 and CD166 are markers that should be present on the cell surface. The products that pass the identity tests should then meet all the other release criteria to be considered an acceptable product lot.

The potency and activity tests measure the biological activity of the product and are attributed to the cells that meet the identity criteria. Tumor necrosis factor receptor 1, or TNFR1, is expressed by the product, and

interleukin-2 receptor alpha, or IL-2R alpha, is measured on T cells that have been activated, then co-cultured with the product. The applicant has based acceptance criteria as minimal threshold level specifications for both TNFR1 and IL-2R alpha.

The regulatory definitions of potency are found in the U.S. Code and Code of Federal Regulations, and a biological license application may be approved on the basis of a demonstration that the biological product that is subject of the application is safe, pure, and potent.

For biological products, potency refers to the specific ability or capacity of the product as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended to affect a given result. The laboratory tests for remestemcel-L are the ones I've described in the previous slide on product-release testing.

In recognition that cellular products are

extremely complex, FDA published a guidance document on potency tests for cell and gene therapy products. Ideally, the potency assay will represent the product's mechanism of action. However, for cellular products such as remestemcel-L, the mechanisms of action may be very complex.

To test for potency of many biological products, we rely on bioassays, including in vivo animal studies; in vitro organ tissue or cell culture systems; or any combination of these. But we can also rely on non-biological analytical assays, which are methods that measure immunochemical, molecular, or biochemical products of the product outside of a living system. We refer to these as surrogate measurements, and these surrogate measurements can be substantiated by correlation to a relevant product-specific biological activity.

Our potency guidance included the use of multiple potency assays also called the Matrix approach. Because of the product's biological complexity, one assay may not be sufficient to

measure potency. In this situation, we consider the use of multiple complementary assays that measure different product attributes associated with quality consistency and stability.

Such matrix assays can consist of combinations of biological assays, or biological and analytical, or analytical assays alone. They also can have a quantitative or qualitative readout, however, a qualitative assay should be accompanied by one or more quantitative assays. The applicant's potency and activity test for TNFR1 and IL-2R alpha constitute such a matrix approach to potency testing.

This slide reminds us of the proposed CQAs for remestemcel-L that are the focus of our discussion today. Identity is assessed by expression of CD105 and CD166, and the activity and potency are attributed to the MSCs in the lot. Tests for TNFR1 and IL-2R alpha comprise the matrix test for potencies. These tests include a quantitative measurement of TNFR1 expression and a qualitative measurement of activity based on the

inhibition of IL-2R alpha expression on the activated T cells that are co-cultured with the MSCs. For both assays, as I said earlier, acceptance criteria are based on a threshold minimum value.

The potency proposed by the applicant is based on a reasonable proposal for mechanism of action and employs a matrix approach that is in line with FDA's guidance. However, as we heard in Dr. Temple's presentation this morning, MSCs are complex and have multimodal biological activities. Also, Dr. Temple's talk and the scientific literature describe considerable heterogeneity of MSCs due to differences in biological activity and donor-related effects.

This raises the essence of the question we wish to discuss today, are the potency tests and other critical quality attributes for remestemcel-L sufficient to ensure the quality of the product from batch to batch? This question is important not only because there have been manufacturing changes during the course of the clinical trials,

but also because if the product is approved for marketing, future manufacturing may need to make remestemcel-L from new donors.

We begin to address this question and look at the evidence to support the proposed MoA of remestemcel-L. The applicant states that remestemcel-L has immunomodulatory properties and a multimodal mechanism of action that counteract inflammatory processes associated with steroid-refractory acute graft-versus-host disease.

Subjects receiving remestemcel-L in MSB-GVHD001 showed reduced markers of inflammation at day 180 post-treatment, but there was no placebo group control for comparison to bolster this proposed MoA.

Also, remestemcel-L has demonstrated in vitro immunomodulatory activity, but this MoA has not been demonstrated in vivo. While remestemcel's MoA might be related to immunomodulatory effects, the FDA believes that the MoA remains unknown.

As you've heard earlier this morning, the

applicant is proposing that there is a positive relationship between TNFR1 levels and overall survival, but here's why we do not agree with that conclusion. In the BLA, the applicant presented data to correlate the potency of lots with clinical outcomes, but some limitations of the data were not discussed in the applicant's briefing document.

In the BLA, the applicant presented data correlating clinical outcomes with mean TNFR1 levels from lots used to individual patients. There were no differences between TNFR1 levels in the product lots received by responders and non-responders in MSB-GVHD001, and now I will refer to this as Study 001. That is the clinical trial that provides the primary evidence of effectiveness.

There was no association between survival on day 100 and the mean TNFR1 levels in lots used in Study 001. Similarly, there was no association between day 28 overall response, the primary outcome in Study 001, and the mean TNFR1 levels in the lots used in Study 001.

Using pooled data from three clinical studies, 275, 280, and Study 001, the applicant found a statistically significant association between TNFR1 results and survival on day 100, however, these studies had different study populations and concomitant medications. This significance is not observed in Study 001 and most subjects received product from multiple remestemcel lots. Therefore, the interpretation of such a pooled analysis is challenging.

Also, there's no clear relationship between TNFR1 levels and the proposed MoA. Since this slide was made, the applicant provided additional data, and the bullet point here about their knockdown study needs some correction.

Although studies in early product development showed that knockdown of TNFR1 expression in the product reduced in vitro immunomodulatory activity, similar knockdown studies using the current version of the product found that inhibition of T cell activation was not affected by TNFR1 knockdown.

These new results suggest the in vitro modulatory activity of the product is largely independent of TNFR1 expression. Also, TNFR1 levels of the product lots without knockdown do not correlate with in vitro immunomodulatory activity. Although these assays are consistent with the hypothesized immunomodulatory mechanism of action, this has not been demonstrated in the clinical trials submitted to support licensure.

While remestemcel-L and other MSC-based investigational products have demonstrated apparent immunomodulatory effects in in vitro experiments, the ability of remestemcel-L to reduce inflammation, as measured by inflammatory biomarkers in humans receiving the product, has not been demonstrated.

Steroid-refractory acute GVHD is thought to be an immune-mediated disorder, but its etiology is complex, and many cell types are likely to be involved in its pathogenesis. Therefore, any efficacy remestemcel-L might have in treating this disease is not sufficient to demonstrate the

product's mechanism of action.

These analyses raise the concern expressed in this first bullet, the CQAs for remestemcel-L may not by themselves ensure adequate control of clinical effectiveness of individual lots of product. Also, although I won't present details, the applicant submitted a product comparability study under the IND and used the same critical quality attributes to assess changes to manufacturing, and we did not accept that this study demonstrated comparability.

FDA understands the complexity and challenges in understanding and developing CQAs for cell therapy products. However, it's important to continue efforts to develop critical quality attributes that demonstrate product quality and predict sustained quality for future manufacturing. Based on our experience as regulators and scientists, we often discuss with stakeholders the importance of characterization studies and how they can support identification and development of release tests for CQAs that are predictive of

effectiveness.

Such characterization studies may be sophisticated, powerful, slow, finicky, labor-intensive, often expensive, comprehensive, and time consuming, and we realize that these characterization studies may necessitate an iterative process in order to reach the goal of developing lot release tests that are predictive.

In the end, what we want is to assist stakeholders to develop release tests that are robust, rapid, GMP friendly, so they're easy to validate and operator independent, and that are economical and focused.

But to help achieve this goal, FDA also supports research on strategies to find CQAs that correlate with in vitro and in vivo assays on safety and effectiveness. This slide gives an overview of FDA's MSC consortium. We manufactured large batches of bone marrow derived MSCs from a variety of commercially available sources and harvested them at passages 3, 5, and 7, then employed a variety of analytical methods to

characterize these MSC batches.

One overarching goal of this research is to correlate product characteristics with bioassay outcomes. Such product characteristics could then potentially be used as predictive CQAs for lot-release testing. At the same, FDA's goal is not to develop tests that must be done by developers of MSCs or any other products, but instead to illustrate strategies that may be useful to find CQAs that are predictive of effectiveness.

One of the key strategies we would use was to develop functional quantitative bioassays for MSCs based on the biological functions shown here, the trilineage differentiation and immunomodulation. So we developed ways to measure the amount of these activities quantitatively. We also developed a method for the immunomodulatory activity as well as the trilineage differentiation.

These methods are sufficiently sensitive and reproducible so that we can detect differences in activity of MSCs from different batches and from different lengths of time in tissue culture or

passaging. These are all specific outcomes based on the way we manufactured the cells that we used so are not necessarily true for all MSCs grown in all conditions. But our techniques were similar to those used by stakeholders who employ MSCs, including the applicant.

