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## P R O C E E D I N G S

## Call to Order

DR. DUBINETT: Good morning. I would like to call the meeting to order.

We will begin with Dr. Dapolito to read the Conflict of Interest Statement.

MS. DAPOLITO: Good morning. Thank you, Dr. Dubinett and welcome everybody. While I am reading the statement it might be a good chance for you to silence your electronic devices. So, good morning.

## Conflict of Interest Statement

MS. DAPOLITO: The Food and Drug Administration convenes the November 17, 2011 meeting of the Cellular, Tissue and Gene Therapies Advisory Committee under the authority of the Federal Advisory Committee Act of 1972. With the exception of the industry representative, all participants of the committee are special government employees or regular Federal employees from other agencies and are subject to the Federal Conflict of Interest Laws

and Regulations.

The following information on the status of this Advisory Committee's compliance with Federal ethics and conflict of interest laws including, but not limited to, 18 USC Subsections 208 and 712 of the Federal Food, Drug and Cosmetic Act, are being provided to participants at this meeting and to the public.

FDA determined that all members of this advisory committee are in compliance with Federal ethics and conflict of interest laws. Under 18 USC Subsection 208, Congress has authorized FDA to grant waivers to special government employees, and regular government employees who have financial conflicts, when it is determined that the Agency's need for a particular individual service outweighs his or her potential financial conflict of interest.

Under Subsection 712 of the Food, Drug and Cosmetic Act, Congress has authorized FDA to grant waivers to special government employees and regular government employees, with potential

financial conflicts, when necessary to afford the committee their essential expertise.

Related to the discussion of this meeting, members and consultants of this committee were screened for potential financial conflict of interest of their own as well as those imputed to them, including those of their spouses or minor children, and for the purposes of 18 USC Subsection 208, their employers.

These interests may include investments, consulting, expert witness testimony, contracts and grants, credos, teaching, speaking, writing, patents and royalties, and also primary employment.

Today, the Committee will discuss and made recommendations on Biologics License Application 125400 for a cellular therapy for the treatment of surgically created gingival and alveolar mucosal surface defects in adults. This is a particular matter involving specific parties. Based on the agenda and all financial interests reported by members and consultants, no

conflict of interest waivers were issued in accordance with 18 USC Subsections 208(b)(3) and 712 of the Food, Drug and Cosmetic Act.

Dr. Michael Bui is serving as the industry representative, acting on behalf of all related industry. He is employed by Bayer. Industry representatives are not special government employees and do not vote. This Conflict of Interest Statement will be available for review at the registration table. We would like to remind members, consultants and participants 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 you may have with the sponsor, its product, and if known its direct competitors.

Thank you. I will turn it over to you,

Dr. Dubinett.

DR. DUBINETT: Thank you. I would like to begin with our introductions, beginning to my right with Dr. Lee.

Introduction of Committee

DR. MEI-LING LEE: My name is Mei-Ling Lee from the University of Maryland, College Park. I am the professor and director of the Biostatistics and Risk Assessment Center.

DR. SNYDER: I am Evan Snyder from the Sanford-Burnham Medical Research Institute in La Jolla and also UCSD. I am director of the Stem Cell Research Center at the Sanford-Burnham, and I am also a practicing pediatric neurologist and neonatologist.

DR. DAHLGREN: I am Linda Dahlgren. I work at Virginia Tech. I am a large animal veterinary surgeon there.

DR. FRATZKE: I am James Fratzke. I am a retired dentist from Portland, Oregon, and I am serving as the Patient Representative.

MS. RUE: I am Karen Rue from Lafayette,

Louisiana. I am with Griswold Special Care, and I am the Consumer Representative.

DR. JEFFCOAT: I am Marjorie Jeffcoat. I am from the University of Pennsylvania, and I am a periodontist.

DR. WITTES: I am Janet Wittes. I am a statistician from Statistics Collaborative in D.C.

DR. REYNOLDS: Good morning. I am Mark Reynolds from the University of Maryland. I am professor and chair of Periodontics.

DR. BUI: Hi. I am Dr. Michael Bui. I am the Industry Representative. I work at Bayer Pharmaceuticals. I am also an adjunct professor at UMDNJ.

DR. DUBINETT: Can we go from my left now for this table.

DR. AHSAN: I am Taby Ahsan. I am at Tulane University. I am an assistant professor in the Department of Biomedical Engineering.

DR. COUTURE: I am Larry Couture. I am the Senior Vice President for the Center for

Applied Technology Development at the Beckman Research Institute at the City of Hope National Medical Center.

DR. HORNICEK: Good morning. I am Fran Hornicek. I am a sarcoma surgeon at Mass General and I am past president of the AATB.

DR. HWU: I am Patrick Hwu. I chair the Melanoma Department at M.D. Anderson Cancer Center, and I work in T cell therapy.

DR. GENCO: I am Bob Genco. I am a professor at the University of Buffalo, Department of Oral Biology and Microbiology. I am also vice provost in charge of technology transfer, and I direct the Periodontal Disease Clinical Research Center at Buffalo.

DR. AMAR: Good morning. I am Salomon Amar. I am professor of Periodontology and Neurobiology at Boston University, and I am the director of the Center of Anti-inflammatory at the same institution.

DR. MARK LEE: Good morning. I am Mark Lee. I am the product reviewer for this BLA. I

am part of the Office of Cellular, Tissue and Gene Therapies.

DR. LIM: I am Agnes Lim. I am the clinical reviewer for this BLA. I am from FDA's Office of Cellular, Tissue and Gene Therapies in the Center for Biologics.

DR. BETZ: My name is Bob Betz. I am a board-certified periodontist. I work for CDRH. I am going to be the background reviewer for this BLA.

DR. WITTEN: Celia Witten, Office Director of the Office of Cellular, Tissue and Gene Therapies at the Center for Biologics.

DR. DUBINETT: I am Steve Dubinett. I am Associate Vice Chancellor for Research at UCLA and direct the Clinical Translational Science Institute, and I am the lung cancer investigator.

Dr. Witten will give the FDA introduction.

#### FDA Introduction

DR. WITTEN: Good morning. I am going to provide the background for today's meeting to set

the stage for the day.

The purpose of today's meeting is to discuss the Biologics License Application submitted by Organogenesis for their product Apligraf Oral, which is a bilayer tissue construct consisting of human cells and bovine-derived collagen.

The applicant proposes an indication for the treatment of surgically treated gingival and alveolar mucosal surface defects in adults.

The Center for Biologics Evaluation and Research is today's lead center for review of the BLA for Apligraf Oral, the topic of today's discussion. The assignment to CBER was based on the determination of the product's primary mode of action, however, Apligraf is currently marketed as a device for certain cutaneous indications, and there is extensive safety history from these indications.

CDRH, the Center for Devices and Radiologic Health has valuable experience with both the marketed Apligraf product and the

clinical development of Apligraf Oral, therefore, CDRH contributed substantially to the current BLA review and in preparation of today's meeting.

We are asking the Advisory Committee members to discuss issues related to product testing, effectiveness, the patient population and safety.

I would like to thank the Advisory Committee members in advance for their thoughtful consideration of these issues and also to thank the FDA staff who have prepared for this meeting.

Now I will turn it over to you.

DR. DUBINETT: We will begin with Organogenesis. We will hold questions until the end of this section unless otherwise pressing.

Organogenesis, Inc.

#### Introduction

MR. BILBO: Good morning. Today, we are here to discuss the Apligraf biologic license application for the oral indication. My name is Patrick Bilbo and I am Vice President of Regulatory Affairs and Government Relations at

Organogenesis.

On behalf of Organogenesis we would like to thank the FDA and this committee for having us here today to present Apligraf, our cell-based combination product for the treatment of gingival and alveolar mucosal surface defects.

After my introduction, Dr. David Cochran will speak to the conditions and treatments for gingival and alveolar mucosal surface defects. Dr. Cochran is the chair of the Department of Periodontics at the University of Texas Health Science Center, and was an investigator for our pivotal clinical study.

He will be followed by Dr. Damien Bates, who will discuss the clinical efficacy and safety data from the pilot and pivotal clinical studies.

Dr. Bates is Organogenesis' chief medical officer. He is a board-certified plastic surgeon, and has a Ph.D. in Developmental Biology.

Next, Dr. Michael McGuire will evaluate the benefit/risk of Apligraf for the treatment of

gingival and alveolar mucosal surface defects. Dr. McGuire is a periodontist in private practice and was the investigator for the pilot clinical study, as well as one of the four investigators for the pivotal clinical study.

Dr. Cochran and Dr. McGuire are past presidents of the American Academy of Periodontology.

Finally, I will conclude the presentation.

The proposed indication for Apligraf is for the treatment of surgically created gingival and alveolar mucosal surface defects. If approved, it will be the third clinical indication for Apligraf.

Apligraf is applied over a vascular wound bed to regenerate site-appropriate oral mucosal tissue. It is important to note that surgically created refers to the wound that is created by the surgeon as the first step in treating the pre-existing defect.

Secondly, site-appropriate as specified

in the indication statement is in the context to the tissue form and appearance, which should be histologically and functionally similar to the surrounding adjacent tissue.

Although Apligraf looks histologically similar to skin, unlike skin, Apligraf is not a graft, but a regenerative approach to wound healing.

Apligraf was originally classified by the FDA as a Class III medical device in 1986. There were many years of development and cutaneous wounds which resulted in the first approval for the venous leg ulcer indication in 1998. An example of a non-healed venous ulcer is shown.

Followed by approval for a second indication in 2000 for the treatment of diabetic foot ulcers, an example of the diabetic foot ulcer is shown on the right.

After Apligraf treatment, these wounds achieve full wound closure. Apligraf has been in human clinical use since 1990 with a total of 19 Organogenesis-sponsored clinical trials for

chronic and acute wounds; 787 patients were treated in these clinical studies.

The left photograph is an example of an acute highly vascular cutaneous wound, which is similar to the acute oral wounds treated in the Apligraf clinical studies. These wounds heal by the same wound healing process, and as shown, both types of acute wounds were healed by Apligraf.

From these studies 496 patients were assessed for humoral and cellular immunological response to the product. Overall, the evidence to date supports no significant humoral or cellular immunologic response to the product. Given this fact, we did not perform an immunological evaluation in the oral clinical studies, and this testing was not required by FDA.

Organogenesis has over 13 years of post-approval marketing experience with Apligraf for chronic wound indications, and over 420,000 Apligraf units have been shipped to date for

patient application.

Apligraf has been well characterized and has a favorable safety profile. After 13 years of use, there have been only 9 adverse events that warranted medical device reports submitted to the FDA. There have been no confirmed reports of immunological reaction, tumorigenicity, or malignant transformation caused by Apligraf, nor have there been reports of disease transmission from the cells or bovine collagen components of the product.

For the proposed oral indication, an initial pilot study was approved in 2005 under an investigational device exemption by the Dental Devices Branch of the Center for Devices at FDA-CDRH.

This was followed by approval for initiation of the Apligraf oral pivotal study in 2007 again by the Dental Devices Branch. Under the guidance of CDRH, Organogenesis submitted a pre-market approval application, or PMA, in 2009.

FDA did not accept the PMA as it had questions

on the jurisdiction of the product for the oral indication.

FDA subsequently reclassified Apligraf for the oral indication as a combination product under the jurisdiction of CBER and specified that Organogenesis submit a Biologics License Application. We filed the BLA for Apligraf in May of this year.

Apligraf is a living cell-based product consisting of 2 layers, an upper and lower, that adhere to form the final product. The upper layer consists of human keratinocytes. This layer imparts structural elements to the product, for example, its mechanical strength, handling, and barrier properties.

The living keratinocytes in the product produce a number of cytokines and growth factors that are known to have a role in wound healing.

The lower layer is comprised of human dermal fibroblasts in a matrix of bovine-derived collagen, as well as human extracellular matrix proteins produced by the fibroblasts during the

manufacturing process.

The fibroblasts also secrete growth factors and cytokines involved in the wound healing process. The functionality of Apligraf is dependent on the upper and lower layers working in concert to form a complete and viable final product.

As shown in the photo on the left, the final product is supplied as a 75-millimeter circular disk, which is on a porous polycarbonate membrane within the shipping tray that contains an agarose and nutrient media. This shipping tray is sealed in a polyethylene bag containing a 10 percent CO<sub>2</sub> environment to maintain the potency and viability of the product.

In this final packaging configuration, the product has a 15-day shelf life when stored at room temperature.

We have a two-tiered cell banking system that consists of a master and working cell banks.

Each master cell bank is a collection of uniform cells derived from a single donated neonatal

foreskin tissue, which is sourced following FDA donation guidelines and informed consent procedures at several participating hospitals.

Following screening of the donor's mother and blood, extensive safety testing is performed on the cell banks to mitigate the risk of disease transmission. Quality and comparability is determined on each cell bank, and that will be discussed in greater detail in the following slides.

Each working cell bank is derived from a single master cell bank, where the cells are serially passaged, or another term subculture to expand the population greatly.

Working cell banks are further passaged to generate seed pools that are then used to manufacture each batch of Apligraf

Our approach to qualifying new cell banks consists of rigorous and comprehensive safety and comparability testing. First, I will summarize the safety testing regimen.

Adventitious agent testing includes

sterility, Mycoplasma testing, and an extensive panel of human, bovine, and porcine viruses all for FDA and international requirements. Our neoplastic safety testing includes assessing karyology, tumorigenicity, and senescence.

Additionally, for each working cell bank, we test sterility, Mycoplasma, and viruses. Collectively, these tests assure that the cells using the product are safe, normal, and healthy.

Our approach to demonstrate comparability for new cell banks involves a combination of functional assays performed on a mature product.

In vitro comparability testing includes percutaneous absorption, MTT, cytokine expression, VEGF production, cell purity, and potency.

For in vivo comparability we utilized the athymic mouse wound model, which evaluates the barrier, integrity and viability of Apligraf by assessing, for example, graft take and integration in this model.

We perform additional characterization

tests to release working cell banks, for example, potency, cell growth, collagen biosynthesis for the fibroblasts, and involucrin for the keratinocytes.

Together, these assays ensure the quality and comparability of new cell banks for their suitability in the manufacture of Apligraf, and are performed numerous times per year.

For each manufacturing batch of Apligraf, we perform multiple in-process and final product test methods to ensure that the product is free of contaminants and will be efficacious for patient use.

Batch release is based on a combination of in-process and final product test results. In the next few slides, I will focus on the potency assay.

The potency of Apligraf is measured by histological assessment and product maturation. This assessment confirms viability, development and functional activity of the two cell types in Apligraf. The potency assay consists of a matrix

of quantitative parameters that are performed on a histological section of the mature product.

Histology provides the most comprehensive and relevant potency assay for the complex and tissue-like structure of Apligraf, and therefore, over the past 13 years of large-scale commercial production, we have determined that histology is the most reliable test for lot release.

This slide outlines the matrix of assessments that comprise the potency assay. The matrix of assays for the upper layer consist of epidermal coverage, epidermal development, and keratinocyte aspects to assess the viability of the basal and suprabasal cell layers.

The development of the stratum corneum barrier is also assessed. Moving down to the lower layer, this assay assesses the thickness of the matrix indicated by the double side arrow. The dark purple circles indicate the fibroblast density which is calculated as the mean number of nuclei of fibroblasts.

Finally, the overall matrix aspect is

evaluated which ensures the entire lower layer is structurally intact.

The histological parameters link to biological attributes, which we measure through a number of functional assays that are included in our product characterization and comparability panel.

As an example, epidermal development assesses the complete development and differentiation of keratinocytes. This biological attribute can be measured by the VEGF assay, percutaneous absorption, and in vivo graft morphology.

When there is a failure mode in epidermal development, as we are able to detect with histology, there is a corresponding decrease in VEGF production, a decrease in barrier function measured by percutaneous absorption, and graft failure in the athymic mouse model.

It is important to note that Apligraf does not engraft in humans. Specifically, there is no vascularization, no integration of product

into host, and only transient cell persistence.

The mechanism of Apligraf is complex and multi-modal. The living cells secrete physiological levels of human cytokines, growth factors, and matrix proteins, and the matrix in stratum corneum provide a barrier upon application in the wound bed.

These two effects stimulate the patient's own cells to regenerate site-appropriate tissue by improving secondary intention wound healing.

Oral mucosal defects lack the quantity and quality of functional gingiva. This photograph illustrates a very common clinical condition where there is insufficient functional tissue, a condition which can be treated either through primary or secondary intention healing strategies. Dr. Cochran will discuss the indication treatment strategies in more depth.

To apply Apligraf, a wound is surgically created at the site of the defect, as shown in the left illustration. As shown on the right, Apligraf is surgically placed over the recipient

site in a non-submerged manner and is anchored to the wound using sutures.

Apligraf is supported by numerous nonclinical animal and in vitro studies. In a battery of toxicity studies required by FDA, there were no overt signs of acute or chronic local or systemic toxicity, irritancy, sensitization, hemolysis, or cytotoxicity.

Safety pharmacology studies demonstrated that the cellular components of Apligraf do not induce T-cell proliferation, and there is no immunological rejection in vivo, which is consistent with the findings to date from the clinic.

In preclinical models, there was no evidence of Apligraf cells migrating from the site of application. Of note, Apligraf DNA does not persist indefinitely in humans, and this will be addressed further by Dr. Bates.

To support efficacy, two prospective randomized clinical studies were designed using a powerful within-patient control design comparing

Apligraf to free gingival graft in the treatment of oral mucosal defects.

The pilot study's primary purpose was to assess safety and proof of concept. The pivotal study's purpose was to address the safety and efficacy of Apligraf in the treatment of oral mucosal defects.

In the pilot study, Apligraf was well tolerated by patients. The pilot study failed to meet the predefined endpoint of non-inferiority to free gingival graft for the generation of attached gingiva, however, there was clinical benefit for the generation of both keratinized tissue and attached gingiva.

The pilot study provided valuable information to optimize the pivotal study design.

The pivotal study demonstrated Apligraf to be clinically and statistically significant for the primary endpoint and multiple secondary endpoints with 95 percent of the Apligraf-treated sites meeting the primary efficacy criteria.

In summary, Apligraf succeeded not only

in regenerating keratinized tissue, but did so while also improving the aesthetics and removing the morbidity associated with the free gingival graft harvest site.

Also, patient feedback showed that they overwhelmingly preferred Apligraf treatment to that with free gingival graft.

These studies also showed Apligraf to have a favorable safety profile. Adverse events were generally mild to moderate, of short duration, and were easily managed.

This oral safety experience is consistent with a large cutaneous clinical trial in postmarket experience with Apligraf. There are three reasons why Apligraf presents an improvement over current therapies.

Firstly, Apligraf has been shown to regenerate site-appropriate tissue meaning that this treatment improves the quality and function of the new tissue and is similar to the surrounding untreated tissue.

Secondly, there is unlimited supply of

Apligraf, therefore, there will be no material constraints for treating patients. In contrast, the free gingival procedure relies on limited supply of palate tissue.

By using Apligraf, all sites requiring treatment could be surgically treated under one procedure, reducing the burden to the patient for multiple office visits. Furthermore, the elimination of the donor site will reduce the morbidity associated with these oral surgical procedures.

In summary, Apligraf is an important new option for regenerating site-appropriate oral mucosal tissue. It offers meaningful clinical benefits with minimal product risk to patients and without donor site morbidity.

Our next speaker this morning is Dr. David Cochran who will address the conditions and treatments that present for oral mucosal defects.

#### Conditions and Treatments

DR. COCHRAN: Good morning. As you have heard, my name is David Cochran, and I teach,

practice, and perform basic animal and human clinical trials in the field of periodontics. I have worked in academics for 26 years, 19 in my present position.

Today, I would like to talk to you about normal anatomy, what the tissues look like in the oral cavity around the teeth. I would like to talk a little bit about what we consider are oral mucosal defects, and then what current treatments we are utilizing to take care of these types of problems, and then lastly, I would like to talk about the opportunities that are there for improvement.

On this slide, we have a schematic view of the soft tissues that surround the teeth or implants and which also covers edentulous areas of the bone.

The top part of the gingiva have a thicker epithelium and is called keratinized tissue, or KT. KT is comprised of both free gingiva and attached gingiva. Attached gingiva is an important functional component of keratinized

tissue and is calculated by subtracting the free gingiva from the keratinized tissue.

The lower part is nonkeratinized and is called the alveolar mucosa. Each of these tissues has very specific functions. Of prime importance is the keratinized tissue which prevents recession of the soft tissues, allows for improved home care, and resists pulls on the gingiva.

The alveolar mucosa, on the other hand, has elastic fibers in it and allows for movement of the jaws and flexibility of the soft tissues.

As you can see in the picture to the right, there is a zone of light pink tissue surrounding the teeth, which is the keratinized tissue, or KT.

Below that is the more loose or stretchy alveolar mucosa, or AM, in this diagram. The yellow dotted line is the border between the keratinized tissue and the alveolar mucosa, and we call that line the mucogingival junction, or MGJ.

It is generally accepted that 2 millimeters of keratinized tissue is adequate for healthy soft tissues around teeth and implants, and from a cosmetic point of view that the mucogingival junction is a continuous line.

So, how do we define an oral mucosal defect? When there is an insufficient amount of keratinized tissue around teeth, implants or edentulous ridge, we generally call this an oral mucosal defect, and not only is there an insufficient amount of keratinized tissue, but it means that the alveolar mucosa is in a location that is not normally there under healthy normal conditions.

The etiology for these types of defects are either developmental or acquired. In the developmental category, we have teeth that are out of alignment, and when these teeth are displaced towards the buccal or the facial, then, usually, the tissues are, in those areas, more in a pathological condition.

Also, in certain areas of the mouth, we

have muscle pulls that actually are muscles that come underneath the tissue and go up to the marginal tissue and pull on the marginal tissue, and pull that tissue away from the tooth or implant.

The acquired etiologies include brushing.

In the past, people have brushed with very hard brushes and scrubbed their teeth, and tend to push their gums away and change the morphology of the tissues around the teeth.

In orthodontics, when the orthodontist moves the teeth outward a little bit, particularly in the lower anterior area, to gain space to allow the teeth, usually that makes the tissues around these recede a little bit and we have a more pathological condition.

And then, of course, in areas where there is calculus accumulation, there is gingival inflammation, and that also is an etiology for these oral mucosal defects.

Now, the prognosis is important, because what happens is that it allows for plaque and

inflammation to develop into the tissues, and very significantly, these defects do not self-resolve, and the conditions can progress, and under certain conditions, this progression can lead to tooth loss or implant loss, and certainly bone resorption.

Furthermore, with the inflammation present, there is a growing body of literature that links periodontal disease to systemic disease, so any chance we have to eliminate inflammation and to create a more normal situation where we can have a more healthy condition in the mouth, it's much better for our patients.

Now, on this slide, you see three pictures. On the left, you have seen the picture earlier where there has been some toothbrush abrasion on that particular tooth in the middle there. You see sort of yellowish down on the root, where the root has been abraded.

But below that there is very little keratinized tissue in that case, and the yellow

dotted line shows you the mucogingival junction there. So, the alveolar mucosa is very high on this tooth, and below that you can see a muscle pull in that area.

In the middle picture, there is an area of the posterior mandible where the teeth were missing in this particular patient, and usually, when the teeth are missing in the posterior mandible, there is only a very small amount of keratinized tissue up on the ridge.

So, when a dentist goes in to put in a dental implant, which is normally 3 to 4 mm in diameter, these implants usually result in being surrounded by alveolar mucosa.

In the picture on the right, you see another area of the posterior mandible where there has been a bone augmentation procedure performed, so that dental implants could be placed, and when we do these bone augmentation procedures, we usually use some sort of bone replacement graft.

We built up that area under the arch, and

then we need to pull the tissues over to cover that, so that the bone formation can occur underneath that tissue. When we pull up that tissue, as you can see in this nice example, the alveolar mucosa then becomes the predominant tissue, and you don't see any keratinized tissue in this particular defect. So, there is an abnormal type, amount and distribution of tissues in these cases.

So, what is our goal? Our goal is obviously to restore those tissues with site-appropriate, what we call site-appropriate functional tissue type, meaning that we would like to have both keratinized tissue and alveolar mucosa back to what it was prior to having any sort of abnormal situation.

We typically see that as a 2-mm zone of keratinized tissue, which then transitions through the mucogingival junction down to the alveolar mucosa, and we would like to see a continuous line of the mucogingival junction congruent with the adjacent mucogingival

junction.

Now, when we talk about doing mucogingival surgeries and corrections of these mucosal defects, we normally depend on -- the surgical procedure depends on what we are trying to achieve.

Today, we are here to talk about augmenting keratinized tissue, but we certainly recognize that in some indications we would like to increase the thickness of that tissue, for example, underneath someone's bridge, we could like to bulk up the tissues, so that it looks very natural under the bridge, and also in many cases, we would like to cover a root if there has been a marginal recession in those areas and exposed roots.

We typically use connective tissue procedures in those cases, but even in those cases, we would like to have sufficient keratinized tissue to be able to coronally position the flap over top of the connective tissue.

So, today, we are going to focus on augmenting keratinized tissue. Now, when we augment keratinized tissue around teeth, dental implants, or on an edentulous ridge, we always start by surgically excising the nonfunctional tissue and creating a wound bed or what we call a recipient site.

After this, there are basically two approaches to treat the recipient site. One approach leads to primary intention healing and is accomplished by placing a graft over the wound where the graft is typically harvested from the patient's palate.

Alternatively, the other approach leads to secondary intention healing and is where the wound site is allowed to heal on its own. In the next few slides, I will discuss each of these approaches and conclude with opportunities for improvement.

So, if you look at this particular slide where we are talking about correcting the oral mucosal defect, what you can see is that the

preparation of the wound bed is the same whether it is on the left side where we are looking at a tooth site, or on the right side where we are looking at the dental implants that have been placed.

What we generally do is make a horizontal incision and remove the keratinized tissue that remains in the area where the defect exists.

We usually extend that horizontal incision over towards the adjacent tissues and then we drop vertical releasing incisions below that, and we remove the loosely adherent and elastic fibers over top of the periosteum, so that we are left with a very tight adherent periosteal bed for correction of the defect.

What is important to note is that the tissue that we tried to regenerate with these types of recipient beds is both the keratinized tissue and the alveolar mucosa.

After the wound bed is prepared, if we are going to do a secondary intention procedure, we then do what is called a pushback type

procedure, and this is where the surgeon removes or strips all or part of the soft tissue, as I have just shown you, in the preparation of the recipient site.

This requires the wound to heal completely without being covered by anything. As you can imagine when this is done in a very large area of the mouth, this is a very painful experience for the patient. It is unpredictable and does not regenerate significant keratinized tissue, so we no longer do this type of procedure.

Currently, our standard of care procedure to augment keratinized tissue is to use what we call a free gingival graft procedure where again a surgical wound is created at the defect site, but in this case, a piece of the roof of your mouth, which is all keratinized tissue, is also surgically excised and placed over the defect wound. This is an autograft and represents a form of primary wound healing.

As you can see in the clinical picture,

this results in a predictably large amount of tissue at the defect site, but in most cases, results in a very unnatural appearance. In fact, this tissue is also known as a tire patch, and many patients that are worried about their looks will not accept this procedure.

While this case is unusually poor in terms of aesthetic outcome that you see on the screen, I use it here to illustrate the concept of primary intention wound healing. The graft has retained the palatal rugae and is unlike the adjacent tissue, and for folks that are not dentists in the room, if you put your finger behind your upper front teeth, you will feel sort of the ridges up there, and those are the rugae that you see in this particular picture.

Furthermore, there is a limit as to how much palatal tissue is available, and that is why we sometimes have to go to where the rugae are, so in many instances, a patient can only have the most severe defects treated and other defects require subsequent surgical procedures once the

roof of their mouth heals.

Secondly, the roof of the mouth is extremely painful after this procedure, and a huge problem is that in some of these cases, the roof of the mouth starts bleeding after the patient goes home. These problems are so important that many periodontists will only treat patients with severe defects.

I can tell you in my practice I don't like having to take this palatal tissue because of the bleeding problems.

I should also mention that it is possible to cover the surgical defect site with other materials like collagen membranes or allografts, but these procedures do not result and is predictable of keratinized tissue as the free gingival autograft.

So, in summary, the standard of care is the free gingival graft. It predictably results in very large amounts of keratinized tissue, in fact, so much so that it does not match the adjacent tissues, and it is not a pleasant

experience for our patients since it involves a sensitive donor site.

The donor site is often described by patients as a severe pizza burn that lasts for a number of days. In addition, the donor site can result in significant bleeding as I just mentioned, and it is difficult to stop.

Together, the limitations of the free gingival graft, including the pain, the bleeding, and the limited donor tissue, negatively influences our ability to treat patients.

So, what are the opportunities for alternatives? Well, obviously, the alternatives should be able to regenerate a clinically relevant amount and distribution of both keratinized tissue and alveolar mucosa.

We would like for that tissue to look natural, to be aesthetically pleasing, and we would certainly like the alternative to improve the overall experience for our patients.

We would like to move away from repair mechanisms and obviously obviate the grafting

from the donor site. We would like to have enough material to be able to treat all the sites that a patient needs.

As you can imagine, if you have a patient that brushes their teeth hard on one side, they usually do it on the other side, or a patient presents with very thin gingiva, and so the problem is usually not localized to one or more teeth. Usually, it is a whole segment of the arch.

Of course, anything as an alternative should be supported by data and should be predictable and evidence based.

