

APPROVED

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APPROVED

By M Serabian at 10:44 am, Nov 21, 2016

I concur with this review memo. B Robinson 11/21/2016

I concur with this review. M. Serabian 11/21/16

FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
Office of Cellular, Tissue and Gene Therapies
Division of Clinical Evaluation and Pharmacology/Toxicology
Pharmacology/Toxicology Branch

BLA NUMBER: STN #125603.000

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PRODUCT: MACI™ (membrane applied characterized autologous cultured chondrocytes)

APPLICANT: Vericel Corporation

PROPOSED INDICATION: MACI™ is indicated for the treatment of symptomatic, full-thickness cartilage defects (single or multiple defects) of the knee with or without bone involvement (b) (4)

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APPROVED

By Allen Wensky at 9:54 am, Nov 21, 2016

¹ Reviewed the biomechanical testing data from the following studies: (1) Characterization of the “Physical and Histological Properties of ACI-Maix Type I/III Collagen Membrane Seeded with Rabbit Chondrocytes” (Study #06GSTR018) and (2) “Investigation of Efficacy of MACI in Horses” (Study # GENZ-09-4417). This consult review memo is attached to this Pharmacology/Toxicology review memo. Analysis of Study #06GSTR018, an *in vitro* product characterization study, is provided in the CMC review memo. t Aspects of the consultant’s review of equine Study #Genz09-4417 are incorporated into this Pharmacology/Toxicology review memo.

EXECUTIVE SUMMARY:

The product, MACI™, is an implant which consists of characterized autologous cultured chondrocytes (ACI) seeded on a resorbable porcine-derived collagen membrane (ACI-Maix), which can be trimmed to fit the size and shape of a cartilage defect of the knee. Studies conducted in horses and rabbits compared ACI-Maix or empty defects to analogous products consisting of ACI-Maix seeded with rabbit or horse cells. These analogous products are also referred to as 'MACI'. A 12-week study in (b) (4) rabbits (Study # (b) (4) 002) was conducted to evaluate the activity of MACI in critical-sized defects of the femoral condyle in a single limb of each animal. Histopathology assessment of the knees at 6 and 12 weeks post-implantation indicated that regeneration of the implanted defects was superior to untreated (i.e., 'empty') defects located in the same limb.

A 24-week study in (b) (4) rabbits (Study # GENZ-06-0147) was conducted to evaluate the activity of ACI-Maix alone or MACI, with or without microfracture (Mfx), in critical-sized defects of the femoral condyle in a single limb of each animal. Histopathology assessment indicated: 1) improved repair of the defects implanted with ACI-Maix alone and MACI in the absence of Mfx and 2) increased inflammation in the knees treated with Mfx which decreased over time. The inflammation was possibly due to immune cell infiltration due to the Mfx procedure. Membrane remnants were still present in the joints at 24 weeks.

A 24-week pilot study in six horses (Study # GENZ-06-0239) was conducted to assess the activity of MACI seeded with $0.9-1.7 \times 10^6$ autologous cultured chondrocytes following implant in critical-sized defects (15-mm diameter) of the trochlear ridge in a single stifle of a hind leg of each animal. Inflammation was present in all knees, likely due to defect creation and possible immune response to the porcine membrane. A trend towards improved repair by 24 weeks was observed in the MACI-implanted defects compared to empty control defects. Membrane was still present at 24 weeks.

A definitive 53-week study was conducted in 27 horses (Study # GENZ-09-4417) to evaluate the safety and the activity of MACI seeded with $0.9-1.7 \times 10^6$ autologous cultured chondrocytes following implant in critical-sized defects (15-mm diameter) of the trochlear ridge in a single stifle of a hind leg of each animal. Overall, the biopsy collection, defect creation, and implantation procedures were well-tolerated. Any observed lameness was mild in nature and occurred early (12 weeks) in the study in approximately 50% of all animals in each group, with full gait recovery by 53 weeks in all animals. A trend towards improved cartilage repair for the MACI-implanted defects compared to the ACI-Maix-implanted defects and the empty defects was observed at 12 and 53 weeks. The MACI-implanted defects approached native cartilage in appearance, and GAG content of cartilage was increased relative to the ACI-Maix-implanted defects. Improved biomechanical testing outcomes were observed in some of the MACI-implanted defects compared to defects implanted with ACI-Maix membrane alone or no agent (empty defect). However, the biomechanical properties of the MACI-implanted defects were not identical to native cartilage. There was no indication of systemic adverse reactions or systemic distribution of MACI, ACI-Maix, or the seeded chondrocytes.

² Reviewed the biocompatibility studies for ACI-Maix membrane (submitted to MF (b) (4) and to the BLA). Refer to the consult review memo for details.

Collectively, the rabbit and equine studies indicate that implantation of MACI secured with a commercially available fibrin sealant resulted in improved repair of critical-sized defects in the knee/stifle, compared to implanted membrane alone or empty defect. There were no significant safety signals identified in the animals. Mild inflammation was observed in the knee of both species, which resolved over time. The ACI-Maix membrane was still present in both species at 6 months post-implant and microscopic fibers from the membrane were noted out to 53 weeks in horses.

Biocompatibility testing of the ACI-Maix (located in Drug Master File (b) (4)) was acceptable. The applicant did not conduct carcinogenicity or developmental and reproductive toxicity studies. This is acceptable considering the local anatomic site of MACI implantation, the absence of systemic MACI distribution following administration, and the lack of genotoxicity or mutagenicity observed for the ACI-Maix membrane in biocompatibility tests.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION: There are no non-clinical deficiencies in the pharmacology/toxicology studies, and there are no outstanding requests for additional non-clinical data. The non-clinical data support the licensure application.

Formulation and Chemistry:

Each MACI implant consists of characterized autologous cultured chondrocytes (ACI) seeded on a resorbable porcine-derived Type I/III collagen membrane (ACI-Maix), at a density of 5×10^5 to 1×10^6 cells per cm^2 , which can then be trimmed by the surgeon to fit the size and shape of the defect (Table 1). The final product is 14.5 cm^2 in size (3 cm x 5 cm with a small orientation notch cut from one edge for directionality). It is packaged in one or two single-dose containers, each consisting of a bottom dish, luer lock lid, and 5-point holding ring designed to ensure proper orientation during shipment and constant emersion in the shipping media. MACI does not require any thawing, reconstitution, dilution, resuspension, or rinsing steps prior to direct application. MACI is implanted with the cell-side down (i.e., facing the bone defect). Implantation is performed using sterile surgical techniques and requires both the preparation of the defect bed and the application of fibrin sealant (which can be from various marketed products according to the applicant) to the base and rim of the defect in order to secure the implant.

