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PRODUCT: SYMVESS™ (acellular tissue engineered vessel-tyod)

APPLICANT: Humacyte Global, Inc.

PROPOSED INDICATION: Urgent arterial repair following extremity vascular trauma (b) (4) when autologous vein is not feasible

PHARM/TOX REVIEWER: David Cantu

PHARM/TOX TEAM LEADER: Feorillo Galivo

PHARM/TOX BRANCH CHIEF: Danielle Brooks

PRODUCT (CMC) REVIEWERS: Jun Sung Hong

DIVISION DIRECTOR: Allen Wensky

OFFICE DIRECTOR: Iwen Wu

CLINICAL REVIEWER: Prateek Shukla

PROJECT MANAGER: Helen Sansone

SUPER OFFICE DIRECTOR: Nicole Verdun

EXECUTIVE SUMMARY:

SYMVESS™ is a tissue-engineered, human acellular vessel (HAV) composed primarily of extracellular matrix (ECM) proteins including collagen (b) (4) that are produced by human smooth muscle cells (SMCs) cultured on a (b) (4) mesh under (b) (4) (b) (4) that is subsequently decellularized. SYMVESS™ is a hollow tube with a 6 millimeter (mm) internal diameter and is 42 centimeters (cm) in length.

In vivo studies to evaluate HAV performance and safety were conducted in non-human primates (NHPs). In an initial study, an earlier HAV prototype was implanted in baboons as an upper limb arteriovenous (AV) graft. Graft patency was maintained in 5 of 8 animals at the planned 3- and 6-month timepoints. HAV suture retention strength pre-implantation and post-explant indicated that the graft retained its mechanical strength during the implantation period. Occlusions were observed in 3 baboons, with 2 HAVs partially resorbed with evidence of a severe inflammatory

reaction and localized infection in 1 of 2 baboons, possibly due to opening of the surgical incision. There were no signs of graft dilation, constriction, or aneurysm observed following angiogram assessment for the grafts that remained patent.

In two subsequent NHP studies using an updated decellularization process, four baboons were implanted with HAV AV grafts and were followed for up to 6 months. In the second week post-implantation, 3 of the 4 grafts were occluded and required clot removal and surgical revision. After surgical revision, the grafts showed favorable blood flow and remained patent for the remainder of the study. A fourth baboon had persistent inflammation and wound infection for the first 2 weeks after HAV implantation. An aneurysm was detected following ultrasound imaging at Week 12 and was subsequently resected and the section replaced with an expanded polyfluoroethylene (ePTFE; Gore-Tex®) graft. The animal was sacrificed at Week 14 due to thrombosis in the ePTFE portion of the AV graft and histological examination showed moderate to severe inflammation and graft resorption throughout the aneurysmal portion of the graft. In the HAV grafts that retained patency, minimal to mild infiltration of host cells including SMCs and endothelial cells were observed in the HAV graft wall and modest accumulation of immune cells were observed. ECM analysis showed increased collagen type I, glycosaminoglycans (GAGs), and elastin with a more circumferential alignment of collagen fibers for the 6-month explanted HAV as compared to the pre-implant HAV.

An in vivo study was also conducted to compare HAV and ePTFE grafts in a porcine hind limb ischemia model with a 0- or 6-hour ischemia time. At day 28, patency rates for grafts implanted within the 0-hour ischemia groups were 85.7% (6/7 animals) for HAV and 66.7% (6/9) for ePTFE grafts, while patency rates in the 6-hour ischemia groups were 100% (8/8) for HAV and 75% (6/8) for ePTFE grafts. In the grafts that remained patent, no aneurysms were observed and animals in the HAV group had similar mean velocity values and Tarlov gait scores as compared to the ePTFE group for the respective ischemia times (i.e., 0- or 6-hour). The clinical pathology results similarly showed no apparent difference between animals receiving the HAV or ePTFE grafts. Histological analysis of the explants showed that both the HAV and ePTFE grafts had some native intimal hyperplasia and host reactivity. Both the HAV and ePTFE grafts showed evidence of immune cell and myofibroblast infiltration, typically from the exterior surrounding tissues, and a subset of the HAV sections showed partial luminal coverage by endothelial cells.

Additional intramuscular implantation tests were conducted in male and female adult rabbits (n = 3) for each timepoint (i.e., 1 week and 4 weeks) comparing the HAV to a US Pharmacopeia (USP) high density polyethylene reference standard (RS) for characterization of biocompatibility in accordance with (b) (4)

The HAV test article was considered a slight irritant based on the histological observations [i.e., macrophages, eosinophils, lymphocytes, multinucleated foreign body giant cells (FBGCs), and fibrosis] as compared to the RS control article.

An additional study in a subcutaneous (SC) abscess model in (b) (6) rats (n = 21 for each bacterial species) compared the bilateral implantation of HAV to ePTFE grafts inoculated with either (b) (4) The HAV showed a lower bacterial bioburden as compared to the ePTFE graft at 2 weeks post-implantation.