This summarizes the method to determine how many cells can undergo adipogenesis after stimulation. In the microscope image on the left, the blue color shows nuclei of the cells and the green indicates the presence of lipid in the cells. These were fluorescent dyes, so we could automate cell counting and determine the percentage of cells that differentiated.

On the right, the bar graph shows that there are large differences in the fraction of cells that turn into adipocytes from different MSC batches, ranging from 0.5 to 14 percent. The purple, orange, and green bars were measurements taken at passages 3, 5, and 7. This data shows that the ability of MSCs to differentiate into adipocytes is different in MSCs from different donors and that

this ability decreases with cell passage.

The results suggest that MSC batches have different numbers of adipocyte precursors. With this degree of difference between batches, one might be able to identify the subpopulation of cells that respond and to find a corresponding molecular signature that correlates with this biological function.

This slide describes a quantitative assay we develop for immunosuppressive activity of MSCs. The assay is based on the co-culture of MSCs with activated T cells. A constant number of activated T cells are co-cultured with increasing doses of MSCs, then 16 different markers associated with T cell activation were measured by flow cytometry and principal component analysis is conducted on this multi-parameter data set.

In principal component analysis, one takes high dimensional data and finds a mathematical description of the variability along the different dimensional axes. If you envision all of the data as a cloud where individual cells fall at different

points within this multidimensional data, principal component 1 describes the line between the farthest separated data points.

So in the graph on the left, we plotted principal component 1 versus the number of MSCs added to the activated T cells, and you can see that the activity of T cells decreases as you increase the number of MSCs. So we calculated the area under this curve from this plot, and we use this as a single number to represent immunosuppression of MSCs.

On the left, you see analysis for one MSC line that was done at an early passage, and the area under the curve, or AUC, is shown in blue, and just to its right, you see the analysis done on MSCs from a late passage, and the area under the curve is shown in red. So in this type of analysis, less area under the curve indicates more immunosuppression. This tells us that the immunosuppressive activity of this MSC line decreased with passage.

On the right, you see a bar graph that shows

the area under the curve value for 6 different MSC lines measured as early and late passage. The take-home message is that MSC immunosuppressive activity can be quantified and that there are differences between MSCs from different donors, and the immunosuppressive activity decreases as the number of passages increases.

We also found that stimulation with interferon gamma could induce greater amounts of immunosuppressive activity and change the morphology of MSCs, so we followed the morphology changes of using up to 96 parameters of cell and nuclear morphology, and then used a machine learning analysis of this high dimensional data to identify subpopulations of cells based on their morphological footprint.

The figure on the upper left shows this multidimensional, the data reduced to two dimensions. We could count the number of cells that fall into the different regions of this display as shown in the middle figure where we were able to gate on cells in these regions. The images

around this show the shapes of cells in three of these morphological subpopulations, including those with the most immunosuppressive cells on the left and images of cells that did not correlate with immunosuppressive activity shown on the right.

In the end, we could correlate the amount of immunosuppressive activity with the number of cells in specific morphological subpopulations present in different MSC batches.

I want to emphasize that we do not expect stakeholders or an applicant to use these specific assays I've described, but I do want to emphasize that there can be approaches and strategies that might be useful in facing the challenge of identifying cell therapy critical quality attributes that correlate with in vitro bioassay outcomes.

This slide shows that the significant functional heterogeneity I've just shown you that was associated with different donors and different passages is not revealed using community consensus cell surface markers for MSC identity. Our

analysis included CD105 and CD166, the two markers used for identity by the applicant.

Shown in these 6 panels that show analysis for six different MSC markers, all of our cell lines at all passages express all of these markers at high levels, and this did not correlate with or reveal any of the functional differences I've shown you in the last several slides or other quantitative assays I did not have time to talk about.

To summarize, the consensus MSC surface markers we used do not identify the significant heterogeneity and biological activity of different MSCs from different donors or cells that were cultured for different numbers of passages.

This slide summarizes the challenging issue for today's discussion. Our research and the research of others touched on in Dr. Temple's talk suggest that the common MSC identity markers and those of the applicant do not capture the substantial functional heterogeneity that has been observed in MSC batches. This raises the concern

that potency tests for remestemcel-L may not detect functionally important differences between batches, or what the applicant calls drug product lots, since they do not correlate with clinical effectiveness.

Our purpose is to facilitate the committee discussion of the applicant's proposed potency assays and to provide some potential alternative approaches to assess quality attributes that may correlate with clinical effectiveness.

We will talk about these questions later. I'll just read these quickly. These are the points for discussion by the advisory committee later. The first is, the product quality attributes measured for remestemcel-L are intended to ensure that key qualities of the drug product are maintained consistently from lot to lot. Please discuss the adequacy of the potency assay established by the applicant for remestemcel-L.

The second is, in addition to discussing potency, please propose and discuss other possible product quality attributes or characteristics that

could be controlled to better assure consistent quality of remestemcel-L with regard to safety or effectiveness of the product.

With that, I'll conclude my presentation and turn back over to the chair. Thank you.

Clarifying Questions to Presenters

DR. HOFFMAN: Thank you very much.

We will now take clarifying questions for the presenters for about 15 minutes, and then we'll probably resume afterwards. Please use your raised-hand icons to indicate that you have a question.

Please remember to put your hand down after you have asked your question, and please remember to state your name for the record before you speak. Please direct your question to a specific presenter if you can. It would be helpful to acknowledge the end of your question with a thank you and end of any follow-up question with "That is all for my questions," so we can move on to the next panel member.

Dr. Morrison?

DR. MORRISON: Yes. Hi. A couple of questions for Mesoblast first. On slides 27 and 28, you suggested that the improved clinical results across all clinical trials correlated with increased TNF receptor expression and IL-2 receptor inhibition, but you had also altered the trial design based on experience to focus on patients more likely to respond in the later trial.

So given those changes in the trials were intended to increase response rates, do you think that the correlation with increased TNF receptor expression can really be interpreted to demonstrate improved product quality based on that attribute, rather than just a change in patient selection?

MS. STORTON: Thank you. It's Geraldine Storton here.

Dr. Itescu, I'll ask you to address this question.

DR. ITESCU: Sure. Thank you very much.

We've done various analyses, but first of all, looking right across the three trials, we've done a regression analysis that took into account

severity of the patients, age of the patients, other attributes, and certainly TNFR1 levels and IL-2 receptor expression level.

If we could go please to MA-23 slide? In a multivariable regression analysis looking at day 180 survival, the only variable that was significantly associated was in fact TNFR1, and neither disease severity nor age of the patients were significant when looking at the analysis by single product lot. That type of an analysis says that irrespective of selecting the type of patient, TNFR1 is the driver of survival outcomes.

Now, in addition to that, we also showed data within one single trial, 275, where all the children were of the same severity. They were all patients who had received multiple biologic agents and had failed where remestemcel was used to salvage therapy.

Here we see that when we looked at a single product lot, as we showed in the earlier slide, patients who received the optimized process with a significantly higher level of TNFR1, approximately

50 percent higher, had a substantial improvement in survival through day 100 in those same children, same severity of disease, and the only difference being the receipt of the older product.

DR. MORRISON: When you did these analyses that showed the strongest correlation with TNF receptor 1, were you looking genome-wide with some dense data set like RNA seq or something like that or were you looking at a modest number of candidates based on your experience with the biology?

DR. ITESCU: The analyses were limited to the existing quality attributes that have been in place for about 10 years, which allows us to have a wide population-based relationship correlation. But there's no doubt that moving forward, it would be good to introduce additional genome-wide analyses as well, as we continue to optimize manufacturing.

DR. MORRISON: If I could just ask a couple of final quick questions.

I assume that the MSCs in each lot that you

manufacture are not clonally derived.

DR. ITESCU: That's correct.

DR. MORRISON: And I don't think you showed us any data on how much variability you observed between donors, between lots of the same donor, or between passages. Can you comment on that?

MS. STORTON: Dr. Itescu, would you like me to take that question?

DR. ITESCU: Certainly.

MS. STORTON: We obviously have strict qualification of our donors, and what we do see in regard to donor variability is attributes that have more of an impact on yield that are impacted by that donor-to-donor variability rather than quality.

In addition to that, we have quality attributes fit and tested at the donor cell bank stage, which gives us an early indication of whether there are substantial differences between donors, and we have an acceptance criteria at that point so that we can determine appropriate donor cell banks that should move forward to

manufacturing of the drug product.

DR. MORRISON: One last question. You describe these cells as being hypoimmunogenic. In the briefing materials that you supplied in advance, the reference that was cited in support of that only did in vitro assays. We know these cells are cleared once they're transplanted in vivo. So what's the evidence that they're actually hypoimmunogenic in vivo?

MS. STORTON: Dr. Itescu, would you like to take that question?