Because of the limitations of the current primary and secondary intention healing strategies, this opportunity for an alternative certainly exists. Ideally, we would like the alternative to move away from the repair strategies of the past and focus on regeneration, and as Dr. Bates will discuss, Apligraf improves secondary intention healing and appears to move the quality of healing from a repair mechanism

more towards regeneration.

So, in summary, there is a need for regeneration of site-appropriate tissue both keratinized and alveolar mucosa, and that is a very significant need for us.

A range of clinical conditions exist. It is not only around teeth, but it is around implants, and it is in those areas of edentulous ridges where we don't have sufficient amounts of keratinized tissue.

But in all these cases, they share the same recipient type bed, which involves the keratinized area as well as the alveolar mucosa, and we want to regenerate both of those types of tissues.

Our current approaches rely on the tissue harvest from a very sensitive donor site, and at this point, no evidence-based alternative exists for us as practitioners.

A new technology which regenerates a clinically significant quantity and quality of these tissues would benefit the clinician and

certainly our patients.

I would now like to turn the podium over to Dr. Damien Bates, who will discuss efficacy and safety of Apligraf.

Thank you.

Clinical Efficacy and Safety

DR. BATES: Thank you, Dr. Cochran.

Good morning. My name is Damien Bates. I am the Chief Medical Officer for Organogenesis, and I will be presenting clinical efficacy and safety.

The clinical efficacy section of my presentation will cover five main areas. I will begin by discussing wound healing, and then move on to another view of study design features needed to test the hypothesis that Apligraf is capable of regenerating site-appropriate tissue in the oral cavity.

I will then present the two clinical studies we conducted and finish with additional efficacy analyses.

The holy grail for regenerative medicine

is to move the quality of healing away from scar and site inappropriate tissue toward complete regeneration of tissue that is perfectly matched to its anatomical location.

Here, we see a spectrum of wound healing which down one end is associated with scar formation and skin, to slightly better wound healing in the oral cavity, to improved wound healing in children, scarless fetal wound healing, and finally, complete regeneration of whole limbs in certain species like the salamander.

Apligraf moves the quality of healing along the spectrum away from scar and further toward regeneration, but in order for Apligraf to do this, there are three important prerequisites for the wound bed. These include location, vascularity, and size.

Firstly, appropriate location for the surgically prepared wound bed include areas around teeth, implants, and edentulous ridges which contain both keratinized and non-

keratinized mucosa.

Secondly, surgical excision of the oral mucosal defect should ensure maintenance of vascular wound bed that supports diffusion of nutrients to prolong Apligraf viability, and finally, the wound bed size should be within a range of 10 to 400 mm<sup>2</sup> that permits timely resolution of wound healing by regeneration and not by scar formation.

Our hypothesis was that Apligraf would not heal the wound by scarring, but would instead regenerate site-appropriate tissue, such as keratinized tissue, or KT, and alveolar mucosa.

To test this hypothesis, there were several important study design considerations. Firstly, the procedure should be commonly performed, and for this reason we chose the tops of mucosal defects treated by FGG.

Secondly, we needed a clinically and ethically acceptable control. While the pushback with a procedure would have been the most obvious comparator from a mode of wound healing

perspective, these procedures are unethical in the context of a clinical trial given their excessive morbidity.

Thirdly, to ensure objective quantification of result, the study design must utilize available anatomical landmarks, such as the cementsoenamel junction present in teeth.

Fourthly, to test the ability of the product to generate site-appropriate tissue, we ideally need to have two different types of oral mucosa, so we can make sure that we were generating the right tissue in the right place, and in these studies, we evaluated two types of tissue, KT and alveolar mucosa.

Finally, the primary endpoint needs to be well accepted and objectively defined, and for this we used greater than or equal to 2 mm of KT in the pivotal study.

The clinical development program supporting Apligraf for the treatment of oral mucosal defects consists of two clinical studies.

A single center pilot study was conducted by Dr.

McGuire and included 25 subjects, and a pivotal, multi-center study with 96 subjects.

The primary efficacy endpoint of the pilot study was non-inferiority between Apligraf and FGG for the change in attached gingiva over the 6-month observation period. While non-inferiority was not established, the pilot study demonstrated that Apligraf-treated sites had a significant increase in the amount of AG from baseline with a mean change of 0.9 mm.

While Apligraf did not produce as much AG and KT as FGG, the pilot study indicated that Apligraf was able to produce a sufficient amount of AG and KT from a clinical perspective. Eighty-two percent of Apligraf-treated sites regenerated at least 2 mm of KT in 6 months with a mean width of 2.5 mm.

We observed an advantage for Apligraf with regard to aesthetic parameters and patient preference. Remember there is no donor site morbidity associated with Apligraf.

Histology demonstrated that while both

treatments generated keratinized mucosa, the longer ridges indicated in the righthand side of this slide suggested formation of tissue with Apligraf that was more anatomically site appropriate. It was more like gingiva and less like the palate.

Finally, Apligraf was well tolerated with a total of 22 adverse events and no SAEs reported in the trial.

This is a representative case from the pilot study. Please look at the righthand most column, which shows treatment outcomes for both FGG and Apligraf at 6 months. The FGG tissue is pale and protuberant, and contrasts sharply with the pinker adjacent gingiva.

In contrast, the 6-month outcome on the Apligraf-treated side in the bottom righthand corner, there is good color and texture match with the adjacent non-treated tissue. This is what we mean by "site appropriate tissue formation."

Given these results in the pilot study,

we embarked on a larger pivotal study to further evaluate Apligraf as a safe and effective treatment, and as a more acceptable alternative to palatal tissue in subjects with recession-type defects not requiring root coverage.

The pivotal study was a prospective, randomized, multi-center study with a within-patient control design. It was conducted at 4 U.S. centers, 2 academic centers and 2 private practices, and included 96 subjects, 8 subjects served as their own control with 1 study receiving an FGG and a contralateral study receiving Apligraf. All subjects were followed for 6 months.

Key inclusion criteria included subjects 18 to 70 years of age, who had at least 2 non-adjacent teeth in contralateral quadrants of the same jaw with an insufficient zone of attached gingiva that required soft tissue grafting.

Key exclusion criteria were Class III recession in the presence of a shallow vestibule or Class IV recession.

Patients were also excluded if they had less than 7 mm of vestibular depth from the base of the recession.

Molar teeth or teeth with Miller Grade 2 or higher mobility were excluded, as was the presence of systemic conditions or use of medications that could compromise wound healing and preclude periodontal surgery.

This included smoking within the last 3 months or presence of acute infectious lesions at the intended treatment sites.

Ninety-six subjects were randomized to treatment at Day 0, 11 training subjects were treated and evaluated per protocol, but were not included in the statistical analysis of efficacy.

The remaining 85 subjects comprised the efficacy cohort. All 96 subjects were evaluated for safety.

Subject follow up was excellent with all 96 subjects completing every required study visit, 1 week, 4 weeks, 3 months, and 6 months.

For the formal test of the primary

endpoint, we wanted to ensure at least 50 percent of Apligraf-treated sites achieved at least 2 mm of KT at 6 months. To achieve this, a one-sided exact binomial test was used.

The 50 percent standard was prospectively defined and was developed in consultation with the FDA's Dental Devices Branch.

The pivotal study also included evaluation of 6 prospectively defined secondary endpoints related to the regeneration of site-appropriate tissue and patient-reported outcomes.

The endpoints were analyzed in the order listed here, so as to preserve alpha.

The first two endpoints evaluated color and texture match comparing Apligraf to FGG.

The third endpoint assessed whether at least 80 percent of Apligraf treated sites regenerated at least 1 mm of KT at 6 months.

The last three endpoints also compared Apligraf to FGG. These included overall treatment preference at 6 months, surgical site sensitivity at 1 week, and absence of pain after

3 days.

To minimize bias in the study, calibrated examiners who were not the investigators we used for measuring KT and probing pocket depth. Although it was not possible to fully blind the study due to the characteristic appearance of the FGG-treated sites, the examiners were not told which tooth received which treatment.

With respect to demographics and baseline patient characteristics, both cohorts were very similar and representative of the general periodontal population in the U.S.

The baseline surgical site characteristics are presented here for both the Apligraf-treated teeth and FGG-treated study teeth. All study teeth had minimal attached gingiva at baseline. While there was approximately 1.4 mm of KT at baseline in both study teeth, it is important to note that this is largely removed during the surgical creation of the wound bed.

There were no statistical differences

observed for any of these characteristics. Overall, the sites were treated in a similar fashion in that both required a vascular wound bed by surgical excision down to the periosteum.

The following are photographs from a single subject in the pivotal study. The yellow arrows indicate the study tooth. Apligraf is on the left and FGG is on the right. These images nicely show the vascular nature of the periosteal wound bed prior to treatment application. Please note that both keratinized mucosa and alveolar mucosa have been excised.

To improve handling and durability, Apligraf was followed to make a 3-layered S or Z fold. Per protocol the minimum size of Apligraf applied was 5 mm width by 10 mm length. A single additional protective layer Apligraf was applied to cover the Z fold.

At the FGG-treated site, the FGG was harvested from the palate on the same side of the subject's mouth at the FGG recipient site. Only a single tooth per side was treated in this

particular patient. On the left you see Apligraf covering the wound bed, and the additional single protective layer applied and sutured in place.

On the right you see FGG. All sites used resorbable sutures.

The mean length of Apligraf was 18 mm, and the mean length of FGG was 17 mm. The length of treatment application was dictated by the number of treated teeth. Width was measured AP coronally. The mean width of Apligraf was 9 mm, and variability in the width of Apligraf varied with the width of the recipient wound bed.

For FGG, the protocol standardized the width at 4 mm. This width specification was based on the clinical experience which indicated that this width would provide a consistently acceptable clinical outcome with regard to KT and minimized the chance of poor aesthetics and donor site morbidity resulting from unnecessarily grafting wider FGGs.

Following treatment application, Co-Pac periodontal dressing was applied covering the

entire treatment area, as well as the FGG palatal donor site.

Now, I would like to move to the result section.

With regard to the primary efficacy endpoint, 95.3 percent of Apligraf-treated sites regenerated at least 2 mm of KT at 6 months. This is statistically significant when compared to the prospectively defined 50 percent success threshold.

These histograms show the distribution of KT responses by treatment site. The red line indicates 2 mm of KT and helps show how many subjects achieved at least 2 mm. Apligraf-treated sites regenerated a mean width of 3.2 mm.

There were 4 Apligraf-treated sites that did not have 2 mm of KT, and in all 4 cases, 1 mm of KT was regenerated at 6 months.

The FGG-treated sites had a mean KT width of 4.6 mm, which is an expected result given the fact that it's a graft.

The baseline 6 months and change from

baseline for both KT and AG are shown for both Apligraf and FGG-treated sites. Apligraf-treated sites regenerated clinically and statistically significant amounts of both KT and AG. Please note the close relationship between KT and AG with respect to change from baseline.

The protocol allowed for treatment of up to 3 adjacent teeth per side. Over half of subjects had a single tooth treated and approximately 25 percent of subjects had 2 teeth treated, and 15 percent had 3 teeth treated. The results on multiple teeth were the same as those for single teeth.

Moving to the secondary endpoints, the first and second endpoints were color and texture match. These are critical measures of site-appropriate healing and are important to the patient by virtue of a superior aesthetic result.

In 92.9 percent of Apligraf-treated sites, there was a color match compared to only 27.1 percent of FGG-treated sites. This was statistically significant.

Similarly, the data showed that in 95.3 percent of Apligraf-treated sites, there was a texture match compared to only 54.1 percent of FGG-treated sites. Again, this was statistically significant.

To graphically illustrate these findings, a subject from the pivotal study, who was seen at 13 months post procedure, is presented here.

On the left side of the image, the FGG-treated site has clear demarcation of the FGG and poor color and texture match with the adjacent non-treated tissue. On the right side, Apligraf has regenerated tissue that is indistinguishable from adjacent gingiva.

All Apligraf-treated sites regenerated at least 1 mm of KT. This secondary endpoint was statistically significant.

For the fourth endpoint, subjects were asked at 6 months to indicate their preference taking into account all aspects of treatment, such as surgery, recovery, and appearance. Seventy-two percent of subjects preferred

Apligraf over FGG. This result was statistically significant, as well.

The fifth and sixth secondary endpoints were related to the subject's assessment of site sensitivity at 1 week and pain at 3 days. Site sensitivity was not statistically significant, therefore, in accordance with the statistical analysis plan, surgical site pain was not formally tested.

So, in summary, 4 of the 6 secondary endpoints - color, texture, KT greater than or equal to 1 mm, and patient preference were all statistically significant.

I will briefly discuss 2 additional analyses based on the pilot and pivotal studies.

These are both more fully described in your briefing package.

Histology was reassessed to see whether there was a correlation to explain the clear differences in clinical appearance between Apligraf and FGG-treated sites. An independent blinded photographic analysis using photographs

from the pivotal study was undertaken to corroborate the unblinded investigator's assessment of color and texture.

We also conducted a biomarker study looking at the quantity of angiogenic proteins at the control and test sites over time with the hope of correlating these protein levels to clinical outcome.

No correlation was found, and for the sake of brevity, I will not discuss this further today.

There were 7 subjects from the pilot study that had 3 mm biopsies obtained from both the Apligraf and FGG-treated sites at baseline and at 6 months. The tissues were processed using standard histological methods by a blinded examiner.

Here, you see the various stains we used to identify the structures of interest. Previous studies suggested the differences in collagen architecture, vascularity, elastin, and tenascin in particular would be able to discriminate

between the different types of mucosa in the oral cavity, and we were interested in discriminating between alveolar mucosa, gingiva, and palatal mucosa.

Although the histology study was not powered for a vast statistical analysis, some trends were observed. Both treatments resulted in a change from baseline, which was mostly alveolar mucosa, and the differences in clinical appearance of Apligraf was possibly related to subtle differences in the presence and distribution of collagen, elastin, and tenascin.

Overall, Apligraf-treated sites were found to result in the formation of tissue more like gingiva, whereas, the FGG-treated site tended to retain the characteristics as expected of palatal mucosa.

Three independent calibrated reviewers assessed the 6-month photographs from the pivotal study in a blinded and randomized fashion. They evaluated four parameters based on a root coverage aesthetic score published by Cairo in

2009.

These included color, presence or absence of scar, mucogingival junction alignment, and marginal tissue contour.

The bottom panels are examples of the 6-month photographs that were analyzed by the blinded examiners. Looking at the parameters of color, mucogingival alignment, and presence or absence of scar-like tissue, these photographs demonstrate again the tire patchlike appearance of FGG-treated sites and the more site-appropriate tissue that is being regenerated as a result of treatment with Apligraf.

All reviewers determined that Apligraf achieved superior results compared to FGG in terms of color, texture, and alignment of the mucogingival junction.

For the assessment of color, which was evaluated in the same manner as the pivotal study, there was strong agreement between the reviewers and the clinical trial data. No significant difference was observed in the

marginal tissue contour.

I will now move on to the safety section of the presentation.

The clinical safety section of our presentation will cover three main areas. I will begin by presenting an integrated safety summary and then move on to a specific safety analysis of the pivotal trial. I will end this section with a summary of the immunology and persistence data for Apligraf.

To put the use of Apligraf and the present indication in the proper context, I would like to remind the panel that Apligraf has been on the market for 13 years and more than 420,000 units have been shipped for patient application.

Since initial approval, a total of 9 medical device reports has been submitted. As Mr. Bilbo mentioned in the introduction, we also have Apligraf clinical experience from 19 clinical trials in various indications including 10 trials in acute conditions involving wound beds that have the same vascularity as in the

present indication.

In the oral clinical program supporting the proposed indication, a total of 121 patients were treated with a single Apligraf application, 25 of which were in the pilot study, and 96 subjects in the pivotal study. All subjects were followed for 6 months.

Overall, 41 of the 121 subjects experienced a total of 65 AEs during the 6-month studies. There were no deaths. There were no unanticipated adverse device effects. There were 3 SAEs, which will be discussed in detail in the pivotal study section.

In the pivotal study, there were 3 AEs at the Apligraf-treated site and 4 at the FGG donor and recipient sites. These AEs will also be discussed further in that part of the presentation.

With the exception of the infections and infestations, and gastrointestinal disorders, system organ classification, or SOC, these had fewer than 5 percent of subjects reporting an AE.

Given that Apligraf comprises viable allogeneic cells, I will also specifically review immune-related AEs and neoplasms.

First, looking at infections, 13 subjects reported a total of 16 adverse events. Most AEs were related to the upper respiratory tract and occurred in 1 or 2 subjects. There were 5 subjects that reported sinusitis during the study. There was a single report of oral herpes -- correction -- the single report of oral herpes was not located at either the Apligraf or FGG-treated sites.

In the gastrointestinal SOC, 12 subjects reported a total of 13 AEs. No single AE was reported in more than 2 subjects.

In the immune system disorders SOC, 4 subjects reported a single occurrence of hypersensitivity. Importantly, the term "hypersensitivity" is a coding term, and not a clinical diagnosis. Three subjects were reported to have one adverse event each of allergies, and one subject reported an adverse event of

environmental allergies. There events were all not related to Apligraf.

There were 2 malignancies reported in the pivotal study, 1 subject with a previous history of hypothyroidism was diagnosed with a follicular thyroid cancer on Day 92. She underwent same day surgery of the left thyroid for removal of a hurthle cell lesion. This was assessed by the investigator as not related to Apligraf, and was not reported as an SAE.

Another patient with a pre-existing mediastinal mass was diagnosed with a metastatic malignant fibrous histiocytoma, or MFH, on Day 154. This was reported by the investigator as unlikely to be related to Apligraf.

Moving to the pivotal study, there were a total of 3 SAEs reported. One SAE was the MFH already discussed on the previous slide. Another patient was hospitalized with pneumonia for 4 days starting on Study Day 100.

The third SAE involved a patient hospitalized with a diagnosis of chest pain on

Study Day 7. Subsequent to the hospitalization, the subject was re-evaluated and diagnosed with bursitis and tendinitis of the left shoulder.

Five anatomical locations were specified per protocol: the Apligraf-treated site, the FGG recipient site, the FGG donor site, and the mouth in general. AEs occurring at any other location in the body were recorded as "Other."

Additionally, in the pivotal study, anticipated post-surgical sequelae were not reported unless a treatment or procedure was required or there was unusual severity or duration.

There were 3 AEs that occurred at the Apligraf treated site. There was 1 report each of gingival pain, gingival injury, and mouth ulceration. All three events were rated as mild in severity. The gingival pain and gingival injuries were both subsequent to inadvertent placement of the Apligraf trans-membrane at the time of Apligraf application.

Both events were assessed as related to

treatment, and the trans-membrane was successfully removed from the subject's gingiva, and no further sequelae were reported. Both of these subjects regenerated at least 2 mm of KT at 6 months.

At the FGG-treated sites which included both the recipient and palatal donor sites, there were a total of AEs reported. At the recipient site, there was a report of severe gingivitis and mild desquamation.

At the palatal donor site, there was one report of post-procedural hemorrhage and one report of thrombosis. The post-procedural hemorrhage was rated as mild and the thrombosis was rated as moderate.

There were 5 AEs that were reported in the mouth. These AEs were general and were not specific to Apligraf, FGG recipient or donor site.

Apligraf is an allogeneic tissue, however, it does not elicit an immune response. This is due to four main reasons. It contains

minimal antigen presenting cells, it has no co-stimulatory molecules or T-cell stimulation, it does not vascularize, and it has a transient persistence.

In a nonclinical model of re-challenge and sensitization, Apligraf did not undergo rejection, whereas, the positive allograft control did.

Repeat application has been performed in the human clinical setting for chronic wounds, and is not associated with any manifestation of hypersensitivity, rejection, or diminution of efficacy.

In 7 highly vascular acute cutaneous wounds clinical trials, immunological tests were performed looking for evidence of sensitization to the components of Apligraf. These included antibodies to Type 1 bovine collagen, antibodies to HLA antigens, and assays to T-cell sensitization.

Apligraf was not found to induce a significant humoral or cellular response, and was

not observed to have any clinical signs of rejection.

Nonclinical studies have also been performed looking at persistence of Apligraf cells. These studies involved grafting experiments of Apligraf onto athymic mice with excision of Apligraf and adjacent tissue at various time points up to 6 months.

Human cells were detected using a DNA probe to the unique human ALU repeat sequence. Human cells were found to remain confined to the site of grafting at all time points.

While there was no evidence of cell migration locally, formal systemic biodistribution studies have not been performed.

There were 2 subjects in the oral pilot study evaluated for persistence of Apligraf DNA at 6 months. Detection of Apligraf persistence was performed using PCR on both 15 short tandem repeat loci and the amelogenin locus. Neither subjects showed evidence of persistence which was consistent with previous studies.

In addition, a periodontal publication by Nevins in 2010 demonstrated no evidence of DNA persistence in 4 subjects that were each evaluated at a single time point, either 20 days, 29 days, 35, and 45 days.

These results are consistent with the fact that Apligraf does not vascularize and persists long term.

These results in the oral cavity indicating transient persistence are consistent with our clinical trial data in acute cutaneous wounds and published data which suggested Apligraf DNA can be detected up to 6 weeks, but in the majority of cases is absent by 4.

In summary, the safety of Apligraf is supported by over 20 years of clinical experience involving 19 clinical trials and over 420,000 units shipped for patient use. Pre-clinical studies suggest that Apligraf is immunologically inert and clinically, there has been no immunological or tumorigenic signal.

Overall, our safety data indicate that

Apligraf can be safely administered to patients.

In the oral studies, AEs were typically non-serious and easily managed.

To ensure safe use in the clinical setting, labeling will provide clear instructions on Apligraf use and medical support will be made available to clinicians who use Apligraf.

Given the favorable clinical safety profile and extensive cutaneous postmarketing experience, we believe Apligraf can also be used safely and appropriately in the treatment of oral mucosal defects.

Dr. Michael McGuire will now address the benefit/risk profile of Apligraf.

#### Benefit/Risk Summary

DR. MCGUIRE: Good morning, everyone. I am Mike McGuire. I am a periodontist in private practice in Houston, Texas. Our practice has an emphasis on periodontal plastic surgery especially as it relates to the resolution of oral mucosal defects.

Along with our private practice, we also

have a practice-based clinical research center. We performed the pilot study, also served as PI for the pivotal trial in which our site was one of the four centers.

I probably have more clinical experience with live cell base devices as anyone else in the world, and it is with that research experience and my perspective as a private practice clinician for over 30 years that I would like to share with you our experience with Apligraf in the oral environment.

As has been suggested this morning, a certain amount of keratinized tissue is necessary in order to maintain health and comfort whether it's around an edentulous ridge adjacent to an appliance or adjacent to implants or teeth.

On the panel that you see highlighted in yellow you see a patient who has already experienced some gum recession. Here, we have just freely movable mucosa, little to no keratinized tissue. This can be a dilemma for the patient because if you don't have keratinized

tissue, and you begin brushing and flossing, oftentimes it makes it sore, it is difficult to do that.

Obviously, if that is the case, our patients don't clean well. That leads to bacterial plaque accumulating at the gun margin.

That can lead to dental decay, can lead to tooth loss. That gingival plaque also leads to inflammation of the soft tissues, periodontal disease, increases our patients' inflammatory burden, and can really be a problem for our patients long term, causing increasing recession.

Now, there is a solution for that problem, and that problem can be fixed in that we have a very effective procedure called the free gingival graft. The free gingival graft has been around for over 50 years.

There is no doubt it is one of the most effective procedures in all of periodontics. It works, but its limitations are also well documented, that there is a limited amount of donor tissue available, and the big limitation is

the secondary surgical site, the donor site, in order to harvest the tissue.

You can see in the panel highlighted in yellow where I have removed a strip of tissue to utilize for the graft. The greater palatine artery and nerve is in that region. I think you can appreciate that there is some bleeding points there that can be problematic.

Here, you see that same patient with a graft. Now, it has worked. It has done a great job. It is unlikely this patient is going to have further recession. You don't see inflammation.

They are going to be comfortable with their normal daily home care procedures, but it would be nice if we had a procedure that would allow us to do that without a donor site and also one that would yield a more aesthetic result.

On the top panel you see one of our patients in the pivotal trial. Now, if you look to the left panel, you see just freely movable mucosa. I think if you look closely, you can

actually see through that tissue. You are seeing the root surface underneath there.

This kind of situation is very prone to recession, again, an area that could be more difficult for the patient to claim because of sensitivity. In the center panel we have created the recipient bed, harvested the tissue from the palate, and sutured it on, and on the right panel you see a very typical look for a free gingival graft. It is very effective. This is successful. This is how we have done it for years.

On the lower panel, again, you see on the left just freely movable mucosa, no keratinized tissue there. We create the recipient bed in the center and place the Apligraf. Then, on the right side you see a nice functional zone of keratinized tissue created. In this case, we have created approximately 4 mm of keratinized tissue, same amount as we have created on the top, but we have done this without a donor site.

If you compare the two slides on the right side, on the top, the free gingival graft,

on the bottom the Apligraf. You will see with the Apligraf that we are achieving one of the major goals of periodontal plastic surgery, and that is to be able to generate tissue that is indistinguishable from what nature intended. You will always see the graft on the top of the free gingival graft, because it is a graft. The Apligraf does not work as a graft, it works for enhanced secondary intention and yields tissue very close to what we would expect in nature.

Now, anytime I sit down to talk to a patient about treatment, I always review benefits, risks, and options.

When it comes to Apligraf, there are many benefits. Apligraf creates a meaningful amount of keratinized tissue. Ninety-five percent of our patients had at least 2 mm of keratinized tissue created with a mean of 3.2 mm. It's predictable, it's aesthetic, 72 percent of the patients preferred Apligraf over free gingival graft when considering the entire experience, and most importantly, again, we did it without a

donor site.

When it comes to risk, yes, there are surgical risks. There are surgical risks anytime you sit down and do an oral surgical procedure. The surgical risks associated with this procedure are the same ones that happen anytime I sit down to do surgery, pain, bleeding, swelling, infections, things that we deal with every day and manage effectively.

There was in the pivotal trial, an inadvertent implantation of the polycarbonate backing with the Apligraf, but it was easily removed. Even those two patients generated 2 mm of keratinized tissue.

There, of course, could be possible product risks, possible allergic reaction to bovine collagen and other proteins. It is also possible that there could be risk of disease transmission from donor cells, but as you have heard today, this product has been used for 13 years and over 400,000 applications, and it has an extremely high safety profile.

If a problem should occur, this type of patient is followed very closely, so if a problem should occur, it will be easily identified and treated. These type of patients are not the kind of surgery, you have the surgery, and the patient is just lost to follow-up. They generally are seen a week later, a month later, and six months later.

The product uses standard surgical techniques. Anybody doing oral surgery is well trained in these kind of procedures. There is a small learning curve related to product handling, but that can be easily taught with probably less than an hour's worth of training.

When it comes to options, they are pretty obvious. I think from the day the very first free gingival graft was performed, both clinicians and patients alike have been looking for an alternative that does not involve harvesting from the palate.

With Apligraf we have that alternative. We have an alternative with minimal and

manageable risk and clear benefits to the patient.

When it comes to the patient, that is why we are here, right? Every week in my practice I have patients who choose not to have the grafts they need because of pain, fear of pain.

When you go to the dental office and see your dentist, what is the dental injection that most of us fear the most? For most of us, it's palatal injection. Translate that from injection to taking a strip of tissue from your palate.

Every week in my office, I have patients who choose to have only those teeth that need the grafts the worst treated, because there is just a limited amount of donor tissue available. It is asking a lot of our patients to have that tissue harvested, let it heal again, come back, harvest it again. That is asking a lot.

Every year I am awakened in the middle of the night with a frantic patient on the other end of the line with palatal bleed. It is one of the few dental emergencies that I cannot manage over

the phone. Almost all of these adverse events can be avoided if we can eliminate the palatal donor site.

With Apligraf, we have that ability. It provides an option that is safe, effective, less invasive, and more aesthetic, it is an option that I hope the Committee will look favorably upon.

Thank you very much.

#### Conclusions

MR. BILBO: Thank you, Dr. McGuire.

Today, we presented Apligraf as an important new treatment option for regenerating site-appropriate oral mucosal tissue. This has been demonstrated through two clinical studies that showed improved quality of tissue, as well as function and aesthetics similar to surrounding untreated tissue.

The oral clinical studies also showed Apligraf to be well tolerated and have a safety profile consistent with a large cutaneous wound healing experience.

Apligraf also eliminates the need for a donor site in the palate, and there is unlimited material to allow for improved patient care.

In conclusion, Apligraf is a safe and effective treatment of surgically created gingival and alveolar mucosal surface defects that provide significant benefits for patients.

We thank you for your attention today. In addition to Dr. McGuire and Dr. Cochran, we have the following experts here available to answer your questions.

DR. DUBINETT: Thank you. We have time now for questions. Yes.

DR. COUTURE: I wonder if we can just start off by asking some questions about the manufacturing process since it is one of the big issues here is about lot release testing, et cetera.

It wasn't entire clear to me exactly how you were doing lot release testing in regards to cytokine expression and the percutaneous water absorption test, and whether that was actually

being done on an expanded sort of equivalent expansion from a master working cell bank or if it is actually being done on the final product.

Let me just throw a second question now to make it confusing, but I wonder if you could define to me exactly what you consider to be a lot, and whether the tests that are performed on the final product are actually being performed on every individual little Apligraf that goes to a clinician or whether it is maybe being performed on a sample of a collection of these things. Again, that is in the context of what you are defining as a lot.

MR. BILBO: Certainly. Just in terms of the definition of a lot of Apligraf, it consists of up to 200 units, and a lot is defined at the starting point of the manufacturing process wound, the first step when we combine the fibroblasts with the bovine collagen.