Table 1

Component	Source (Derivation or Manufacturer)	Amount Administered	Function
Cultured chondrocyte cells	<u>Biologic Component:</u> Autologous chondrocytes	5×10^5 to 1×10^6 cells/ cm^2 of membrane used	Active ingredient for generation of cartilage membrane within defect
ACI-Maix	<u>Device Component:</u> Porcine-derived collagen type I/III membrane	Cut to fit the shape and size of the patient's cartilage defect	To ensure correct localization of chondrocyte cells within the defect

Component	Source (Derivation or Manufacturer)	Amount Administered	Function
(b) (4)	(b) (4)	(b) (4)	Transport media to maintain the chondrocytes in a physiologically stable environment during shipping

Abbreviations:

ACI	Autologous Chondrocyte Implant
BLA	Biologics License Application
CDRH	Center for Devices and Radiological Health
DART	Developmental and Reproductive Toxicology
(b) (4)	(b) (4)
(b) (4)	(b) (4)
FRCPath	Fellow of the Royal College of Pathologists
GAG	Glycosaminoglycan
GLP	Good Laboratory Practice
(b) (4)	(b) (4)
IA	Intra-Articular
IFN	Interferon
IHC	Immunohistochemistry
IL	Interleukin
MACI	Membrane applied characterized autologous cultured chondrocytes
MF	Master File
Mfx	Microfracture
MOA	Mechanism of Action
OA	Osteoarthritis
OUS	Outside of the United States
PGE2	Prostaglandin E2
QA	Quality Assurance
ROA	Route of administration

Cross Referenced Files:

(b) (4) – Gordon MacFarlane; Matricel GmbH; Device MF ACI-Maix **Membrane – ACTIVE**

(b) (4)

Related Files:**Pre-submissions related to MACI:**

PTS #PS002102 – Pre-IND - Genzyme Corp.; Autologous Chondrocytes Expanded *Ex Vivo* and Seeded onto a Porcine Collagen-Based Carrier (MAIX membrane) –**Aug 2005**

PTS #PS002103 – Pre-IND - Genzyme Corp.; Autologous Chondrocytes Expanded *Ex Vivo* and Seeded onto a Porcine Collagen-Based Carrier (MAIX membrane) – **Nov 2005**

PTS #PS002106 – Pre-BLA - Genzyme Corp.; Autologous Chondrocytes Expanded *Ex Vivo* and Seeded onto a Porcine Collagen-Based Carrier (MAIX membrane) –**May 2009**

PTS #PS002661 – Pre-BLA – Vericel Corp.; Autologous Chondrocytes Expanded *Ex Vivo* and Seeded onto a Porcine Collagen-Based Carrier (MAIX membrane) –**Feb 2015**

Note: No IND(s) is associated with this file. All clinical studies were conducted outside of the United States (OUS).

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INTRODUCTION

Critical-sized defects in acute articular cartilage damage of the knee lack the ability to self-repair or regenerate tissue. These defects result in significant pain, loss of function, and could eventually lead to osteoarthritis of the knee. Cartilage and chondrocyte replacement of the damaged tissue has been an area of intense interest in these injuries, but has met with limited success. In addition to limited availability, whole cartilage replacement has proven difficult to integrate into load-bearing tissue. Chondrocyte isolation, expansion, and implantation may address the availability issue; however, technical challenges such as graft loss, inappropriate tissue architecture, and delamination still remain a concern. Application of the ACI-Maix, consisting of a resorbable bi-phasic porcine collagen membrane combined with ACI, is hypothesized to stabilize the cells, provide a framework for appropriate cell growth and collagen disposition, and provide support for maintaining the implant in the defect.

The applicant proposes to conduct a two-step procedure in which: (1) cartilage from a non-load bearing portion of the knee is harvested, processed, seeded, and cultured on the ACI-Maix membrane (See 'Formulation and Chemistry' in this review memo) before (2) implantation into one or two prepared critical-sized defects (3-15 cm² diameter). The implant is sealed with a fibrin sealant that is approved for hemostasis and sealing. The applicant's clinical data indicate that at 3 years post-procedure MACI is superior to microfracture (Mfx), which is the current standard-of-care for defects between 2-4 cm², when assessing subjective pain and function. However, there does not appear to be a difference between Mfx and MACI by MRI at the time points evaluated (Refer to the clinical review memo for details).

NON-CLINICAL STUDIES

PHARMACOLOGY STUDIES

Summary List of Pharmacology Studies

The following pharmacology studies were conducted to support the rationale for the administration of MACI to treat the proposed clinical indication.

In Vitro Studies: No *in vitro* Pharmacology studies were conducted

In Vivo Studies: *In Vivo* studies in cartilage defect animal models

Study Number	Study Title / Publication Citation	Report Number
1	<i>Membrane-induced autologous chondrocyte implantation in rabbits using matricel membrane</i>	(b) (4)
2	<i>Histopathological investigation of the safety of ACI-MAIX scaffold (seeded and unseeded with autologous chondrocytes) in a rabbit osteochondral defect.</i>	GENZ-06-0147
3	<i>Investigation of 24 Week Efficacy of MACI in Horses: Final Report at 6 months Post Implantation</i>	GENZ 06-0239
4	<i>Investigation of Efficacy of MACI in Horses</i>	GENZ 09-4417

Note: Studies #1-4 are briefly summarized in this review memo under ‘Overview of Pharmacology Studies.’ No individual animal data were provided for Study #1. Therefore this reviewer can only review the summary information provided for this study.

Overview of Pharmacology Studies

Overview of In Vitro Studies: N/A

Overview of In Vivo Studies:

Study #1

Report Number		(b) (4) 002
Date Report Signed		September 29, 2002
Title		<i>Matrix-induced autologous chondrocyte implantation in rabbits using matricel membrane</i>
GLP Status		No (GLP was claimed by the study sponsor but this study and resulting report are not considered GLP by this reviewer. See this reviewer’s ‘Comments’ below) An unsigned Quality Assurance (QA) Statement was provided ((b) (6)), with no associated QA report.
Testing Facility		(b) (4)
Objective		To evaluate a porcine-derived type I/III collagen membrane for autologous chondrocyte implantation in rabbits.
Study Animals	Breed	(b) (4)
	Species	<i>Oryctolagus cuniculus</i> (Rabbit)
	Age	Adult (age not specified)
	Body Weight	Males = 3-4 kg Females = 2.2-3.5 kg
	#/group	8/group (4/group sacrificed at 6 and 12 weeks post-implantation) Note: Although the study sponsor states that male and female animals were used, the numbers of each sex included in the study and the number allocated to each group were not provided. This is a potential source of bias.
		Total # 16
Test Article		ACI-Maix membrane (Lot #((b) (4))) seeded with rabbit chondrocytes – referred to as ‘MACI’ <ul style="list-style-type: none"> Chondrocyte source - autologous rabbit cartilage similar to the human source of non-load bearing cartilage Cultured chondrocytes ((b) (4)) were seeded for ((b) (4)) on a 1 cm² membrane Samples of ACI-Maix were returned to the study sponsor for characterization and verification of seeding.
Control Article		Saline
Route of Administration		Intra-articular (IA)

Description of the Disease/ Injury Model and Implant Procedure	Tissue for production of MACI was harvested from non-load bearing cartilage tissue in the index knee of all rabbits. The cells from the biopsy were (b) (4), cultured, and seeded on the ACI-Maix scaffold for (b) (4) before implantation. A single femoral condylar defect was created by an osteochondral punch. In the same surgical procedure, MACI was then press-fit into the defect after fibrin sealant/glue was applied to the defect. Fibrin glue was also applied subsequent to MACI implantation for further sealing.
Study Groups and Dose Levels	Group 1 – Saline control (designated as ‘Untreated’) Group 2 – MACI – Single implant (~1cm ²) at ~1 x 10 ⁶ cells/implant
Randomization	No
Description of Masking	N/A
Scheduled Sacrifice Time Points	6 and 12 weeks post-implant

Key Evaluations and Assessments:

In-life Assessments: Observed for morbidity only.