DR. ITESCU: Sure. Thank you.

We've performed extensive in vivo studies across different trials from indications where we've followed anti-HLA antibodies, for example. We see in some cases a low level of anti-class 1 antibodies that are transient, not associated with any kind of clinical sequelae, and generally within 12 months are no longer observed, of the order of less than 10 percent of patients develop such antibodies. We do see anti-class 2 antibodies. And more importantly, in those situations where

we've measured T-cell responses against donor cells, we do not see donor T-cell responses.

So given that these cells express class 1 HLA but not class 2, this suggests that the class 1 HLA antigens can induce, in some patients, alloantibody response and does not appear to have any clinical sequelae and do not appear to be T-cell or antibody responses against class 2 antigens.

DR. MORRISON: Thank you.

DR. HOFFMAN: Dr. Robey?

DR. ROBEY: Yes. This is Pam Robey. I have two questions.

When you measured the TNF receptor 1, you're measuring it in the whole population. You add this as the whole population, but it could be that there are cells that are very actively producing that receptor and other cells that are not.

Do you have a sense of how homogeneous TNFR1 expression is across your donor population?

MS. STORTON: Dr. Itescu, would you like to comment on that question?

DR. ITESCU: Look, that is an excellent question. It's a very complex question. I think you're suggesting that within a heterogeneous cultured population there may be different levels of expression from cell to cell to cell. That's certainly possible. We're not analyzing single cells here; we're analyzing population-based analyses.

It's certainly possible there are differences, but those differences are unlikely to be very large given that the population of cells that we have, they're all treated the same way and cultured the same way. At the end of the day, this is an overall cultured process targeting the entire population that's harvested.

MS. STORTON: And I will add to that. Geraldine Storton here. I'll just add that we have a specific dose per vial. So even though there may be differences between cells, what we're looking at is an amount per dose for the patient that has been dosed based on their body weight.

DR. ROBEY: Right. But the point is that

you could have one cell in a hundred making tons and the others not making very much, and that could impact upon the effectiveness. It's just some food for thought.

Another question that I have about your presentation is that even though you've mentioned colony-forming efficiency, you don't really have that as one of your critical quality attributes, and it would seem to me that that would be a very important measure of the viability and healthfulness of the cell cultures.

Do you actually do colony-forming efficiency on a routine basis?

MS. STORTON: I will start and see if Dr. Itescu wants to add anything. It is a measure that we use as part of our extended characterization panel at both the donor cell bank stage.

Dr. Itescu, would you like to add?

DR. ITESCU: Yes. We certainly do routinely measure see CFU-Fs, absolutely, and it's part of the criteria we use to select the appropriate

donors that go through the donor cell bank stage. And then of course we verify the CFU-F levels at the donor cell bank stage, as well as the final lot release; a very important attribute, no doubt. We agree.

DR. ROBEY: Okay. Thank you very much. I have no further questions.

DR. HOFFMAN: Okay. Dr. Cheng?

DR. CHENG: Good morning. Jon Cheng, industry rep. I appreciate the presentation. I have two clarifying questions. The first is, how many distinct drug product lots were used in Protocol 01, GVHD001, and how many donors does that represent? I have a similar question for 280, your randomized study. How many drug product lots were used and how many donors?

Then my second question is Dr. Bauer stated that the TNFR1 levels did not correlate with response or clinical outcome in GVHD 1, and I was interested in the sponsor's perspective as to why that may be.

MS. STORTON: I will address the first

question. The number of lots in GVHD001, I don't have the specific number, but I can bring that back after the break. But those lots were made with three distinct donors. That was for the pivotal study, GVHD001. The second question in regard to the correlation of TNFR1, I will turn to Dr. Itescu to address that question.

DR. ITESCU: Sure. I think this is a very, very important question. When one looks and tries to provide correlations between biomarkers and clinical outcomes, it's essential that you have sufficient patients, large numbers of population-based patients, to be able to make those correlations.

We have a very, very large database. We're fortunate, in fact, that we've treated more than 400 patients with remestemcel across multiple trials over 10 years. We can only demonstrate and we can only identify correlations when you have enough patients. By the fact that changes in manufacturing and optimization has occurred, we're able to look across those timelines and learn.

In fact, when you do that, you see that the product that was used in some of the failed trials 10 years ago, trial 280, 265, et cetera, you used a product that had about a 50 percent lower level of TNFR1.

When you have that degree of variability, you can actually see correlations in patients. The optimized product, which has now got a 50 percent higher level of TNFR1, was used entirely in the pivotal trial phase 3 and in a good portion of patients in the Expanded Access Protocol 275.

When you have such a high, high level of consistency, as we saw in 001, the phase 3 trial, and I showed you that between lot variability it was very low, when you have that degree of consistency and reproducibility in a level that is 50 percent higher than a previously used product, it is of course very difficult to show within only 50 patients a relationship between minor differences in TNFR1 and survival outcomes. Those are very, very important clinical outcomes and requires hundreds, if not thousands, of patients to

relate.

The fact that we've been able to demonstrate that in 400 patients is precisely because we've improved the potency by 50 percent, and those variabilities between old product and new product allow us to make those conclusions. But within 50 patients only, or 54 patients only, in the pivotal phase 3 trial where the product has been significantly improved and the variability is very small, it is not possible to show a relationship with survival. Nonetheless, we have very significant outcomes in terms of biomarkers and immunomodulation that appears to be very important and related to the outcomes in these patients.

DR. CHENG: Thank you.

DR. HOFFMAN: This is Dr. Hoffman. I have a question. I apologize if this was covered at the very beginning for the sponsor. How many donors go into producing one lot, or does one lot represent multiple donors, or does one donor's cells, which are then expanded, cover multiple lots? I'm just not sure. Are there thousands of donors or there

are a few? It's a very basic question, I realize.

MS. STORTON: Geraldine Storton here. I'll address that question. One bone marrow aspirate generates a number of donor cell bank vials. So one donor cell bank, of which there are numerous vials, and one vial is used for every manufacturing lot, and then you obviously result in a certain number from that manufacturing lot. So one bone marrow aspirate can provide enough finished product to treat over 400 patients.

DR. HOFFMAN: So within a given lot, a given lot only represents one donor.

MS. STORTON: Yes, always you can trace back to one donor from a given lot.

DR. HOFFMAN: Okay. Thank you.

Dr. Singec?1

DR. SINGEC: Yes. Hi. This is Ilyas Singec from NIH. I have a question regarding the use of animal serum, such as fetal bovine serum. Also, based on the provided materials, it seems that animal serum is required to be used.

Could you please clarify at what stage

throughout manufacturing or I saw that it's being used also in some potency assays?

MS. STORTON: Yes. I will address that question for you. Fetal bovine serum is used in the culture media both for the expansion of the cells for the donor cell bank and the expansion passages for the drug product. So it is a component of our growth medium.

Does that answer your question? 1

DR. SINGEC: Yes, it does. How do you control lot-to-lot variability across animal serum batches?

MS. STORTON: Sure. We actually have quite strict acceptance criteria in relation to the fetal bovine serum, and it needs to meet, obviously, certain tests. We ensure that it's safe and it's been irradiated, et cetera. We look at things like the proliferative capacity of the fetal bovine serum as well to try and ensure as best consistency as possible each time.

DR. SINGEC: Okay. Thank you.

DR. HOFFMAN: How about if we take the last

question before the break. Dr. Garcia?

DR. GARCIA: Thank you, Dr. Hoffman.

Jorge Garcia. I have a clarifying question for Dr. Bauer from the FDA. I do understand the heterogeneity and the concerns perhaps raised by the group as to, again, the difference in manufacturing each lot or product at the time and the concerns that there may be variation between the products used in clinical trials and protocols against the commercial product.

My question relates simply to the release testing. The applicant used phenotype, the CD166 and the 105. For potency they used a TNF receptor 1 expression and for activity they used the inhibition of IL-2 receptor. Are these acceptable for the FDA as release testing or is the FDA, the agency, asking for additional testing to be done prior to release of the product?

DR. BAUER: Yes. We don't describe or prescribe what tests the manufacturers have to rely on for these assays, so we don't really say we can't use these or we need to use something in

addition. We're asking the question in a different way. Are those assays the way the applicant has used them sufficient to say each batch is going to be the same?

Our concern is as we stated the issue. We don't see a correlation of individual patient outcomes with individual lots. Are they doing this assay or are they using a quality attribute that will associate or predict that quality attribute with a clinical outcome? We're not saying that those aren't useful or informative assays as a general class of assay, but in the particular case here.

Does that answer your question?

DR. GARCIA: Yes. Thank you.