I will bring up Dr. Pitkin to elaborate on the testing that we do, but in terms of lot release, the tests that we use primarily for lot

release is histology as a potency assay. We do that because it provides extensive information on multiple parameters. Each of those parameters are associated with biological attributes to the product.

So, for this complex tissue-like product, we have determined that the histology is the most appropriate test for the product.

DR. COUTURE: I am sorry, then, my question wasn't really quite clear. In the monograph here that we have, you do some cytokine testing, and you do a bunch of other tests, the percutaneous water absorption test, and I believe that was done on cells from the master cell bank, is that correct?

Is that part of the lot release testing or master cell bank release testing, is that done on every master cell bank?

Is it only done in the master cell bank, and again, are those tests that you do, for example, H&E stain done on every single Apligraf that goes in, or is it only on the representative

sample of your lot?

MR. BILBO: In terms of the actual testing for percutaneous absorption, VEGF, that is done on the finished product. In terms of the cell bank testing, we would evaluate at the end of that testing on the finished product, those tests. I would like to bring up Dr. Pitkin to elaborate on that.

DR. PITKIN: Thank you. Good morning. My name is Zorina Pitkin. I am Vice President of Quality Systems at Organogenesis.

I would like to answer first your first question regarding testing for cytokines and percutaneous absorption. These tests are performed as part of our characterization or comparability panel for every single cell bank that is created for both keratinocytes and fibroblasts.

These tests are performed on the product that is made -- slide up, please -- these tests are performed on the product that is made from the cells that are being evaluated, so as part of

our comparability testing for each cell bank.

With regard to lot release, we perform multiple in-process testing, and due to the nature, destructive nature of histology, we cannot perform this test on every single unit, therefore, the histology technique is used to assess potency with multiple quantitative parameters on representative samples from each lot of Apligraf that is released for clinical use.

Slide up, please.

I would like to further elaborate on our in-process and final release testing for each lot. As Mr. Bilbo described, a lot represents up to 200 units. We perform in-process testing at multiple steps that are considered to be critical steps of our manufacturing process, and are listed on the left side under "In process safety Testing."

To further minimize the risk of potentially introducing contamination in the product, we have implemented in rapid sterility

test method that has enabled us to detect any contamination very, very rapidly.

The testing that is performed, is performed on both cells upstream of the process and on spent media or we can call it supernatant or any media that has been in contact with the cells or with the construct for a period of time.

DR. COUTURE: If I can go on, or follow up anyway. So, cytokine testing done on cells from your master cell bank, it is effectively an equivalent product, is that correct?

DR. PITKIN: That is correct.

DR. COUTURE: So, that is not a nondestructive test, is that correct, cytokine testing?

DR. PITKIN: Cytokine testing is a destructive test.

DR. COUTURE: It is?

DR. PITKIN: Yes, it is performed on the product that is made from the cells that are being evaluated on cell bank, so it is on the product that is made as part of that evaluation.

DR. DUBINETT: Dr. Snyder.

DR. SNYDER: I was wondering, in terms of the comparison between -- actually, I have two questions -- so, the first question is in comparing Apligraf with FGG, you talked about pain and texture and things of that sort, are there functional tests that can be applied to show that the outcome is functionally equivalent, and in the briefing package, there was one allusion to resistance to muscle pull.

That, to me, would sound as if what one really is looking for is, is the product giving you what you want, which is immobilization of the teeth and diminishing the loss of teeth.

So, I was wondering how that is tested and how those compare.

Then, I wanted to hear a little bit more about the mechanism of action of the Apligraf, and is that the same, or how does that differ from FGG in terms of mechanism of action.

I found it interesting, for example, that you look for VEGF production, but yet you

indicate that there is no vascularization of the graft.

So, if you could maybe address those two issues.

MR. BILBO: So, two questions, one related to possible functional tests for evaluating the outcomes, and then the second, understanding the mechanism of action of the product.

Dr. Bates.

DR. BATES: Dr. Snyder, I will give you a brief answer to the first part of your question.

I think it would be beneficial to have one of the clinicians speak about the type of keratinized tissue that is being produced, and how that compares to the type of keratinized tissue that you get with FGG.

The first thing I would like to say is that the amount of KT that we generate at 3 months is stable through to 6 months, and the sixth month amount of KT is at least in the periodontal literature, a very good indicator of

long-term keratinized tissue.

We did look at muscle pull, and I can show that as data, if we can have slide up, please.

This slide summarizes muscle pull resistance. I would like to say at the outset that there was uniform confusion, if you will, with the interpretation of the terms "no resistance" and "resistance." Let me explain that.

When you are talking about resistance, basically, the manual maneuver that you do is to grab the cheek and pull it, and what you are looking for is resistance to that force, because you want the tissue to stay where it is supposed to be. For the keratinized tissue, we kind of adhere down next to the root surface and not move.

If we are looking at the terms, resistance is the correct term, but if you look at the percentages achieved, that outcome, it is almost as if you would flip the terms of

resistance and no resistance about FGG, that would make more clinical sense.

So, if you understand what I am describing in terms of resistance to that pull, look at the percentages, for instance, on Apligraf on the lefthand column, where you see an increase from 70.6 percent to 85.9 percent.

On an FGG, you see 74.1 to 96.5 percent, so on both cases you see an increased resistance to muscle pull even though the terms have been inconsistently applied in this particular table.

The results that you can see are statistically significant.

I will bring up a clinician to talk about the quality of keratinized tissue in just a moment, but I would like to address the second part, the second question, because it is a really important question and fundamental.

FGG is a graft, so basically, you take tissue from the palate, and it retains its characteristics of origin. You need a vascular wound bed. It has got its pre-existing

vasculature inside it. You put it on the wound bed and then you get inosculation, which is linking up with the blood vessels, and you get angiogenesis, as well, and it is going to retain its characteristics.

Apligraf, in contrast, doesn't have a pre-existing vasculature in its matrix, and, in fact, it has a bovine collagen layer, and it seems like that the bovine collagen layer is a barrier, if you will, to vascularization, so it never gets vascularized. It has a transient resistance.

Your question is about VEGF is still relevant, because the mechanism of action is complex, we don't fully understand it, but we consider that the elaboration of cytokines and growth factors, things like VEGF, are important in terms of their effect on the wound bed in terms of how rapidly the epithelial cells migrate and underneath it, how much vascularization granulation tissue that you would have.

If I could sort of illustrate that by

bringing up another slide, there was a publication back in 1983, which looked at secondary intention healing alone, if you will, because we are talking about what the effect of Apligraf is on secondary intention healing.

This particular publication by Wenstrom looked at gingivectomy, which is essentially creating the same type of wound bed and just letting it heal on its own.

If we could have this slide up, please. On the very top row you can see the results in that Wenstrom paper. The tissue basically, it heals, but it goes back to baseline conditions for both KT and AG, whereas, with Apligraf, the effect of the barrier function, the elaboration of cytokines, we see a change from baseline for both KT and AG, so you are not just accelerating healing back to the baseline conditions, you are actually changing through the trajectory of healing, so you are actually generating new tissue, new phenotype of tissue different from baseline.

If I could have Dr. Nevins address your first question.

DR. NEVINS: Thank you. Dr. Mark Nevins, one of the pivotal investigators.

I would like to present two points to try to answer your question. I think the first point in demonstrating the longevity of the tissue, if I can have the clinical follow-up slide.

We have had the observation of the patients in the pivotal trial in practice beyond the six-month time point, and we have been able to observe what appears to be even increased stability of the site.

If I could have the slide up, please.

What is of note here is that as you are at the 3-month to 6-month time point, there is even some difficulty in determining the mucogingival junction. We use the Schiller's iodine stain to aid in this measurement during the trial, perhaps even some crossing over at the layers between the keratinized tissue in the alveolar mucosa.

As the tissue matures it appears to be in two-year photograph even a more apical displacement at the mucogingival junction representing the maturity of the tissue that was augmented during the clinical procedure.

In addition, in regards to the method of action, it is definitely a paradigm shift from a clinician's point of view, the fact of the method of application, and that is where the persistence data from my own clinical observations that I had done previously demonstrating the lack of persistence to make myself understand that.

The second point is, and some other surgical experience is using the Apligraf product in sites where there is edentulous sites outside of pivotal trial to experience it, I have been able to go back to those sites surgically once it is healed even after two months and create other surgical flaps either for implant placement or other procedures, and have not had any wound healing problems with the product.

DR. DUBINETT: Can you tell us if there

are patients with chronic smoking histories included in the studies?

MR. BILBO: Patients with --

DR. DUBINETT: Chronic smoking histories.

MR. BILBO: -- chronic smoking histories.

Dr. Bates.

DR. BATES: Dr. Dubinett, we excluded patients that hadn't smoked within the last three months, but if you ignore that, further back in history, from memory, it was around 40 percent of the subjects had a smoking history.

DR. DUBINETT: And was there a difference in the outcomes for smokers versus nonsmokers?

DR. BATES: If you just give me a moment.

[Pause.]

DR. BATES: We didn't specifically do that analysis.

DR. DUBINETT: Okay. Were there patients included that had a history of oral premalignancy?

DR. BATES: Obviously, patients would be excluded if they had a concurrent history with

respect to a history of oral -- I am not aware of any.

DR. DUBINETT: But patients with a history of oral premalignancy were excluded?

DR. BATES: Excuse me for a second.

[Pause.]

DR. BATES: Yes, if they have a history of it, that had been treated, they were able to be included in the study.

DR. DUBINETT: They were able to be?

DR. BATES: They were able to be.

DR. DUBINETT: But you don't know how many patients had that history?

DR. BATES: I don't have those data at the present time.

DR. DUBINETT: Dr. Lee.

DR. MEI-LING LEE: Thank you.

Could you give us more information on the non-inferiority of Apligraf in comparison with free gingival graft? Thank you.

MR. BILBO: Dr. Lee, are you referring specifically to the pilot clinical study where

that was the statistical analysis?

DR. MEI-LING LEE: Yes, and also the other study. Yes, the pilot study.

MR. BILBO: The results of the clinical experience relative to free gingival graft?

DR. MEI-LING LEE: Right.

MR. BILBO: As already noted in the presentation, the success rate of free gingival grafts is very high, you know, almost 100 percent. We determined based on the pilot study that with the different modes of healing with the product, Apligraf not functioning as a graft, we used a 50 percent threshold as the primary efficacy endpoint, so we weren't directly comparing to free gingival graft for the pivotal clinical trial.

I would like to bring up Dr. Bates to expand on that.

DR. BATES: Dr. Lee, if I understand your question correctly, you are specifically interested in the pilot study, and you want to look at the amount of AG for Apligraf versus FGG,

is that correct?

DR. MEI-LING LEE: Yes. First, the pilot study and then if you also can extend to the general.

DR. BATES: If you would just give us a moment, we will pull up those data for you.

Could we please have slide up.

This slide, Dr. Lee, summarizes the results from the pilot study where we are looking at attached gingiva, the baseline measurements, the six-month measurements, and the change.

DR. MEI-LING LEE: Thank you. Can you also go to page 38?

DR. BATES: Of my presentation, the efficacy?

DR. MEI-LING LEE: Yes.

DR. BATES: Yes. Slide up.

These are the results from the pivotal study where you can see the baseline, divided up into keratinized tissue and attached to gingiva, and you see the baseline, the 6-month results, and the change from baseline.

What I wanted to highlight in my co-presentation is that if you look at the change in baseline of both KT and AG, the measurements are the same, so we are using UKT, which is effectively AG is attached.

DR. MEI-LING LEE: So, was the confidence interval about the same for non-inferiority for both treatments?

DR. BATES: I would like to call up Dr. Gene Poggio to address that question.

DR. MEI-LING LEE: If you look at FGG, it is 4.6 for the 6-month result, it is 4.6 plus or minus 1 and compared with the Apligraf 3.2 plus or minus 1.

DR. POGGIO: Hi. My name is Gene Poggio. I am the chief biostatistician at Biostatistical Consulting, Inc. I think in the pivotal, it wasn't designed as a non-inferiority study.

DR. MEI-LING LEE: It was not designed.

DR. POGGIO: It was not. I think there was a recognition in the pilot study that I think from my point of view, two things were learned,

one, that it isn't non-inferior, but that sufficient amounts of KT and AG were generated, and so an effective product nonetheless. Based on what was learned in the pilot study, the pivotal was designed more with an objective performance criterion.

DR. MEI-LING LEE: But for this case, for the 6 months, KT, would the FGG generate more? I mean do you have the comparison whether they are significantly more than the Apligraf?

MR. BILBO: Yes, there was certainly significantly more keratinized tissue with the free gingival graft procedure at 6 months.

DR. MEI-LING LEE: For Apligraf.

MR. BILBO: It wasn't a direct comparison for the final primary efficacy analysis for the product.

DR. MEI-LING LEE: Yes, it is just not clear from this table. Thank you.

DR. DUBINETT: Dr. Hwu.

DR. HWU: I have some questions about the immunologic studies. The alloresponse is such a

tone response normally, could this possibly be an explanation for some of the transient nature of the survival of the graft? And how many donors were there per lot? That will tell you about how much mismatch you might expect between the recipient HLA and the cells that were actually in the material in the product.

There is a lot of detailed studies about the past cutaneous experience, but really no experience here, and there could be differences between -- we know there are differences in the immune response to cutaneous surfaces compared to mucosal surfaces in terms of chemokines and trafficking of immune cells, and a lot of those studies were circulating serum antibodies, circulating T-cells, and not really at the tissue site where you wouldn't really see the action. It would be infiltration of immune cells and antibodies at the tissue site itself.

Can you address some of those issues?

MR. BILBO: I think you have three questions. One relates to possibly the transient

persistence of the product may be related to immunologic response by the patient.

Second, mismatch of donors, so you are trying to understand in terms of the tissue that we use to create the cell banks, is there matching.

Thirdly, how the cutaneous immunological experience is representative for the oral environment.

Just in terms of the donors that we use for our cell strains, we take the tissue and we separate, disaggregate the tissue and we create - - fibroblastic cell banks, we create keratinocyte cell banks, and we don't have any matching within the process, so a particular lot of product may have one strain of keratinocytes, and a separate strain obviously of fibroblasts, but unlikely to be from the same donor.

DR. HWU: I see, but it is one donor, it is not pooled, multiple donors for keratinocyte -

MR. BILBO: No, for each cell type it is

one donor.

DR. HWU: Each product is one donor.

MR. BILBO: I would like to bring up Dr. Bates to respond to your other two questions.

DR. BATES: You are absolutely correct. In the case of the skin allograft, you would expect a vigorous immune response. I think that is a good segue for me to again highlight to the panel the differences between a skin allograft and Apligraf.

The big difference is the fact that the skin allograft has got antigen presenting cells, Apligraf has a minimal number of antigen presenting cells in it. There are no co-stimulatory molecules as far as the keratinocytes, and the fibroblasts are concerned necessary for T-cell stimulation.

The question about the limited persistence, you know, vascularization versus immunology, taking a page from my own clinical experience, if you graft something and it doesn't vascularize within a 5-day window, those cells

undergo ischemic cell death.

A publication by Griffiths in 2004 firmly established that there is no vascularization of Apligraf, and we think that is the most probable explanation for the limited persistence of the product.

So, there are some of the product attributes. We have conducted three clinical studies, most notably a paper published in 1999 by Briscoe, which was a humanized scid mouse model, where we had a positive control, which was the skin allograft, and we had Apligraf grafted.

Please note that it is interesting, the smaller wounds in the back, on the dorsum of the mouse. Apligraf actually engrafts, which is not what happens in the human clinical situation, and we believe that is because of bridging rather than angiogenesis from the center.

But in those studies, the skin allograft rejected by Day 21, whereas, the Apligraf didn't, and we tried very hard to sensitize the mouse model to Apligraf, and we did that in a number of

ways where we exposed Apligraf to interferon gamma to try and upregulate Class II HLA expression, and that didn't lead to rejection.

We also injected keratinocytes that were stimulated with interferon gamma before application of the Apligraf to try and sensitize that, and again we didn't see any evidence of rejection.

We also put sensitized PBMCs after engraftment had occurred, so there was a vasculature in the Apligraf, and that didn't lead to rejection either.

As I tried to provide an overview in my presentation, we have looked at 242 subjects in acute studies. Cutaneous wounds, highly vascular wounds, and we haven't seen any significant humoral immune response to it, and the most likely explanation is because it doesn't get vascularized.

DR. HWU: That's circulating, right? Humoral, you are talking about, you checked the blood. Have you looked at the tissue site

itself?

DR. BATES: In terms of the biopsy?

DR. HWU: Yes.

DR. BATES: We have, and what we see there is mostly a neutrophil infiltrate, not T-cells. We see a limited, in the nonclinical model that I was describing to you where Apligraf had already been engrafted, and then it stays within infused. We saw a limited CD3-positive T-cell infiltrate to the base, but it was nothing like what we saw in the positive control.

Yes, so the humoral, my use of the term "humoral" is antibodies to bovine collagen, antibodies to HLA antigens, et cetera.

With respect to your question about differences in oral mucosa versus skin as far as the immunity is concerned, I think Professor Wood would be best qualified to address that question.

DR. WOOD: Kathryn Wood, Professor of Immunology at the University of Oxford.

I think the Company has done extensive studies on the both cellular and humoral response

after application at cutaneous sites, so they have looked for the development of antibodies circulating in the serum, anti-HLA antibodies and anticollagen antibodies, as Dr. Bates suggested.

There is no evidence for a response.

They have also looked for T-cell responses after application at cutaneous sites, and again there was no evidence for T-cell stimulation by the alloantigens presented in the Apligraf construct. So, I think they have got extensive evidence that there is no significant cellular or humoral response at the cutaneous sites.

As you quite rightly say, there may be differences in the cutaneous sites versus the oral sites, but actually, if you look at the evidence, the antigen presentation at oral sites, there is some data to suggest that actually it is less responsive than at the cutaneous sites, and therefore, although there is no direct evidence from the clinical studies performed, I think it is reasonable to assume that there is going to be

no more response than was observed in the extensive analysis at cutaneous sites, and therefore unlikely to be an adverse event associated with the oral application.

DR. DUBINETT: Dr. Couture.

DR. COUTURE: Again, one of the major questions we are being asked to address is whether H&E staining is an adequate measure of potency for this product, so I think it is probably worth focusing on that issue for a moment.

I appreciate that a lot of these tests -- these are presumptions, so correct me if I am wrong -- were established, the clinical trials were established when this product was considered a device, and then the Agency, I think rightfully so, recognized this is probably more of a biological, leading component of it, the primary component of it is the cells that are involved in this.

So, while I can understand that H&E staining would certainly be an indicator of

structure of the material, and certainly a test or an indicator, a surrogate for percutaneous absorption, it is not entirely clear to me how we go from that to testing the true potency of their product with an H&E stain, which is supposed to be measuring some of the other characteristics of the material, which is cytokine release, et cetera.

I understand that some of the master cell banks from your document, or at least one in one example that was presented, failed percutaneous absorption because by H&E staining, it was not, or it failed H&E staining.

It seems to me that if the structure completely fails to form, it would be pretty much straightforward that it is going to fail percutaneous absorption.

It is not clear to me whether a structure that has H&E staining also has all the other biological characteristics that it needs to have its function in vivo, which is cytokine release at least according to the way you have described

the potential or presumed mechanism of action.

So, the question, it's a complex question, but the real question then, the functional question that you can probably answer is -- we can sort of talk about that a little bit -- but more importantly, so I understand that a lot of lots have passed testing by H&E staining.

Is there any correlation to an intact H&E stain, percutaneous absorption positive sort of a lot and activity engraftment in either animals or in the human setting? So, would you help me to try to understand how H&E staining acts as a surrogate for what would otherwise be a surrogate, which is cytokine release for potency of the product?

MR. BILBO: Certainly. Again, Apligraf is a complex tissue-like product, multiple potential modes of action. So, we have determined that histology is the most appropriate comprehensive potency test.

I would like to bring up Dr. Baksh to really directly address your question related to

how histology relates to, and is representative of, the biological function of the product.

DR. BAKSH: Thank you for your question.

Good morning, Dolores Baksh, Director, Research and Development, Organogenesis.

During our development studies, we aimed to take the fundamental parameters on our potency assay and correlate those two biological functions, and what I would like to do is share data this morning with you to demonstrate how we have established this link.

If I can have the slide which demonstrates how we have, for example, linked epidermal development to one of the parameters that assess biological function, which is barrier function. Slide up, please.

So, the slide here summarizes results from one of our developmental studies, looking at two experimental groups, a control group which is the living Apligraf T-cell type product, and a test article where we purposely compromised the Apligraf product to initiate a failure in a

number of the parameters we test for potency.

So, what you are looking at here are results from epidermal development, as well as corresponding results from the percutaneous absorption assay. What is highlighted in red here shows failure in the compromised Apligraf unit with respect to epidermal development, as well as corresponding failure with percutaneous absorption.

We have also demonstrated a link between the number of parameters that we assess viability that we use in the potency assays specifically epidermal aspects that we measure for basal and suprabasal aspects.

I would like to share data for this, as well. Slide up, please.

This data here shows a relationship that we observed in a product, an expired product where we would expect to see viability compromised in the unit. Data shown here highlighted in red demonstrates where we see failure in the basal aspect and suprabasal aspect

that assess viability of the keratinocytes in the upper layer.

We see a corresponding failure in our MTT assay, which measures the biochemical activity of our viable cells.

So, taken together, as these examples are shown here, what we are attempting to demonstrate is that each of these parameters that we assess in our potency assay are linked to the biological function of how we believe this product to work in this indication.

DR. COUTURE: Thank you very much, but the question is, if it passes H&E staining, so it has that intact bilayer, and everything you want in there, is there any other evidence that you have that all those are functionally equivalent?

I understand when you have a total failure it doesn't form, MTT fails, et cetera, that it is not going to be a useful product, but that is not really the question.

The question is when they do form, is that the definitive test, and will all of those

grafts function equivalently or comparably once they have been shown to be H&E form positive and/or have passed, therefore, percutaneous absorption?

So, are there differences in intact grafts that pass H&E staining by any parameter as evidenced by animal studies or in the human condition, i.e., have you tested more than one lot in the clinical trials, have you tested multiple lots, all of which have passed H&E and MTT, and you see exactly the same function of these grafts?

I realize that your earlier -- well, maybe I should ask -- in your cutaneous wound indication where you have got 400,000-plus samples, you must have some data from some of those patients where intact grafts again function the same, don't function the same, have some cytokine release profiles from the master cell banks that are equivalent, et cetera.

MR. BILBO: Histology was used as a release test for the clinical studies, the

definitive clinical studies for both cutaneous as well as the two oral clinical studies, so obviously, only those lots or batches that passed the histology assay were utilized in those clinical studies.

We have further preclinical evidence that when they -- and I think that is really what Dr. Baksh illustrated -- that when they don't meet, lots don't meet the histological criteria, they don't function properly in these grafting models, in both in vitro and in vivo methods of evaluating the function of the product.

DR. DUBINETT: Dr. Snyder.

DR. SNYDER: I had a question that I think maybe elaborates on what Larry was getting at.

In a way, we are being asked to compare this product with the standard of care, which is FGG. My understanding is that the whole purpose for any procedure is to eliminate the risk of tooth loss, pain, and inflammation.

I guess the fundamental question is given

that the two mechanisms of action of FGG and Apligraf are different, when you follow patients long term, in fact, is there a decreased incidence or at least a comparable incidence of tooth retention, no pain, no inflammation, in other words, on long-term follow-up, do they behave the same, are the patients' outcomes the same.

MR. BILBO: The follow-up that we have from the clinical trials is 6 months, which is standard for periodontal research, the standard for soft tissue wound healing studies submitted to the FDA, so in terms of determining the efficacy and the generation of KT that was appropriate endpoint.

In terms of longer follow-up, we haven't systematically done that. We have some experience with our clinical investigators here, Dr. Nevins spoke to his experience.

DR. SNYDER: So, realistically, you need more than 6 months to know whether this is equally as efficacious as FGG, isn't that

correct? You have to see how the patients do, because even though they may love the appearance better, if they lose teeth at a greater rate, then, it's not such a great benefit.

MR. BILBO: Six months is the appropriate endpoint. I would like to bring Dr. Cochran to elaborate.

DR. COCHRAN: Thank you for your question. There were a series of studies done back at the Medical College of Virginia a number of years ago by Kennedy, Bryd, and Dorfman, that looked at patients who had one side that was treated, and the other side was not treated, patients that had two different types of lesions.

The patients then were followed over a longer period of time, and for those patients that were not treated, their sides that were not treated, in those cases, there was more plaque and inflammation in those areas, and there was more ongoing recession in those cases.

So, that was sort of a classic series of studies that showed the longer term effects of

not being treated versus being treated.

Obviously, in the results of our pivotal trial, we generated I think it is 3.2 mm of keratinized tissue, which provided that adequate zone of tissue that we feel is important, that met the criteria that Kennedy, Byrd and Dorfman were sort of going after as well, too.

DR. DUBINETT: Dr. Wittes.

DR. WITTES: I have three questions. One, a follow-up in EF-116 slide, I didn't understand it. The second has to do with the discussion that has been going on about given that the FGG is so effective, the objective performance criterion seems kind of laughable to me. If you have something that is basically, over 95 percent effective, to only ask for 50 percent efficacy doesn't make sense to me.

So, what I would like to see is three things about efficacy. The 95 percent confidence limit for the KT greater than or equal to 2, that is the proportion, and then the millimeter is the differences, I want to see the difference between

the Apligraf and the FGG, the mean for the attached gingiva, and the mean for the KT. So, that is the second question.

The third has to do with pain, that one would expect from everything one hears, that there would be more pain in the standard therapy, but the design of the study in a sense precludes the ability to assess pain, because it's the same mouth.

I think this issue is going to come up in other things, too. Insofar as you are comparing redness or things that you can see in the two different places, then, this is a very good design.

When you are trying to look at anything systemic like safety, like pain, you can't, so if you have data that actually show pain for the Apligraf.

MR. BILBO: You certainly hit on an important point, that a possible drawback within patient control design is that we have got three treatment sites or two treatment sites and the

palatal donor site within the same mouth, so there is the difficulty for the patient in reporting to discriminate where the pain is coming from.

I want to bring up Dr. Bates to discuss your questions related to the data and confidence intervals.

DR. BATES: Dr. Wittes, I will address your first two questions with the data, and I think Dr. McGuire would be in the best position to address the question around pain.

If I could have slide up, please. This slide illustrates the confidence interval for the proportion of patients achieving at least 2 mm of KT at 6 months.

DR. WITTES: No, that's not the question. The question is the confidence interval for the difference between the two treatments. What I am interested in knowing is how much loss is there in benefit. I think that is what Dr. Lee was asking.

DR. BATES: Dr. Wittes, we can provide

you with that, but we will need a bit of time to provide you with those data. Perhaps we could give those to you after lunch, if you will just give me a moment.

[Pause.]

DR. BATES: Yes, I believe the slide will address the second question. Please have slide up.

This is from the pivotal study, Apligraf, FGG. We have got the baseline measurements, the KT at the top row, and 6 month results and the changes from baseline, and you can see those results presented in the same way for attached gingiva in the bottom section of the slide.

DR. WITTES: But again that is not the question that I have asked. The question I have asked is what is the difference between the two in the 95 percent confidence for the difference.

DR. BATES: We will have to get that for you after lunch.

If I could call Dr. McGuire to address that.

DR. MCGUIRE: I think you addressed really the challenge and the split mouth design, it is very difficult to differentiate that, because the patient has difficulties in picking all this out. We did try to make sure the donor site was the same side as the free gingival graft, but nonetheless, it is very difficult for the patient to do that.

I have been involved in I think eight RCTs with grafting studies in the mouth, and all were designed in the split mouth design because of the way they performed, being able to compare different modalities of treatment, but I think as this particular study, the results of this I think what you need to look at is that 72 percent of the patients felt that they would rather have the Apligraf than the free gingival graft.

Now, that doesn't just look at pain, it looks at the entire experience, but certainly I think it would include the pain, and I think that is the best that we can give you based on the design of the study. We chose the split mouth

because of the ability to look at the different procedures but it doesn't do a good job in discriminating pain.

DR. WITTES: Can you answer 116, the EF-116 question, which was my first question? It was just that I didn't understand it. I needed clarification.

MR. BILBO: Dr. Nevins, can you address this?

DR. NEVINS: Yes. Slide up, please.

If the site is resistant to muscle pull, then, there is lack of movement of the free gingival margin when you are moving the tissues in the vesicular area. There was improvement for both the Apligraf sites with resistance dropping from 25 to 12, and there was more improvement in the FGG site, dropping from 22 to 3.

As we know, we have a much wider zone of keratinized tissue gained in the FGG sites, and that would be consistent that sites that had heavy muscle pulls, we would have certainly more obviation of that. Does that clarify the

question?

DR. WITTES: Let me just ask it really simply, okay? Is that 007, is that benefit for Apligraf or benefit for FGG? That is what I am asking. I basically don't know which is better. That is my problem.

DR. NEVINS: I would presume that is a benefit towards FGG.

DR. WITTES: That is what I thought. So, in fact, what this slide is showing is a very significant benefit for FGG.

DR. NEVINS: I would like, if it is possible, to answer your first question, which was about demonstrating the statistical data, but looking at it from perhaps a clinician's point of view, is that at the beginning of the trial, these patients presented with 0, and to 0.0 to 0.1 mm of attached gingiva in extremely limited keratinized tissue, 95 percent of the patients in the Apligraf treatment section presenting with a mean of 3.2 mm keratinized tissue postoperatively, and 1.8 mm of attached gingiva.