Post-mortem Assessments:

- Histological ((b) (4)) assessment of the defect at 6 and 12 weeks using an in-house scoring system. This system included scores (0-4) that assessed the percentage defect filling, articular surface continuity, restoration of architecture, tissue integration, cellular morphology, and membrane staining. The system was weighted toward defect fill percentage and cellular morphology. This system has not been used previously.

Key Results:

- No morbidity or mortality observed throughout the study.
- Scoring of defects:
Table 1 outlines the scoring system used to assess defect repair. Tables 2 and 3 document the raw scoring data for each study group: Group 1 Control (Untreated) and Group 2 (MACI) scores for each animal are provided for each category. Rabbits suffering graft failure (due to inflammatory reaction) and/or osteoarthritis (OA) are denoted by an ‘F’ for their score.

Table 1: Replicated from page 14 of the protocol for Study # (b) (4) 002

Comprehensive Scoring System for Articular Cartilage Repair	
CATEGORY	SCORE
(1) Percentage defect filling:	
100%	4
75%	3
50%	2
25%	1
0%	0
(2) Articular surface continuity:	
Continuous and smooth	2
Continuous but rough	1
Discontinuous	0
(3) Restoration of osteochondral architecture:	
Clearly differentiable	2
Unclear (heterologous)	1
Poor	0
(4) Repair tissue integration:	
Complete	2
Partial	1
Poor	0
(5) Cellular morphology of articular cartilage regeneration:	
Hyaline with zonal architecture	4
Hyaline without zonal architecture	3
Hyaline/ Fibrocartilage hybrid	2
Fibrocartilage	1
Fibrous tissue	0
(6) Matrix staining (Alcian Blue):	
Higher intensity than normal	2*
Normal intensity	2
Less intense	1
Far less intense	0

Table 2: Replicated from page 23 of the results section for Study # (b) (4) 002

Scoring Outcomes of Untreated Regeneration Tissue 6 and 12 weeks Postoperatively

Categories:	6 week (rabbit #)				12 week (rabbit #)			
	1	2	3	4	5	6	7	8
Percentage defect filling	4	4	4	4	4	4	4	4
Articular surface continuity	0	2	1	0	1	0	0	0
Restoration of osteochondral architecture	1	0	0	0	1	0	0	1
Repair tissue integration	1	0	2	0	1	1	0	1
Cellular morphology of articular cartilage regeneration	2	2	1	2	2	1	1	2
Matrix staining (Alcian blue)	2	2*	1	2*	2*	1	2*	1
Totals:	10	10*	9	8*	11*	7	7*	9

Table 3: Replicated from page 23 of the results section for Study # (b) (4) 002

: Scoring Outcomes of MACI Regeneration Tissue 6 and 12 weeks Postoperatively

Categories:	6 week (rabbit #)				12 week (rabbit #)			
	9	10	11	12	13	14	15	16
Percentage defect filling	4	F	4	4	F	4	4	4
Articular surface continuity	2		2	2		2	2	1
Restoration of osteochondral architecture	2		2	1		1	1	1
Repair tissue integration	2		2	2		2	2	2
Cellular morphology of articular cartilage regeneration	4		4	3		3	2	2
Matrix staining (Alcian blue)	2*		2*	2*		2	1	1
Totals:	16*		16*	14*		13	12	11

Note: A notation of “*” apparently indicates intense Alcian blue staining; however, the attributed score only reached ‘2’. This reviewer is not able to interpret the meaning of this notation.

Descriptive histological assessments were provided based on representative (black and white) slides included in the submission. The pathologist (not identified) reported better repair in animals receiving MACI compared to controls, but that the quality of the tissue in MACI-implanted defects at 6 weeks was not similar to native tissue. In addition, two animals from the MACI group were not scored (see above) due to signs of OA and/or graft failure, according to the study report.

Comments:

- No randomization or masking was used in this study, which are notable confounding parameters.
- The leg/knee in which the defect was created was not indicated.

- The number of males and females in each group was not indicated, although per the report both genders were used in the study.
- A rationale for the sacrifice and assessment time points post-implantation was not provided.
- The comparability of test article manufacturing and final product characterization to the proposed final clinical product manufacturing and characterization was not provided, therefore, the relevance of these data to the final clinical product is not known.
- Individual animal data were not provided, thus this reviewer is unable to independently verify the summary results.
- The defect scoring system was not validated, thus, its usefulness is questionable. However, this system seems to be reasonable to this reviewer.
- Results from the scoring assessments may indicate that MACI is superior to empty defect (saline control) for healing out to 12 weeks. However, this may be due to the kinetics of healing, which is slower in the empty defect controls. In addition, the animals listed as graft failures due to inflammation should be included in the final assessment comparing MACI to empty defect. Thus, the MACI implant itself and/or the implant procedure as a cause for the observed OA and/or infection and thus graft failure cannot be ruled out.
- Inadequate sterile processing or handling is indicated due to 2 of 8 animals exhibiting graft failure due to inflammatory reactions. It cannot be ruled out that the inflammation was caused by MACI as well. Thus, excluding these animals from analysis was inappropriate.
- This reviewer cannot make any definitive conclusions from the study summary provided. The safety and activity profile of MACI will thus be entirely based on the 53-week horse study (Study #4) and the clinical data.

Study #2

Report Number	GENZ 06-0147
Date Report Signed	Study report was not signed
Title	<i>Histopathological investigation of the safety of ACI-MAIX membrane (seeded and unseeded with autologous chondrocytes) in a rabbit osteochondral defect</i>
GLP Status	No (No QA report or statement was provided.)

Testing Facilities		<p>Cell Isolation, Culture, and Seeding Genzyme Corporation Carticel® Operations 64 Sidney Street Cambridge, MA</p> <p>In-life Genzyme Corporation 78 New York Avenue Framingham, MA 01701</p> <p>Histopathology Michael L. Hawes, D.V.M., Diplomate A.C.V.P. Senior Scientist Genzyme Corporation</p>
Objective		To better characterize local tissue response to implantation and determine the role of factors, such as autologous cell seeding and/or subchondral bone plate breach, on the safety and degradation profile of the ACI-Maix membrane.
Study Animals	Breed	(b) (4)
	Species	Rabbit
	Age	8-12 months
	Body Weight	>3.4 kg on the day of implantation
	#/sex/group	Not reported
	Total #	20
Test Article		<p>ACI-Maix membrane (Lot #'s (b) (4)) seeded with autologous cultured chondrocytes – referred to as ‘MACI’</p> <p>Approximately 5 weeks before implantation, all rabbits had a cartilage biopsy performed on the knee joint contralateral to the knee joint (not specified) that received the implants. Under general anesthesia, a 2-3 mm wide by 8-10 mm long cartilage flap or multiple fragments were harvested from the lateral trochlear ridge of the distal femur. The retrieved cartilage samples were placed in transport medium and transported on wet ice to the Carticel manufacturing facility in Cambridge, MA, where chondrocytes were isolated and expanded. Approximately 1×10^6 live cells/cm² (acceptable cell count range: (b) (4) live cells/cm²) were seeded onto a 3-mm diameter ACI-Maix porcine-derived collagen membrane and cultured for 3-5 days.</p>
Control Article		Unseeded membranes (sterilely packaged) were utilized for defects not receiving cells. A (b) (4) was applied to the membrane to allow for easier defect application.
Route of Administration		IA