Perhaps if I can expand my question for the applicant. It wasn't really clear to me throughout the morning that I truly understand -- I understand GVHD is a complex and multifactorial, and perhaps multifaceted, biological issue, but does the company really know what is the true MoA of this agent? MSCs

MS. STORTON: I'm going to ask Dr. Itescu to respond to that question in regards to mechanism of action.

DR. ITESCU: Certainly.

So as we tried to show you, remestemcel has surface receptors that are able to sense inciting inflammatory cytokines. The TNF receptor is a very important one. Interferon gamma receptor is another one. You put the cells into an inflammatory micro environment where you have high levels of TNF alpha, and through these receptors, the cell senses the inflammation and is activated. The activation pathway goes through TNFR1, through NF-kappaB, which is a master regulator of multiple factors, and it's well established that this cell then secretes the anti-inflammatory mediators that are downstream, resulting from NF-kappaB activation.

Those factors, and they include CCL2 and M-CSF, act on the proinflammatory macrophage, which has made TNF in the first place and is the M1 macrophage, and switches it off, and turns it into

an M2 macrophage, which is an immunomodulatory long-lived cell that produces interleukin 10. Interleukin 10 is a very important cytokine that immunomodulates an inflammatory micro environment as occurs in GVHD.

In addition, through parallel pathways, including interferon gamma, the remestemcel cell also secretes factors that switch off T-cell activation. So a downstream measurement of multiple factors, some through regulators through TNFR1 and some through additional pathways, is the measurement of a matrix that measures the ability of a cell to inhibit T-cell activation. T cell activation is important also in disease pathogenesis.

So we're dealing with a living cell that is able to sense inflammation, respond to inflammation, and through multiple secretory factors switch off the inflammatory process that is at the core of damaging tissue in GVHD, and that causes death. So yes, we do understand the mechanism of action very well, and it's a question

of how best to measure those assays in vitro to ensure that we've got a safe and effective and reproducible product for use in this very bad disease.

DR. GARCIA: Thank you. No further questions.

DR. HOFFMAN: Okay. Thank you.

We will now take a 10-minute break. Panel members, please remember that there --

MS. STORTON: Sorry. Can I just follow up on the question from Dr. Cheng? Because I have the details here.

In the 001 study, there were 40 batches of product used, manufactured from three separate donors, and in Protocol 280, there were 227 batches made from 9 separate donors. Thank you.

DR. HOFFMAN: Okay. Thank you.

DR. CHENG: Thank you.

DR. HOFFMAN: We'll now take a 10-minute break. Panel members, please remember that there should be no chatting or discussion of the meeting topic with anyone during the break. We'll resume

at 10:30 a.m. with the open public hearing. Thank you.

(Whereupon, at 10:31 a.m., a recess was taken.)

Open Public Hearing

DR. HOFFMAN: Both the Food and Drug Administration and the public believe in a transparent process for information gathering and decision making. To ensure such transparency at the open public hearing session of the advisory committee meeting, FDA believes that it is important to understand the context of an individual's presentation.

For this reason, FDA encourages you, the open public hearing speaker, at the beginning of your written or oral statement to advise the committee of any financial relationship that you may have with the sponsor, its product, and if known, its direct competitors. For example, this financial information may include the sponsor's payment of your travel, lodging, or other expenses in connection with your participation in this

meeting.

Likewise, FDA encourages you at the beginning of your statement to advise the committee if you do not have any such financial relationships. If you choose not to address this issue of financial relationships at the beginning of your statement, it will not preclude you from speaking.

The FDA and this committee place great importance in the open public hearing process. The insights and comments provided can help the agency and this committee in their consideration of the issues before them.

That said, in many instances and for many topics, there will be a variety of opinions. One of our goals today is for this open public hearing to be conducted in a fair and open way, where every participant is listened to carefully and treated with dignity, courtesy, and respect. Therefore, please speak only when recognized by the chairperson. Thank you for your cooperation.

Speaker number 1, your audio is connected

now. Will speaker number 1 begin and introduce yourself? Please state your name and any organization you are representing for the record.

DR. CAPLAN: This is Arnold Caplan. I'm a professor at Case Western Reserve University, and I have no financial relationship with Mesoblast, and I'd like to thank the FDA for this opportunity to make a comment.

In the late 1980s, I named a special class of cells isolated into cell culture from bone marrow. I called them mesenchymal stem cells, MSCs. Importantly, we could show that these cells in culture could be made to form bone or cartilage or fat, as Steve Bauer actually has already commented. These cells, therefore in culture, appear to be multipotent.

In the context of today's proceedings, in the mid-1990s, we were the first to infuse autologous cell culture expanded MSCs into cancer patients who were undergoing bone marrow transplantation procedures. Because of these cell culture observations of multipotency, some people

have incorrectly referred to MSCs as stem cells, and I have begged people to stop using the stem cell nomenclature because we believe that this is a capacity that these cells do not exhibit naturally in the body, nor when they're introduced after cell culture expansion.

We now know that all MSCs come from in situ to habitats, which are around and just outside blood vessels, and therefore are referred to as perivascular cells. When such perivascular cells are detached from injured or inflamed or broken blood vessels, they then form activated MSCs.

These MSCs function, first, to sense their unique and distinctive surrounding micro environment. Second, they respond to these micro environmental signals by secreting a spectrum of emergency molecules that are immunomodulatory intropic. These molecules are naturally secreted to tune down the naturally over-aggressive immune system so it does not interrogate or destroy the injured tissue. And in the case of GVHD, it can modify the over-aggressive immune response current

directed against the hosts.

Third, the secretory MSCs, which I've renamed in 2010, medicinal signaling cells, MSCs, can arise and be isolated from any disrupted vascularized tissue in the body. Their natural function is to initiate site-specific cell progeneration, meaning that they activate the innate regenerative capabilities of the injured tissue in which they're embedded.

Last, by adding exogenously culture-expanded MSCs to diseased or injured patients either in the autologous or allogeneic study, we can medicinally supplement the normal and inadequate titers of host MSCs, indeed, endogenously added MSCs, home to broken or inflamed blood vessels where they set up shop and respond to that specific local environment.

This response is the basis for all MSC cell-based therapy. I believe that these exogenously added cells function as they normally do by providing medicinal molecules and signals at sites of injury.

The website clinicaltrials.gov has over 1100 different clinical trials using MSCs. These MSCs, when added back to patients, set up a local curtain of molecules that are secreted by the MSCs, again, either autologously or allogeneically, and this curtain provides a barrier so that the host immune system doesn't immediately see the donor MSCs. Thus, MSCs are immunoevasive initially, and they're not immunoprivileged.

I urge you, the committee, to allow the use of MSCs for therapeutic purposes, and I urge you to judge them as an aspect of cell-based therapy, not as a single purified drug. These cells are alive. They do what they do when they're isolated and expanded from marrow or other tissues and are implanted back into diseased individuals.

These perivascular medicinal cells are unique therapeutic entities because they adjust their responses to the micro environment in which they have land. Probably on the order of 50,000 people have been infused with MSCs, and they have been documented in various clinical trials to be

safe. I urge you to positively consider the evidence-based information provided by Mesoblast this afternoon of MSCs' medicinal capabilities for pediatric patients with graft-versus-host disease. If a BLA is granted, it is a watershed moment for MSC technology and for the whole concept of cell-based therapy. I look forward to your deliberations. Thank you.

DR. HOFFMAN: Thank you.

Speaker number 2, your audio is connected now. Will speaker number 2 begin and introduce yourself? Please state your name and any organization you're representing for the record.

MR. KOOSHESH: Hello. My name is Kameron Kooshesh. I do not have any financial relationship with Mesoblast or any of its competitors. Thank you for allowing me to speak today. It means the world to me.

I am a survivor of acute lymphoblastic leukemia and graft-versus-host disease, and I received remestemcel for the treatment of steroid-refractory graft-versus-host. At age 9, I

was diagnosed with ALL and then underwent two and a half years of chemotherapy. For most of this time, I wasn't able to go to school and to see my friends, and to live a normal life. However, on my long awaited last day of chemotherapy and final evaluation with the bone marrow aspiration, I was told that I had relapsed and that I would have to start all over again.

Statistically, the survivorship odds are considerably worse after relapse. My best chance, and maybe only chance, for a cure was a bone marrow transplantation. I was told that I was very fortunate to have a perfect 10 out of 10 bone marrow transplant match. By that point, I had been in and out of hospitals for so long, it was starting to feel as if I would always have to live with cancer.

My bone marrow transplant went well and I was able to leave the hospital after a 30-day stay. Finally, after so long, I felt like I was on the road to recovery. My doctors had told me that graft-versus-host disease could be a serious

complication, and the road to recovery may yet still be lengthy, but I thought, what could possibly be worse than what I just went through? That was graft-versus-host.