These patients would no longer be candidates for an individual soft tissue grafting procedure. That would be enough tissue to clinically evaluate them as being stabilized.

DR. DUBINETT: Dr. Amar.

DR. AMAR: It was unclear to me as to the number and particularly when we deal with fibroblasts, not so much for keratinocytes, as the number of passages that were used over time for the delivery of a certain lot, and what would be the threshold of the number of passages for which you would consider, quote, unquote, "senescence" are not applicable to be used in this kind of craft. That would be the first question.

The second question, and coming back to my colleagues, if histology is going to be taken as a gold standard for efficacy, I wanted to know whether there was any attempt after the procedures of quantifying the level of inflammation between free gingival graft and Apligraf particularly when it comes to the

connective tissue and whether or not there were any difference branched into the spent medium that I saw in the analysis of cytokine expression.

I was surprised by the selection, but be that as it may, there was basically one cytokine that was proinflammatory, which classically that we consider as proinflammatory, which was IL-1 alpha.

I wanted to know what went on into the design of determining the number of cytokines pro- and anti- inflammation given the fact that this issue will remain as do we or does this procedure get the system into some kind of chronic inflammation underneath into the connective tissue.

The last one was -- I am sorry -- I suppose that we have different donors, and those are male and female, am I correct?

MR. BILBO: It would be male, neonatal foreskin tissue.

DR. AMAR: Excuse me?

MR. BILBO: It's male, neonatal foreskin tissue.

DR. AMAR: Absolutely. What I meant by this is that in the female donor -- in the female recipient, we can follow up on XY chromosome as to the behavior of the donors, because that is very easy. On karyotyping staining -- and that is what I meant, I didn't mean that the donors were -- I know that circumcision occurs at least in my book only male.

MR. BILBO: Okay. So, I think I heard four questions, one related to at what passage level do the cells senesce and at what passage level are we utilizing the different cells within the manufacturing process, which is a very controlled aspect of the manufacturing, and histology, you had mentioned histology as the gold standard for efficacy for the product. It is really better positioned as histology is the most appropriate evaluation for the potency of the product to release the product.

Again, Apligraf is a complex tissue-like

product with multiple potential modes of healing, so we are not necessarily trying to evaluate efficacy with histology, but it is a measure of the appropriate structure development related to biological attributes.

Then, quantitatively, I think you were asking me, you know, have we evaluated cytokines when the product is applied to patients.

DR. AMAR: After the placement particularly, after placement of the graft, was there any monitoring of the amount of inflammation quantitatively, what do we find, do we have.

MR. BILBO: And lastly, related to persistence. I will bring up Dr. Pitkin just to briefly discuss the passage levels that we used in the manufacturing process at which stage these cells senesce.

DR. PITKIN: Thank you. As Mr. Bilbo stated, we have very controlled manufacturing process, that results in a product at which cells, keratinized cells, are at passage 5, and

fibroblast cells are at passage 7.

In comparison, the cells, we perform senescent testing for all our cell banks and keratinocytes senesce at passage approximately 12 to 14, and that is a 5 versus 14, and fibroblast cells senesce as a passage approximately 18 to 20 versus passage 7, which product is produced.

Slide up, please.

This is a graphical representation on the Y axis, you see cumulative population doubling. On the X axis is the number of passages. This is a representative of 4 cell banks that have been tested and released for production of manufacturing product.

In the first column, this column signifies the passage level at which product is made for keratinocytes, and this is senescence for keratinocyte cell banks, again from passage 12 to 14.

Any questions, additional questions, or did I answer your question?

DR. REYNOLDS: For clarification, are

these constructs made from a single donor, the keratinocytes and fibroblasts, or are these from separate donors?

MR. BILBO: Each cell type used in a particular lot or batch of product would be from one donor, so one cell strain of the keratinocytes or one cell strain, but there could be matching between the two cell types.

DR. REYNOLDS: So, in many instances, then, the construct will actually represent cells from different donors.

MR. BILBO: Correct.

DR. REYNOLDS: Thank you.

MR. BILBO: I think I will bring up Dr. Bates to respond to I think your related questions about cytokine expression, proinflammatory and persistence.

DR. BATES: Dr. Reynolds, I just want to clarify there is no more than two donors for any product is the limit.

Dr. Amar, your question is around inflammation and persistence. I would like to

take the opportunity to remind the panel that the product has a complex mechanism of action we don't fully understand.

There is no one particular cytokine either anti-inflammatory, pro-inflammatory, angiogenic, et cetera, that we can say this is the one that is responsible for what we see.

We tried to look at a variety of angiogenic proteins in a biomarker study and correlate that to either KT, aesthetic outcomes, and we weren't able to find any close correlation, and this is another reason why we consider histology to be the most appropriate potency measure for the product.

With respect to inflammation, we did look at that, and the conclusion from this is that any inflammation is short lived with Apligraf, and this is consistent with our knowledge that it has transient persistence, that it probably doesn't last very long, it doesn't get vascularized, and it goes, it gradually degrades, so any inflammation is within the first week or so.

So, if you just give me a moment. I can present and share with you some data on the inflammation scores during the study. If we could have the slide up, please.

We have divided this up in terms of Apligraf and FGG. Again, this is from the pivotal study. We have got scores of inflammation as zero being none or mild, and the relative proportions, and you can see that there is no substantial difference between FGG and Apligraf.

DR. AMAR: Is this clinical inflammation or --

DR. BATES: This is clinical inflammation. In the histology study that I presented also in the efficacy section of my co-presentation, we did some additional histology analysis looking at just basic H&E staining at baseline and then at 6 months, and there was no statistically significant increase in inflammatory cell.

DR. AMAR: Was there any quantitation of

inflammation?

DR. BATES: It was a qualitative, it was a blinded histopathologist looking at it.

DR. AMAR: Was there any evidence of multinucleated cells?

DR. BATES: Not in the oral studies. In the cutaneous studies, there is one study published by Betty Arvis which has seen the presence of some multinucleated cells consistent with a foreign body type reaction in the acute phase in cutaneous wounds that has been published.

DR. AMAR: A host response.

DR. BATES: A host response.

Then, for your last question, with respect to the Y chromosome, you are absolutely right. We actually used the fact that you can track the Y chromosome or even the amelogenin locus and the fact that you put it into a female patient, you get a little bit different migration on the gel as a marker of persistence to see how long is the produce persisting.

DR. AMAR: Suppose also.

DR. BATES: We haven't done any in-situ hybridization, chromosome in-situ hybridization.

What we have looked for is DNA evidence of either the Y chromosome or the amelogenin locus.

DR. AMAR: I think that Dr. Nevins presented or published a paper on the DNA persistence over a certain --

DR. BATES: Correct.

DR. AMAR: Can I have clarifications on that?

DR. BATES: Certainly. If we could have Dr. Nevins.

DR. NEVINS: These were a K series of patients that were treated in my practice, and biopsy was taken post-treatment at different time points, and it was just in the four patients, 20, 29, 35 and 45 days, and that was my own personal experience in the paradigm shift of not understanding how something that I am using like a graft really can persist.

In those four examples, those four time

points with DNA analysis, we did a biopsy of the gingival tissue and then we did a salivary swab from the patient, and there was 100 percent consistency between it, there was no other DNA present.

DR. DUBINETT: We are going to take three more questions from Drs. Hornicek, Ahsan, and Jeffcoat, and then we will go a break and have more time to answer questions later.

DR. HORNICEK: Thank you. I had a quick question regarding the technical aspects of the application. You showed a slide showing the placement of the Apligraf, and I was curious about the anchorage and suturing of it, and is there a standard way of doing that, is there a standard way of also covering the graft, and then is the Apligraf maybe easier to use in terms of application than the FGG?

MR. BILBO: I would like to bring up Dr. McGuire to respond to your question.

DR. MCGUIRE: It is a relatively standard way we go about suturing it. We typically suture

at each of the papilla, which is the tissue between the teeth, and then we also try to stabilize it by doing a periosteal suture which we run through the periosteum, below the graft, up around the neck of the tooth, back around, and tie it, and that just suspends it.

It is pretty much the exact same suture technique that we use for a free gingival graft or if you are placing a connective tissue graft so it's basically the same, to my point earlier, that we are using the same surgical techniques with this that we do with our other procedures.

DR. HORNICEK: How important is the covering of that after you do the repair, because in certain tissues, the covering of the repairs are maybe more important than the actual anchoring of the graft?

DR. MCGUIRE: You know the mouth is a very -- it's a tough environment to work in, and the coverage is probably important just to help stabilize the graft and also to help prevent a toothbrush from accidentally hitting it, and that

type of thing.

We cover the free gingival graft in the same way, but, you know, I think it is primarily just as a protective kind of thing, that's all the covering does.

DR. DUBINETT: Dr. Ahsan.

DR. AHSAN: I had a quick question about the elimination of the product in this oral environment. According to the presentation by 6 months, the DNA is largely absent. Could you tell me whether this is due to incremental erosion or whether this is a bulk loss of the Apligraf?

Then, also, does that mean that there are earlier time points at which you could predict that the procedure was not going to be successful in leading to 2 mm of KT?

The third question being that if it failed according to that criteria, what is the status of the site, because you created the wound, is the patient worse off, or is there also the opportunity for re-application of Apligraf or

to move them to a subsequent approach with FGG?

MR. BILBO: To respond to your last question, there is nothing about the Apligraf procedure that would prevent subsequent re-treatment with another modality if that was required.

I will bring up Dr. Bates to respond to your first two questions.

DR. BATES: I think the best way for me to answer your first question about the elimination or the degradation of the product is to show some illustrations. It doesn't vascularize, it degrades quite rapidly, it's a hostile environment in the oral cavity, the mouth is moving a lot, there is lots of salivary enzymes, it gets degraded.

I will show you two images, representative images from one week, if we could have slide up, please, to show you that in one case, there is no evidence that the Apligraf is even there, and on the righthand side you can see it is in a stage of gradual degradation.

What is really fascinating from a biological point of view is that you can have a viable product like Apligraf placed on the wound, and it will affect the course, the trajectory of healing by secondary intention to achieve the types of KT measurements that we are seeing at 6 months rather than going back to baseline that you saw in those historical studies.

Your point about the DNA being largely absent, I just wanted to make a clarification in the two subjects that we did test it, there was no evidence, it wasn't largely, there was no evidence, and that is again consistent with the fact that it doesn't engraft, and it is not integrated into the host tissue.

Predicting at earlier time points, from a surgical or clinical perspective, these tissues take a while to evolve, to create mature tissues, but by 3 months, we saw tissues that were pretty indicative of what we would see at 6 months.

I could have one of the clinicians speak to it, but in my own clinical experience, I

wouldn't make any decisions prior to that time period about what is best for the treatment,, and to add to what Mr. Bilbo said, there is nothing about Apligraf treatment that after 6 months, if the clinician thought it was in the patient's best interest to re-treat, they could be re-treated.

It is the same for the FGG, if the FGG doesn't work correctly, they can redo it.

With respect to outcomes, all patients achieved, while all of them didn't achieve 2 mm, all achieved at least 1 mm, so there was an improvement.

Does that satisfactorily answer?

DR. AHSAN: Yes.

DR. BATES: Thank you.

DR. DUBINETT: Dr. Jeffcoat.

DR. JEFFCOAT: Thank you very much.

Dr. Wittes raised the important questions about the analysis of the study. I want to raise a question about the control, which the control group was a positive control, FGG, and I don't

know how many people are still using that frankly.

I mean connective tissue graft, you know you are going to get the outcome you are going to get on a free gingival graft. So, this study has been designed for success rather than designed for the null hypothesis. I think that's a question. I said it like I am lecturing to a bunch of students which isn't appropriate.

But the question is did you do that, and did you mean to do that, because you get a lot less pain, you get a lot less of those rugae. If you do a connective tissue graft, which is again an autograft, and it's a question I would like to pose to the sponsors who have brought the product to us.

MR. BILBO: Sure. As a reminder, the primary enrollment criteria for the study were patients that had 1 mm or less of attached gingiva where root coverage was not desired. Just generally in terms of I think you were asking whether this is still a procedure that is

performed and in terms of free gingival graft.

DR. JEFFCOAT: Actually, that is not what I was asking. What I was asking was why do you feel this is the appropriate control for this product given that you could do the same thing with a procedure that is out there that has less morbidity for the patient.

MR. BILBO: I would like to bring up Dr. McGuire to respond to your question.

DR. MCGUIRE: Thank you, Dr. Jeffcoat.

I think the most appropriate control, as was mentioned earlier, would be just a pushback secondary intention, and we obviously for ethical reasons can't do that.

But today in the United States at least, and pretty much throughout the world, but in the United States for sure, the free gingival graft remains the number one procedure being done to generate keratinized tissue, not to cover roots, remember we are not trying to cover roots.

Typically, connective tissue grafts are done when root coverage is indicated. The ADA

statistics show that about 275,000 free gingival grafts were performed last year, and that is probably a low estimate.

But certainly for root coverage and other procedures, connective tissue grafts have taken the lead, but not for generating keratinized tissue.

DR. DUBINETT: We will now take a 10-minute break and come back for the FDA Presentation.

[Break.]

DR. DUBINETT: I would like to call everyone back to the table, and we will begin again.

Okay. Before we begin the FDA presentation, we will have Gail remind us of the questions.

MS. DAPOLITO: I just wanted to note to the Committee that as you are listening to these presentations -- and I know you are thinking about the questions later on in the day -- there is copies of questions in your folders. They are

slightly revised from the previous copies that you have received, so you might want to refer to them as you go through the day. Thank you.

DR. DUBINETT: We will begin the FDA presentation. Dr. Lee.

FDA Presentation

Product Quality

DR. MARK LEE: Good morning. My name is Mark Lee, and I would lead off the presentations by the FDA this morning regarding Apligraf, a product that contains allogeneic cultured keratinocytes and fibroblasts in bovine collagen for the achievement of surgically created gingival and alveolar mucosal surface defects in adults.

It should be noted first that an official name has not been designated for this product at this time, however, for the purpose of the Advisory Committee meeting presentation, and following discussion this afternoon, the FDA presenters will be referring to the product as "Apligraf."

These FDA presentations are designed to provide the Committee with a summary of FDA's review of the BLA to this point, but focused primarily on the particular areas for which FDA is seeking the Advisory Committee's expertise and input.

There will be three presentations this morning from the FDA. I am chair of the BLA Review Committee and will be first presenting information regarding product manufacture.

Dr. Robert Betz will then follow with an overview of the disease and treatment considerations.

Lastly, Dr. Agnes Lim will present the design of the clinical studies performed in support of the BLA, and a summary of the available efficacy and safety information for Apligraf.

Before I begin my presentation, I want to acknowledge the important contributions from other members of the BLA review team who are listed on this slide. This BLA is being reviewed

by the Office of Cellular, Tissue and Gene Therapies within the Center for Biologics Evaluation and Research in collaboration with the Division of Anesthesiology, General Hospital Infection Control, and Dental Devices, and the Division of Surgical, Orthopedic, and Restorative Devices within the Center for Devices and Radiological Health of FDA.

Now, I will begin my part of the presentation with basic product information and some relevant regulatory background.

To briefly describe the product that is the subject of this Advisory Committee meeting, Apligraf is a bilayered tissue construct consisting of an upper layer of human keratinocytes and a supporting lower layer constructed of bovine derived Type 1 collagen and human neonatal foreskin-derived dermal fibroblasts.

The upper and lower layers of the product make up approximately 33 percent and 67 percent of the construct.

Apligraf in this BLA is being evaluated for the treatment of surgically created gingival and alveolar mucosal surface defects in adults.

Development regulatory history of the approved Apligraf product is briefly summarized on this slide.

The approved product is identical in characteristics for the product being evaluated for BLA approval. The approved product has received FDA approvals in 1998 and 2000 for two cutaneous indications. These include uses with certain venous-like ulcers and diabetic foot ulcers described on this slide.

Apligraf is manufactured by combining viable allogeneic human fibroblasts and keratinocytes with Type 1 bovine collagen by material. The product is manufactured using a 3- to 4-week process resulting in a bilayered structure that resembles the dermal and epidermal structures found in skin.

Apligraf, however, does not contain Langerhans cells, melanocytes, macrophages,

lymphocytes, blood vessels or hair follicles.

The key steps in the manufacture of the cell- scaffold construct are described on the slide. Initially, fibroblasts and keratinocyte master and working cell banks are established by isolation of these cells from neonatal foreskin donor tissue.

The cells undergo limited expansion in culture prior to being cryopreserved for further manufacturing.

The next four steps describe key steps in producing the construct from the starting cellular and culture matrix components. These include sequentially production of a dermal equivalent layer, production of an epidermal layer, differentiation, and cornification.

After these production steps, the resulting final cell-scaffold construct is stored under conditions to maintain product quality, so it can be packaged and shipped to the clinical site within a 7-day window.

This slide shows an overview of the main

manufacturing steps and the quality control tests that are performed at these steps. Product quality for Apligraf is designed to be ensured by a combination of final product and in-process testing performed at key manufacturing steps.

The testing includes sterility, mycoplasma, bioburden purity and potency. In the interest of time, my presentation from this point on will only focus on information relevant to the two product quality topics for discussion by the Advisory Committee today.

Therefore, the following slides will concentrate on the information regarding testing for cell bank qualification and for the final cell-scaffold construct that is Apligraf.

I will now describe the cell bank qualification use for Apligraf manufacturing in more detail.

This slide lays out some of the main points regarding fibroblasts and keratinocyte cell banks used for product manufacture. Because expansion of each cell bank is limited, new cell

banks from new donor tissue must be generated on a periodic basis.

In the testing approach used by the applicant, the quality and comparability of these cells are supported in general by testing at master and working cell bank levels, combination of direct testing of cells, and of the cell-scaffold construct produced by the cells, and incorporates both in vitro and in vivo testing.

I will provide more detailed information regarding the testing performed at the master cell bank and working cell bank levels in the next two slides.

It is important to note that for Apligraf testing conducted at the master and working cell bank levels for both cell types are critical in ensuring that the different cells used in the manufacturing of different lots of Apligraf are safe and possess comparable characteristics.

Therefore, FDA is requesting scientific discussion from the Advisory Committee today regarding the testing approach used by the

applicant.

Master cell bank qualification testing includes a panel of tests to assess microbiological and viral safety and tests to assess neoplastic safety, which includes isoenzyme analysis, karyology, senescence, and tumorigenicity.

Cell purity is also assessed using markers to distinguish potential cellular impurities that may be present after the cell isolation process. These include markers for total leukocytes, monocytes, Langerhans cells, endothelial cells, and antigen-presenting cells that includes macrophages, activated T-cells, and dendritic cells.

Master cell bank qualification also includes testing to assess the functional capacity of these cells to produce the final cell-scaffold construct. These include in vitro tests, such as percutaneous absorption which provides a measure of the product's ability to function as a physical barrier, a profile of key

growth factors and cytokines expressed by these cells including those along the wound healing process, such as PDGF alpha, PDGF beta-1, IL-1 alpha, and proinflammatory cytokines like L-4.

Mitochondrial tetrazolium test, or MTT, which provides a measure of some metabolic activity, and quantification of vascular endothelial growth factor secreted by the final construct, and the histological analysis of the final product, which is the potency assay.

This also includes an in-vivo functional assessment of the final cell-scaffold construct in an athymic mouse model as a part of master cell bank qualification.

The subset of tests that are boxed in red on this slide represent the tests that are used by the applicant to assess the cell bank comparability and are submitted to the FDA for review each time a new substrain is released.

The working cell bank qualification testing also includes a panel of tests to assess microbiological and viral safety, as well as

isoenzyme analysis. Several tests to assess cell function including cell growth, viability, collagen biosynthesis for fibroblasts, and involucrin content for keratinocytes.

Similar to the approach used for master cell bank qualifications, some of the testing for the working cell bank is also performed on the final cell-scaffold product that are produced by these cells, which is the histological analysis of final product using the potency assay.

Now, I will move on to describing the proposed potency assay for Apligraf in more detail. It should be noted that during the previous review of Apligraf as a PMA, considerable emphasis was placed on identifying in-process and final product release specifications that predicted the safety, consistency, and performance of the device.

As a product review as a BLA, we revisit these issues to ensure compliance with potency requirements of 21 CFR 610.10. I will therefore be presenting detailed information regarding the

histological potency assay that is proposed by the applicant as well as other relevant testing information in the following slides.

The regulatory requirements for potency are included on this slide. Potency is defined as "the specific ability or capacity of the product, as indicated by appropriate laboratory tests to effect a given result," and that is a part of 21 CFR 600.3(s).

In the BLA, the applicant proposes to measure Apligraf potency using a set of histological parameters, which is supported by other biological assays. Scientific discussion from the Advisory Committee is requested on this topic.

The applicant proposes to measure product potency using a set of histological parameters which collectively assess the quality of the epidermal and dermal layers present in the final product.

The proposed histological potency measurement utilizes hematoxylin and eosin (H&E)

staining to distinguish fibroblasts and collagen within the lower dermal matrix, as well as keratinocytes within the upper epidermal layer.

The stains distinguish the basophilic and eosinophilic cellular structures within the tissue layers. The applicant describes the relevance of the morphological structures observed through the histological parameters of the potency assay to the function of Apligraf product as follows:

The lower layer is believed to provide both a structural matrix for the fibroblasts and the substrate for the development and maintenance of the upper layer. The upper epidermal layer is believed to impart important structural elements for the construct, which contributes in turn to its biomechanical strength, handling properties, and barrier properties.

This table shows the set of histological parameters of the potency assay and the currently used acceptance criteria. The parameters include those relevant for assessing the quality of the

epidermal layer including epidermal coverage, epidermal development, basal cell layer keratinocyte viability and suprabasal cell layer viability.

Parameters for assessing the quality of the dermal layer including dermal thickness, fibroblast density, and matrix aspect. It is noted that in this assay, cell reliability is determined indirectly by using histological assessment of a basophilic cytoplasm in the absence of severe vacuolization and necrosis.

The histology assay and specifications that are described on this slide are identical to that used to demonstrate device performance for approved medical device. The specifications were initially supported by histological studies in 1996, and are related to the ability of Apligraf to serve as a fiscal barrier and its persistence in vivo.

A sample of the H&E stained image that is used to assess Apligraf potency is shown on this slide. The distinct structures that are observed

in the H&E image are identified. The samples used for the assay are obtained from biopsy punches taken from units from each lot for different testing. The punches are fixed, sliced into 3 mm strips, and further processed according to established standard procedures.

The proposed histology-based potency assays supported by the applicant with earlier developmental studies and a set of in vitro and in vivo biological assays that are currently used for cell bank qualification. It is important to note that these tests are not currently performed on a lot-by-lot basis, and is not used for product release.

As previously mentioned, some of the function assays used for cell bank qualification and provide information regarding the function of the final cell-scaffold construct Apligraf, and are relevant to the potency discussion.

Testing performed on the construct included percutaneous water absorption, a measure of the construct's ability to function as a

barrier; profile growth factors and cytokines relevant to wound healing, such as PDGF-alpha, TGF-beta 1, IL-1-alpha, and inflammatory cytokines, such as L-4, mitochondrial tetrazolium testing around TTSA, measurement of cell metabolic activity.

Concentration of vascular endothelial growth factor, which is a growth factor that is believed to be indicative of relevant biological factors produced by the keratinocytes as a part of the cell-scaffold construct.

Also, of relevance to the potency discussion is the in vivo assessment of the product function in an athymic mouse graft model.

The test is also performed as a part of cell bank qualification and evaluate its functional parameters, such as pre-graft morphology, graft take and integration, graft contraction, graft morphology, graft remodeling, and immunohistochemistry.

In the last set of slides, I have provided information regarding the potency assay

and other assays of relevance to the product potency and function that are performed on the final cell-scaffold construct.

Prior to the discussion, I also wanted to make available to the Advisory Committee, relevant information from CBER's Guidance on Potency Tests for Cellular and Gene Therapy Products, which provides clarification regarding the characteristics that all potency assays used for release testing of licensed biological products must have.

I will not read the individual items on this list as the guidance was also included as a reference from the FDA briefing document and is available to the public on the FDA web site.

In summary, FDA has thus far presented a relevant subset of information from the BLA submission regarding the two issues related to the quality of the product Apligraf for the oral indication.

These are current approach to qualify and demonstrate comparability for new cell banks used

for Apligraf manufacture, and usage of histology as basis for product potency and the relevance and applicability of the other in vitro and in vivo assays relevant to product potency that are performed on this final cell-scaffold construct.

In considering these topics for discussion in the afternoon session, please also consider development information in the following presentations by the clinical reviewers.

In particular, there were clinical studies performed by the applicant during their ID studies regarding histology, DNA persistence, and levels of angiogenic biomarkers expressed by Apligraf in the oral wound that may be of relevance to the potency discussion.

That concludes the presentation on product quality. Now, we will move on to the next FDA presentation by Dr. Robert Betz. Thank you.

Overview of Periodontal Conditions and Treatments

DR. BETZ: Good morning. My name is Bob Betz. I am a board-certified periodontist. I

work in the Center for Devices and Radiologic Health. I was the primary reviewer for both IDE studies.

The applicant states that Apligraf is intended for the treatment of surgically created gingival and alveolar mucosal surface defects. Because most of my presentation has already been presented by Dr. Cochran, what I will do is I will try to minimize any duplication.

This slide was shown by Dr. Cochran. It shows the anatomy of the dental gingival complex.

It shows the keratinized tissue, the alveolar mucosa.

This slide demonstrates for the non-periodontist how you use a periodontal probe, and the left View A shows pretty much a normal situation where the attachment of the tissues to the tooth is at the cemento-enamel junction where the enamel of the crown meets the cementum of the root surface. Slide C or View C is a depiction of gingival recession.

This slide is a stylized diagram

depicting the possible differences between the zone of attached gingiva and the zone of keratinized gingiva. A to C is the free gingiva, A to B is the attached gingiva, B to C is the keratinized gingiva, and C to A is the probing depth.

This may or may not occur in real life situations. Please note that if there is significant pocket probing depth, there may be a significant difference between measurements in keratinized gingiva and attached gingiva. This is important because the pilot study evaluated test gingiva, and the pivotal study evaluated keratinized gingiva.

This slide basically shows what mucogingival problems are, because gingival defects are soft tissue defects that involve both keratinized attached gingiva and nonkeratinized alveolar mucosa at the mucogingival junction.

Usually, there is insufficient zone of attached gingiva. Most of the time there is gingival recession. Historically, they have used

less than a millimeter of keratinized gingiva to indicate that you have a mucogingival problem.

What happens is you develop inflammation in the area, you develop bone loss, and the lesion can progress. Eventually, if untreated, the current loss of the tooth.

Possible causes and effects are listed on this slide. Toothbrush abrasion, as was mentioned before, scrubbing the teeth with a medium or hard bristle brush to try to remove plaque can cause damage especially to thin gingiva.

Anatomical considerations, such as crowding, can occur. Sometimes it is hereditary related to a discrepancy between the arch length and the widths of all the teeth added together, you just don't have a match there, and it develops crowding. This pushes teeth forward, creates a thin alveolar bone on the facial side of that area, and also a thin soft tissue covering.

Periodontitis, as we all know, can cause

loss of alveolar bone around any tooth. Faulty restorations, whether they be over-contoured or under-contoured, can affect the gingival margin adversely.

Orthodontic treatment, as mentioned before, can push a tooth out to the facial aspect, creating thin bone and dead tissue. Obviously, a combination of these effects can occur.

Gingival recession is defined by the American Academy of Periodontology as the location of the gingival margin apical to the cemento-enamel junction, and the sponsor used the Miller classification, which is basically a depth measurement classification.

There are other classifications that take into consideration defect, width, but this is a very good one to use. The Miller Class I defect basically is the gingival margin apical to the cemento-enamel junction, but does not involve the mucogingival junction.

Class II does involve the junction.

Class III and IV were not used in the studies because they are rather complex issues related to marginal bone loss between the teeth, and they usually require complex procedures to treat them.

This slide is a slide of gingival recession with toothbrush abrasion. Dental recession does not have to be treated necessarily, however, when there are aesthetic issues, as shown here, there are sensitivity and root surface issues. Possibly, it involves the root canal containing the dental nerve. Then, these defects really should be treated.

Treatment options include soft tissue grafting or autogenous grafting, which includes free gingival graft or a soft tissue autograft. The subepithelial connective tissue graft, which is a cousin to the free gingival graft, the lateral and rotated pedicle graft procedures, the double papillae graft, which is basically doing a pedicle graft on both sides of the defect, and a coronally positioned flap.

When you have a coronally positioned flap

procedure, you do need to have some margin of attached gingiva at the base of the defect to be able to pull the tissue up. There is also kind of tissue regeneration procedures where you use a barrier membrane to exclude connective tissue and epithelial tissue components from the periodontal complex.

There is also Apligraf. Newer stuff include the platelet rich fibrin membrane from PRP procedures, tunneling procedures using a connective tissue graft, using just a dental restoration only is a possibility, or you can do nothing.

In the clinical studies, Apligraf was compared to soft tissue free gingival graft. This was because soft tissue graft was considered mainly a historical benchmark against which all other grafting modalities are usually compared.

The soft tissue autograft is not necessarily the best, most successful, or most aesthetic treatment for gingival recession, however, many times it is used to create or

increase the zone of attached gingiva.

The zone of attached gingiva is the important part because the zone of attached gingiva is what basically dissipates muscle pull, which precipitates inflammation, which precipitates bone loss, and eventually it's loss of the tooth.