Study Groups and Dose Levels	<table><tr><th>Species</th><th>No. of Animals</th><th>No. of Defects</th><th>Treatment</th><th>Orthopedic Setting</th><th>Length of Treatment</th></tr><tr><td rowspan="2">(b) (4) Rabbit</td><td rowspan="2">5</td><td>5</td><td>MAIX + Cells + Fibrin Glue</td><td rowspan="2">No Microfracture</td><td rowspan="2">12 weeks</td></tr><tr><td>5</td><td>MAIX + No Cells + Fibrin Glue</td></tr><tr><td rowspan="2">(b) (4) Rabbit</td><td rowspan="2">5</td><td>5</td><td>MAIX + Cells + Fibrin Glue</td><td rowspan="2">Microfracture</td><td rowspan="2">12 weeks</td></tr><tr><td>5</td><td>MAIX + No Cells + Fibrin Glue</td></tr><tr><td rowspan="2">(b) (4) Rabbit</td><td rowspan="2">5</td><td>5</td><td>MAIX + Cells + Fibrin Glue</td><td rowspan="2">No Microfracture</td><td rowspan="2">24 weeks</td></tr><tr><td>5</td><td>MAIX + No Cells + Fibrin Glue</td></tr><tr><td rowspan="2">(b) (4) Rabbit</td><td rowspan="2">5</td><td>5</td><td>MAIX + Cells + Fibrin Glue</td><td rowspan="2">Microfracture</td><td rowspan="2">24 weeks</td></tr><tr><td>5</td><td>MAIX + No Cells + Fibrin Glue</td></tr></table>						Species	No. of Animals	No. of Defects	Treatment	Orthopedic Setting	Length of Treatment	(b) (4) Rabbit	5	5	MAIX + Cells + Fibrin Glue	No Microfracture	12 weeks	5	MAIX + No Cells + Fibrin Glue	(b) (4) Rabbit	5	5	MAIX + Cells + Fibrin Glue	Microfracture	12 weeks	5	MAIX + No Cells + Fibrin Glue	(b) (4) Rabbit	5	5	MAIX + Cells + Fibrin Glue	No Microfracture	24 weeks	5	MAIX + No Cells + Fibrin Glue	(b) (4) Rabbit	5	5	MAIX + Cells + Fibrin Glue	Microfracture	24 weeks	5	MAIX + No Cells + Fibrin Glue
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Dose Level: ~1 x 10 ⁶ cells/cm ² scaffold. The scaffold and defect were ~3 mm in diameter x 1 mm in depth																																												
Description of Implant Procedure	<u>Single defect administration</u> - Rabbits were randomly assigned to each study group prior to creation of the cartilage defects. A 2-4 cm arthrotomy was performed on the femoropatellar joint of a single randomly selected hind limb. Two 3-mm diameter osteochondral cartilage defects that were ~1 mm in depth (penetrating lightly through the subchondral bone) were made on the trochlear groove. Within 12 hours of product receipt, and in the same surgical procedure, the membrane was secured to the base of each defect with (b) (4), such that the surface of the repair construct was recessed below the surface of the articular cartilage. Cell-seeded membranes were oriented with the cell-seeded (rough) surface adjacent to the base of the defect, leaving the smooth unseeded surface facing the joint cavity. Mfx was performed by drilling three holes, evenly spaced, through the subchondral bone in the defect space.																																											
Randomization	Yes																																											
Description of Masking	Masked histopathology																																											
Scheduled Sacrifice Time Points	12 and 24 weeks post-implant																																											

Key Evaluations and Assessments:

Clinical Observations	Daily for duration of the study
Body Weights	Prior to implantation and at necropsy
Histology on Index Knee	Masked pathology examination of the defect and synovium

Key Results:

- No reported major abnormal clinical observations for any animal.
- Body weights increased in all animals.
- Per the pathology report:
 - ACI-Maix membrane was still detectable at the 24-week time point in all defects which received membrane and was made up mostly of elastin.
 - Membrane remnants were associated with histiocytic infiltrates and inflammation, which were diminished, but not completely resolved, by 24 weeks compared to 12 weeks.

- Defects injected with the test article in the absence of Mfx (with and without cell seeding) exhibited significantly better repair by 24 weeks compared to 12 weeks.
- Inflammation was more pronounced in the knees of animals receiving test article + Mfx that were sacrificed at 24 weeks compared to the knees of animals sacrificed at 12 weeks. There was no explanation for this finding in the study report but it was likely due to the increased access by immune cells to the test article due to the Mfx procedure.

Comments:

- The appropriate controls consisting of: 1) defect only (i.e., no Mfx, no test article, and no ACI-Maix membrane), 2) defect + (b) (4), or 3) defect + Mfx were not included. Thus, determining the effects of defect creation and subsequent fill on healing and inflammation cannot be separated from the ACI-Maix membrane effects.
- The study data indicate that implanted ACI-Maix membrane (without cells) may cause inflammation until it is fully degraded. However, this cannot be confirmed since the appropriate controls were not included in this study. Every animal was implanted with the membrane and every animal was sacrificed at 24 weeks displayed reduced inflammation compared to the knees of animals sacrificed at 12 weeks. It did not appear that: 1) the presence or absence of cells or 2) exposure to Mfx greatly influenced the level of inflammation. Therefore the membrane is likely the cause of the inflammation observed, although the surgical procedure itself cannot be totally ruled out. However, this inflammation did not result in systemic effects manifested in animal weight loss or abnormal appearance/observations.
- Section 12.1 of the proposed label states that the ACI-Maix membrane promotes proliferation and re-differentiation of chondrocytes. However, the applicant has not provided *in vivo* data to support this statement.
- Section 12.2 of the proposed label states that the membrane is resorbed over a period of 3 months following implantation. This statement is not supported by the data generated in rabbits (Study #3, Report #GENZ 06-0147) and horses (Study #4, Report #GENZ 06-0239) that indicate the presence of the ACI-Maix membrane for at least 4-6 months after implantation.

In conclusion, this study indicates that the presence or absence of autologous rabbit chondrocytes seeded on the ACI-Maix membrane with or without Mfx, do not appear to exacerbate the local inflammatory reaction due to defect creation and membrane-specific inflammation following IA administration in the rabbit knee cartilage defect at 12 and 24 weeks post-implantation.