Graft-versus-host disease started with what I thought was one of the worst sunburns of my life. I was awake all night itching, and what followed was unbearable abdominal pain and diarrhea. I was spending a lot of time in the bathroom with uncontrollable pain. I was in so much pain that I could not walk around our neighborhood block. It caused me to be in and out of the hospital constantly, as I was not well controlled on steroids.

This was the gastrointestinal graft-versus-host disease that I had heard so much about. Days turned into weeks, weeks became months, and I still teetered on the edge of an upset every day. I ate a controlled diet approved with my doctors to ensure that there was nothing that could upset my graft-versus-host disease. Worst of all for me, this meant no ice cream.

This is when I was told that graft-versus-host disease was starting to damage my liver in spite of the steroids that I was taking, and that meant that the progression of my graft-versus-host disease was starting to take a very serious turn. I'd heard the whispers about a few of my friends that had received bone marrow transplants and also were developing liver graft-versus-host, and I knew that they did not make it. After all I'd been through, I thought to myself was it really possible that it would come to this?

My doctor said that there was a possible solution, a drug called remestemcel. Fortunately, I was able to receive a course of the drug for compassionate use, and within a matter of weeks, my rashes receded, my abdominal pain abated, and my liver function returned to normal. After a while, I was able to go kayaking with my dad and hiking with my mom, and I was even able to have ice cream.

To this day, I have not suffered further complications of graft-versus-host disease or any side effects of remestemcel. Remestemcel provided

me the opportunity to be a kid again and a second chance at life that I would likely not have had otherwise. Looking back years later from the other side, I believe that my experience with remestemcel and the inflection points that it created in my life encouraged me to pursue medicine as a career.

As a current Harvard medical student, I have been inspired by the difference remestemcel made in my life to study cellular therapies as the new revolution in personalized medicine. It continues to give me hope. Thank you, again, to the committee for letting me speak today. This means the world to me.

DR. HOFFMAN: Thank you.

Speaker number 3, your audio is connected now. Will speaker number 3 begin and introduce yourself? Please state your name and any organization you're representing for the record.

DR. GALIPEAU: This is Jacques Galipeau. I'm going to ask if you could just move my slide forward because I don't have any control -- sorry. I take that back. I'm a professor of medicine at

University of Wisconsin-Madison, where I serve as the associate dean for therapeutics development at the School of Medicine in Public Health, and I'm also the director of the Program for Advanced Therapy at the school. I do not have any conflict of interest, professional or financial, with Mesoblast or any of its competitors, and the views here expressed are all my own.

I'm internationally a recognized expert in the MSC space having sponsored three phase 1 clinical trials, as well as published a number of papers. I have recently published an invited editorial commenting on the clinical trials conducted by Mesoblast that are being discussed today as noted on this slide.

The approach we use to think about functionality and potency of MSCs is a comparative biology approach. Secreted factors from MSCs, captured in the box with the red factors here, identify their competencies that are shared between mouse and human MSCs in vitro. These have been shown to block T cells and monocytes in vitro, but

importantly these selected factors shown here have been further shown in mice to be central in vivo by using gene-targeted knockout donor MSCs.

I'm highlighting the monocytes and M2 macrophages because of their central importance in the host response, because pharmacological depletion of endogenous macrophages, clodronate [indiscernible], abolishes MSC functionality in vivo. Though these factors are necessary, none of them are sufficient. For example, IL-6 knockout MSCs, which retain CCL2 competency, also lose functionality, which speak to the aggregate or matrix function of MSCs.

I'd like to draw your eye to the right. Not only are host macrophages important, but also host IL-10 competency, in particular, as derived from host macrophages are important. Subject matter that's of importance here is the in vivo fate of IV-administered MSCs.

Labeling of human MSCs injected IV in mice -- you'll see the blue boxes on the left -- will aggregate in the lung and will

disappear promptly within 24 to 72 hours. Dead MSCs also aggregate the lung but will redistribute to liver because they get preferentially phagocytosed by MSCs, which leads to their IL-10 polarization.

These pieces of information inform two functionalities. On your left are the cell function, fitness-dependent functionality as you've heard of today, but on the right is also a cell, autonomous functionality, where MSCs can get phagocytosed, which triggers an efferocytotic response, where macrophages become IL-10 competent in vivo.

The single most important quality attribute one can think of for MSCs would be viability. Dead MSCs do not antagonize live MSCs, but they're also significantly less potent when they significantly are void of potency. So MSC viability at infusion also was found in clinical trials done by academic centers to correlate with outcomes.

Mesoblast has done a series of pragmatic studies in pediatric steroid-resistant graph versus

host, and they found imperatively that children, especially with severe disease, respond better. And I'd like to emphasize to this committee that this reflects results erupting also by European academic collaborative groups that had similar outcomes in pediatric GVHD.

I'd like to point out that placebo controlled is likely unfeasible. There was a large study in Europe, the RETHRIM study, that had to be stopped because they could not enroll patients to the placebo arm due to subject-parent resistance.

I would conclude the main quality attributes in the disease are viability, first and foremost, and you would need at least one, and preferably many, functional attributes that are informed by comparative biology to determine a likely MoA in human subjects. Post-derived predictive biomarkers of response are something that need to be looked at.

In conclusion, I'm bringing these closing remarks which restate some of my points, but I would like to state that, in my opinion, the

benefit-risk ratio of MSCs favors a clinical utility for pediatrics steroid-resistant graft-versus-host. Thank you very much for your attention.

DR. HOFFMAN: Okay. Thank you.

The morning open public hearing portion of this meeting has now concluded and we will no longer take comments from the audience. The committee will now turn its attention to address the task at hand, the careful consideration of the data before the committee, as well as the public comments.

We'll now proceed with the questions to the committee and panel discussions. I'd like to remind public observers that while this meeting is open for public observation, public attendees may not participate except at the specific request of the panel. I'll ask a member of the FDA to read question number 1.

Questions to the Committee and Discussion

DR. BAUER: Steve Bauer here. This is question number 1 for discussion. Product quality

attributes measured for remestemcel-L are intended to ensure that key qualities of the drug product are maintained consistently from lot to lot. Please discuss the adequacy of the potency assay established by the applicant for remestemcel-L. Thank you.

DR. HOFFMAN: First, if there are no questions or comments concerning the wording of the question, we'll now open the question to discussion.

(No response.)

DR. HOFFMAN: Please indicate your interest in speaking by raising your hand.

(No response.)

DR. HOFFMAN: Do any of our guest members of the committee have any comments about this question? Dr. Robey?

DR. ROBEY: In going back to Dr. Bauer's slide about the no clear relationship between TNFR1 levels and proposed mechanism of action, do you have any thoughts about how you can address this issue or if there are additional factors that could

be used to bolster the potential mechanism of action in vivo?

DR. BAUER: Is that a question for me, Steve Bauer?

DR. HOFFMAN: Yes, I believe so.

DR. ROBEY: No. I'm sorry. That's a question for Mesoblast. In other words, I think this is a major question that Dr. Bauer has raised about the fact that there is no correlation. It's an in vitro assay, and we're concerned about what's happening in vivo. So what are your forward-looking thoughts about how you will address this issue or can you address this issue?

MS. STORTON: Yes. Geraldine Storton here.

Dr. Itescu, I'll get you to speak to that question, and if possible, we would like to put up some material to support the argument.

DR. ITESCU: Certainly. Thank you very much.

First of all, if we could have slide MA-2 up, please? Thank you.

Shown on this slide is the relationship

between TNFR1 on the X-axis and production of phosphorylated NF-kappaB on the left, M-CSF in middle, and CCL2 on the right by remestemcel lots that have had TNFR1 specifically knocked down with siRNA. You can see that in the level of TNFR1 that spans precisely the level expressed by clinical grade product. We can see a direct correlation between the production of each of these factors.

So all of them over time could be established as additional assays of measurement of potency, and what this demonstrates is the umbrella nature of the TNFR1 sensor that results in intracellular activation of the master regulator of these factors NF-kappaB and the downstream secretion of these factors. This is really important.

If we could have slide MA-5, please? MA-5, which we have shown previously, we are able to demonstrate that there is a clear correlation between a second potency assay, IL-2 receptor inhibition in vitro and the reduction over a 28-day period in activated CD4 T cells as defined by

expression of IL-2 receptor in HLA VF, a direct significant correlation. That therefore allows us to validate this as a potency assay.

If we could move on to slide MA-6, the next slide shows the in vivo biomarkers that are associated with outcomes. In the left two panels, you see the proportion of activated CD4 T cells or CD8 T cells significantly decline particularly in the first 28 days during the treatment with remestemcel, but they significantly decline over the 180-day period of follow-up. This is in now the pivotal phase 3 trial, demonstrating bioactivity in vivo in patients relating to the potency of the product.