Soft tissue autografts heal primarily by primary intention and are part of the healed recipient site, and this cannot be said about Apligraf as stated before. One of the sponsors referenced Griffiths stated that Apligraf could be considered a biological bandage.

Neither the cells of Apligraf or soft tissue autograft generally survive the procedure.

This slide you have seen before. The sponsors adequately described it. The only point I want to bring up with this slide is that the sponsor used releasing incisions on the left and the right side of the defect site, and some people choose to use a semilunar incision instead of that, and it is a social choice, and the

sponsor chose to use releasing incisions.

Soft tissue autografts from the IDE were harvested using traditional palatal soft tissue grafting harvesting procedures. The periodontal literature states that soft tissue autograft thickness usually ranges from 1 to 2 mm. In the IDE, soft tissue grafts and Apligraf membranes were tacked down to the periosteum by using a surgical blade to fenestrate or cut to the alveolar bone, and this helps to tack these products down.

There is a difference between the periosteum, the nature of the periosteum of under test gingiva as opposed to alveolar mucosa. The periosteum and the alveolar, because they tend to be parallel to the surface, and they are easily reflected, the ones sub or below the test gingiva are basically perpendicular to the surface of the bone, and actually attach into it, and are much harder to remove.

Root coverage was not provided during the surgical procedures performed in IDE studies, as

stated before, if root coverage was desired it could have been done at a later time.

A discussion issue is if probing depth is significant, there may be a measurable difference between attached and keratinized gingiva.

Could the change from measuring attached gingiva in the 05 study to keratinized tissue in the 06 study be clinically significant?

Is keratinized tissue a surrogate for attached gingiva?

That is pretty much my presentation.

Dr. Lim will continue with her presentation.

Clinical Study Design and Safety and Efficacy

DR. LIM: Thank you, Dr. Betz.

Good morning. I am Agnes Lim. I am from the Clinical Evaluation Branch in the Office of Cellular, Tissue and Gene Therapies in the Center for Biologics.

My presentation this morning will cover the study design, efficacy, and safety results from the two studies that support this BLA,

Studies 05 and 06, as well as additional safety experience from the use of Apligraf for chronic cutaneous wounds.

The study design for Pilot Study 05 will be presented in the next few slides.

The title of the study is shown here, I am not going to read it, but the objectives for the study were to assess the safety and efficacy of Apligraf in establishing a functional zone of attached gingiva.

This was a randomized, within-subject controlled study conducted at a single center in 25 subjects. Controlled treatment consisted of a palatal autograft, also called a free gingival graft.

The graft treatment sites were matched for condition of the teeth and gingiva.

The primary efficacy endpoint was the change in the amount of attached gingiva at 6 months, comparing Apligraf to control treatment.

There were 8 secondary efficacy endpoints shown here. There was no sequential order of

testing specified and there was no adjustment made for multiplicity.

Major inclusion criteria included subjects with an insufficient zone of attached gingiva that required soft tissue grafting in at least two non-adjacent teeth where root coverage was not desired at the time of grafting.

Excluded were subjects who needed root coverage, had an infection at the intended surgical site, or had health condition or were on medications that could impair wound healing, and current smokers were excluded from the study.

The study plan was to treat 25 subjects.

Following randomization for treatment site in order of treatment, each subject was treated with Apligraf and a soft tissue palatal autograft on contralateral side of the mouth in the same jaw.

The soft tissue palatal graft treatment was the control. It is also called a free gingival graft or FGG. In my presentation, I shall refer to the palatal graft treatment site as either the control or the control site, and

refer to the site from which the palatal tissue was obtained as the donor site.

The first three subjects participated as training subjects and were excluded from the efficacy analysis, but were included in the safety analysis. Neither the subject nor investigator could be blinded in this study.

The primary efficacy evaluation was performed at Month 6, with schedule interim visits as shown on this slide. There were 2 adjunct laboratory studies conducted with Study 05, a histology study and a DNA persistence study.

The primary efficacy endpoint was the amount of attached gingiva, was evaluated using a calibrated probe measured to the nearest 1/2 mm.

Assessment methods for the secondary endpoints are shown here. Color and texture were compared to adjacent tissue and were rated as more, less, or equally red or firm at the time point shown. Inflammation, probing depth, KT width, clinical attachment, level, and resistance

to muscle pull were assessed by examiners or investigators by the method shown here.

Patient satisfaction was based on responses to two questionnaires, a subject aesthetic questionnaire and a subject discomfort questionnaire. The perceptions of sensitivity and pain were recorded as none, mild, moderate, or severe in the patient discomfort questionnaire.

For the statistical analysis of efficacy, the primary endpoint was the absolute change in the amount of attached gingiva over 6 months between Apligraf and control using a non-inferiority comparison at a 5 percent significant level in 22 subjects.

The non-inferiority margin was a 1 mm difference in change from baseline between the two sites. As mentioned earlier, no detailed statistical analysis was provided for the secondary efficacy endpoints, and no adjustment was made for multiplicity.

The efficacy results with Study 05.

Twenty-five subjects were enrolled and treated. The mean age was 49 years. Sixty-eight percent were female. Eighty-eight percent were Caucasian.

Results for the primary efficacy endpoints are shown on this slide. At the Apligraf site at 6 months, 64 percent showed an increase in attached gingiva. The average increase was 0.85 mm from baseline.

At control sites at 6 months, 95 percent showed an increase in attached gingiva. The average increase was 2.43 mm from baseline. Thus, Apligraf failed to demonstrate non-inferiority to control for the primary efficacy endpoint.

There were 8 secondary efficacy endpoints. I shall highlight selected endpoints in the next few slides. Apligraf was superior to control for 3 of the secondary endpoints: for tissue color, tissue matching, and patient satisfaction. There was no difference between the two groups in probing depth, recession, or

clinical attachment. There was also no clinically meaningful differences in resistance to muscle pull, inflammation, or bleeding on probing.

The width of keratinized tissue, KT, was a secondary efficacy endpoint. There was a larger increase from baseline to 6 months in KT width in control sites compared to Apligraf sites. A mean increase of 3.33 mm for control versus a mean increase of 1.37 mm for Apligraf.

Additionally, but not shown here, at 6 months, 100 percent of control sites established a KT width of 2 mm or greater compared to 82 percent at Apligraf site.

The perception of pain at Week 1 was one of the variables in the subject discomfort questionnaire in the patient satisfaction assessment.

Shown on this slide at Week 1, more subjects reported severe pain at the Apligraf site than at either the control or donor site.

In summary, Apligraf failed to

demonstrate success in the non-inferiority primary efficacy endpoint of establishing a zone of attached gingiva compared to a control palatal graft.

There was some indication of success in 3 of the 8 secondary endpoints, for tissue color, tissue matching, and patient satisfaction. However, there was a greater increase in KT width at control sites, and there was more severe pain reported at Apligraf site at Week 1.

The results from this study were used to guide a study design for the pivotal Study 06, in that the width of KT tissue was chosen as the primary efficacy endpoint for the pivotal 06 study.

Two adjunct laboratory studies were conducted with Study 05. Tissue specimens from 7 subjects at baseline and at 6 months from both Apligraf and control sites were histologically evaluated to examine cellular composition and tissue architecture.

In general, the presence of both gingival

and alveolar mucosal phenotypes with a transition shown between them was seen in the 6-month biopsies, however, the origins of these cell types were not determined.

The second adjunct laboratory study conducted was a DNA persistence study performed from 2 biopsy specimens. The Apligraf specimens, as well as subjects' buccal samples were analyzed by PCR.

At Month 6, there was no evidence of allograft DNA persistence at the Apligraf sites.

The control sites were also negative for allograft DNA.

The second study that I am going to show was Pivotal Study 06, and I will start with the study design.

The primary objective for Study 06 was to assess the ability of Apligraf to achieve a clinically acceptable KT threshold at 6 months of 2 mm or greater. This was a randomized, multi-center within subject control study in 96 subjects. The graft sites were also matched for

teeth and gingival condition.

Major inclusion criteria included adults with an insufficient zone of 1 mm or less of attached gingiva that requires soft tissue grafting for at least two non-adjacent teeth where root coverage was not desired.

The major exclusion criteria, also shown here, was similar to those for Study 05.

The primary efficacy endpoint was the proportion of subjects with a KT of 2 mm or greater at Apligraf sites versus a 50 percent success rate. This was a single arm comparison.

The six secondary efficacy endpoints are listed on this slide. Five of the 6 were superiority comparisons between the two groups.

Endpoint No. 3, a KT of 1 mm or greater for Apligraf after 6 months was compared to an 80 percent success standard.

The study plan was similar to Study 05 except the first two subjects per investigator participated as training subjects. The schedule interim visit time points was slightly different,

and the adjunct laboratory study conducted in Study 06 was an angiogenic biomarker study.

Evaluation of the width of keratinized tissue for assessment of the primary efficacy endpoint was measured at 3 and 6 months using a roll technique. Schiller's iodine was applied to aid in visualizing and distinguishing between keratinized and non-keratinized tissue.

Measurements were taken with a 15-gauge probe and rounded to the nearest 0.5 mm.

Methods for the secondary endpoint assessments are shown here. In this study, patient preference was assessed at 6 months by asking subjects to respond to the question: "Taking into account all aspects of treatment, surgery, recovery, and appearance, which treatment is preferred?"

Sensitivity at the surgical site was assessed at Week 1 using a 3-second puff of air to obtain the subject's response at the Apligraf control and donor sites. Pain and general sensitivity were recorded on a study-provided

diary each day from Day 1 through Day 14.

Analysis of the primary efficacy endpoint was the superiority of Apligraf relative to a pre-defined standard of 50 percent success for a 2 mm KT threshold after 6 months at a 5 percent level of significance.

The analysis population for effectiveness was 85 subjects.

The efficacy results for Study 06. Ninety-six subjects were enrolled and treated. All 96 completed all required visits. The mean age was 47 years. Fifty-four percent were female, 91 percent of study subjects were Caucasian.

Of the 96 enrolled, 11 participated as training subjects and were not included for efficacy analysis.

The primary efficacy endpoint, the criterion of 2 mm or greater KT at the Apligraf site at 6 months in at least 50 percent of subjects was met by 95 percent of the study subjects.

The exact binomial 95 percent confidence interval was 88.4 to 98.7 percent. All 11 subjects in the training cohort also met the primary endpoint at the Apligraf site. All 96 subjects met the primary endpoint at the control site.

Sequential order of testing was pre-specified for the secondary endpoints.

Results for the secondary endpoints show that Apligraf was statistically superior to control for 3 of the 4 secondary endpoints.

Endpoints No. 1, 2, and 4, shown on this slide, these were color matching, texture matching, and patient preference. Apligraf also met criterion No. 3 for KT of 1 mm or greater. This criterion was a single arm comparison to an 80 percent success standard. There was no significant difference between Apligraf and control in sensitivity at the surgical site, the fifth secondary endpoint.

Due to the pre-specified order of testing, the sixth endpoint, the absence of pain

after 3 days was not tested, and although it was not tested statistically, the results summarized in this table showed no important differences between Apligraf and control sites.

At FDA's request, other effectiveness endpoints were analyzed post hoc. Changes from baseline to 6 months were performed for each of the endpoints shown on this slide.

I will highlight results of 3 of these, because these were the endpoints for which Apligraf showed some improvement from baseline.

The 3 were recession depth, the KT width, and attached gingiva.

For Apligraf site, there were improvements from baseline to 6 months in recession depth, KT width, and attached gingiva width. No other endpoints yield clinically significant important changes.

For control sites, there were improvements from baseline to 6 months in the KT width and attached gingiva. There was no clinically important improvement in the recession

depth.

Results for KT width and attached gingiva between Apligraf and control sites are shown in this table. Control site has greater KT width and attached gingiva width than Apligraf at 6 months. There was no other difference between Apligraf and control for the other effectiveness endpoint at 6 months.

In summary, Study 06 met its primary efficacy endpoint. A KT of 2 mm or greater at 6 months in at least 50 percent of the subjects, 95 percent met the success criteria at the Apligraf site. Apligraf also met its secondary efficacy endpoints for color matching, texture matching, KT of 1 mm or greater, and patient preference, but it did not meet the fifth endpoint for sensitivity at the surgical site, and pain was not tested due to the pre-specified sixth testing sequence.

Study 06 adjunct laboratory study was evaluated and the rate of angiogenic biomarkers, such as angiogenin and fibroblast growth factor-2

from wound fluid samples taken from Apligraf and control sites.

There were no biomarker correlations to the quality of healing results, such as color, texture, pain, or inflammation in either treatment group.

The safety of Apligraf will be presented in the next few slides.

The safety database was derived from three clinical studies for the application of Apligraf in the oral environment. Studies 05 and 06, and a pilot Study 07.

Study 07 will not be discussed in detail here since its study objectives and treatment procedures were different than those presented for Studies 05 and 06, however, the safety results from Study 06 are applicable and will be included in the safety discussion.

There is extensive additional safety information from the clinical studies of Apligraf for the treatment of chronic cutaneous wounds, as well as postmarketing safety information from the

FDA MAUDE safety database.

In Study 05, there were no SAEs or deaths reported during the study. There were no related AEs that were clinically significant. There was also no infection reported at Apligraf-treated sites and no evidence of clinical immune response was reported.

In Study 06, three SAEs were reported. The cases of pneumonia and chest pain were assessed as unrelated by investigators, and the relatedness of the case of metastatic malignant fibrous histiocytoma was unlikely.

An additional case of follicular thyroid neoplasm, a Hurthle cell lesion was reported as moderate and was described as resolved after removal of the lesion. It was not reported as an SAE, and it was assessed as unrelated by the investigator.

The local AEs by Apligraf, control, and donor sites were unremarkable.

There were no important AEs identified in Study 07.

Pre-market studies for the treatment of chronic cutaneous wounds exposed a total of 273 subjects to Apligraf. There were no significant or related AEs from the two studies, and no clinical evidence of Apligraf rejection was reported.

Since the FDA approval of Apligraf for the treatment of venous leg ulcer in 1998, more than 400,000 units have been shipped commercially. A total of 9 medical device reports (MDRs) have been submitted to the FDA MAUDE safety database.

No allergy or acute rejection of Apligraf have been identified, and no malignancy has been attributed to Apligraf.

In today's Advisory Committee meeting, the clinical topics for discussion and for voting for some questions will center on issues concerning the effectiveness, the intended patient population, and the safety of Apligraf.

This concludes my conclusions for this morning.

DR. DUBINETT: Thank you. We now have time for questions from the Committee.

DR. AHSAN: I have a quick question about comparing the effectiveness or the efficacy between the pilot and the pivotal study. If I am correct in reading the document the sponsor gave, the pivotal study included an additional layer of Apligraf, and so my question is gearing toward the questions we have to discuss this afternoon in terms of potency and dose, it did seem like the numbers, but I don't know what the statistics are between the efficacy measurements between those two studies.

Can either the FDA or the sponsor speak to that?

DR. WITTEN: I don't think we have a comparison between the effectiveness of the two studies. Maybe the sponsor would like to comment on that.

MR. BILBO: Dr. Bates can provide some information on that, that will be helpful.

DR. BATES: There were a lot of learnings

for the Company between the pilot study and the pivotal study, and some of these related to a better understanding of the mode of healing.

Apart from the covering layer, which is correct, there was a difference between the two studies, the amount of Apligraf actually applied to the wound was also different, the suturing technique was different, the patient population was different, as well, and we are not sure which of those variables would have contributed to the results.

I can share with you a slide, if we could have slide up, just to show you the differences between the results, the keratinized tissue at six months for the pilot and the pivotal study, but as I said, with those variables, we are not sure which of those is attributable.

We consider the role of the covering layer to be more of a protective layer for the product.

With respect to I think you were alluding to the amount of Apligraf that was applied to the

wound, the relevant part of Apligraf is the part that is in contact with the wound, it is then folded back on itself, and all the other parts are quite some distance away.

We have a non-schematic to show you that with the Masson's trichrome stain, just to show you the distances that are involved. Slide up, please.

If you just focus your attention on the righthand image, you can see the wound bed just down at the bottom, and it is that part of the Apligraf that is in contact with the wound, and if you think about cytokines, et cetera, that are diffusing from other parts, that there is a huge diffusion distance from it.

Again, this construct does not get vascularized, that under surface of the Apligraf is a barrier. We have a scanning electron micrograph to share with you, and that also I think helps the panel understand why this product does not get vascularized.

Slide up, please.

You have got the standard H&E, actually, it is the Masson's trichrome stain on the lefthand side, and you can see in brackets the bovine collagen layer, and then we used SEM, that would be looking at the under surface with and without the bovine collagen layer, you can see that this is a barrier to cell infiltration and cell migration.

MR. BILBO: Dr. McGuire would also like to add a comment relative to the two studies.

DR. MCGUIRE: And also just on a practical basis, one of the reasons or the primary reason we changed and put the fourth layer on top in the pivotal trial was on the pilot study, there were a couple of instances when the Coe-Pak, we showed you a picture of the dressing was coming off prematurely at the very beginning, and we could actually kind of peek underneath that, and it appeared that the Apligraf was attached to the under base of that, and we wanted to make certain that should that occur in the pivotal trial, if the Coe-Pak were

to fall off, it would not take the Apligraf with it. So, as a practical basis, that is why we made the change.

DR. DUBINETT: Dr. Wittes.

DR. WITTES: I have a regulatory question. So, this move from CDRH to CBER, and I wonder whether -- I find using success standard for these KT widths sort of peculiar, the 50 percent and 80 percent for the primary and secondary.

I wonder why it switched to CBER and whether the use of the success standard rather than what seems like more natural comparison to the control, is that a consequence of the movement?

DR. WITTEN: Well, it is in CBER for this indication because of the combination products rule that was I think passed in 2005, so that is why it is here.

As far as the study, this is the study that was designed and I think that the question that we have to look at, and also the Committee,

is looking at the data, does this show effectiveness of the product.

One thing that wasn't commented on except I think somewhat briefly by the sponsor, but it may be worth asking the periodontal experts on this Committee, which is there may also be an issue of, you know, there is a concept of there may be enough of backup tests, there may be a certain amount that is effective enough, so whether you have that much or whether you have a lot more than that much, you have enough. It is not really like looking at a survival study where obviously, 8 months is twice as good as 4 months.

But I mean that is something that maybe we will hear discussion from our Committee on that question, but I think that is a difference not -- it is a different study, but that also may be related to a difference in what is the goal of the product compared to the goal of some of the other products, but I think that is a question we would like to hear from the Committee about.

DR. DUBINETT: Dr. Hwu.

DR. HWU: Because there is such an effective standard therapy, I think the secondary endpoint of patient preference is an important one. Was any granularity collected in that data to see why patients prefer the Apligraf?

MR. BILBO: No, there was no more specific data than what we presented.

DR. HWU: Also, a question about the potency assay. It is H&E staining, and there is always some subject to when you are interpreting an H&E stain, I saw there was some validation and training period for people interpreting that assay, but, you know, there are some very sophisticated image analysis programs now available, and was that ever tried to make the interpretation of the potency assay more objective?

MR. BILBO: We haven't evaluated them in image analysis techniques, but I can bring up Dr. Pitkin to elaborate on the training, validation qualification program we have for our histology assay.

DR. PITKIN: Thank you. Our histology assay, as was stated earlier, evaluates multiple parameters in quantitative manner. Each parameter has been validated in accordance with ICH guidelines for validating analytical method. It has been validated for accuracy or reproducibility and sensitivity.

Moreover, each analyst that performs this type of an assay has to undergo a very rigorous training program. The training program takes at least six months and what it includes is multiple evaluations of the technique, comparability to subject matter expert, and it ends up with a very rigorous exam that analysts have to pass.

After that, they are qualified and certified to perform this assay. In addition, on a regular basis, they have to perform proficiency tests to demonstrate that they can perform this type of technique in a reproducible and consistent manner.

DR. AMAR: Can I just follow up on this? In the standardization, there is ways right now

to standardize the staining. We know there is a great level of variability with how long you stain a section.

Was there any attempt to have -- there is a robot right now that are available, and they stain for a certain period of time, and all the sections are done the same way.

MR. BILBO: We have a standardized histological processing methodology that results in uniform results. It is also important to note that there is a USP monograph for evaluation of Apligraf histology as an analytical method.

DR. DUBINETT: Dr. Couture.

DR. COUTURE: I actually have three questions. The first one is very simple, and the first is that fourth layer or that outer layer, is that expected to be part of the BLA, so that that would be actually how it will be used in the clinic? A fairly simply question, yes or no, I guess.

MR. BILBO: Yes.

DR. COUTURE: Okay. The second is there

wasn't any explanation given to why there is that Z fold, why they need three layers, and whether anything else has been tested, if that is just a clinical issue about being able to suture it down or something, or is there a dose effect, as it were, between 1, 2, 3, and then really 4 layers if you add the outer layer.

MR. BILBO: Correct. You hit on it exactly. In terms of working with our clinical investigators, we developed the C fold or S fold simply for handling purposes to improve the durability of the product in a harsher oral environment, but as Dr. Bates noted, it is really that initial layer that is a primary contact with the wound bed that is providing the clinical benefit. The other two layers are separated by two layers of stratum corneum barriers that we don't anticipate that they are providing clinical benefit.

DR. COUTURE: Actually, I lied, there were four questions, but the second and third one is how many lots of material, which I presume

were two different donors put together to make the product, were used in the clinical trial?

DR. PITKIN: There were a total of 39 lots of material that were used in the pivotal trial for the oral indication, 39 batches, or I would say material from 39 batches.

DR. COUTURE: Okay. Then, would you tell me what a batch is versus a lot?

DR. PITKIN: This is the same thing, batches and lot, we use it interchangeably.

DR. COUTURE: All right. So, you did your productions of 300 or so individual little units, you did that, you had 39 of those runs that you used in this?

DR. PITKIN: Thirty-nine lots were used in the pivotal trial for the treatment of 96 patients.

DR. COUTURE: Okay. The last question goes, is a completely different track, but it goes to the comment that was made -- I am sorry, I apologize for forgetting the clinician's name who did the study -- that said the big issue here

is that patients right now won't do the FGG because of pain, and yet the evidence here is that there is really little difference between the pain in the Apligraf and overall pain in the FGG method.

So, how does that translate into this actually being an even more useful product if where patients are going to have to be told it is still going to be painful?

MR. BILBO: I would like to bring up Dr. McGuire to elaborate.

DR. MCGUIRE: Thank you for the question.

As we discussed earlier, with the split mouth design, it is very difficult to pick up pain. I think that it is hard to believe that if you have, in the surgical, you know, a slice of tissue taken out of your palate, that that does not carry with it some pain.

Again, I go back to the fact that 72 percent of the patients said they would prefer Apligraf over the free gingival graft as a global experience, and pain has to be part of that.

Picking out pain, if you go to any of -- it is very difficult, in fact, I can't think of hardly any, if any, RCTs in the dental literature comparing soft tissue grafts that have a panel donor site that you can pick up pain in when they are split mouth, so that is a difficulty, but I think it is just common sense that we are eliminating an area that has potential for bleeding and pain.

Also, to speak to the fact of what benefit we are giving here, one of the real challenges here is that we are asked to compare this to the free gingival graft, and we are seeing the free gingival graft as producing more keratinized tissue, but this is one of those situations where more is not necessarily better.

With the free gingival graft, if you put 4 mm on there, you are going to get 4 mm, if you put 8 mm, you are going to get 8 mm. In fact, just a couple of weeks ago, in my office, I had a patient come in who had had a previous free gingival graft placed on a lower molar, and asked

me to come in and thin it out because he had chronic food impaction underneath it. So, there is problems with that.

What we are trying to do here is to create enough keratinized tissue to maintain health, and we are doing that.

DR. COUTURE: But then would you comment on the relevance of attached gingival for which this product doesn't provide as much as the FGG?

DR. MCGUIRE: Well, the attached gingiva is made up as was talked about, of a zone of keratinized tissue from the mucogingival junction to the free gingival margin, and then you subtract the probing depth, and that equals the attached gingiva.

The reason why we moved from the attached gingiva to the keratinized tissue to the pivotal trial is because there is reduced measurements, therefore, there is a chance for reduced -- there is less chance for error because we are measuring fewer things, and also what we are trying to do is create keratinized tissue, not attached

gingiva, and that is what we want to measure.

You can have a broad zone of keratinized tissue, but if you have deep probing depths, it is not a great deal of clinical value.

DR. DUBINETT: Dr. Lee.

DR. MEI-LING LEE: Thank you.

I have questions on pages 71 to 77. On page 71, the primary efficacy endpoint, the result looks good, however, I am just curious, it is not compared with the control FGG. On page 77, there is a difference on the Apligraf and FGG on this. There is two different widths.

Can you give some comments?

MR. BILBO: Well, I think it is important here to really emphasize that the endpoint for the study was generation of 2 mm of keratinized tissue which is clinically beneficial, not direct comparison to free gingival graft for the generation of keratinized tissue.

I would like to bring up Dr. Bates to discuss it further.

DR. BATES: The measurement of 2 mm is

based on a landmark study published in 1972 by Lang and Lowe where the publication found that if you had at least 2 mm of KT, then, that is going to halt further progression of periodontal disease.

As Dr. McGuire mentioned, the FGG is a very reliable procedure, and if it takes, you get what you graft, so if you have a 10 mm graft, you are going to get 10 mm. Is that necessary? There is no data to support the idea that once you get beyond 2 mm of keratinized tissue, any incremental keratinized tissue gives you incremental benefit.

So, the important point that the panel would benefit from considering is are we producing enough keratinized tissue that is clinically relevant, are we producing enough attached gingiva that is clinically relevant, and to help, I would like to show a slide that summarizes the changes in both KT and AG with Apligraf between baseline and 6 months.

Could I have a slide up, please.

You may recall this slide. The important -- it is the change from baseline, so we are not going back to baseline, we are producing more, and looking at the 6-month result. You are ending up with, if you look at attached gingiva in the bottom half, you are looking at 1.8 mm of attached gingiva. Dr. Nevins can comment on the clinical relevance of that amount.

DR. NEVINS: It gets back to the last question, as well, that we don't have a procedure beyond the tissue graft of today where we can predictably offer our patients an ability to go from zero mm of attached gingiva to 1.8, and repeat the statement I made earlier that a patient who is presenting with almost 2 mm of attached gingiva and 3 mm of keratinized tissue is unlikely to be in need of a soft tissue grafting procedure at that point in time.

That really shows that an adequate zone of attached gingiva, as well as keratinized tissue, was gained through the results of the pivotal setting.

DR. DUBINETT: In order to stay on time, we are going to have four more questions from Drs. Dubinett, Snyder, Bui, and Ahsan, and then we will pick up after lunch with additional questions to stay on time.

I have a question for Dr. Lim, and it has to do with the safety endpoint regarding neoplasm, and we noted the results for malignancies in your slides as presented from the two studies.

But I really wanted to preface my question by this, that is, there is a rich literature, of course, in the way in which inflammation synergizes with minimum mutational events in the development of malignancies is particularly true in tobacco-related malignancies in which inflammation has been shown to be an important cofactor.

In the data that we have from these two studies, because oral malignancy, of course, has specific risk factors and is quite a bit different from the application of the oral

Apligraf as opposed to the cutaneous setting, do you think with the number of patients that we have and the amount of time that they have been evaluated, that we would see a signal for the development of oral malignancy as a function of that time and number of patients?

DR. LIM: I would agree that, in general, a 6-month follow-up is certainly inadequate if you are going to assess the malignancy potential of product. So, ideally, more than 6 months is required in terms of how many years even is required is an open question.

You mentioned that the cutaneous environment and the oral environment are different, and we recognize that, but I think we can still draw some information from the large body of information we have from the postmarketing of Apligraf in the cutaneous setting.

We look at all the different factors, and we gave it our best judgment, but in our review of this BLA, if we approve it, then, certainly

something that we will take into consideration in terms of assessing whether additional follow-up time would be required.

DR. DUBINETT: I think I am asking the question in the context of do cytokines and the growth factors that we have heard about, PDGF, IL-1 alpha, and the others, those are the exact cytokines that have been found to be important cofactors in the development of epithelial malignancies particularly those that are tobacco related.

We heard that 40 percent of these individuals had a smoking history, some of whom had oral pre-malignancy, but we don't know their follow-up. It would be surprising to me that we would have enough patients and enough time of those 40 percent to have any meaningful knowledge of the natural history of those patients at risk.

So, the second question I have for Dr. Betz, and that relates to smoking and the need for FGGs, is there a relationship between smoking and the requirement or outcome with the FGGs?

DR. BETZ: None that I am aware of.

DR. DUBINETT: The design in which patients are not smoking for three months prior to the surgery, why is that? What role does smoking play in the outcome?

DR. BETZ: I believe it is related to how the tissues heal intraorally generally. The sponsor may want to comment on that also.

MR. BILBO: Yes, Dr. Bates would like to take an opportunity to comment on that.

DR. BATES: For wound healing studies it is pretty standard to exclude patients that are smoking when you are looking at events like angiogenesis, which we know are going to be impacted by smoking, so we don't have that additional confounder, and it would probably be beneficial for Dr. McGuire to discuss in the field of periodontology compared to odontological studies whether smoking exclusion is normal.

DR. COCHRAN: In the field of periodontics, we have looked at a number of therapies and the outcome in smokers versus

nonsmokers, and generally, I think as a rule of thumb, you can say particularly for almost every therapeutic option that is done in periodontics that if you include smokers, they are still going to respond, but they don't respond quite to the same degree as a nonsmoker.