Study #3

Report Number	GENZ 06-0239
Date Report Signed	May 29, 2012
Title	<i>Investigation of 24 Week Efficacy of MACI in Horses: Final Report at 6 months Post Implantation</i>
GLP Status	No

Testing Facility		(b) (4)
Objective		To provide, in a smaller, shorter term study, the rationale for a larger, longer term equine plausibility of efficacy/mechanism of action/safety study in horses that will support filings for the MACI product.
Study Animals	Breed	Thoroughbred or mixed breeds
	Species	<i>Equus feris</i> (Horse)
	Age	1.5-3 years (skeletally mature)
	Body Weight	300-500 kg
	#/sex/group	3 animals/sex/group Note: The groups consisted of: 1) defects implanted with MACI and 2) untreated defects. Thus essentially both 'groups' existed within each animal.
	Total #	6 (3 castrated males and 3 females)
Test Article		ACI-Maix (Lot # not specified) seeded with autologous equine cultured chondrocytes (MACI; autologous horse analog). Autologous chondrocytes were isolated from healthy femoral articular cartilage biopsies obtained approximately 10 weeks before implantation and expanded in culture according to Standard Operating Procedures (SOP) for the final product. The cells (b) (4) were seeded onto the ACI-Maix membrane, implanted in the defect, and secured with (b) (4) of (b) (4).
Control Article		Empty defect proximal or distal to the lateral trochlear ridge. (b) (4) was not administered to the empty defect.
Route of Administration		IA
Description of the Injury Model and Implant Procedure		Two 15-mm diameter critical sized proximal and distal trochlear ridge defects were created in a single knee/stifle of the hind leg for each animal. The index knee/stifle was contralateral to the randomly selected biopsy knee (designated as the 'control knee/joint'). Each defect was randomly selected to receive MACI or no agent. The MACI implantation surgeries were performed 10 weeks after the cartilage biopsy/harvest and during the same surgical procedure as defect creation. MACI was inserted next to the empty defect with the cell side toward the bone and secured with (b) (4). Twelve weeks (3 months) after implantation, second-look arthroscopies of the index knee/stifle were performed. Note: The report does not state whether the MACI product shipped from Genzyme was (b) (4) or fresh.
Study Groups and Dose Level		Refer to '#/sex/group' for the description of the study groups Dose Level: <u>MACI</u> - approximately 1.77 cm ² ACI-Maix seeded with autologous chondrocytes for each defect. Note: The cell dose level was not specified in the study report. The study sponsor states that the membrane was seeded according to standard operating procedures, which would be 5 x 10 ⁵ - 1 x 10 ⁶ cells/cm ² .
Randomization		Yes, the MACI implant was randomized to the distal or proximal defect, and the knee/stifle selected for implant was also randomly chosen.
Description of Masking		Only the second-look arthroscopy assessments performed at 12 weeks post-implantation were reportedly masked to the assessor.
Scheduled Sacrifice Time Point		24 weeks (6 months) post-implant

Key Evaluations and Assessments:

Assessment	Time point
Lameness scores	Pre-study, pre-implant, 3 and 6 months post-implant
Clinical pathology	3 and 6 months post-implant
Second-look arthroscopy	3 months post-implant; Note: The First-look arthroscopy occurred at the time of defect creation.
Synovial fluid analysis	3 and 6 months post-implant
Synovial membrane histology	3 months post-implant; Note: This analysis was not conducted at 6 months.
Prostaglandin E2 (PGE2) of synovial fluid by ELISA	Collected from the hind leg containing the defects and the contralateral legs at 3 and 6 months post-implant
Gross and microscopic exams	6 months post-implant
Immunohistochemistry (IHC)-Collagen Type II	6 months post-implant

Key Results:

Assessment	Key Results
Lameness scores	No lameness was reported in any animal at any time point except in the acute time interval post-implant (expected as a result of the surgical procedures).
Clinical pathology	No marked changes
Second-look arthroscopy	There was a slight increase in the tissue material filling the defects implanted with MACI compared to control/empty defects.
Synovial fluid analysis	<p>Mild lymphoid accumulation and increased fluid were observed in synovial tissue obtained from several horses at 3 months. At 6 months, per the study report, the following was observed in the synovial fluid collected from the index knee:</p> <ul style="list-style-type: none"> One horse had evidence of chronic inflammation as indicated by numerous vacuolated macrophages. Three horses had evidence of mild mixed inflammation. Two horses within normal limits (Note: the normal limits of cells in fluid were not provided). The protein concentration in the synovial fluid was within normal limits (<2.5 g/dL) for all six index joints. <p>Synovial fluid analysis of control joints revealed:</p> <ul style="list-style-type: none"> One horse with mild lymphocytic inflammation and elevated protein. One horse with mild mixed inflammation. One horse with mildly elevated protein. Two horses with blood contamination of an otherwise normal sample. One horse with values with normal limits and no other abnormalities.
Synovial membrane histology	<p>Increased fibrosis and inflammatory cell infiltrates were observed in the synovial membranes obtained from the index knee compared to the control knee of the same animal.</p> <p>Note: This would be expected from the trauma from surgery at the 12 week time point.</p>

Assessment	Key Results
PGE2 levels	PGE2 was elevated at both 3 and 6 months in the index knees compared to control knees. Note: PGE2 is a marker of ongoing inflammation and was used by the study sponsor as a surrogate for healing.
Gross and microscopic exams	No notable differences between untreated vs. the MACI-implanted defects were observed. The pathology report notes that there were still substantial differences between the MACI-implanted cartilage and native cartilage (i.e., surrounding healthy cartilage) both grossly and histologically. Although the residual ACI-Maix membrane was not specifically examined, the pathology report noted membrane presence in the MACI-implanted defect out to 24 weeks.
IHC – Collagen Type II	Collagen type II was not dense or uniform in all defects for all animals.

Comments:

- This study indicates that the creation and assessment of two 15-mm diameter critical-sized defects in a single knee is feasible in horses.
 - The surgical biopsy procedure and subsequent MACI final product generation were consistent both intra-and inter-animal and seemingly well-executed.
 - The defect creation and MACI implantation procedures were well-executed and consistent both intra- and inter-animal.
 - The in-life (including arthroscopic), and terminal results were relatively consistent for both intra-and inter-animal. The findings demonstrated that MACI was present in the defect out to at least 6 months in all animals. The majority of animals exhibited inflammation at the defect site that was present to some extent for the duration of the study. Regardless, per the study sponsor, MACI trended toward improved repair compared to empty defect.
- In this model, healing by 6 months post-MACI implantation was marginal.

Study #4

Report Number		GENZ 09-4417
Date Report Signed		May 29, 2012
Title		<i>Investigation of Efficacy of MACI in Horses</i>
GLP Status		No
Testing Facility		(b) (4)
Objective		To assess long-term efficacy, mechanism of action (MOA), and safety in horses
Study Animals	Breed	Mixed breeds
	Species	<i>Equus ferus</i> (Horse)
	Age	1.5-6 years old
	Body Weight	290-450 kg
	#/sex/group	Group 1 – 12 animals (3 males and 9 females) Group 2 – 12 animals (3 males and 9 females) Group 3 – 3 animals (1 male and 2 females)
	Total #	27 (7 castrated males and 20 females)