In addition, on the two panels to the right, we measure soluble ST2 and the composite of the magic biomarkers for which incorporate ST2. These are validated biomarkers that reflect the severity of epithelial gut damage, and the higher the level, the greater the severity of the GVHD disease and the greater the likelihood of death.

What you see, again, in both of these panels

is a significant reduction within the first 28 days of therapy, but continued reduction over 180 days of follow-up in the cohort of patients in the phase 3 trial treated with remestemcel, a significant reduction in these biomarkers, which reflects healing of the gut and ultimately reduction in risk of mortality. Again, these are the sort of biomarkers that we will be following in the real world in patients who receive our therapy.

If we could go to slide MA-10, please? When we looked at our phase 3 trial patients, we evaluated outcomes based on severity score and, again, the NBS biomarker score developed by the MAGIC Consortium, the international consortium, which has demonstrated that a score of greater than 0.29 is a validated biomarker for severity and mortality in GVHD.

It turns out that 18 out of 29 of our patients, approximately two-thirds, who were measured for this were at a baseline level greater than 0.29, which is associated with higher mortality. When you compare three separate disease

cohorts on the left of steroid-refractory disease patients, published in 2018, who have an MBS score above 0.29, the day 28th overall responses of the order of 18 to 32 percent. In contrast, in our phase 3 trial, patients with this degree of severity, MBS scored on 0.29, had a 61 percent day 28 response, demonstrating that we can predict based on biomarker severity outcomes.

Next slide, please. That correlates with a significant improvement in survival. These same -- MA-11, please -- three cohorts, published cohorts, demonstrate on the left that patients with MBS biomarker score greater than 0.29, the validated marker of severity, results in a survival of between 20 to 40 percent at 6 months, extremely poor survival.

In contrast, if you look at the Kaplan-Meier on the right, in our phase 3 Trial 001, what you see is that the patients with highest risk or severity, which is two-thirds of our patients, have a survival level that's approximately 60 percent at 6 months, and brings them in line with patients at

low risk for mortality, a very different outcome than what we've expected with best available standard of care.

These are the sort of outcomes that we will be continuing to monitor in the real world, correlating survival and responder rates with severity scores at baseline and with various biomarkers. Thank you.

DR. HOFFMAN: Dr. Robey, does that help with your question?

DR. ROBEY: Yes.

DR. HOFFMAN: Okay.

DR. ROBEY: Thank you. Sorry. I'm having a problem with my new mute here.

DR. HOFFMAN: Okay. No worries.

Can I get a sense from the members of our committee -- I mean, this is not a voting question here, of course -- whether we are comfortable with the potency assay that the applicant has proposed, the key part of this discussion question, without reviewing everything again? I realize it's complicated.

Dr. Morrison, do you want to comment?

DR. MORRISON: Yes. The points that Dr. Bauer made are well taken. The Mesoblast argument that the mechanism of action is likely complex is also well taken. I think they're right that this involves effects on multiple cell types and multiple cell cytokines. It's really tough because most of the data related to mechanism are based on experiments performed in culture. It's just a lot harder to do these experiments in vivo, but we do have much less data on what these cells are actually doing in vivo.

We've also had a couple of clinical trials that didn't meet prespecified endpoints and reason to believe that there's heterogeneity in the product. So with such a complex mechanism of action, I think there are real concerns about knowing how to measure potency and to predict activity.

DR. HOFFMAN: I think --

DR. MORRISON: If I --

DR. HOFFMAN: Please, go ahead.

DR. MORRISON: Sorry. May I just ask a question of Dr. Itescu?

Do you know how long the cells actually persist in vivo after they're injected, and what impact does that have on how you think about mechanism of action?

DR. ITESCU: Sure. Thank you very much.

What we do know is that the cells do not engraft. They're allogenic, not autologous, so they do not engraft. They do not persist long term. From animal studies, we certainly know that they last days to sometimes weeks, and then they're certainly gone. So they're short-lived. They're surely short-lived.

So therefore, how do we account for long-term durable effects? Well, it's precisely by their interaction with long-lived cells, importantly macrophages and regulatory T cells. What these cells do is they hand over and they educate long-lived tissue resident macrophages to become immunomodulatory and not to be proinflammatory, and that's why we talk about the

M1 to M2 macrophage polarization, for example.

How they do that precisely remains an area of further research, but they clearly are able to do that, and they're able to hand over an immunomodulatory effect through a variety of factors, including CCL2 that we've demonstrated and that has been shown by others, including TGF beta that are important in regulatory T-cell stimulation.

But ultimately, it's other cell types that are critical in inducing an immunotolerant state that takes over and are responsible for the durability of the effects. I think that's the way to think about this. Their mechanism by which they do that is multifactorial, but their ability to do that is triggered by their receptors, which sense the proinflammatory state of the M1 macrophage that drives a lot of this process. Their ability to sense TNF alpha production by the M1-producing macrophages is critical initiating the handover process.

DR. MORRISON: Thank you.

DR. HOFFMAN: Dr. Garcia, do you have a comment?

DR. GARCIA: Yes, Dr. Hoffman.

Jorge Garcia. Just perhaps for the committee members to think and perhaps discuss among ourselves, I think the data that we have may be perfect, at least in my view and how I see it. I'm not a hematologist and I don't do GVH, but I think the bigger question that I have is, it sounds like this is the best that we're going to be able to get with the data that has been presented.

I understand the concerns from the FDA and I understand the applicant position in context. I think the question for us as a group and perhaps for my committee colleagues would be do we feel that there is an ideal clinical trial or at least a basic or in vitro -- clearly, in vivo, as alluded to before by Dr. Morrison, it's going to be very challenging for us to prove that.

But the question is, is there such a thing as an ideal study, whether it's in vivo, in vitro, or what have you, that will actually overcome the

concerns that have been raised today? Because if there is such a study, then I think that we can discuss that, but if there's no viable study because of the complexity of the product -- and obviously with the heterogeneity, specifically with the manufacturing controls, whether it's the [indiscernible] or the process, then I think even if another company or the applicant does another trial or another study, I got a feeling that we're going to be in the same position where we are today.

So I'm just trying to actually see if you have any other thoughts as to how we can overcome the concerns that have been raised today.

DR. HOFFMAN: Well, I think that's actually, I think, a good summary of where we are, or some of us are, in our thinking. Maybe we should actually put up the second discussion question because these certainly are two related ones, if I could suggest that, because that's basically the second question.

Would somebody from the FDA read that, please?

DR. BAUER: Yes. Thanks, Dr. Hoffman.

Steve Bauer again. This is question 2 for discussion. In addition to discussion of potency, please propose and discuss other possible product quality attributes or characteristics that could be controlled to better assure consistent quality of remestemcel-L with regard to safety or effectiveness of the product. Thank you.

DR. HOFFMAN: If there's no discussion about the wording of the question, I think Dr. Garcia introduced this appropriately, and we welcome some discussion about it among are committee members here.

DR. GARCIA: Dr. Hoffman, Jorge again. If I may comment?

DR. HOFFMAN: Yes, please, please.

DR. GARCIA: In the real world, we would like to actually assure that if indeed this agent gets approved, how we're going to control the quality of the product, obviously, after it's commercialized. I don't know if the company can comment.

Is there any plan or any strategic plan for the company to actually do -- in addition to what has been described as the release test in the phenotype, the potency and activity, is there any other plan in place for the future that could actually minimize or at least relieve that concern as to the heterogeneity? Perhaps the product that was tested is not consistent with the product that you're going to release commercially.

MS. STORTON: Geraldine Storton here. I'll make a comment, and then I'll pass over to Dr. Itescu.

As we mentioned in our presentation, we have set out acceptance criteria at this point of our discussions with the FDA -- obviously, the BLA is still under review -- in order to ensure that the any commercial product will be reflective of the same level of attributes as the product that was used in the GVHD001 study. So that's the first step that we've taken.

I'll ask Dr. Itescu to talk to any future plans we may have for the product that may provide

opportunity to evaluate potentially some other attributes that may be helpful.

DR. ITESCU: Sure. Thank you.

We of course continue to refine and optimize and learn; that's without doubt. For example, we will evaluate our donors in a way that we now understand what a high-quality donor cell bank needs to look like and the attributes that we would be looking for from every donor product to be consistent and to be reproducible.

Already we have a much higher level of consistency and reproducibility across a number of quality attributes. That is the reason that this trial has been successful. The reason that this trial is successful is precisely because we've learned and optimized manufacturing.

The attributes that we've shown you today look at the two ends of the spectrum, both of which reflect matrix production of multiple factors of the cells, at one end, the ability to ensure that the cell is built with a machinery that is able to sense the micro environment well. TNF receptor is

one; interferon gamma receptor.