So, what that means clinically for our patients is that we have to just have better informed consent and let them know that that is a confounding variable and that their expected response will not be what it would be if they did not smoke.

Now, how long it is if they stop smoking, we really don't know. As a general rule of thumb, we consider 10 cigarettes or less a day as sort of a minimum amount of a smoker, and a number of implant studies segregate smoking more than 10 cigarettes a day versus less, and that seems to be a breakout point.

I don't think there is any good rationale for it, but that's the way the studies are done, and if you stop generally 3 months prior to that,

we consider most of the effect gone in those particular patients at least from a periodontal studies point of view. Thank you.

DR. DUBINETT: Dr. Snyder.

DR. SNYDER: I just had maybe two and a half questions that I wanted to just quickly pose to the FDA examiners just to clarify for myself.

One of them may have already been answered, to Dr. Betz as the clinician, so is it your view, as maybe Dr. Witten said, that it is not a reasonable clinical comparison is, for example, at least 2 mm of keratinized gingiva, and there is a threshold, and it doesn't matter as long as you hit the threshold, your clinical outcome is acceptable, and following that for 6 months should be reasonable, and it is not simply that more is better, that you just have to hit threshold, 6 months is good enough to be able to do that.

I guess that is my first question, and then my second question was probably I guess to Dr. Lee, and trying to get a sense of how long

does the Apligraf, how long do the cells really last. They are clearly gone by 6 months.

Is there some way from your review of the data that indicates when do they really disappear, in other words, how long are they producing these cytokines that may actually get to some of the concerns that Dr. Dubinett legitimately raised?

I guess a corollary of that is, is the mechanism by which the same complex works in the mouth, the same as the way it works in the foot ulcers, are the mechanisms of action the same for repairing ulcerated legs as you would expect, as we think it may be working in the mouth?

Maybe I will take Dr. Betz's assessment first and then Dr. Lee's.

DR. BETZ: As far as the minimum amount of keratinized tissue, there is a study out there that basically says it is more of a function of how the tissues respond to the perioral musculature.

Some people could have a mm, or maybe

even less of what appears to be keratinized or attached gingiva, and if you pull on a lip and the margin just doesn't move, it is clinically acceptable.

DR. SNYDER: So, you would be happy saying that the two procedures are identical in terms of clinical outcome, therefore, they can simply be now influenced by patient satisfaction?

DR. BETZ: I am going to pass the buck and say that is what we want you guys to talk about.

DR. MARK LEE: Regarding your questions, so the first question was regarding how long are these cells producing the growth factors and cytokines, we probably more limited data set from review, so I would actually bounce that over to the sponsor, maybe they could speak more to that.

MR. BILBO: We would like to have Dr. Bates discuss in terms of the production of cytokines and growth factors and clinical effect.

DR. SNYDER: But particularly in vivo, not in vitro.

DR. BATES: So, learning from our experience with skin grafting, if a skin graft does not take within 5 days, those cells die. We have no evidence that Apligraf vascularizes, so linking the two knowledge sets, our understanding is that these cells would undergo cell death within that type of time frame.

We have that dense bovine collagen layer, we don't have any evidence of vascularization. We are surviving for the first few days on plasmatic inhibition and then there is no antigenesis in those cells, if you recall, and I showed some images of sort of the degradation profile of the product. It doesn't last very long, and the Apligraf DNA detection that we performed is also obviously not necessarily a marker of cell liability either.

So, that is on the vascularization. With respect to the mechanism of action, our understanding is the mechanism of action is very similar. We have got a wound healing situation in the mouth, we have got a wound healing

situation on the skin. Apligraf is applied topically to a wound bed. In both situations, we are relying on a modification of secondary intention healing.

We focused in the skin more on the rate of healing, so the rate of re-epithelization, the rate of granulation tissue formation. We focused here more on the quality of the tissue that is being formed, but we have also got data from our acute cutaneous clinical studies where we see an influence on the quality of healing as well.

So, there is a bridge. We know that the phases of wound healing are very similar in both.

There are subtle differences between oral mucosa wound healing and cutaneous wound healing.

We know that the mucosa heals very rapidly and usually heals with less scar than the skin, but the similarities overall and especially from a clinical perspective far outweigh the differences.

I also wanted to take the opportunity, if I may, for addressing some of the points raised

by Dr. Dubinett regarding inflammation, and I wanted to remind the panel that Apligraf is short lived, we don't see any difference in the inflammatory profile, clinical profile, between FGG and Apligraf.

We have measured angiogenic cytokines of the type that you were describing, PDGF, et cetera, and we also measured those in the roof of the mouth from where the graft is taken, and in fact, the amount of the cytokines released in the roof of the mouth are higher than what we see with Apligraf.

So, the cytokines are within the physiological range, it's not a supraphysiological release, and the longevity of Apligraf is finite and very small, and our understanding is that the cells are most likely nonviable by a week.

DR. DUBINETT: Just to clarify the point on this, it is not so much the cytokines from the graft itself, but the cytokines they induce, so in light of that, I would ask you what is the

number of cytokines and angiogenic factors that you measured from the host.

DR. BATES: Exactly. So, we measured the in vivo, we took gingival crevicular fluid, and we also took fluid from the roof of the mouth, and I can show you a summary of those data. Slide up, please.

This just shows you FGG and Apligraf. We have data also from the roof of the mouth, and where Apligraf tends to net out, typically, is halfway between -- the concentration is maximal at one week overall, and we see, in terms of the patient's response to either the FGG, Apligraf, or the donor site wound, which is secondary intention healing alone, is that Apligraf nets out somewhere between the graft and the secondary intentional or open wound alone.

If we could have the next slide up, this illustrates it with respect to VEGF where you have the donor site at one week higher than Apligraf, and Apligraf higher than the FGG, but again the one point here is to reiterate that it

is short lived and growth factor is within physiological concentrations.

DR. DUBINETT: Thank you.

Dr. Bui.

DR. BUI: There were 3 independent reviews to evaluate several characteristics. The first question for the sponsor is did you see any discordance between these 3 independent reviews, and if you see it, can you provide any data to the panel?

MR. BILBO: I am sorry, I didn't hear your question.

DR. BUI: The discordance between the three independent reviews, is there any discordance that you see?

MR. BILBO: I will bring up Dr. Bates to respond to your question.

DR. BATES: We will have that slide up for you in just a second. Slide up, please.

This is looking at the color measurement.

If you remember, we also looked at mucogingival contour, texture, and the marginal contour. On

the righthand side you have got the clinical site results, 92.9, the same for Apligraf at 27.1 percent, and then you can see the blinded independent photographic assessment where you have -- the rating is 95.3, so there is a range, but it is overall pretty consistent with what we are seeing in the clinical studies, and then you can see the results for the FGG-treated sites below, you can see the difference between Apligraf and FGG.

DR. BUI: The second question I have is for the FDA. I know that the sponsor submitted the pharmacovigilance plans to the FDA at the end of July, and two potential risks identified was gingival injuries and gingival pain.

Can the FDA provide the panel some details about pharmacovigilance plans and what is the status and the review of that right now?

DR. WITTEN: Do we have our OBE representative here?

DR. BARASH: The pharmacovigilance plan was actually acceptable. We are not planning on

requesting any postmarketing requirement, but we did request that any source of malignancies be reported as a 15-day report.

The contracting agency should be identified and trained prior to marketing, but we didn't have any major problems with the pharmacovigilance plan.

DR. BUI: Okay. Can I just follow up with that question? Will there be any pharmacovigilance I guess similar -- will that be required in the labeling?

DR. WITTEN: I think we can't say what we are going to do or what we are going to require until we complete our review, so if there are some specific recommendations you have, we would be interested in hearing what they are.

DR. DUBINETT: Dr. Ahsan.

DR. AHSAN: I have two questions, and they are both really about information leading us to the afternoon discussion.

The first one is about the master cell banks. So, you have two different cell types,

they don't necessarily need to be from the same donor, right, and so are there specific pairings that you use for the master cell banks, or you can use mix and match as you like?

MR. BILBO: That is correct. Once a cell strain is approved, fibroblasts or keratinocytes, there is no matching required between the two cell types.

DR. AHSAN: Okay. So, in terms of historical data with Apligraf, how many master cell banks of each cell type have you gone through and actually made lots with, that have passed, and how many have failed?

MR. BILBO: Bring up Dr. Pitkin to review that data.

DR. PITKIN: Over the past almost 20 years of our experience we have generated 17 keratinocyte cell banks, and we have generated 5 fibroblast cell banks that have been tested and approved.

DR. AHSAN: And how many failed?

DR. PITKIN: I would defer to my

colleague, Dr. Baksh.

DR. BAKSH: Thank you for your question.

In terms of our cell bank qualification and regimen that we use for testing, we screen a number of tissues during this process, and typically what we see here is at least 1 out of 5 tissue actually go through and make our ultimate product, so that is typically what we expect to see, and that is simply based on the biological performance of the cell banks.

DR. AHSAN: And then you make MCBs as necessary, right?

DR. BAKSH: That is correct.

DR. AHSAN: My second question is actually about -- thank you for that, about the master cell banks -- my second question is about the data related to potency. So, you have potency specifications and you have a characterization panel of assessments.

There is this attempt to make these connections. I understand that you had compromised samples and you also used an expired

sample, and you looked at assessments.

Do you have any correlation data? It seems like everything is a 1 point variation or a threshold bit of data. So, do you have any correlation to show on any of these metrics that relate the histology to these more characterization type assays?

MR. BILBO: So, correlation of the numerous histological parameters to biological function.

DR. AHSAN: Right, as you have mapped them, if you could speak to the sensitivity and the resolution of the histology metric that you would like to substitute in versus the biological assay, and then also the range in the robustness of each, just so that -- I understand if one change, when you show the compromised or the expired one changed, and the other changed, but how much of a change do you need in one in order to observe the same difference, or a marked difference, or an observable difference in the other correlated study?

MR. BILBO: Your question really goes to the sensitivity of the assay. Dr. Baksh.

DR. BAKSH: I completely appreciate your question. We haven't actually done any direct correlation between the values that we generate in the histology assay versus the biological measurements that we take from a number of these assays.

If you can imagine, it is very difficult to simulate the range in a number of these different parameters, and so the best that we have been able to do to show at least an association or a link is to create conditions that we would simulate, like say, for example, in manufacturing, and those are the types of conditions that we would see, and we would be able to assess the quality of the product based off the measurements we obtained.

DR. AHSAN: Right, so there is two ways to do it, one is more difficult, as you mentioned, which is any of the levels of compromised sample.

DR. BAKSH: Correct.

DR. AHSAN: I appreciate that that sometimes ends up being a little bit more binary, but even within your normal lots, do you have scatter plots to show the correlation between these?

DR. BAKSH: Actually, we do. I would like to bring up Dr. Pitkin. She can share with you the historical trending data for our batches of Apligraf.

DR. PITKIN: First, I would like to share with you one representative data on just histology, only one specific parameter being epidermal development. This data represents a year, 439 batches that were made or lots that were made within the one year 2010. Slide up, please.

It did show that we have, on the Y axis, we have epidermal development, on the X axis is all 439 batches of Apligraf that were made within one year 2010 to specification for this particular parameter is greater or equal to 70

percent.

What it demonstrates is that we had 8 failures over the course of this year, which constitutes 1.8 percent, so this method itself is sensitive enough for each parameter to detect failure modes, and as compared to other methods that we use for product characterization, this is the most sensitive.

The second data set that I would like to share with you is a table of historical data on multiple cells banks that were produced, and characterization panel or test results from characterization panel for multiple cell banks. Slide up, please.

Just again representative cell banks, keratinocyte cell banks, and on the far left column, and across the top of this table, the header, the table listed all characterization testing that was performed, cytokine production, expression, MTT, cell purity, senescence, in the far right column we have epidermal development again as an example of potency.

Specifications for each parameter is listed down below under the header of this table.

I would like to draw your attention to the last row of this table where averages, plus or minus standard deviation are presented for each parameter. That demonstrates a great deal of consistency across multiple, in this case, 4 cell lines, keratinocyte cell lines that were produced.

For each parameter, as well as the potency parameter, as measured by our histological techniques.

Did I answer your question?

DR. AHSAN: You did, but I think this points to my concern, which is you see a 97 plus or minus 2 on the potency metric, and yet you see, let's say, on the VEGF, 467 plus or minus 130, so the levels of sensitivity are different.

So, my question, which we can discuss in the afternoon, is whether using histology as a potency indicator is sensitive enough to actual changes in bioactivity.

DR. PITKIN: Let me just pull up -- indeed, the potency assay is more sensitive, it is the only assay in our panel that is able to detect the failure mode. With regard to VEGF where we have 467 plus or minus 130, that is the range that we have observed across multiple cell lines, and although VEGF or other tests are performed as part of characterization, they are able to detect major departures from the process. We also use this characterization panel for major process changes, but for minor departures from manufacturing process histology is the most sensitive.

I can also share with you other parameters like basal aspect that would speak to viability of the cells again showing that we have had several failures over the course of the same year. We just chose the full year 2010, '11 is still not there. Slide up, please.

Again, the same 439 batches. In this case, it is basal aspect that has been evaluated for the specification of greater or equal to 95

percent. We have had 5 failures, which is 1.1 percent, but in previous years, it ranges from up to 2 percent we have seen failures.

It is important to remember that these batches are generated from cell banks that have already been approved, in other words, they have met all specifications, full characterization parameters, as well, so potency parameters, so it is a very sensitive method that we use to detect failure mode. Thank you.

DR. DUBINETT: Dr. Genco, can we have your question this afternoon, can it wait?

#### Open Public Hearing

DR. DUBINETT: At this point, we would like to ask the audience if they have any questions to address to the Committee, comments to the Committee?

[No audible response.]

DR. DUBINETT: Hearing none, we will now move to lunch and reconvene at 1:45.

[Luncheon break.]

DR. DUBINETT: Good afternoon and welcome

back. We are going to get started.

What we will do is we had two short questions from Dr. Genco and Ms. Rue to begin, and then we will be going back to the sponsor, because they had data that was asked for previously from Dr. Wittes.

So, to start, Dr. Genco.

DR. GENCO: Thank you. Actually, I don't have a short question, but a rather lengthy question.

DR. DUBINETT: Do you want to hold it for our discussion?

DR. GENCO: Sure.

DR. DUBINETT: Okay. Ms. Rue.

MS. RUE: My question is since it seems like one of the major advantages of the Apligraf is cosmetic issues, I am wondering if there is any data on dissatisfaction of people from having the free gingival graft and the appearance over the years.

DR. DUBINETT: Do you want to ask Dr. Betz to address that first?

DR. BETZ: I am not aware of any at all, however, I do know that as the sponsor indicated, people do not like the appearance of the free gingival graft because the entire patch, quote, unquote, appearance.

MS. RUE: Well, that is what made me ask, because when they are comparing both of them, but I just meant since it has been around for so long, if practitioners have heard of people complaining about the grafts that they received and the appearance of them.

DR. DUBINETT: Okay. I think because Dr. Wittes is not back yet, we are going to wait for your information that we have, and at this time begin our questions.

The way we want to do this is try to confine ourselves to 15 minutes per question and then have the discussions, so that we can stay on time. The questions will be projected, and so rather than read this whole thing that is projected here, I am going to read the question.

Dr. Wittes is here, so before we begin the first

question, we will hear from the sponsor that has the data that you asked about.

DR. WITTES: Thank you.

MR. BILBO: Dr. Bates would like to present the response to your question regarding confidence intervals.

DR. BATES: Could I please have slide up.

Dr. Wittes, I would like to focus your attention on the part of the slide that is highlighted in yellow, and what we did is calculated the confidence intervals for the Month 6 results and also for the change in baselines, and you can the differences between FGG and Apligraf with that confidence interval.

What I also wanted to take the opportunity of saying is that providing that margin or that interval creates 2 mm in the majority of patients, and if I could have the next slide, please.

That is the clinically relevant amount of tissue, and even if we were to move that threshold that you mentioned previously to 85

percent, we would have exceeded that threshold, so it's a very reliable, very predictable procedure generating a clinically relevant amount of KT.

DR. WITTES: What about the other two confidence intervals?

DR. BATES: For?

DR. WITTES: For the proportion and for the -- what was the endpoint?

DR. BATES: For the attached gingiva?

DR. WITTES: Yes.

DR. BATES: We don't have that. I think it's an opportunity to address the question again, are we producing enough KT irrespective of what FGG is doing from a clinical perspective, is this relevant, and we believe that it is.

DR. DUBINETT: Thank you.

Before we begin the discussion of the questions let me remind the Committee that if you have a question, and you just raise your hand, Gail is watching and she is keeping a queue list here as we go through, so everyone will have a

chance to have their questions heard up to the 15-minute maximum.

Commission Discussion of Questions and Vote

DR. DUBINETT: We will begin with the first discussion question, and you see it here. This is to discuss the applicant's approach to qualify and demonstrate comparability of new cell banks used for Apligraf manufacture.

Our lead discussant for this is Dr. Couture.

DR. COUTURE: I will try not to take up the whole 15 minutes.

This is a combination product and which makes it somewhat complex. It has been developed all through the years up until fairly recently as a device. Devices and biologics are handled somewhat differently and how one characterizes components of a biologic, and a biologic is part of a combination product, is sometimes different or not essentially the same or always the same as how a device might be measured.

The device has, according to the sponsor,

two different properties. It acts as a barrier, which means it is something of a structural component, and it has a biological activity. That biological activity is very likely, although not entirely demonstrated by the sponsor to be related to cytokines, growth factors, or what other things being produced by the biological component of the material.

So, I won't get into potency testing, because I think that will come up next. The questions are sort of what tests actually define a well characterized biologic product. In that sense, one would want tests that would measure both the structural properties of the combination product, as well as tests that would measure the bioactivity of that product.

The sponsor uses H&E testing in general to test the product and uses that as one of the final product tests for tests coming off of the master cell bank. The master cell bank stays qualified -- that is really what the question is focused on here -- the master cell bank is tested

by a number of parameters including, at the end, an actual product being formed and tested by H&E staining as for potency.

What is not really tested in a great way are the biological activities of either the individual cell banks themselves or the combination product. The sponsor reports that they test VEGF, which you saw some data before the break, and then they tested some cytokines.

The cytokines are tested, as I understand from the literature that was provided by the sponsor and more or less how it was presented here as a pass/fail, whether those individual cytokines exist or not by many criteria that is kind of a low bar to get over if there is any cytokines at all, and so the sponsor doesn't really provide a lot of correlative data between cytokine expression of their biological product and the actual activity of the product or efficacy or potency of the product when used in the clinical setting.

So, there is not a lot of testing done

that actually relates directly or has been shown to correlate directly to H&E staining or the activities demonstrated by H&E staining of individual lots.

What confounds the issue is that the sponsor makes master cell banks of the fibroblasts from one donor and then makes master cell banks, and, of course, working cell banks, as well, of the keratinocytes from the same donor, but also from other donors, as well, and so then combines those two together in some way.

The only correlative studies between to show, or comparability studies between various master cell banks is a fairly simple test, you know, viability, growth, et cetera, and then the cytokine profile once they actually form a combination product at the end, but that is a test of one master cell bank and another master cell bank. As far as we can tell, there is no other evidence in the literature other than documentation that says that that is tested with a broad panel of others.

So, one of the arguments, and one of my suggestions to the Agency, would be to look very carefully at just how well characterized those individual master cell banks are in regards to their variability of being able to produce cytokines when they make a combination product at the end, not just that they can do it once with one master cell bank from a keratinocyte and one master cell bank from a fibroblast, but these are complex products and a complex structure that is formed between these two cell types, and there probably should be a little more assessment of the amount of cytokines that are produced in that combination product with multiple banks, and then some correlation made between that and H&E staining if they want to not do that H&E -- excuse me -- that cytokine study on individual products or lots of products.

I think I will just stop there.

DR. DUBINETT: Do we have discussion questions from the Committee?

DR. REYNOLDS: If I could just make a

comment. The sponsor commented on this earlier. The correlative studies are dependent on range, and if the product, if the lots have a relatively constrained range in terms of cytokine levels, you really are going to have a very difficult time showing a correlation.

That, I think resonates with me, because as that range becomes increasingly more truncated because they are within a tight band, you won't really be able to see that unless you create deviations in the protocol to artificially create it.

DR. COUTURE: That is absolutely completely true. Without any data being presented, it is not clear how that broad those ranges are, and it is not clear how broad the range of cytokine expression is for an H&E-positive cell layer, and there is no data presented on the variability seen in the clinical trials, whether it is KT or any other parameter and cytokine expression.

Since cytokines are a part and parcel of

how this thing is likely to work, the biological activity, again, which is completely in the dark in terms of whether those cytokines really matter or not and what ranges would be applicable, it is normal for release of a product to have an assay matrix especially the combination product that would include potentially physical properties like the H&E, but then also some other biological activities.

And then, of course, an important part is normally, those ranges are defined and honed down through the course of clinical study, so one doesn't get to a BLA and say, you know, if we don't know, we usually get to the point of BLA and say, well, here is all the data, and then sometimes you come up with a BLA point and say, well, what we have learned is that the range is so variable, and there is no correlation between expression beyond, it just has to express some in biological activity, but at least what has been presented to us, I don't know everything that has been presented to the Agency, maybe this is it,

there has been no data that suggests that range of cytokine expression doesn't correlate with biological activity or potency.

DR. DUBINETT: Dr. Dahlgren.

DR. DAHLGREN: My question has to do with we are making the assumption that the cytokines are important, but is there data somewhere that shows whether we have looked at acellular constructs in comparison to the cellularized constructs?

I mean there is a paper in Tissue Engineering that is recent, but, of course, I can't pull it up because I can never get Tissue Engineering to pull up, but I guess my question is. is the collagen scaffold alone capable of producing the response that we are talking about.

DR. COUTURE: Well, or you could even go further, because the sponsor does present data about their VEGF, and they show that you can look at a fibroblast later and don't get VEGF, you can look at a keratinocyte layer, you don't get VEGF, but as far as I know, those are never tested in

the clinic to demonstrate that you actually need the combination in VEGF, so maybe the sponsor can comment. I don't know if you are allowed to do that or not, but it's an excellent question.

DR. DUBINETT: You want them to address that?

DR. COUTURE: Well, maybe that is good question, we can do that? Okay. I think that is an exceptional question to give the sponsor an opportunity to weigh in.

DR. DUBINETT: With very brief answers.

MR. BILBO: I think it is helpful to point out that the product doesn't exist until its final form, going through, combining the two cell types with the bovine collagen and going through the 20-plus state manufacturing process where we are developing this three-dimensional, fully differentiated epidermal layer, so to try to tease out different parts, it is just not feasible. The product is what it is, so to speak.

DR. COUTURE: I suppose a more simple

part of the question is does the product have to be that complex, do you know that you need both layers to do this, in either mouse studies or --

MR. BILBO: Dr. Bates can respond.

DR. BATES: It is a great question. There are some studies looking at acellular membranes in the oral cavity, and top line conclusions are that it is less reliable and you get scar formations, so if you remember the spectrum of wound healing responses I was showing you, scar down one end and regeneration down the other, you tend to get more scarring.

If we look also to the wound healing studies in the cutaneous environment, the rites of wound healing or the rites of re-epithelization that were seen with the cellularized product exceed an acellular matrix alone. So, we don't fully understand the mechanism of action, but seeing the clinical difference we are postulating that these are due to the viable cells and cytokines.

DR. COUTURE: I think that is a very good

answer, and I think that is a very acceptable answer to me in any case.

The mechanism of action is always very difficult to prove, and in a product like this, it could be extremely difficult, if not impossible to actually prove, but it does beg the question, if you are arguing, and we would all agree that there is probably, since these cells don't hang around, it has to be that early barrier effect or it's cytokine expression, and if it's cytokine expression, there should be some measurement of those cytokines or some correlation between those cytokines and the potency assay that you want to use to release the product and demonstrate that cytokine levels aren't important, because right now all you have is a pass or fail.

I mean there are some cytokines and that's it, which is counter to any other biologic I have ever worked with where if we think cytokines are important, you tend to measure those cytokines and ask some question about

those.

DR. DUBINETT: Dr. Ahsan.

DR. AHSAN: Actually, the question we are addressing at the moment is about the cell decks, and so one of the things that I kind of wanted to point out again was something that Dr. Couture mentioned, which is that the product actually uses a combination of two cell banks.

So it is that pairing that led to a construct that passed or failed, and so I am not quite sure how it should be handled, but there is some argument to be made about keeping track of those pairings and the success of the clinical outcome and whether or not, you know, you always keep the pairing together and that even if only one master cell bank is depleted, that both master cell banks be retired.

So, I think in talking about the cell banks, I think that that is something that might be important for the FDA to think about in terms of moving forward.

DR. DUBINETT: We will now ask Dr. Witten

if we have had adequate discussion for the FDA.

DR. WITTEN: Well, I do have a follow-on question, which is that I think it is important for us to understand as much as we can about the testing and what it means about the outcome, but I don't think that the suggestion is to look at, or at least I don't -- maybe you could clarify -- it would be hard to envision having a new clinical trial for every different combination of cells in a construct or every new construct, so I wonder if you could clarify what it is that you think would be helpful or that we might ask the sponsor to do.

DR. AHSAN: Well, I am not sure that I know exactly. That is why I left it to you. But I think just keeping track of that a little bit might be important as we see. I mean maybe it is more in the best interests of the sponsor in terms of successful pairings that lead to lots that pass their potency assays.

But I think thinking of cells as plug and play is a little bit naive, and there might be a

nuance there. Again, it may not be an FDA issue, because if they meet their metrics they meet their metrics, it might be something that the sponsor thinks about as they keep track in the future.

Of course, they already have many years of history on this, so they might even already have a sense of how to manage that, which wasn't part of this BLA.

DR. WITTEN: Maybe I will ask a more general follow-on question. Did you have an answer for that?

DR. COUTURE: Yes, I just wanted to say I think my comments are actually directed towards the cell bank is what I am really trying to get to potency, but it is separable completely, and my personal view is I don't think there is a need for more clinical trials to address these questions. I think the question is whether or not, or even whether the particular assays that the sponsor has done are inadequate, it is just how it is being reported.

Right now cytokines aren't being reported in a very quantitative way, it is more qualitative, and my recommendation is that the Agency look into cytokines, quantitative cytokine analysis as part of a well-characterized product assessment.

So, that is not more trials, that is just more data around their cell banks, and correlating that information from one cell bank to the next cell bank, to the next cell bank, and then making correlative data about whether their form their complete bilayer as well, and then ultimately correlating whether or not they actually work in the clinic. That would be maybe postmarketing I suppose.

DR. SNYDER: I suppose one way to get around that would be -- maybe you have mentioned this already -- whether there is a bioassay that could be used to just screen the cells.

Do you have a bioassay, not just measuring cytokines, but some readout that is informative as to something that will work?

MR. BILBO: If we could bring up our slide, just to review again the characterization panel and comparability panel that we are performing on our cell banks, which does -- it includes the sort of plus or minus for the cytokines, but it also is measuring separately VEGF levels quantitatively.

Then, of course, in terms of a bioassay, we believe the ultimate bioassay is the athymic mouse grafting model that evaluates the performance of the product. We do that on every cell strain for approval.

DR. SNYDER: In the athymic mouse, you are looking for integration, right?

MR. BILBO: We are looking for -- it's a measure of barrier function, it's a measure of viability of the product, and, of course, graft integration.

DR. SNYDER: Which is different than what you actually get in the mouth, though, where it doesn't integrate.

MR. BILBO: Sure. The athymic mouse

model is not intended to be a measure of the clinical indication that we are evaluating. It's a measure of the biological function of the product. It has been a very reproducible test for that.

DR. SNYDER: Have ever found any differences -- so, you talked about the epidemiology of the recipients, have you ever looked at the profile of the donors of the skin, for example, what their ethnic background is, and then whether there is any variability based on ethnic background, or based on meeting a release criteria and then any correlation between a source of a cell and maybe the ethnic background of the recipient, that kind of thing?

MR. BILBO: We certainly have that information. We focus on all the multiple measures and parameters that we are looking at in our cell banking process where we qualify our cell banks, so they must meet performance and quality criteria for them to be released.

DR. SNYDER: How often do you need to get

a new donor, for example, how many patients typically can you treat before you have to go back to the well?

MR. BILBO: Typically, with our current level of production, we are sourcing something like 5 or so new keratinocyte cell banks per year. The fibroblasts are much more proliferative, so we are able to produce many more units of Apligraf with a single fibroblast cell bank.

DR. COUTURE: I think part of the issue that I think as was just said, it is really a matter of understanding how one cell bank of one type and another cell bank of another type work together.

You have done various tests, engraftment in a mouse model and whatnot to show that that works, and the question is how does that demonstrate that that one cell bank with any of the other cell banks of that same cell type would work equivalently, and as far as we understand that, you are not doing that test.

So, there is no real correlative data or comparability data between then other than somehow, at some point, these other cell banks were tested, so all the keratinocyte banks at one point were tested with at least one fibroblast bank and vice versa, but the pairs aren't always tested, for example, so you might use multiple keratinocyte banks with one fibroblast bank, but that fibroblast bank was only lot release tested with one keratinocyte bank.

DR. DUBINETT: I think that that discussion, I think we have finished this question unless Dr. Witten has something else.

DR. WITTEN: No, thank you.

DR. DUBINETT: Thank you.

We are moving then to Question 2, which you see projected here, and we will ask Dr. Ahsan to lead off the discussion.

DR. AHSAN: I think we have already been talking about potency in different forms, so just to outline again a little bit of what we are talking about, so there is a guidance on potency

tests for cellular and gene therapy products.