Test Article		MACI – ACI-Maix Lot # (b) (4) (12/2010) seeded with autologous equine chondrocytes isolated from healthy femoral articular cartilage biopsies obtained approximately 10 weeks before implantation (MACI; autologous horse analog). The cells were expanded in culture and frozen at the Cambridge, MA facility (clinical manufacturing facility). The cells (b) (4) and seeded onto the ACI-Maix membrane (4x5 cm) for (b) (4) prior to implant, and secured with (b) (4) of (b) (4). Test article was administered during the same surgical procedure as defect creation.
Control Article(s)		<ol style="list-style-type: none"> 1. ACI-Maix Membrane only - re-hydrated in transport media and handled in a manner identical to the test article for shipping to the testing facility. . 2. Empty defect only
Description of the Injury Model and Implant Procedure		The cartilage biopsy was performed on all animals at 28 days after the acclimation and screening periods. At least 4 weeks following biopsy, two 15-mm diameter critical sized proximal and distal trochlear ridge defects were created in a single knee/stifle of the hind leg for each animal. This process occurred in a staggered manner (i.e., procedures did not all occur on the same day). The implantation surgeries were performed 4 weeks after the cartilage biopsy/harvest. MACI was inserted next to the designated defect with the cell side toward the bone and secured with (b) (4). As a control, ACI-Maix membrane alone was inserted next to the designated defect and secured with (b) (4). For the second control (Group 3), two defects were created via arthrectomy and left empty (empty defects).
Study Groups and Dose Levels		<p>Group 1 – MACI (one defect) and ACI-Maix (one defect) Group 2 – Empty defect (one defect) and MACI (one defect) Group 3 – Both defects received no agent</p> <p>Dose Level: <u>MACI</u> - approximately 1.77 cm² ACI-Maix seeded with (b) (4) autologous chondrocytes /defect <u>Membrane alone</u> - approximately 1.77 cm² ACI-Maix/defect</p>
Randomization		<p>Yes. <u>Randomization to study group:</u> Based on weight, gender, then hind limb balancing (i.e., equal numbers of left and right hind limbs)</p> <p><u>Randomization of defects:</u> Within a group, the two defects/stifle/horse (proximal and distal) were randomly assigned to receive either ACI-Maix or MACI. Group 3 defects were all left empty.</p>
Description of Masking		Groups 1 and 2 - Masked to MACI versus control defect assignment and whether the implant was MACI or ACI-Maix
Scheduled Sacrifice Time Point		53 weeks post-implant
Study Parameters	Clinical observations and mortality	Throughout the study, including; 1) entry into the study, 2) before biopsy and before implant surgery, 3) 5 days after each surgical procedure (biopsy and defect/implant surgeries), and 4) immediately prior to sacrifice.
	Lameness	Measured on a 5-point scale advocated by the American Association of Equine Practitioners. This included assessments at: 1) entry into the study, 2) before biopsy and before implant surgery, 3) before second-look arthroscopy at 12 weeks, and 4) immediately prior to sacrifice.
	Clinical pathology	Hematology and clinical chemistry - pre-biopsy and Weeks 12 and 53 post-implant
	Synovial fluid analysis	Protein content (inflammatory measure), white blood cell (WBC) counts, and PGE2 levels were measured at post-biopsy/pre-implant and 12 and 53 weeks post-implant. <u>Synovial fluid from the biopsied hind leg was only assessed at sacrifice (53 weeks).</u>

**Second-look
arthroscopy**

- Gross visualization when harvesting tissue for scoring
- Subjective mechanical probing of the repair site and synovial membranes when harvesting tissue for scoring
- Scoring of the repair tissue using a cartilage scoring system to assess repair tissue coverage, percent filling, integration, color, and Pannus (fibrous tissue overgrowth)

Note: The scoring system for tissue repair is in the table below:

Analysis	Score	Qualifications
Area covered with smooth white repair tissue (0-3)	0	100%
	1	>75%
	2	50% - 75%
	3	<50%
Defect fill (0-4)	1	overfill (considered less than ideal, and scored similarly to >75% fill)
	0	100%
	1	>75%
	2	50% to 75%
	3	25-49%
	4	<25%
Repair tissue integration (0-4)	0	100% of perimeter
	1	>75%
	2	50-75%
	3	25-49%
	4	<25%
Tissue Color (0-4)	0	100% white
	1	>75% white
	2	50-75 % white
	3	25-49 % white
	4	<25% white
Pannus (0-2)	0	none
	1	slight attachment
	2	heavily overrun

	Terminal assessments	<ul style="list-style-type: none"> Gross pathology - index knee and major organs Histology of lymph nodes and major organs Assessment and cartilage scoring of the repair tissue by histology* Glycosaminoglycan (GAG) and collagen content at the repair site by histology using toluidine and type II collagen-specific immunohistochemistry Measurement of GAG and DNA content in the defects Biomechanical testing of defects in index limb (aggregate modulus, Hydraulic permeability, shear modulus, accurate friction coefficient) <p>Note: Refer to the CDRH consult review for details on methods and results.</p> <p>*Note: The histology scoring system used is in the table below:</p> <table border="1"> <thead> <tr> <th>Analysis</th><th>Score</th><th>Qualifications</th></tr> </thead> <tbody> <tr> <td rowspan="5">Defect fill (%)</td><td>0</td><td>91-110</td></tr> <tr> <td>1</td><td>76-90 or > 111 (overfill – considered less than Ideal)</td></tr> <tr> <td>2</td><td>51-75</td></tr> <tr> <td>3</td><td>26-50</td></tr> <tr> <td>4</td><td>0-25</td></tr> <tr> <td rowspan="4">Chondrocyte predominance</td><td>0</td><td>All</td></tr> <tr> <td>1</td><td>Many</td></tr> <tr> <td>2</td><td>Few</td></tr> <tr> <td>3</td><td>None</td></tr> <tr> <td rowspan="4">Perilesional chondrocyte cloning</td><td>0</td><td>None</td></tr> <tr> <td>1</td><td>Seldom</td></tr> <tr> <td>2</td><td>Occasional</td></tr> <tr> <td>3</td><td>Frequent</td></tr> <tr> <td rowspan="5">Subchondral attachment (%)</td><td>0</td><td>91-100</td></tr> <tr> <td>1</td><td>76-90</td></tr> <tr> <td>2</td><td>51-75</td></tr> <tr> <td>3</td><td>26-50</td></tr> <tr> <td>4</td><td>0-25</td></tr> <tr> <td rowspan="3">Perimeter attachment</td><td>0</td><td>complete</td></tr> <tr> <td>1</td><td>1 gap, 1 side</td></tr> <tr> <td>2</td><td>gap, both sides</td></tr> <tr> <td rowspan="4">Toluidine staining (%)</td><td>0</td><td>91-100</td></tr> <tr> <td>1</td><td>76-90</td></tr> <tr> <td>2</td><td>26-75</td></tr> <tr> <td>3</td><td>0-25</td></tr> <tr> <td rowspan="4">Collagen type II (%)</td><td>0</td><td>>90</td></tr> <tr> <td>1</td><td>76-90</td></tr> <tr> <td>2</td><td>26-75</td></tr> <tr> <td>3</td><td>0-25</td></tr> <tr> <td rowspan="4">Surface Fibrillation</td><td>0</td><td>none</td></tr> <tr> <td>1</td><td>slight</td></tr> <tr> <td>2</td><td>moderate</td></tr> <tr> <td>3</td><td>severe</td></tr> <tr> <td rowspan="2">Tidemark</td><td>0</td><td>complete</td></tr> <tr> <td>3</td><td>none</td></tr> </tbody> </table>	Analysis	Score	Qualifications	Defect fill (%)	0	91-110	1	76-90 or > 111 (overfill – considered less than Ideal)	2	51-75	3	26-50	4	0-25	Chondrocyte predominance	0	All	1	Many	2	Few	3	None	Perilesional chondrocyte cloning	0	None	1	Seldom	2	Occasional	3	Frequent	Subchondral attachment (%)	0	91-100	1	76-90	2	51-75	3	26-50	4	0-25	Perimeter attachment	0	complete	1	1 gap, 1 side	2	gap, both sides	Toluidine staining (%)	0	91-100	1	76-90	2	26-75	3	0-25	Collagen type II (%)	0	>90	1	76-90	2	26-75	3	0-25	Surface Fibrillation	0	none	1	slight	2	moderate	3	severe	Tidemark	0	complete	3	none
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Key Results:

Note: The study parameters were evaluated and reported by group (i.e., Groups 1-3). However, one of the two defects/horse for Groups 1 and 2 animals was implanted with MACI. Therefore, the study report's presentation of the data, as well as the interpretation of the results, cannot

provide clear attribution for the findings. Only the Group 3 animals (empty defects) are relevant controls to Groups 1 and 2.