There are other receptors, IL-17 receptors, IL-1 receptors. They're all relevant to the ability of the cell to sense the inflammatory micro environment. We believe the TNFR1 is probably the most important because TNF is implicated so centrally in the diseases that we're targeting of inflammation, and GVHD is a major disease that is driven by TNF alpha. But ensuring the health of the final product is a major focus for us.

You've heard how important viability is. We ensure that this cell is very viable at the end, a high level of viability at the 95 percent level, and we will continue to learn from the science. Genomics, proteomics, and matrix approach to the health of the product and the reproducibility of the product, both at the donor cell bank level and at the final release stage, is critical. However, the two attributes that we have in place already have demonstrated, through over more than 400 patients worth of clinical data, how best to approach the relationship between manufactured cell

therapy and clinical outcomes.

I think this is going to take more patient exposures and ongoing relationship analyses between optimization of manufacturing and clinical outcomes, and that's what we're going to do.

DR. HOFFMAN: Dr. Robey, do you have a comment?

DR. ROBEY: Yes, going back to one of the comments that Sally Temple made this morning about taking retention vials and looking at the quality attributes of those vials in comparison to clinical response. Nowadays, RNA seq and even single cells, transcriptomics is getting so much cheaper.

Are you considering doing that? Are you going to be looking at responders' and non-responders' lost durability in certain factors, that kind of an approach; and also, as a certificate of analysis to have a transcriptomic profile or a transcriptome [indiscernible] profile that you can attach to that product and track how effective that product was?

MS. STORTON: Dr. Itescu, would you like to

respond?

DR. ITESCU: Yes. Thank you. I think characteristics of products are critical. Characteristics of the recipient are just as critical. To determine respond and non-responder outcomes is going to require, again, large data sets. We can look at things like baseline severity scores, baseline biomarkers.

I showed you earlier that the sort of responses we're getting with this product give us substantially improved outcomes in the most severe patients based on biomarker criteria. That would allow us, for example, to do further evaluation by stratifying the most severe patients against other therapeutics and be able to demonstrate these types of relationships.

In terms of genomic characteristics of the recipient, yes, they need to be evaluated in outpatient settings. And in terms of tracking the product itself by genomic analysis, I think those are the sort of studies that we'd want to do longitudinally over time.

DR. ROBEY: Thank you.

DR. HOFFMAN: Thank you. Dr. Morrison?

DR. MORRISON: Yes, a couple of questions for Dr. Itescu.

There's been an explosion in the last few years of basic science studies done in mouse models that have shown a lot of unanticipated heterogeneity among mesenchymal progenitors around different kinds of blood vessels in the bone marrow, functional heterogeneity in vivo, as well as a lot of new markers that hadn't been taken into account when the field was based mainly on characterization of cells that grew out in culture.

Do you want to say anything about whether those studies have influenced the way that you think about heterogeneity of the product that you're growing out?

DR. ITESCU: Yes. I think that's critical. In fact, we use that science precisely in what we do as a company. For example, we are very much aware of the STRO-1 antigen being critical to identify the earliest progenitor of this cell, and

in fact the chief scientific officer, Professor Paul Simmons, identified this antigen originally as being critical to the earliest precursor of this lineage. CD271 and a number of other markers have been identified as being on the surface of the earliest progenitors of the mesenchymal lineage.

So we use those types of markers to identify at a very pure level when we isolate and extract these cells and start the whole process because we do agree that by starting with relatively homogeneous populations. If selection for cells that express these markers gives us greater homogeneity, then we have the ability to end up with cells that are very well characterized by lineage and ultimately by function; because we're talking about the earlier progenitors, and we maintain the earlier progenitor phenotypes through the culture process, et cetera.

So yes, that is a major focus of the company in terms of optimizing products right through the manufacturing process.

DR. MORRISON: I'm also thinking about the

more recent studies, though, where people have started doing extensive fate mapping in vivo of different subpopulations of mesenchymal cells in the bone marrow, where it's not exactly clear yet how the markers -- the markers probably change to a certain extent when you put these cells in culture, so it's not exactly clear how the recently characterized populations compare to populations that have been characterized primarily in culture in earlier studies.

But it has become clear that there really is a lot of heterogeneity within the bone marrow, cells that at least have different properties in vivo and that could potentially be similar when grown out in culture but might ultimately have different impacts once they're put into a patient.

DR. ITESCU: Look, I think the point you make is excellent, and it cuts across cell therapy more broadly. This is not something that is limited to the mesenchymal lineage. Neural cells and other cell types share this type of issue between single-cell specificity versus -- and we're

talking about clonal single-cell specificity versus cultured progeny that contains some degree of in-process heterogeneity, yes.

I think this is the nature of the cell therapy field as we're evolving and as we're building out the ability to develop products that can do good in terms of clinical outcomes. I think it's an evolution, and we are committed as a company to continuously optimizing and improving and raising the bar to maintain scientific excellence linked with clinical excellence.

I think we have already brought to the table, currently, a high-level product that is reproducible, that is consistent, that we understand, at least in part, its mechanism of action, and that translates into the ability to generate clinical outcomes that are clearly beneficial to patients in a way that other approaches do not exist. We're talking about patients who are the most refractory with a severe risk of death that we are changing the outcomes for.

I think we will continue to optimize. And I agree with you. I think that there needs to be ongoing scientific and rigorous development to continue to optimize product potency, but today we have a safe product. We have a product that has no serious adverse events and a clear safety profile with a very potent clinical outcome in a patient population that has no alternatives.

DR. MORRISON: One last question. You showed us data indicating that the best correlation in terms of predicting potency was with TNF receptor. You also, I think, made the correct point that the mechanism of action is likely multifactorial complicated, involving multiple cytokines.

In the analyses where you arrived on TNF receptor, did you try doing multifactorial correlations to see if you could get better predictive value from incorporating some combination of multiple factors?

DR. ITESCU: We looked at the factors that had already been incorporated as part of release

criteria across more than 400 patients that the product had been used in through clinical trials. Beyond TNF receptor viability, inhibition of T-cell proliferation, gender, age, and disease severity, we're not yet in these trials. We hadn't been measuring, I suppose, other quality attributes or other factors.

Moving forward, of course we will, but these were what were available to us. One would have expected in these kind of multivariable regression analyses that the strongest predictors of outcome, in particular, survival, would be disease severity or would be potentially age. In fact, we were really, really surprised that the predictor of survival, at least using these parameters, the strongest was the level of TNFR1 expression, the degree of expression.

The reason that we were able to detect that was precisely because we had such high variability from the older process that was used 10 years ago in two trials that did not meet their primary endpoint versus the optimized process that is being

developed and manufactured today.

That kind of large variability within a single key parameter allows us to them to determine its relationship to outcome. Its strength came out through the regression analyses, and the fact that it was more related to outcome than traditional GVHD severity grade -- grade 3/4 CD, et cetera -- tells us how important this quality attribute is on this product. But over time, we will evaluate additional CQAs.

DR. MORRISON; Thank you.

DR. HOFFMAN: Dr. Halabi?

DR. HALABI: Yes. Susan Halabi. Thank you, Dr. Hoffman.

I'm also still struggling on the whole issue of variability in vivo versus in vitro. In one of your slides, I believe MA-2, you showed correlation, a proportion of variability range from 0.72 to 0.8, but then in the in vitro, I believe IL-2R alpha, you looked at that correlation with the CD3, et cetera. But that correlation, as measured by the proportion of variability, was

really small, was only 0.38. I'm wondering if you also looked at other correlations similar to what you've done in MA-2 for in vitro versus in vivo.

That was one of the questions. I think my other question you've already addressed, based on what Dr. Morrison asked in terms of other analyses that takes into account other factors.

DR. ITESCU: Thank you very much.

If we could have MA-2 slide up again, please?

What's important about this slide is it shows strong correlations, as you've noted, between the absolute level of TNFR1 on the X-axis and three different intracellular characteristics of the cell. What's important between these TNFR1 levels on the X-axis is that they encompass the absolute level that is seen in our clinical trials, so they relate very precisely to the levels of TNFR1 that we talk about in our correlations with survival across the three trials to date.

The fact that by knocking down TNFR1 to levels that encompass what's been in the clinic

gives us a very good sense of the ability of the cell to respond when it has a low level of TNFR1 and when it has a 50 percent higher level of TNFR1 with respect to factors that are likely to be relevant in vivo and we know are biologically critical, such as CCL2, that is very important in macrophage polarization to M2.

By doing these in vitro assays and then taking the levels of TNFR1 that here you can see relates to intracellular bioactivity of the cell, and then showing that these very levels of TNFR1 then correlate with survival benefits in patients I think links the surface attribute of the cell with its bioactivity with long-term survival of the patient, and that's the way we will continue to optimize our product.