No dedicated carcinogenicity or tumorigenicity studies were conducted. However, an Ames test of HAV extracts did not show evidence of bacterial mutagenicity when compared to the negative control. Additionally, an in vitro mammalian chromosome aberration test conducted using human peripheral blood lymphocytes exposed to HAV extracts showed no evidence of genotoxic activity.

No animal reproductive or developmental toxicity (DART) studies were conducted with SYMVESS™. These studies are not warranted based on the product characteristics and intended use.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:

There are no nonclinical deficiencies identified in this submission and no outstanding requests for additional data for nonclinical evaluation of SYMVESS™. The nonclinical information provided in the submission supports approval of the biologics license application (BLA).

Formulation and Chemistry:

SYMVESS™ is an acellular tissue engineered vessel primarily consisting of collagen (b) (4) (b) (4) which are fibrillar, structural proteins that provide mechanical strength to the HAV. The HAV is implanted using standard surgical techniques to replace a patient's damaged blood vessel after vascular trauma. The HAV has a 6 mm internal diameter and is 42 cm in length with the usable length for vascular anastomoses being approximately 40 cm. The HAV is manufactured by seeding human SMCs onto a (b) (4) mesh scaffold surrounding a supportive silicone tube.

(b) (4) in (b) (4) supplemented with (b) (4)
(b) (4)
(b) (4) in individual bioreactor bags. During a portion of the manufacturing process, (b) (4)

The HAV is kept submerged in (b) (4) Phosphate Buffered Saline (b) (4) within the primary packaging.

Abbreviations

APTT	Activated Partial Thromboplastin Time (PTT)
AV	Arteriovenous
AST	Aspartate aminotransferase
(b) (4)	(b) (4)
BLA	Biologics License Application
cm	Centimeter
CD45	Cluster of differentiation 45
CFU	Colony forming unit
CK	Creatine kinase
DTH	Delayed-Type Hypersensitivity
(b) (4)	(b) (4)
(b) (4)	(b) (4)
<i>E. Coli</i>	<i>Escherichia coli</i>
ePTFE	Expanded polytetrafluoroethylene
ECM	Extracellular matrix
FBGC	Foreign body giant cell
GAG	Glycosaminoglycans
H&E	Hematoxylin and Eosin
HAV	Human Acellular Vessel
IgG	Immunoglobulin G
IHC	Immunohistochemistry
IL-6	Interleukin-6
ISO	International Organization for Standardization
ID	Intradermal
IV	Intravenous
kg	kilogram
LDH	Lactate dehydrogenase
mm	Millimeter
NHP	Non-human primate
PRA	Panel Reactive Antibody
PTT	Partial Thromboplastin Time (PTT)
(b) (4)	(b) (4)
PTFE	polytetrafluoroethylene
POD	Post-operative Day
pH	Potential of hydrogen
RS	Reference Standard
<i>S. typhimurium</i>	<i>Salmonella typhimurium</i>
SMC	Smooth Muscle Cell
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
TNF- α	Tumor Necrosis Factor- α
US	Ultrasound
USP	US Pharmacopeia

Related File

IND#16746: Human Acellular Vessel (HAV) formed with Allogeneic Smooth Muscle Cells onto a (b) (4) Mesh Scaffold; For urgent arterial repair following extremity vascular trauma (b) (4) when autologous vein is not feasible; Humacyte Global, Inc.

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INTRODUCTION

Traumatic vascular wounds require prompt medical care to repair and salvage the injured limb in the distal vascular bed and to prevent life-threatening hemorrhage. The current standard of care for treatment of traumatic injuries relies on interposition or bypass grafting either using an autologous vein derived from the patient (i.e., saphenous vein), or a synthetic graft such as ePTFE or Dacron®. However, the use of these grafts may not always be feasible or appropriate if: 1) the patient does have an adequate autologous vein for harvest; 2) the urgency of the repair may preclude additional operating time for vein harvest; and 3) traumatic injuries may be contaminated or highly prone to infection (A. George Akingba 2013). SYMVESS™ is an HAV composed of human ECM proteins intended for urgent arterial repair following extremity vascular trauma when autologous vein (b) (4) graft are not suitable for use.

NONCLINICAL STUDIES

PHARMACOLOGY STUDIES

The majority of the submitted studies were intended to investigate the mechanical/functional integrity and remodeling of the implant, as well as any toxicological manifestations following implantation. Since these evaluations were investigated together, all nonclinical results pertaining to the functionality and remodeling (i.e., pharmacology) of the HAV are included in the toxicology section summarized in Section 2.6.6 and Section 2.6.7. The following pharmacology study was conducted to evaluate the infection resistance of SYMVESS™.