In that, they talk about potency assays and thinking about things such as accuracy, precision, specificity, linearity, and range, system suitability and robustness, so I think some of the conversation that we have had so far has really focused on that, and whether the potency assays that are proposed are sensitive enough to detect changes that would result in changes in outcome.

Also, something else to think about is, well, what is potency as defined for this application in particular. I think we all agree that this is a very complex system. There are potentially, in using the language of the guidance, multiple active ingredients, and it is difficult to know what are the active ingredients versus what are adjuvants in the outcome that we observe.

There is also really a lack of reference standards for potency, of course, in this, and we have nothing to really compare it to. We compare

the clinical outcome, but the potency itself doesn't have good standards with which to compare.

Of course, we also have to acknowledge that there is a complex mechanism of action. It is highly speculative, we don't really know what that is, and so defining potency assays themselves are difficult, and then correlating the potency assays to the mechanism of action adds another layer of complexity.

But I think one of the things that the histology assessment does is it allows you to assess for spatial arrangement, which in a lot of these classic bioactivity assays, is not really part of it, where you are looking at whole population, so I think that that is actually an important part that should really remain as we move forward, and I don't think anyone is proposing that we get rid of it, but I think that that is highly valuable and should be acknowledged.

The sponsor really proposes potency and

has potency assays and then surrogates for which they think the potency assay is -- or the potency assay acts as surrogates for these other characterization panel assessments, and I think we have gone through them, and there is lots of them, but there is not really a 1 to 1 match, and that is understandable.

But again going back to the mechanism of action, we have these bioactive assays, how do we correlate that to the potency assays is really difficult. So, there has been discussion of correlations.

It has been brought up that it is hard to do correlations when you have such tight processes, and many of these parameters don't vary much, and that is true, and I don't think, as Dr. Couture said before, we are not asking necessarily, we are not asking for more clinical trials or even more assays, but the data that has been reported has been quite limited, so clearly the data is there.

When we asked for questions, some tables

came up that weren't in the document and weren't in the presentation. Maybe the FDA has that data.

But I think it is important to look at that data in terms of the resolution and sensitivity and the linearity, and the correlations that you would get there, because I think it can provide insight in terms of predicting clinical outcome and mechanisms of action that really needs to be understood as much as possible.

Nothing is thoroughly understood before BLAs are approved, but as much as possible before we move forward. So, I think that is kind of some of the questions I think we need to talk about in the next hopefully 12 or 13 minutes that we have left for the question.

I think it is a complex question this potency, I don't think that there is a perfect or ideal answer, but there has to be, we have to have a satisfying answer before we move forward.

DR. DUBINETT: Thank you.

The specific question before us, then is: Please discuss the use of H&E staining as a product potency measure for Apligraf. So, we have discussion points from the Committee at this point.

DR. HORNICEK: I think it is challenging to try to figure out the mechanism and link potency with actual effectiveness, but here is where more work on the animal model might help. If you do have an animal model going, there are ways to block cytokines in the animal, there are ways to figure out that mechanism, and that might help inform a potency assay that really correlates with effectiveness.

But I think short of that, use of H&E, you know, we really don't have any additional data to say it should be something else. We don't have a level of VEGF, we don't have a level of IL-1 alpha that we need. You know, I think it is the best at this point that we have.

DR. DUBINETT: I would really agree with that in that one of the problems with measuring a

few cytokines, it represents this tiny fraction of the universe of what is being produced, and I always feel uncomfortable when we link activities to a paucity of proteins and having, as you said, bottled what actually is important functionally.

There was an additional question here.

Yes.

DR. AMAR: I think the suggestion of keeping H&E and the histology is very well taken, that should remain, but at least -- and I did suggest this morning -- to try to standardize the staining, so at least if there is an appreciation of viability and efficacy, at least this is going to be done from lot to lot, and the variability between different lots is going to be reduced, and not subject to investigator and technician on their day and timing, et cetera.

The question of the cytokines, I think granted there is a slew of and now I can tell you that we test similar things, 32, 34 cytokines at the same time, and that is not the point here, but there is the actual, the cytokines that do

play in general most of the role TNF, IL-1, that many of us will agree that it is a good representation of proinflammation and anti-inflammation of IL-10 and IL-4.

So, there is a short list of cytokines by which we now are confident and a process is ongoing or not ongoing.

DR. DUBINETT: Dr. Couture.

DR. COUTURE: First of all, I don't know of anybody who is suggesting that we drop H&E staining. I think the H&E staining is as good assay, and it tells us a lot about the product, but I think what it tells us about the product is the structural integrity of the product.

I don't know if it says anything about the actual biological activity of the product. I would certainly be the last one on the Committee to actually tell you what cytokines one should measure to assess bioactivity or if there is not a completely different assay one should do.

But I can tell you that is normally the responsibility of the sponsor through the

clinical trial program to develop a panel of assays that measure the biological activity of their biological product. I would say that H&E staining isn't a measure of biological activity other than as a barrier.

It doesn't indicate whether or not that surface, that structure is going to produce whatever it is that the sponsor thinks is critical in effecting the treatment that it is supposed to provide, in this case, they think, and I think we would all agree, it would probably have something to do with cytokines.

I would not suggest that cytokines is the answer, but until they know otherwise, it seems like something that should at least be measured in a quantitative sense, and perhaps something that should have been done before the point of a biological license application for the product, so that we would know whether the cytokines are correct.

The question that should be at the table today is are the cytokines that the sponsor has

proposed for a bioassay the appropriate cytokines, but that is not the question. We are looking at simply is H&E staining enough.

DR. DUBINETT: Dr. Snyder.

DR. SNYDER: I guess I would agree with Steven and Larry that we don't have to know what cytokines are working. It is probably a whole cocktail of things, and to me it is astounding that essentially a 5-day exposure can have this long-lasting an effect, which raises some other questions about safety, that this little hit and run can do that much.

What we probably need is a dynamic, some kind of dynamic readout even if we don't know what is doing it, at least an informative bioassay that says this will be potent, and this will be safe if X, Y, and Z happened when we observe the cells doing this, and that is a reasonable release lot.

H&E, while it can be part of it, it doesn't have the dynamic readout of a biologically informative system.

DR. DUBINETT: Dr. Genco.

DR. GENCO: I would like to know what the correlation is between the H&E result and the nude mouse result. I mean that might be a way to assess. I mean you could argue that maybe the nude mouse could be the potency measure.

I don't know how difficult it is, and if it can be done lot to lot, has to be practical, too, but I would ask the question what is the correlation between H&E, the failure of H&E, and do you also get failures of the nude mouse that correlate.

DR. DUBINETT: Dr. Ahsan.

DR. AHSAN: Just to kind of sum up a couple of the points that have been just made, which is that the H&E, by acting as a pass/fail metric can act as a single point calibration as to whether or not to move forward, but any kind of dynamic biological assay is much more informative.

I think the panel, or at least myself, laments that we haven't been able to see some of

that data to educate us in terms of how this product is variable in those biological assays, and how it might be correlated to the clinical outcome or even the preclinical outcomes as was just asked for.

So, I think it is a desire for more data that is already obtained, but the presentation hasn't been made to the panel.

DR. DUBINETT: Dr. Witten.

DR. WITTEN: I am just wondering if the sponsor has anything brief that they could provide to that last comment.

MR. BILBO: We do have data that correlates histology with the athymic mouse model, so I think that would be helpful to illustrate that information.

DR. BAKSH: So, earlier in our development studies, we generated some data that we then published in 1996, and the intent of the study was to create different versions in maturation states of the Apligraf product, and then graft these onto the back of the athymic

mouse.

The goal here was to determine whether or not these grafts would, in fact, take or would be sloughed off, rejected in this model.

I would like to show just the results taken right out of the study that showed the histological sections, just the H&E slide. Thank you. Slide up, please.

This data here shows, on the lefthand side, grafts that were 4 days post-airlifted into our sample, so we would have expected to see the quality and the maturation state of the epithelium would be less than adequate than what we would see in our finished product, which is that 10-day post-airlift, which is the data set that you see on the far righthand corner.

The first row shows the pre-grafted unit.

You can clearly see that there is poor quality in the epithelium development on the 4-day post-airlifted sample, and a full development on the 10-day post-airlifted sample.

These were then grafted in the back of

the mouse and after 14 days of being on the mouse, we see that -- and this is 7-day data, but then we also generated 14-day data -- the immature grafts did not take, and were essentially sloughed off while the grafts that were fully mature took.

So, what this data is suggesting here is that the full viability, the maturation state that is achieved by the 10-day post-airlifted product was able to graft and integrate into this mouse model.

Then, just to go back on the discussion about our cytokine and growth factor data, we actually did two types of analysis. We used traditional gene expression on the finished product assessing the detection of the mRNA transcript of PGDF, TGF-beta, IL-1 alpha, and the lack of expression of IL-4, but we also quantify VEGF in the spent media, the conditioned media of the finished product.

I would like to show some data, trending data that we have generated over the course of 12

to 14 months during the development of this assay to demonstrate the variability that we see over the lot. Slide up, please.

This data here shows, on the X axis, the 14 lots, the Apligraf lots that we have used in this data collection, and this is the quantification of the VEGF at the finished product.

What you are seeing here is the level of variability that we see across the lots, and I just wanted to point out that these lots were created with at least 4 different keratinocyte banks, and at least 3 different HDF banks, they were all paired in various different combinations, so you get to appreciate the level of variability, heterogeneity, but also consistency across these different lots.

DR. DUBINETT: Yes, Dr. Wittes.

DR. WITTES: A question about that. The X axis is what? Why is there a tendency to go down? Is that a temporal --

DR. BAKSH: Yes, it's a temporal.

DR. WITTES: So, it seems to me that there is actually a trend downward, is that true, and why?

DR. BAKSH: No, no, no, I think if we grafted a trend line to the data, I think you would see there was a general tendency down, but I don't think it's -- it's essentially the noise in the assay itself.

DR. DUBINETT: Dr. Hornicek.

DR. HORNICEK: I just had one comment. As you propagate those cells, those cytokine levels like most of these benign sort of cultures as they change in terms of their ability to proliferate, cytokine proliferation will also change as well. So, you know, I am not sure again what all that means.

DR. DUBINETT: Okay. Dr. Snyder.

DR. SNYDER: I am curious as to why did you choose VEGF to look at, is that cytokine known to play a role in even any degree of spontaneous repair in this process regardless of the mechanism?

DR. BAKSH: Sure. So, the choice, our selection of VEGF actually came from trying to understand the level of or the types of growth factors and cytokines that are produced by the products simply as a measure of product quality, and we screened a number of different cytokines, a number of interleukins, FGS, for example, and we determined that VEGF was the most relevant and meaningful to manufacturing this product.

I would like to actually show you data that demonstrates this. Slide up, please.

This data here shows the level of VEGF that we detect along the continuum of the manufacturing process of the product, and in every step of the process where there are media changes, there are cells being added, et cetera, and you see that the VEGF production is associated with changes in the process.

As we have the fibroblasts, we add in the keratinocytes, et cetera, we see that the VEGF rises and ultimately at the final finished product level, we see that there is a level of

leveling off an equilibrium of the VEGF.

So, our choice for VEGF in the context of this product was simply because it had relevancy to manufacturing the product, and it suggested something about the quality of the product at its finished form.

DR. DUBINETT: Thank you.

We are now moving to the next question, which is our efficacy question. Please discuss the effectiveness of Apligraf for the proposed indication, particularly considering the study results for KT, appearance, texture, patient preference, pain, and attached gingiva.

Our lead discussant for this is Dr. Genco.

DR. GENCO: The way I understand efficacy is that it has at least, or effectiveness, it has at least two components of efficacy, which is clinically significant function in ameliorating a disease.

The second component, and we have been asked to address this, I think, is indication.

So, clinical efficacy, an indication. Define effectiveness in my view.

I would like to direct the attention to the indication, but before that, I want to answer your question here. I think that the efficacy, the clinically significant function has been shown by both the pilot and the pivotal study. There is enough attached gingiva to prevent further attachment loss, further pocket depth to probably reduce sensitivity and to allow for adequate plaque control in those lesions.

That is my opinion based upon the data that I read and also what I have heard today and the FDA analysis of the data.

However, with respect to the indication, what is the population? The patient population that would be appropriate for this therapy, I would like, if I could, to address that issue, if that is appropriate.

DR. DUBINETT: Yes.

DR. GENCO: Okay. One of the rationales for this product is to prevent further gingival

recession, gingivitis, pocket depth, periodontitis, bone loss, and eventual loss of the tooth. I mean that is what we heard this morning, and that is the conventional wisdom in the field.

I looked at the data, and if you look at Slide CE-19, the lesions, if you look at bleeding upon probing, about a fifth of them had bleeding upon probing. That means 80 percent of them didn't bleed on probing, probably pretty healthy.

So, these were probably pretty healthy sites, 80 percent of them to begin with.

The same with the presence of plaque. About 20 percent, unless I am misreading this, had plaque, and the rest had little or no plaque.

So, again, that is consistent with a healthy site.

I am a little concerned that there is some literature -- and it's controversial -- there is 2 studies in particular, and they were mentioned. One of them was mentioned this morning, the Kennedy study, which showed that if

you carry out good plaque control in these sites with minimal attachment level, there is a percent of breakdown, but it is a small percent.

There is another study, the Wenstrom study, 1987. These are old studies often forgotten, but again -- and I will read the conclusion -- "It appears that in patients maintaining a proper plaque control, the lack of an adequate zone of attached gingiva does not result in increased incidence of soft tissue recession." But if you look at his paper, he does have recession in about 10, 15 percent of the cases

So, I bring that up as what is the indication, and I was struck with the indication for the leg, the venous lesion, as well as the foot ulcer, and the indication was in those patients who had conventional therapy, and it didn't work, then, this therapy would be used. So, I am just wondering if that might be something we could discuss, the indications.

DR. DUBINETT: Let me gain clarification

on this, Dr. Witten. It is a discussion question that we have moved a little bit from effectiveness to indication.

DR. WITTEN: Well, I think those comments are very valuable. Yes, you are right, we have a separate discussion question on patient population. There may be other comments about the patient population, and could be made then, but I think those are important comments, you know, that we want to hear those, but perhaps at this point, we could hear further discussion about effectiveness or any other views or comments on effectiveness.

DR. DUBINETT: Other discussion from the Committee? Dr. Wittes.

DR. WITTES: I am still having trouble with pain, because I am not convinced that just because there should be less pain, there actually is less pain. I am not that impressed with patient preference, because patient preference was asked 6 months out. They had an old-fashioned, something old-fashioned in one side of

their mouth, something brand-new in the other side of their mouth.

You kind of forget bad things that happened 6 months ago, the intensity of stuff, so when I asked a question about pain, and the answer was, well, pain is included in patient preference, well, that is the memory of pain 6 months ago, which I don't think really asks the pain question.

So, it seems to me that what was convincing was the appearance, the texture, but the others were not, and I need to understand from the people around here that the cosmetic stuff was better, clearly better, but I don't understand how serious it is that both the KT and the sensitivity and the attached gingiva, all three of them were actually worse in the new treatment.

DR. DUBINETT: Perhaps we could have some discussion of those points from our experts on the Committee.

DR. REYNOLDS: If I may, the clinical

efficacy in terms of change in qualitative based on appearance and the limited histology, is consistent with the efficacy that we would like to see.

The parameters related to pain, pain perception, are one that I think clinically, we have a lot of experience with, and patients are very consistent in letting you know what their experience is like, particularly when you have to harvest tissue from another site in the mouth, and that is regardless of the procedure. Secondary sites often create more problems for us clinically than the primary site that we are trying to treat.

I don't know of that answers the question. As a clinician, the pain side of the story falls into place. The model doesn't really allow you to assess it with the clarity that you would like to, but based on clinical experience, when you go to a second site for autogenous material, patients remember that, and that is something that they would like to avoid if given

the option.

If I may follow it with a second question, and the sponsor may only be able to answer this, is if you look at Slide CE-23, the question that I have is if you place a 4 mm width with free gingival graft, you get a certain predictable zone of keratinized tissue.

What is the label going to read in terms of the amount of Apligraf that is necessary to achieve an increase in the zone of keratinized tissue?

And the reason I ask is that if you look at the amount that was used, it ranged considerably more than that for the autogenous graft, and the question then is simply is there a minimum width of the construct that needs to be used clinically to achieve a therapeutic result.

DR. DUBINETT: I would like to hold the answer for that until we finish the Committee discussion, because we have several other questions.

Dr. Dahlgren.

DR. DAHLGREN: So, this is related to pain. In the FDA presentation, Slide 57, that table to me actually suggests that in the pilot study, the patients that got the Apligraf could have actually had more pain, and that doesn't seem to have been part of the discussion.

So, if there is a way to just clarify there were 5 patients or nearly 23 percent in the pilot study that had severe pain associated with the Apligraf, and we haven't talked about that at all.

So, I don't know whether that is a difference in how it was applied or -- if somebody could clarify, that would be great.

DR. DUBINETT: Dr. Ahsan.

DR. AHSAN: My question is not on pain, so I don't know if we need to finish the pain.

DR. DUBINETT: That's fine. We will come back to the pain.

DR. AHSAN: Okay. My question is actually about the effectiveness, is a little bit to what I was asking about before, which is if

you try the Apligraf and it doesn't work, there seems to be some improvement, but is there anything that hinders you from doing the FGG in the future.

So, is this a situation where the effectiveness is couched in the concept of why not give it a go aspect of it. I would be interested in knowing what the experts in the field would think about that mentality as they were to use this product or not.

DR. DUBINETT: Dr. Reynolds.

DR. REYNOLDS: In answer to your question would it preclude a second procedure, absolutely not. We have to use a variety of constructs or materials in most instances, in fact, I can't think really of any where you would not be able to go back and re-treat and often we end up having to.

DR. DUBINETT: But if I could rephrase your question, it sounded to me as if both the mentality of the practitioner and the patient would be geared toward trying something that is

apparently less invasive. Is that your question?

DR. AHSAN: Yes, a little bit to that effect, and where the cost is really the 6 months, and not any other aspect of ultimate outcome.

DR. DUBINETT: We are going a little bit out of order, but Dr. Fratzke.

DR. FRATZKE: Yes, I would just like to - - I think pain is many times subjective, and so somebody, let's say you are in a test or a trial, and perhaps, gee, this is the new deal going and might think that this is perhaps -- anyway, their evaluation of the pain may be completely different sometimes in those situations.

DR. DUBINETT: Thank you. Ms. Rue.

MS. RUE: I was going to make one comment the same as Dr. Fratzke about pain, but we also have a society that is very focused on the cosmetics, and if this offers an option for them, that maybe they have chose for their overall wellness because it does affect cosmetics, and pain is very subjective, and I think that is

something that we do need to consider in the spectrum of overall health.

MS. DAPOLITO: Okay. Dr. Snyder.

DR. SNYDER: I think to help me get my arms around the question of efficacy, I am going to restate a question that I said this morning, and I think I probably need some input from the periodontologists on the Committee.

Typically, when you are judging a new intervention, you compare it with standard of care, and you judge whether the new intervention is at least as good as the standard of care. Certainly, you hope it is not worse than standard of care.

So, my question is, is it a reasonable metric to say that this intervention reaches at least a minimal threshold, 2 mm of growth at 6 months, so that that is a reasonable surrogate to say this has reached a threshold and therefore will have the same long-term outcome that standard of care would have had, which is FGG.

If the answer to that is yes, it meets

the threshold, and anything beyond that is unnecessary, then, I think you could say it has met efficacy and now it is simply a matter of patient and physician preference to determine that.

However, if that is not a reasonable readout, or if the readout is not long enough, or it suggests that it is maybe not as good a standard of care, then, I would say that it has not met the efficacy, so I just need some feedback from -- you know, I am in a different field of medicine, and usually, you know, you really look for long-term outcome as to whether the patient does better. So, I need some education from the experts who deal with these patients.

DR. DUBINETT: So, it is right in time, it is Dr. Jeffcoat's turn.

DR. JEFFCOAT: You all may want to kill me, but I have a difference of opinion here. Frankly, and it is probably just in the way you all presented the data, the data is presented as

dichotomous, so we can't tell what in the world was found.

Patients don't from a little bit of recession around their teeth, they very rarely lose a tooth unless they have periodontitis, which these patients did not according to the -- we are going to discuss that later -- and the amount, the continuous, and one of the statisticians will correct me if I have got the words wrong, okay, but you have got continuous data, that they have thrown most of it away. FDA may have it, we just may have not seen it, but I haven't seen it.

So, I can't be sure what it meets, what criteria it meets, okay. These are such good clinicians, and I have seen these clinicians work, that part of the reason you may be seeing - - I am going to give you another reason that you may be seeing -- no effect on pain, and no effect is no effect still, it is because they are so good at what they do, and they are so confident at what they do after they go to the first

couple, which is what these data would fit that hypothesis, it doesn't make it right, just because it would fit it, that may be why we are not seeing a pain effect.

It would be nice to measure all the aesthetic things quantitatively in some way, but that would be nice from my point of view, because the dichotomous variable they have is such a low bar that it is hard not to meet it.

There are a lot of other things on the market, and for our medical colleagues, our patients pay for these products, okay, so this is not getting thrown into a -- so patients are not necessarily going to choose to do it.

DR. SNYDER: But even though it's a low bar, if it meets it, is it clinically relevant.

DR. JEFFCOAT: In my opinion? You are just getting one person's opinion. I mean you are okay with it, but you are going to get a bunch of different opinions around this table.

DR. DUBINETT: Okay. We have an order here unless, Dr. Reynolds, you are going to

specifically address Dr. Snyder's question?

DR. REYNOLDS: If I may, this is a highly controversial topic, in fact, it is one of the topics that is discussed every year in our residency programs. There are some who believe that you need any amount to be able to preserve the architecture.

So, the threshold that they have established, that the sponsor has established, really exceeds what others would consider to be necessary. So, I think it fulfills the criterion of having a minimum threshold that is above what most would consider to be acceptable.

We have this discussion, we don't always agree on it, but most are in agreement without exception that you have to have at least some.

DR. SNYDER: And 6 months follow up is long enough?

DR. REYNOLDS: Six months follow up is our standard. Our experience in other soft tissue procedures is that we tend to think that improves actually, it doesn't get worse, it

improves as you go out over 12 to 24 months.

DR. DUBINETT: Thank you.

Dr. Amar.

DR. AMAR: Just to support this, the reason we haven't spoken so out loud as you wanted is because the field is so divided, there are schools that maintain that no keratinized tissue is necessary and you can maintain cases, and there are schools that maintain that you need some kind of keratinized tissue.

Where do we stand? I do believe that some keratinized tissue is necessary in that area, because perfect oral hygiene is not possible, and that could allow and help the patient maintain this kind of, quote, unquote, "protection" for further disease.

Having said that, if we base our opinion on these premises, there is efficacy, no one is going to disagree with that. Where the issue is going to remain is patient selection and indication.

DR. DUBINETT: Thank you.

We have three additional points. Dr. Hornicek.

DR. HORNICEK: My understanding is with the FGG, that you have to harvest the graft, and with the Apligraf you don't. So, no matter how much pain is associated with the harvest, that we do for other procedures around the body, that is a morbidity that you are going to assume.

So, I would say that if the Apligraf has -- and it does, they have shown some effect -- we don't know the mechanism of action per se, but then it avoids that harvest of the graft and the morbidity associated with it, whether it is 2 out of 10, or 8 out of 10, or whatever.

But the harvesting of a graft I would imagine is some, you know, it's harvest, and no matter whether it's from the mouth or from the hip or from wherever, there is some morbidity associated and pain associated with it.

So, I would say that the Apligraf, you know, it seems to work.

DR. DUBINETT: Thank you.

Dr. Couture.

DR. COUTURE: I actually have more of a request for clarification from Dr. Witten down there, because I am having a hard time, thinking that I have to vote here in a few minutes, associating this question from Question No. 5, because this question is whether efficacy has been showing in the proposed indication, which is in all adults in both alveolar and gingival mucosa.

Of course, we know that the study didn't test all of those indications, and so voting for efficacy here, are we voting for efficacy in any of these indications, and then address the broader patient population later?

DR. WITTEN: You are voting in response to the questions about efficacy, effectiveness, but then after the voting question, I mean this comes up all the time where people want to vote yes except they are concerned about something in the label about, for example, as the initial speaker talked about, making sure the label had

something about conventional therapy tried first or people may have some reservations about the indication or how broad it is.

So, after the vote, whether you vote yes or no, we will be going around the table and you can explain your vote, so if you have any caveats or anything where you say, well, I voted yes, but I would have voted no except that I thought this, or I voted no, but I would have voted yes if this had been included, you can explain that.

You have to take your best shot at voting on the question, but then when you explain the vote, you can put in all your qualifiers.

DR. DUBINETT: Dr. Genco.

DR. GENCO: I would say in answer to Dr. Snyder's question, that there is very strong evidence that the Apligraf meets the standard of care, free gingival graft, not only primary outcome, but in most of the secondary outcomes including -- and we haven't seen the data -- but that there is no difference in gingivitis plaque, and we heard the data about sensitivity, so I

think that it meets it in those clinical criteria.

The cosmetic, I am a little conflicted with because I thought we were dealing with a disease, and the treatment of a disease, the cosmetic is something I am not used to dealing with at the FDA. At the Dental Products Panel, we don't really deal with cosmetic effects.

DR. DUBINETT: Thank you.

We will move on, Dr. Witten, unless you have other questions and points.

DR. WITTEN: No. Are there any other comments before the vote?

[No audible response.]

DR. WITTEN: No.

DR. DUBINETT: We are now moving to vote on Voting Question 4, which is: Based on the data provided, is Apligraf effective for the treatment of surgically created gingival surface defects in adults?

I will remind you that we have voting on your microphones as Yes, Abstain, and No. We

will be voting simultaneously. The green flashing lights now say we can do so. So, can we vote then on Question 4.

MS. DAPOLITO: I will read the tally for the record. There are 15 voting members, and the vote for Question 4 was 15 Yes. 0 Abstain, and 0 No.

The individual poll of the votes are:

Dr. Reynolds, Yes; Dr. Wittes, Yes; Dr. Genco, Yes; Ms. Rue, Yes; Dr. Fratzke, Yes; Dr. Dahlgren, Yes; Dr. Snyder, Yes; Dr. Lee, Yes; Dr. Dubinett, Yes; Dr. Ahsan, Yes; Dr. Couture, Yes; Dr. Hornicek, Yes; Dr. Hwu, Yes; Dr. Genco, Yes; and Dr. Amar, Yes.

Thank you.

DR. DUBINETT: We will now go around and each of us will explain their votes. We will start to the right and go around. Dr. Lee.

DR. MEI-LEI LEE: Well, after all the discussion this morning, I think this seems to be effective. The primary endpoint, although it is not compared to the standard, but it looks pretty

good with this 2 mm point.

DR. DUBINETT: Dr. Snyder.

DR. SNYDER: Based on the input from my colleagues in the field, I was persuaded that it reaches a clinically significant and predictive surrogate threshold, that there seem to, if it failed, one could follow up with standard of care, and that one could defer to the preference of the patient and his health care provider.

DR. DUBINETT: Dr. Dahlgren.

DR. DAHLGREN: I agree with Dr. Snyder.

DR. DUBINETT: Dr. Fratzke.

DR. FRATZKE: I agree, and also as a Patient Representative, I think it is important probably to have access to the aesthetic value even posed in the anterior perhaps, and that would probably appeal to patients, and also the talk of not having to take the sample from the palate, and be free or less pain.

DR. DUBINETT: Ms. Rue.

MS. RUE: I feel that it was demonstrated that it was effective and just needs to be

adoption for the patient.

DR. DUBINETT: Dr. Jeffcoat.

DR. JEFFCOAT: I keep doing that, I apologize. I agree with what has been said.

DR. DUBINETT: Thank you. Dr. Wittes.

DR. WITTES: I agree also.

DR. REYNOLDS: Yes, I also agree with what has been said.

DR. DUBINETT: Dr. Bui, you are allowed to tell us how you would have voted and why.

DR. BUI: I would probably vote yes as well. I think the data met the primary and secondary endpoints.

DR. DUBINETT: Thank you. Dr. Amar.

DR. AMAR: Yes, for all the reasons that I made in my last comment.

DR. DUBINETT: Dr. Genco.

DR. GENCO: I voted yes for those reasons, but I look forward to the discussion of the indication and the population definition.

DR. HWU: I think the product appears effective although I think it could be simplified

or improved if going forward more characterization biomarkers are followed and linked with clinical outcome.

DR. HORNICEK: I have nothing else to add.

DR. COUTURE: Yes, obviously, with two caveats. One, I wasn't convinced that it was actually as good or better than FGG, but that may or may not be relevant to licensure for the product, and second, I think it is very important to have the discussion that is going to come up next about what particular indication we are voting for and to have been efficacious.

DR. AHSAN: I agree with what has been said.

DR. DUBINETT: I voted Yes with the particular concerns that Dr. Hwu just mentioned, that in the future we look for predictors of efficacy in the biology of the product.

We are now moving on to Question 5. Our discussant leader is Dr. Jeffcoat. The discussion question is: Please discuss the

applicant's approach to quality and demonstrate comparability for the new cell banks used for Apligraf manufacture.

DR. JEFFCOAT: I hope not.

DR. DUBINETT: Pardon me?

DR. JEFFCOAT: I hope it is please discuss whether the studies are applicable. Have I got it right or wrong?

DR. DUBINETT: Actually, let me read what is on the paper, not on the screen.

The question is: Please discuss whether the results of these studies are applicable to the broader patient population as described in the proposed indication is the real question.