When you mention the other correlation between the proportion of CD4 activated T cells in vivo with the ability to suppress activation of T cells in vitro, you are correct. The correlation was less tight. And that's why we are using the ability of the cells to suppress T-cell activation

and proliferation in vitro as really a qualitative bioassay, because it does not correlate quite as well with in vivo reduction of activated T cells.

This is really the way we're going to continue to optimize and learn about which characteristics are best for quantification in relation to in vivo outcomes versus which are relevant to ensuring that the product has a biologic activity that is reproducible but is not necessarily able to measure a quantitative in vivo outcome quite as precisely.

This is the slide that shows that, yes, we are able to demonstrate that in vitro inhibition of T-cell activation has a correlation with reduction of activated T cells in vivo, but that correlation, you are correct, is not as strong as the correlations seen between TNFR1 and the production of factors by the remestemcel factors that are relevant to immunomodulation.

DR. HALABI: Thank you. The follow-up question also regarding slide MA-10, with that, I know the sample size was small, but in one of your

graphs -- I believe the one to the extreme right where you had an MBS score -- I think you were looking at MBS score with survival.

DR. ITESCU: Yes.

DR. HALABI: Well, not this slide. You had four Kaplan-Meier curves. It wasn't this one.

DR. ITESCU: MA-11, next one.

DR. HALABI: Right. If you look at the last Kaplan-Meier -- yes, that's exactly right -- my understanding is this is what you're likely going to see in patients, so the Kaplan-Meier curve on the right.

DR. ITESCU: That's right.

DR. HALABI: If you look at the difference, even though you did see, based on cohorts A and validation cohorts 1 and 2, there is a huge gap between the area in terms of high and low, but in terms of what you're expecting to see in the clinic, it looks like really a high MBS does not reflect the benefit here.

DR. ITESCU: Let me explain. No, I think you misinterpreted the slide. Let me explain it.

In the three Kaplan-Meiers on the left, they represent the outcomes of steroid-refractory population of patients treated with best available therapy today, and what you see is that those patients with a high MBS score above 0.29, they are in blue, those patients with the best available therapy today have a survival of no better than 20 percent to 40 percent through 12 months. That's what the blue line shows. In contrast, today, if you have a low biomarker score, you have an excellent survival.

So this is a validated biomarker score by the Levine Group that is now well accepted in the GVHD community. It's validated based on these three cohorts as being able to predict patients who otherwise are going to have a very high mortality to 12 months.

If you look at the Kaplan-Meier on the right, which is the results of our phase 3 trial, where we've also looked at the same biomarker MBS greater than point 0.29 or less than 0.29 at baseline, now we see in blue those patients that

have high MBS scores greater than 0.29 no longer have a poor survival. Those patients now have a survival at day 180 at 6 months, approximately 60 percent, which is not significantly lower than patients with low MBS score at baseline.

So we've shifted the survival curve of the high-risk patient from what would have been expected to be a 20 to 40 percent survival to a 60 percent survival at 6 months. That's the point.

DR. HALABI: Okay. Thank you; although the sample size is really small. You have only 29 patients, and then the follow-up, it seems you needed a longer follow-up.

I assume this is ongoing; correct?

DR. ITESCU: That's correct.

DR. HALABI: Okay. Thank you.

DR. HOFFMAN: Dr. Singec?

DR. SINGEC: Yes. Hi there. I have a question regarding TNF alpha receptor expression. Has it changed as you culture the cells, the early passage verses late passage?

MS. STORTON: I will start the response to

that, and then I'll ask Dr. Itescu to comment. We test the levels of TNFR1 at the donor cell bank stage and at the drug product stage, and we see similar levels at passage 2 and then again at passage 5.

Dr. Itescu, would you like to add anything to that comment?

(No response.)

MS. STORTON: Dr. Itescu, are you there?

(No response.)

MS. STORTON: I think we've lost him.

DR. SINGEC: Can I ask a follow-up question?

DR. HOFFMAN: Yes.

DR. SINGEC: I think it's still rather risky. Functional heterogeneity has been brought up several times. Also considering powerful technologies like single-cell analysis, you could really get an idea of heterogeneity at the single-cell level and potentially have taken advantage of that and prospectively isolating the cell type that could be most beneficial in this context.

So I'm still wondering if and how the approach could be tested so that you basically don't simply rely on cells attaching differentially to a plastic surface. If I understand correctly, this is the current method of basically filtering out the cells initially after the bone marrow aspiration and plating cells on plastic, and those that seem to attach are then being further propagated.

So overall, it could be very useful to have really a comparison of what you have initially and what happens over the course of isolating the cells. Again, the use of animal serum will also introduce confounders potentially; so basically having some better handle on prospectively isolating cells could be of value here so that you could really pinpoint better the cell product that you eventually want to develop.

Any comments on that?

DR. ITESCU: Yes. May I add to this, Geraldine?

MS. STORTON: Yes.

DR. ITESCU: I think, look, now that we understand how important the cell surface receptors are for the integrity of the cell and its ability to respond to the micro environment in vivo, we now have levels that we will use through the entire manufacturing process at the level of the donor cell bank, at the level of each passage, at the level of harvesting, and final freeze/thaw, for example.

So we are able to now protect the integrity, ensure that the surface receptors are intact, are functional, and are able to signal internally and result in the release of these important factors. Certainly, I think the entire manufacturing process will be designed around ensuring that levels of TNFR1, amongst other receptors, are maintained at optimal levels for cellular function, and we're building outputs that will allow us to continue to ensure that is a reproducible process.

DR. HOFFMAN: Thank you.

Dr. Garcia, do you have your hand up or that was from before?

DR. GARCIA: I apologize.

DR. HOFFMAN: No worries.

I think if we've learned nothing else, the complexity of this subject is enormous, and I think we've had a very thorough discussion from experts, not including myself, who know more about this technology than I do. I think of one of the comments that Dr. Garcia made earlier, which is that this is the state of the art at the moment, and obviously we have what we have, and obviously improvements and modifications and other changes are being made to perfect this. I think this has been helpful for us to have a sense of the complexity of this subject matter.

Before we adjourn the morning session, are there any last comments from the FDA?

DR. BAUER: Yes. This is Steve Bauer. I wanted to express my appreciation for the comments and questions the committees discussed so far and the applicant's responses. I think, as Dr. Sally Temple first brought up, this issue of continually addressing the complexity that you just mentioned,

Dr. Hoffman, and that we all acknowledge is important and a very iterative process. I think Dr. Itescu has mentioned that several times, and we've brought it up as well, and I think it's important to keep that in mind going forward.

Our committees asked the applicant many questions, but I do want to ask one last time if any members or panelists have any specific proposals for specific analyses or ways to improve the state of the art and to continue a short consistent quality of remestemcel-L going forward.

DR. HOFFMAN: Dr. Hinrichs, do you have a comment?

DR. HINRICHS: Yes. I think part of why there's not a giant wave of answers to this question is that with a complex and somewhat unclear mechanism of action, it's really difficult to think about how you would want to see the potency determined and what attributes and what characteristics you would want to see tested. For me personally, that is a bit of an obstacle to thinking clearly about exactly what I would want to

know about the product.

DR. HOFFMAN: Dr. Robey?

(No response.)

DR. HOFFMAN: Did you have your hand up? I'm sorry, Dr. Robey

(No response.)

DR. HOFFMAN: Perhaps not. Okay.

I just want to be sure I don't miss anybody before I conclude our morning session.

DR. BAUER: May I ask if any of our FDA --

DR. ROBEY: I --

DR. BAUER: -- oh, go ahead. Sorry.

DR. ROBEY: Sorry. This is Pam Robey. I lost connection there momentarily. We've talked about the need for better analyses, and I just want to say very specifically that I think that each lot of cells should have a transcriptomic profile associated with it, and that it would come in extremely handy for future evaluations and also address the issue of lot-to-lot variability. So I think specifically that is a recommendation, from me anyway.

DR. HOFFMAN: Okay.

Dr. Hinrichs, your hand is up or is that left over?

(No response.)

Adjournment

DR. HOFFMAN: Left over. Okay.

I think this can conclude the morning session. We'll now break for lunch. We'll reconvene at 1:00. Maybe I could suggest that we maybe try to come on even 5 or 10 minutes before 1:00 if we can because I suspect there'll be a lot of discussion this afternoon as well, so that we can start at least sharp at 1:00 for the afternoon session of today's meeting.

Panel members, please remember that there should be no discussion of the meeting topics during lunch or with other panel members. Thank you very much.

(Whereupon, at 11:41 a.m., the morning session was adjourned.)