Assessment		Key Results
Mortality		No animals died during the study
Clinical pathology		No significant differences between all groups at any time point.
Lameness		<p>Variable but mild, hind limb lameness was observed in 12/27 horses at 12 weeks (Group 1 = 6/12, Group 2 = 4/12, and Group 3 = 2/3). However, these numbers were not statistically more prevalent in any one group. No lameness was detected in any horse at 53 weeks.</p> <p>Note: Although the study reported no statistically relevant differences, Group 3 horses with empty defects are trending to increased prevalence of lameness which may be expected.</p>
Synovial fluid analysis		<p>Mean PGE2 levels in the hind leg with the defects for Group 3 appeared to be notably increased at Weeks 12 and 53 compared to 'pre-implant' (i.e., baseline) levels. Mean PGE2 levels in samples collected from the implanted limb were not significantly different between and within Groups 1 and 2 at any time point.</p> <p>Total protein and WBC counts were slightly elevated at 53 weeks in biopsied and implanted limbs of some animals, with no correlation to group.</p>
Second-look arthroscopy		There were no differences between implants within each group [i.e., MACI vs membrane (Group 1) only or MACI vs. empty defect (Group 2)] at 12 weeks post-implant. However, the Group 3 horses seemed to display reduced healing (increased inflammation and repair tissue scores) compared to the horses in Groups 1 and 2.
Terminal assessments	Gross visualization at necropsy	<ul style="list-style-type: none"> No membrane migrated away from the defect site. The use of the fibrin glue likely supported retention of MACI/ACI-Maix at the implanted site. MACI-implanted defects had better regenerated tissue integration compared to ACI-Maix-implanted defects and empty defects MACI had better attachment to the subchondral bone compared to ACI-Maix. There was no gross pathology or inflammation observed in any animal for all major perfused organs assessed, except for incidental findings.
	Scoring for repair tissue	MACI-implanted defects had better overall total healing and histologic scores compared to ACI-Maix-implanted defects and empty defects.

Assessment		Key Results
	Histology and DNA content for GAG and collagen at the defect site	<ul style="list-style-type: none"> Defects implanted with MACI trended toward increased GAG content in cartilage compared to empty defects; however, this difference did not reach the level of statistical significance. There was a trend toward improved GAG content in cartilage repair tissue in MACI-implanted defects compared to defects implanted with ACI-Maix membrane alone. All defects had less total membrane GAG content than biopsy or normal cartilage (i.e., cartilage tissue adjacent to the defect and harvested as part of the histology protocol) GAG. However, the GAG content of MACI-implanted defects was the closest to the contralateral biopsied joint and to normal cartilage. DNA content assessment indicated less cellularity in the MACI implanted defects than membrane alone indicating less cellularity, but the significance of this result is unknown other than a correlation with improved outcome. Microscopic fibers from the membrane were observed.
	Biomechanical testing of implanted grafts and healthy cartilage (positive control)	<p>The following points were obtained from the CDRH consultant's [Aric Kaiser, MS] review memo:</p> <ul style="list-style-type: none"> <u>Aggregate modulus (implant stiffness)</u>: MACI-implanted defects were equivalent to control* and ACI-Maix-implanted defects were less than control <u>Hydraulic permeability</u>: MACI and ACI-Maix-implanted defects had similar absolute differences (larger than control), but MACI had higher variability so were not significantly different from control compared to less variable ACI-Maix-implanted defects. MACI- and ACI-Maix-implanted defects had significantly lower mean <u>shear modulus</u> compared to control. Because of large edge effects associated with small diameter of samples (3 mm), it was not possible to get accurate friction coefficient measurements. The observed values were all similar to the controls. <p>Note: For the biomechanical testing, 'control' refers to normal/healthy cartilage harvested from the contralateral knee.</p>

Comments:

- This study provides long-term (one year) safety and activity data for MACI in the setting of critical sized articular cartilage defects in the limbs of a load-bearing animal.
- Overall, the biopsy collection, defect creation, and implantation procedures were well tolerated. Any observed lameness was mild in nature and occurred early (12 weeks) in the study in approximately 50% of all animals in each group, with full gait recovery by 53 weeks in all animals.
- Gross visualization during necropsy at 53 weeks and histological assessment of tissue showed a trend towards improved cartilage repair for the MACI-implanted defects compared to the ACI-Maix-implanted defects and the empty defects. The MACI-implanted defects approached native cartilage in appearance, and GAG content of cartilage was increased relative to the ACI-Maix-implanted defects.

- There were no indications of systemic adverse reactions or distribution of MACI, ACI-Maix, or the seeded chondrocytes to draining lymph nodes or other examined tissues.
- The CDRH consultant [Aric Kaiser], who reviewed the biomechanical testing conducted on tissue harvested from the defects and healthy tissue (control), concluded that in general, this study demonstrated that implantation with MACI is capable of repairing cartilage defects. Improved results were observed in some biomechanical testing outcomes over implanted ACI-Maix membrane alone or no agent (empty defect). However, he also noted that biomechanical properties and repair with MACI are not identical to native cartilage. This reviewer concurs with Mr. Kaiser's assessment. See the CDRH consult review for additional details.

SAFETY PHARMACOLOGY STUDIES

Summary List of Safety Pharmacology Studies:

No safety pharmacology studies were conducted.

PHARMACOKINETIC STUDIES (Cell Distribution)

Summary List of Pharmacokinetic Studies

The following cell distribution studies were conducted:

In Vivo Studies:

Study Number	Study Title / Publication Citation	Report Number
4	<i>Investigation of Efficacy of MACI in Horses</i> Note: This evaluation was submitted as part of the combined definitive pharmacology and toxicology study. See the Pharmacology section of this review memo for an analysis.	GENZ 09-4417

TOXICOLOGY STUDIES

Summary List of Toxicology Studies

The following toxicology studies were conducted to evaluate the safety of MACI following administration in various animal species:

Toxicology Studies:

Study Number	Study Title / Publication Citation	Report Number
4	<i>Investigation of Efficacy of MACI in Horses</i> Note: This evaluation was submitted as part of the combined definitive pharmacology and toxicology study. See the Pharmacology section of this review for an analysis.	GENZ 09-4417

Developmental and Reproductive Toxicology (DART) Studies:

Per the applicant, studies were not conducted to evaluate this safety endpoint because the product is administered locally in the knee with minimal to no product entry systemically. As MACI is derived from autologous, mature, terminally differentiated cells, the concern for potential DART, as well as adverse effects on lactation, are mitigated. In addition, genotoxicity testing of the ACI-Maix membrane did not raise concerns about possible mutagenicity.

Genotoxicity Studies:

These studies are a component of the biocompatibility testing conducted on ACI-Maix. The data for the two studies initially submitted in the BLA (Report #GENZ 05009 and GENX 05029) were reviewed by the CDRH consultant, Joseph Nielsen, PhD. Additional genotoxicity testing was requested. The reports for the two additional studies were subsequently provided by the applicant (Report #16-01526-G1 and #16-01526-G2). Refer to the Biocompatibility section below in this review memo, for a summary analysis of these studies.