DR. JEFFCOAT: Right, okay

DR. WITTEN: This is Question 5.

DR. JEFFCOAT: Thank you, because I don't fail to qualify.

DR. DUBINETT: There was a disparity between what was written.

DR. JEFFCOAT: You may not think I am qualified to discuss this one, but I am going to

give it a go, and I want to thank you all for having settled the other one before we do this one.

The issue here is always when we do a study, we have to choose the patient population to do our study, everybody does, everybody around this table does who is doing a study, and around that table, too.

In particular, there is some wording that was in the proposed indication that, frankly, was not in what we at least received for their inclusion criteria, which were that alveolar mucosal surface defects, and I think what they really mean is gingival defect that goes into the mucosa, because they sometimes do that, but, frankly, that needs to be reworded in my opinion so that it is clear to anybody reading it, because if you have a defect, say, from a fistula, that is going to give you a mucosal defect, this isn't what you are going to want to use, or at least there is no data here, you haven't in any way implied that we should be

thinking in that direction.

There are some other things that we want to consider here. One is do patients need to not have periodontitis, is this for aesthetics in patients, not for patients who do not have periodontitis.

Mike, you used the term -- and I am sorry I keep turning around on everybody, have my back to you, I apologize for that, Mike, you used the term "periodontal plastic surgery," and that is exactly what they are proposing here.

That is a good term to describe what these studies were to stick, but you wouldn't do that in the absence of periodontitis, or at least they don't present data to say we should do it in the presence of periodontitis would be more accurate.

Then, the one that I really think has to do with broader populations, okay, has to do with children, and children isn't in the proposed indication, but the pediatric rule -- and FDA is supposed to tell us whether or not we are

supposed to discuss the pediatric rule, because I did call last week on this -- this is likely going to be used in children off label no matter what, because as everybody has mentioned, orthodontics, in the presence of orthodontics, you are likely going to get stripping -- you will in certain circumstances, not all the time -- get the stripping of the gingiva, and this, if I am a parent, it sounds like just the ticket. I want to know it's safe, I want to know it's effective.

There is no date here, and they haven't proposed that they say it, but we have to discuss it and at least give FDA some advice as to what that label insert should say, that package insert should say. If I am incorrect, you can tell me.

That is what I thought I heard on the phone last week.

DR. WITTEN: Yes, I was going to say we would be interested in your comments for the label, but also, if you have comments specifically about studies you would like to see, you could throw that information in, too. We

would be interested in hearing that.

DR. JEFFCOAT: Yeah, I would like to see a study, personally, the size of a pilot study, not a huge study, that is going to -- I am really concerned, not so much that efficacy is going to be different, but, you know, all the things that we have talked about that could turn on oncogenes or anything, these growth factors that we are throwing in the gamish here. You throw them into a young child, we may have a very different mix, and we may have a very different result.

So, the outcomes you would want to look at, and personally, if you are asking me if I would design it, I would follow those children longer than 6 months, because they are children, they are growing, things are changing. That is different from an adult where things are pretty much stable.

We know how long we have looked at growth factors in adults in periodontitis, which is a different disease, it's a different thing, and I do not want to take up the whole 15 minutes just

doing that, but I think this is a really important issue, and it is not that they have done anything wrong, it is just that we have to make sure that the practitioners and then, by extension, parents don't think they are in effect buying something that they are not.

Frankly, I doubt that people sitting in back of me want to have a problem on their hands five years from now anyway.

DR. DUBINETT: Thank you.

Dr. Genco.

DR. GENCO: To expand a bit on what I brought up before about indication and defining the appropriate patient population for this therapy, there is evidence in the literature, it's just not complete, and proper studies haven't really been done as to the percent of the adult population, that if they had reasonable plaque control, would not have further recession, would not have gingivitis, would not have periodontitis, would not have bone loss, would not have tooth loss.

So, it is not the occasional, it is probably a large segment of the population. In this day and age of cost containment and concern about unnecessary surgeries, I am not saying this is unnecessary, but there might be situations where it could be. I would think very, very strongly, we would have to have that data, somebody has to have it. There may be databases where it can be looked at, longitudinal databases. Nobody has looked at it that I know.

The small studies of the 100 patients of Kennedy and 26 patients of Wenstrom show that maybe about 10 percent over a 5-year-period will have further recession, so that is why I am a little concerned that it might be a subset of the population that really gets worse with time.

I know the feeling. I am a periodontist, I did many of these procedures, but I also watched a lot of these and they didn't get worse, so I think that that has to be a consideration, what is the population, how do we know who needs this procedure, and one way to do it is to define

the population as one who, after whatever, 3 months or so of conventional scaling and root planning, and oral hygiene, they do get worse, therefore, use the procedure on that population, very similar to the labeling that you use now on your venous leg application and your diabetic ulcer application.

DR. DUBINETT: Thank you.

Other comments? Yes.

DR. WITTES: Ask a question. So, this patient population was actually not particularly sick. Does the fact that you are losing some efficacy in the measurements, is that more or less relevant to people who would be more severely affected at baseline?

DR. GENCO: The problem was -- and Dr. Snyder brought it up this morning -- he would like to see tooth loss as the endpoint. It is very difficult to use tooth loss, almost no periodontal study uses tooth loss because it takes decades.

So, the surrogate is pocket depth or

attachment loss, so that might be a way to look at this, does the pocket depth get worse, does the attachment and loss continue, and on the buccal plates and the lingual plates you could probably measure the bone, too, because the plate is thin.

DR. WITTES: But I mean in terms of the population, the population right now is everybody, right? Should it be limited to the people who are similar to the population in this study, or do you feel it doesn't need to be? Can it be the people who come in with more disease?

DR. GENCO: I feel it should be limited to the population that does get worse after having a trial of more conservative therapy. That would be my recommendation.

DR. DUBINETT: Dr. Amar.

DR. AMAR: Don't all our patients have a trial of conservative therapy before getting on to do surgeries? I guess my question -- and it was brought up this morning -- there is still an effect for aesthetic no matter how we look at

those studies, there is an improvement on the patch aspect that we have with free gingival graft.

The question that I have for the FDA is very simple. Is this a condition, or a disease, or how do we classify aesthetic when it comes to those approvals?

DR. WITTEN: The indication proposed is not aesthetic, it is to use in the surgically created defects, but I would consider the aesthetics to be part of the claims that they would make, which we would evaluate for the label. Is that helpful?

DR. DUBINETT: Dr. Genco, directly relates to this?

DR. GENCO: Yes, I think in terms of other indications, certainly there might be other indications like if the tooth is sensitive, this might be a good treatment. They really didn't address that, but they did show there was very low sensitivity.

The other is aesthetics. I mean

certainly if a patient has a high lip line with a lot of root exposed, it is very unaesthetic, and I could understand that that would be, that is something that you would consider as an indication. I think that that is a perfectly adequate indication, but it is not disease.

DR. AMAR: Not as a treatment of a disease, as an amelioration of the aesthetic, and I am confused as to where aesthetic fits within -

DR. DUBINETT: Dr. Witten reviewed the specific indication under discussion.

DR. WITTEN: The indication is for surgically created defects. It is a little more specific than that, and so the specifics of what those procedures are is not defined by that indication. So, one option is to leave the indication general, and then I think it would cover all those different -- I would call those treatment objectives. That is how we would look at that. There might be the objective of the periodontologist treating the patient. Yes,

okay. Dr. Betz wants to comment.

DR. DUBINETT: So, we have Dr. Bui followed by Dr. Jeffcoat -- oh, I am sorry, Dr. Betz.

DR. BETZ: I forgot what I was going to say. Come back to me tomorrow.

DR. DUBINETT: Dr. Bui.

DR. BUI: In certain therapeutics area, when you design a trial, you want to select a patient population that represent the U.S. population overall. I work in oncology, that is something that we usually look at.

Looking at slides here, the populations here is mostly Caucasian patients. I am not sure it is something that the FDA would be interested in asking the sponsor to probably recruit patients from different racial ethnic groups and whether that would have impact on efficacy as well, but certainly in other therapeutics area, that is something we look at, and I think the FDA actually have a guidance on this, and that is something that might be helpful to have in the

label, as well.

DR. DUBINETT: Thanks.

Dr. Jeffcoat.

DR. JEFFCOAT: I mean I think you can -- I agree with you, Bob, that scientifically, the beautiful pristine way, you and I would love to do that study, however, we will be doing it 20 years from now, everybody who isn't retired will review it I think -- excuse me, Bob, that was a crack, I didn't mean it to be -- but I do think you need to go through the inclusion and exclusion criteria and statements like its effect on current smokers have not been determined, its effect on pregnant women have not been determined.

One thing is, you know, you don't expect anybody is going to do this in pregnant women, so that one isn't a big, that one doesn't -- I mean I am a periodontist, so it doesn't really seem as gee, we have to really get on that one, but the children one we do, because children will end up being treated with this.

I see little kids all the time, and every periodontist around this table does, to perform grafts of some sort, and how much they hurt is in the eye of the beholder. You tell a little kid, they tell their mother they can have ice cream, and they will never tell you it hurts. They will ask if they can have it done next week.

DR. DUBINETT: Some adults as well.

DR. JEFFCOAT: Me, too.

DR. DUBINETT: Dr. Witten, do we have adequate discussion at this point?

DR. WITTEN: I would appreciate hearing from the other periodontologists if they have any comments about the pediatric issue and their suggestions about whether or not additional data would be needed and what they would like to see, and also any other general comments on the label.

I mean the question is specifically about indications, but if there are other comments about the label, that would be a good time to let us know.

DR. REYNOLDS: If I may, I would like to

come back to the specific indication first, and it would be helpful to have some clarification in the description of the indication beyond what is provided here, because I don't know what this means.

I mean if you were to ask me, I mean I understand what it means, but I don't understand where I would create a surgically created gingival surface defect. I understand it, because I have seen the studies, I would like to have greater clarification on what indications it would be appropriate for, such as gingival recession defects with the intent of increasing the zone of keratinized tissue, and if you thought appropriate to expand that to any mucogingival zones or sites that the therapeutic objectives increase the zone of keratinized tissue, that would be something that I, as a clinician, would understand easily with the caveats of Dr. Genco, if I may put out, I think that there are parameters of care certainly before we would like to use a procedure we would

want to adhere to widely accepted guidelines.

But I think the specific indications typically are listed in a language that we use, such as interosseous defects, recession defects, that type of descriptor, that would be I think very helpful for the practitioner.

DR. WITTEN: I appreciate that comment, and that leads to actually a question from me, which is it is possible to put in an indication specifically something that calls out the procedure that was performed in the study. Sometimes the clinicians will tell us that they think the procedures performed in the study support a more general indication.

So, I am wondering what your view is on this, you know, do you think the data supports specifically the intended indication for the procedure that they performed, do you think that it supports a more general indication, if so, how would you characterize what it does support.

DR. REYNOLDS: The model was a recession defect model. I think it would not be

unreasonable to extrapolate that to mucogingival deformities or defects, which would include the alveolar ridge, dental implants. That is broader, but I am worried that this description could end up being used to treat mucosal defects, which I don't think was really the intent here.

It may have applicability, therefore value, but I think I would be comfortable with the management of mucogingival deformities or deficiencies where an increase in keratinized tissue is desired.

DR. WITTEN: Are there other --

DR. AMAR: This would include root coverage in this case.

DR. REYNOLDS: Well, root coverage, you are absolutely right. It would imply that it could be. The question was whether in a more general sense. I would not want to limit its use to recession defects because clearly, we have significant need adjacent to dental implants, and there is no biologic reason to assume that it would behave differently.

Similarly, with deficient zones of keratinized tissue on the edentulous ridge, we don't have data, but there is really no reason that you would anticipate a difference in wound healing.

So, I guess the question is, can you capture that in a language that would not suggest, for example, that it would appropriate for root coverage, which is an indication that has unique needs in terms of meeting it therapeutically.

DR. DUBINETT: Dr. Genco, you had additional --

DR. GENCO: I would agree with Mark that that wording "in surgically created defects" is a little confusing. Why would you create the surgical defect unless you weren't going after a problem? So, I would phrase it a little differently, but I am sure they had good reasons for phrasing it that way.

I would like to address the question Dr. Witten asked about children, and ask a question.

Can the sponsor tell us, is there any contraindication to using these graft materials in children, theoretical or practical, and if not, I would think that they -- I mean obviously, you would want to be a purist, you want them tested in children, but I would like to know if there is any theoretical or practical implication or barrier to using them in children.

DR. BATES: Dr. Genco, first and foremost, we don't have any data for children. Diabetic foot ulcers and venous leg ulcers tend to occur in a much older population, but from a biological point of view, the cells are from neonates, they are neonatal cells, so I don't see any particular biological reason why it wouldn't necessarily work, but we don't have data.

DR. DUBINETT: Dr. Snyder.

DR. SNYDER: The only thing, I would want to echo what Dr. Jeffcoat said, you know, that I think since we don't know the mechanism, we know that -- and we will discuss this I think during the safety discussion -- that somehow there is

this change from a very short transient exposure to a cytokine.

In a growing mouth, I think there may be some concerns and longer follow up than 6 months would be my recommendation, as well, and I agree with Dr. Jeffcoat with regard to that.

DR. DUBINETT: Dr. Bui.

DR. BUI: Going back to Dr. Reynolds' comments, I think the indications and what the sponsor proposed here are very broad. I would agree with that. I think the problem when you start going to specific details, you know, you are going to have a laundry list, where are you going to stop.

Being in industries, I think that is one thing, whenever we negotiate with the FDA for indications, tend to be very broad. If you want to be more specific, then, you cross reference to other sections of the label, and you will provide more data, and the physician can go there and read more about it.

I would keep the indication the way it is

right now very broad, because the more technical, you know, you try to provide more details, add more detailed indications, it will just cause more confusion down the line.

You have got to keep in mind the indication is not just for the label, but it has an impact on reimbursement, as well, so that is something to keep in mind.

DR. DUBINETT: Dr. Witten, anything else?

DR. WITTEN: No, that is very helpful.

Thank you.

DR. DUBINETT: We are now to the point of a 10-minute break, at which point we will reconvene for the safety question.

[Break.]

DR. DUBINETT: We are now convening to talk about safety. We are to Discussion Question 6. Our discussant leader is Dr. Reynolds.

The discussion question is: Please discuss the safety of Apligraf for the proposed oral indication, considering the available nonclinical and clinical study results including

both premarketing and postmarketing experience.

Dr. Reynolds.

DR. REYNOLDS: Good afternoon. Thank you.

The pilot and pivotal trial today come forward with a long history with Apligraf, and so there is a large database with respect to its application for the management of chronic cutaneous lesions, 13 years of postmarketing data, so we have that.

I will go from that to the pivotal trial here where there were 3 serious adverse events and 3 AEs at the Apligraf-treated sites, and 4 at the pre-gingival graft-treated sites.

I think that as you look at these on pages 48 and 49 of the morning presentation, none of these resonate as being of particular concern to me. I think the questions that were raised earlier that we have to discuss relate to the comparability of the cutaneous wound to an oral mucosal wound.

We talked about differences in

inflammatory and immune responsiveness including differences in cellular trafficking. I think that is a fair question to ask.

We have some histologic evidence to suggest that at least early there is an inflammatory component which we would expect to see with, for example, bovine-derived acellular matrix, so that is not a surprise, and we have some evidence to suggest that the persistence of these cells is relatively short, so that the mechanism of action, whether it's cytokine or otherwise, is transient.

So the question then with respect to the cytokines is what potential impact might they have given that we recognize that they are, by design, transient mediators that are cleared very rapidly.

My perspective from a clinical side is that there is no compelling evidence to suggest that there is a significant safety concern. I think from an academic perspective, it is worth talking a bit about the inflammation to explore

whether that is something that would require some level of pharmacovigilance in terms of following certain patients up over time to ensure that there are no concerns.

DR. DUBINETT: Thank you, Dr. Reynolds. I will start off with a few comments and then hope there will be others.

I think that my concern regarding the safety of any inflammatory response is not the inflammatory response alone, but the sequelae for individuals who are at risk for oral malignancy.

So, what we do know about the epithelium of both the oral epithelium and the entire respiratory tract following tobacco exposure, and these events continue in many patients for years following that tobacco exposure, is that the mutations in the oral and airway epithelium that are not necessarily evident histologically, and that they form a baseline risk in the current and former smoker.

Added to that is the substantial literature to suggest that the inflammatory

response serves as a cofactor and synergizes with those mutations in carcinogenesis. So, what does that mean?

It means at some level the many million of former smokers in this country have a risk for oral malignancy, and why should it concern us, because the cytokines we saw earlier today go up and down in a relatively short time frame.

I have two concerns about that. One is that the cellular response and the generation of other cytokines in response to that blip can be substantial. One example, and we happened to focus on VEGF, is that VEGF is really at the nexus of angiogenesis and dysregulation or suppression of cell-mediated immunity that can both predispose to cancer and certainly encourages premalignancy to progress.

I have some ideas about how one might study that, but certainly I would want to know initially if patients receive this therapy and were either at high risk or had a history of oral premalignancy, you know, what happened to them

would be a first step.

Unfortunately, it is not something that we would typically see in this number of patients and in this time course. So, those are my concerns.

DR. REYNOLDS: If I may, just to put a context for others, and your points are I think very important, we create in our efforts to tissue engineer, lesions that are chronic, and I mean chronic, not a week, or two weeks in some instances, months, and these are lesions that are angry and they look unpleasant, and so we have a history of creating these insults, and so from my perspective, and others, please comment.

What I see here is clinically, I can't get excited about it. It's not that the relationship and the need to be sense of that, but when we use barrier systems, for example, to isolate the alveolar ridge, they become exposed, we leave them exposed in a majority environment with highly inflamed tissue -- Marjorie, do you disagree?

DR. JEFFCOAT: No, can get infected.

DR. REYNOLDS: They do, but we leave them as long as we can frequently, but they are infected, you are right, the biofilm is there and significant.

The question is at what level does it become a concern, and I think that, as I look at the transient nature, and we went from using cortex, which you had to go back and surgically retrieve, which in the vast majority of instances remained exposed for six weeks to eight weeks, just for context.

So, this is I think of concern, but doesn't rise to the level of what we do routinely and inadvertently sometimes.

Marjorie.

DR. JEFFCOAT: That light is blinking.

MS. DAPOLITO: Dr. Jeffcoat, please use your mike.

DR. JEFFCOAT: Oh, I was just saying that light is blinking, but I didn't turn it on. I don't know why it is blinking. I don't think

everybody had to hear that.

DR. DUBINETT: Other comments to this or other points related to safety?

DR. SNYDER: I just wanted to reiterate something that Steve said, and that I mentioned earlier, that, in fact, it is a little bit of the transient nature of the survival of the cells and the seemingly short exposure of the cytokines that kind of intrigues me.

Cells that are present expressing some cocktail of factors after just as little as five days can have such a profound effect on the environment makes me wonder what other changes are going on, and perhaps if those changes are superimposed onto the wrong substrate, maybe a smoker or somebody predisposed to a malignancy or some other problem, or to an immune reaction or to a developing mouth as in a kid, that this could be a problem.

Now, maybe it won't be, but I think six months is simply too short a period of time to assess that. We would never use six months to

look for tumorigenicity in almost any cell-based therapy.

Also, the persistent, as far as I can tell from the briefing documents, this notion of the cells persisting is based on only two patients in this study and four in a published study, so this is not an extensive look at how long these are persisted, and it is simply based on ALU sequences.

DR. DUBINETT: Yes, Dr. Wittes.

DR. WITTES: I just want to say that the very large database doesn't really comfort me, because if there were an agarose effect that were caused by changes in whatever, and it occurred later, nobody would think of it, nobody would associate it with the product.

So, I think only if something occurs very close to the time of administration but it seems to me that there is no way of answering this, that the sample size that would be necessary, and the length of time that would be necessary, to say this is not a concern or this is a concern

would have to be very large.

So, it seems to me this is a sort of situation where it is kind of faith-based safety.

DR. DUBINETT: Dr. Hornicek.

DR. HORNICEK: I think it is nice that we have the data from the venous stasis and the diabetic feet, I know it is not the mouth, but we have relatively long-term experience, and some of those patients are immunosuppressed and have various other issues, and it has been relatively safe, so I would say that is in support of the safety of this. I mean it's not the mouth, but I think that is a positive.

DR. DUBINETT: Dr. Reynolds.

DR. REYNOLDS: I may also point out that we use cell-based technologies in allogeneic grafts now, so we are implanting presumably live tissue or tissue that has cells from a donor, so I think that the concerns remain, the question is we don't have the profile, but we do have other evidence. I would agree that it is substantial in length.

The association is one that the timing is one that you would hope that would be reported, but we do use live cell-based products now.

DR. SNYDER: What are those cell-based interventions that are done now in the mouth?

DR. REYNOLDS: They are tissue banks, the use of fresh frozen in essence cryo-preserved allogeneic bone.

DR. SNYDER: But not passage cells, right?

DR. REYNOLDS: Not passage cells.

DR. SNYDER: Not passage lines or things of that sort.

DR. REYNOLDS: Which raises another question as to just in terms of bookkeeping, on the tissue bank side, all of those grafts or all of these constructs would have to have an ID that would allow the manufacturer to alert clinicians if there was a problem, and they could link it to the patient.

DR. SNYDER: I am not even saying that it has to be a hugely long follow up, maybe even a

year, which, quite frankly, may already have been done. These patients probably, they were followed officially for six months, but that was probably six months ago, so we may already have those data.

DR. DUBINETT: I think what Dr. Wittes is telling us is that they don't have enough patients to tell us the meaning of the data, but I do think it is of interest to put into context actually all of the other things that are utilized, and the way I am interpreting that the comments between you, Mr. Reynolds, and Dr. Snyder, is actually that, on the one hand, there is great experience with inflammation in general in the mouth, of course, but yet there is a concern about passage cells, that we may be into an area that is somewhat different from current practice and perhaps posing some unusual risks.

I think that the cutaneous issue is somewhat different in that we don't have specific risk factors for malignancy for those wounds necessarily, whereas, in the mouth we do have a

defined risk population for whom we know there are mutations there, and so they become a susceptible population that is distinct in some way.

DR. HORNICEK: I would argue that for many, for example, squamous cell carcinomas that occur in the skin, they occur after multiple episodes of injury to that area.

DR. AHSAN: When it comes to safety, I wonder, and maybe the clinicians can speak to this, about if the indication is a little bit more widespread, whether we are talking about increasing the length of Apligraf that is supplied, or, you know, the number of different Z stacks that are put, and how that affects the safety and the bioburden if they are either simultaneously applying to multiple regions or repeatedly applying over the course of years at different time points to different regions. Maybe the clinicians could speak to that.

DR. DUBINETT: Dr. Couture, I think in the absence of a clinician, maybe you could

comment.

DR. COUTURE: If there is nothing better, right? Actually, I can't respond to that because I am not a clinician, so obviously, if there is a clinician that wants to respond to that, I will just defer to them.

DR. DUBINETT: Dr. Genco.

DR. GENCO: Yes, I think that there is a possibility that it would be used multiple times in a single individual, and the dose, the area might be maybe 3- or 4-fold what you saw today, so that is a possibility.

I am not too concerned about that especially if the sponsor has evidence that multiple applications to the same patient doesn't induce an immune response, and I think they have that data.

In other words, if it is used on a leg ulcer or foot ulcer multiple times in the same subject, and there is no immune response, then, I wouldn't worry about there being an immune response in the oral cavity to multiple use.

DR. DUBINETT: Other comments?

DR. COUTURE: If we can move on to a non-clinical point. I actually share Dr. Snyder's intrigue with the very short window that this product actually had to have biological activity, but I even come to maybe a different conclusion that that is actually the feature that kind of mitigates that oncology risk, because in most cell therapies where we are concerned, they are usually talking about cell therapies we intend to be resident for a long, long, long period of time, and that is just not the case here.

These cells are clearly gone by six months, and the evidence is they are probably gone after a week or two weeks at the most, so I would weigh that in, in terms of deciding whether or not we consider this to be a safe product even if multiple administrations.

DR. JEFFCOAT: We have seen this before in other graft materials that have been used, sometimes now approved, sometimes now still undergoing research, but I think it is fair to

say the short period of effectiveness -- I am not sure if effectiveness is the word I want -- but it has a short period acting on the cells that we are seeing here, and this is now going out up to 15 years, some of these studies, so again, where I would be worried, people who drink alcohol, who smoke, and children, and I guess I have said it enough.

DR. DUBINETT: Dr. Amar.

DR. AMAR: I share the concerns about the cells, and I heard that this concern should be mitigated by the fact that there was a turnover.

What I am concerned is during this turnover -- and I asked the question this morning about the multinucleated cells -- what causes the general mounting of the inflammatory response, it is not probably the cell, but the remnant of this digestion of cell and cell debris, and we have evidence right now that DNA stimulates, fragment of DNA stimulate a total response, which is associated with heavy immune consequences.

So, one aspect is related to this. The

second aspect is during this process, the apoptosis programmed cell death or the degradation of the cell could allow some kind of deviation.

DR. SNYDER: One other aspect, I think a little more information would be helpful in the long run, because it is possible that if these cytokines and inflammatory mediators, we know what a lot of them are, Type 1 interferons, IL-1, all the things the toll-like receptors will trigger.

You can measure all those, and it is possible that what you are going to see at these applied sites is going to be no greater than what you would see if you biopsied an anthus ulcer or a little surgical procedure or something, and if that is the case, then, I think that would allay a lot of concern.

Actually, some of what Dr. Amar brought up also triggered some thinking about, you know, patients who have quiescent or herpesvirus, or other kinds of viruses, once you actually assault

these cells, and even if they go through cell cycle transiently in response of these cytokines, does that reactivate a quiescent virus.

That was one aspect of safety that I don't recall being looked at, and once this is used broadly, there will be lots of patients who have herpes simplex or other kinds of quiescent viruses in the trigeminal nerve and other places.

In terms of looking at the consequences of putting cells back into the cell cycle even transiently for repair, I think the concerns might be mitigated perhaps by longer follow up and a better understanding of the mechanism of action.

DR. AMAR: And probably this long-term follow up which could fall within the postmarketing surveillance will allow us to understand very clearly whether the gain of keratinized tissue, be it 1 mm or 4 mm is maintainable over time in those patients, which is a critical aspect.

Do patients with plaque accumulation

maintain and defend themselves the same way that we have with the free gingival graft as opposed to with a treatment like that?

DR. DUBINETT: Okay. Additional comments?

Dr. Reynolds, as the discussant, do you want to have the last word?

DR. REYNOLDS: No, I thought the discussion was thorough and excellent.

DR. DUBINETT: However, we have to wait and see if Dr. Witten concurs.

DR. WITTEN: I agree.

DR. DUBINETT: With that unanimity, then, we are able to move to Voting Question No. 7. Do the data presented demonstrate the safety of Apligraf for the proposed Indication?

You will remember that the buttons before you are for Yes, Abstain, and No will be flashing.

MS. DAPOLITO: There are 15 voting members, and the tally is 14 Yes, 0 Abstain, 1 No for a total of 15. The one No vote was Dr.

Snyder. The Yes votes were all the other voting members who were listed in the prior question.

Thank you.

DR. DUBINETT: Should we go around in the same order? Dr. Lee.

DR. MEI-LEI LEE: I don't have any concern.

DR. SNYDER: I think I articulated what my concerns were, I would like longer follow up and a better understanding of some of the mechanisms, but principally longer follow up.

DR. DAHLGREN: No concerns.

DR. FRATZKE: No concerns.

MS. RUE: Nothing different to add.

DR. JEFFCOAT: I don't have no concerns, because what I have said 45 times, which I don't think you want to hear again.

DR. WITTES: I also don't have no concerns, and I actually don't believe in the word demonstrate. I voted Yes because I think if you change the word demonstrate to something softer, I think the data do not demonstrate.

They indicate consistent with, so it is a qualified Yes. But I agree with Dr. Snyder about the other issues.

DR. REYNOLDS: I voted Yes obviously. I have nothing to add from what has been discussed. Thank you.

DR. BUI: I would vote Yes. I think the product has a good safety profile.

DR. DUBINETT: Dr. Amar.

DR. AMAR: I share the concern of Dr. Snyder, however, the data in the materials has been in use in other areas, such as diabetic ulcers and venous, and as a result of that, I would strongly advise a very, very strong program of postmarketing surveillance for all the issues that we raised.

DR. GENCO: I would just again reiterate I have no safety concerns except the issue of repeat use, and I asked if sponsor had data, I am assuming they do, and the remote possibility of an adverse immune response with repeat use, which would probably occur in the oral cavity.

DR. HWU: I think this is likely safe, but agree that longer term follow up especially in the pediatric population is indicated, as well as a better quantitative characterization of the potentially pro-carcinogenic factors that are produced like the PDGF and the VEGF.

DR. HORNICEK: Nothing else to add.

DR. COUTURE: I just agree with the postmarketing surveillance comments.

DR. AHSAN: I agree with safety for this indication as the inclusion and exclusion criteria are stated. If we broaden the application, I don't think necessarily the translation is there at the moment, looking at longer term, looking at other applications, also looking at repeated use.

DR. DUBINETT: Okay. I agreed to the safety with the caveats that I had mentioned previously and has been added by others here.

Dr. Witten.

DR. WITTEN: Yes. I would just like to thank the Advisory Committee, the FDA staff who

prepared for this meeting, and particularly our  
Chair. Thank you.

DR. DUBINETT: Thanks, everyone for  
participating. We are adjourned.

[Whereupon, the meeting was adjourned.]