Study Number	Study Title / Publication Citation	Report Number
N/A – Biocompatibility	(b) (4)	GENZ 05009 (b) (4)
N/A – Biocompatibility	Genotoxicity: (b) (4)	GENZ 05029 (b) (4)
N/A – Biocompatibility	(b) (4)	16-01526-G1
N/A – Biocompatibility	(b) (4)	16-01526-G2

Carcinogenicity/Tumorigenicity Studies:

Per the applicant, studies were not conducted to evaluate this safety endpoint because of the avascular local implantation site, the terminally differentiated state of the seeded cells, and the lack of systemic distribution of MACI following implantation. No tumors were detected in the one-year animal study, thus indicating that tumor formation is unlikely. In addition, per the applicant there has been no obvious or reported incidence of increased tumor formation from the clinical trials using MACI and collagen membranes in general. The applicant also stated that the lack of any findings from genotoxicity assessments of the membrane as a rationale for not conducting additional carcinogenicity testing.

Other Safety/Toxicology Studies:

Study Number	Study Title / Publication Citation	Report Number
5	Evaluation of Toxicity of (b) (4)	RR08028

Comment:

- Only summary information from this study was provided. However, since this study is not critical for determining the safety and activity of MACI in the context of the other non-clinical and clinical data submitted, a complete study report was not requested.

Study #5

Report Number		RR08028
Study Title		Evaluation of toxicity of (b) (4)
Date Report Signed		May 4, 2011
GLP Status		No
Testing Facility		Genzyme Biosurgery
Objective		Fibrin glue will be used during implantation to adhere MACI to the injured surface. Therefore this study tested (b) (4) commercially available fibrin glues for toxicity to human chondrocytes.
Test Article(s)		(b) (4)
Control Article(s)		
Methods		
Dosing Regimen		N/A Note: The actual amount of glue added to each culture dish was not specified.
Randomization		No
Description of Masking		N/A
Study Parameters	Cell Toxicity	Visual observation of cells.

Key Results: The study sponsor concluded that (b) (4) are not toxic to cultured human chondrocytes.

Comments:

- This *in vitro* study to assess the toxicity of various fibrin glues/sealants on human chondrocytes was poorly designed, conducted, and reported. Thus, this reviewer is not able to independently verify the study sponsor's conclusion.
- It is not clear if the chondrocytes were from a clinical lot of cells, and obtained from healthy tissue. In addition, the cell culture conditions were not provided. The time of exposure of the cells to the fibrin glues and the amount of fibrin glue used in relation to the volume of media, concentration of cells, and other factors were also not documented.

- The rationale for the choice of cell incubation times and assessments chosen for this study is not presented.
- The data presented (copies of representative photographs presumably of chondrocytes in dishes were of extremely poor quality.
- Effects of the various fibrin glues/sealants on MACI may affect the clinical outcomes and thus efficacy of the final product. Per a discussion with the clinical reviewer [Michael Yao, MD], the clinical team has determined that use of any fibrin glue commonly used in orthopedic practice is acceptable. This reviewer defers to the clinical team on this point.

Biocompatibility Studies:

The biocompatibility studies conducted on the (b) (4) ACI-Maix porcine-derived collagen membrane were reviewed by the CDRH consult reviewer Joseph Nielsen, PhD. The “Summary Results” column in the table below, are the conclusions taken from his consult review memo. All studies submitted to (b) (4) were also submitted to the BLA.

Study Report #	Title (provided by the applicant)	Summary Results
02-1975-G2 (b) (4)	(b) (4)	The test article met the acceptance criteria with no evidence of irritation.
GENZ 05009 (b) (4)	(b) (4)	Under the conditions of this assay, (b) (4) were considered to be non-mutagenic to (b) (4)
GENZ 05029 (b) (4)	Genotoxicity: (b) (4)	Under the conditions of this assay, (b) (4) were considered to be non-mutagenic to (b) (4)
16-01526-G1	(b) (4)	Under the conditions of this assay, there was no evidence of genotoxicity for the (b) (4) ACI-Maix collagen membrane.
16-01526-G2	(b) (4)	Under the conditions of this assay, there was no evidence of genotoxicity for the (b) (4) ACI-Maix collagen membrane.
GENZ 05013 (b) (4)	(b) (4)	ACI-Maix Collagen Membrane was classified as a slight irritant as compared to the negative control and non-irritant as compared to the biomaterial control.
GENZ 05014 (b) (4)	(b) (4)	ACI-Maix Collagen Membrane was classified as a non-irritant.
GENZ 06015 (b) (4)	(b) (4)	The test article was a non-irritant when compared to the biomaterial controls and a moderate irritant as compared to the negative control.
UWA Orthop004 (b) (4)	<i>Long Term Subcutaneous Implantation Test of Matricel Membrane in Rats</i>	No obvious inflammatory response induced by the implant. No necrosis, hemorrhage, or generation of fibrosis was observed.
GENZ 05040 (b) (4)	(b) (4)	Under the conditions of this assay, both extracts of the ACI-Maix Collagen Membrane were classified as a weak sensitizer (grade 0).

Study Report #	Title (provided by the applicant)	Summary Results
GENZ 06006 (b) (4)	<i>Cytotoxicity Study Using the</i> (b) (4)	The test extract showed no evidence of causing cell lysis or reactivity.
GENZ-GT-361-TX-1 (b) (4)	(b) (4) <i>Cytotoxicity Assay</i>	None of the ACI-Maix membrane extracts showed cytotoxic potential to (b) (4).
GENZ 06008 (b) (4)	<i>Acute Systemic Toxicity of ACI-Maix Membrane Extracted in</i> (b) (4) <i>in a Mouse Model</i>	ACI-Maix Collagen Membrane did not demonstrate systemic toxicity or induce a biological response significantly greater than the corresponding control extracts.

APPLICANT'S PROPOSED LABEL

Section 8 ('Use in Specific Populations') should be revised to comply with 21 CFR 201.56(d)(1), 201.57(c)(9), and 201.57(c)(14)³.

Section 12 ('Clinical Pharmacology') should be revised to reflect: 1) the unknown mechanism of action of MACI (Section 12.1; 'Mechanism of action'), and 2) the ACI-Maix membrane resorption time interval observed following implantation in the animals (Section 12.3; 'Pharmacokinetics').

Section 13 ('Nonclinical Toxicology') should be revised to reflect: 1) the biocompatibility results for the ACI-Maix membrane (Section 13.1; 'Carcinogenesis, Mutagenesis, Impairment of Fertility'), and 2) the resulting data following implantation of the animal-derived MACI in critical-sized defects in the knee/stifle of rabbits and horses (Section 13.2; 'Animal Toxicology and/or Pharmacology').

CONCLUSION OF NON-CLINICAL STUDIES

Review of the non-clinical studies did not identify any safety concerns that could not be adequately addressed in labeling (see above recommendations regarding the label). The non-clinical data support approval of the license application.

KEY WORDS/TERMS

MACI, ACI-Maix, porcine membrane, Type I/III collagen, OA, cell therapy, membrane, critical-sized defect, cartilage repair, knee, fibrin sealant, rabbits, horses

³ Pregnancy and Lactation Rule (PLLR), at:

<http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/actsrulesregulations/ucm445102.htm>.