Summary List of Pharmacology Studies

Study Number	Study Title / Publication Citation	Report Number
1	Preclinical Evaluation and Biological Mechanisms of Infection Resistance in Bioengineered Human Acellular Vessels Compared to Synthetic ePTFE Grafts	DEV-RPT-0328-01

Overview of Pharmacology Studies

Study #1

Study objectives:

1. To evaluate the susceptibility of the HAV versus ePTFE vascular grafts using a controlled bacterial inoculation model involving SC implantation in (b) (4) rats
2. To characterize neutrophil viability and function when cultured on HAV versus ePTFE vascular grafts

Study design:*In vivo study*

Male (b) (4) rats (~3 – 5 months old) were implanted with 1 cm² sections of HAV or ePTFE (n = 21 for each bacterial species) within bilateral dorsal SC pockets, followed by inoculation of the implanted material with either (b) (4) prior to wound closure. Two weeks post-implantation/inoculation, evaluation of abscess formation, microbial recovery and histological characterization was performed.

In vitro study

Freshly isolated human neutrophils were seeded onto 10 mm-diameter discs of either ePTFE, HAV, or tissue culture plastic. Neutrophil viability was measured via the lactate dehydrogenase (LDH) assay and microscopic (b) (4) cell imaging at 1, 3, and 5 hours after seeding. Immunohistochemistry (IHC) analysis of protein expression was conducted to identify phenotypic markers of neutrophil function and viability.

Results: The (b) (4) rat abscess model showed that the ePTFE samples had significantly greater bioburden with larger abscesses and increased bacterial colonization as compared to the HAV. Dense populations of cluster of differentiation 45 (CD45)+ leukocytes, primarily neutrophils, were observed surrounding the contaminated ePTFE implants at the time of explant, which was not observed for the HAV implants. Based on the LDH release and (b) (4) staining, significant loss of neutrophil membrane integrity and viability was observed when seeded upon ePTFE samples. Conversely, neutrophils retained high viability after exposure to the HAV, with similar neutrophil viability as compared to the control material (i.e., tissue culture plastic).

SAFETY PHARMACOLOGY STUDIES

No nonclinical safety pharmacology studies were conducted as this is an implanted engineered vascular graft.

PHARMACOKINETIC STUDIES

No pharmacokinetic studies were conducted as this is an implanted engineered vascular graft.

TOXICOLOGY STUDIES**Summary List of Toxicology Studies**

The following studies were conducted to evaluate the safety and functionality of SYMVESS™ following implantation in baboon (*Papio sp.*), porcine (b) (4) and canine vascular graft models. Study nos. 8 - 9 were not summarized separately in this memorandum since they contained immunological and histological analysis from animals in study nos. 2 - 4 and the data are summarized under those studies. Study nos. 10 - 14 were not summarized in this memorandum since they were non-pivotal pilot studies using earlier HAV prototypes and mainly

evaluated the surgical procedure and technical feasibility of HAV implantation. Study nos. 15 – 16 were not summarized in this memorandum since these (b) (4) studies are included in the CMC reviewer's memorandum.

Toxicology Studies:

Study Number	Study Title / Publication Citation	Report Number
	<i>Primary Studies</i>	
2	Implantation of the Humacyte Vascular Graft in a Baboon (<i>Papio sp.</i>) Arteriovenous Graft from the Axillary Artery to the Brachial Vein: Evaluation of Graft Function, Toxicology and Immunological Response	B-005-RPT
3	Implantation of the Humacyte Vascular Graft in a Baboon (<i>Papio sp.</i>) Arteriovenous Graft from the Axillary Artery to the Brachial Vein: A One Month Study to Evaluate a New Decellularization Process on Graft Function and Inflammatory Response	(b) (4) 2011-B-009-V-RPT
4	Implantation of the Humacyte Vascular Graft in a Baboon (<i>Papio sp.</i>), Arteriovenous Graft from the Axillary Artery to the Brachial Vein: Evaluation of Graft Function, Toxicology, and Immunological Response	(b) (4) 2011-B-10-V-RPT
5	Comparison of Humacyte Acellular Vessels (HAVs) to Polytetrafluoroethylene (PTFE) Grafts Implanted in a Porcine Model of Hind Limb Ischemia/Reperfusion Injury	RAD-STY-H091
6	Intramuscular Implant Test One Week Duration	157404
7	Intramuscular Implant Test Four Week Duration	157405
	<i>Supplementary Studies</i>	
8	In-vitro Immune Response Assessment of Baboons B5-B16: T-cell Proliferation and IgG Evaluation	RAD-STY-H008
9	Remodeling of the Humacyte Human Acellular Vascular Graft after Implantation as an Arteriovenous Graft in the Baboon Arm	RAD-STY-H025-RPT
10	Initial Testing of Canine Acellular Grafts in a Canine Model: Arterial Patches	RAD-STY-H015
11	Initial Testing of Canine Grafts in a Canine Model: Carotid Artery Bypass	RAD-STY-H016
12	Initial Testing of Canine Acellular Grafts in a Canine Model: AV Carotid to Jugular Model	RAD-STY-H017
13	Initial Testing of Canine Grafts in a Canine Model: Coronary Bypass	RAD-STY-H018
14	Initial Testing of Human Acellular Grafts in a Xenogeneic Primate Model; Aorto-Caval & Iliac Artery Implants	RAD-STY-H019
15	(b) (4) (b) (4) Assay With (b) (4) Fibroblast Cells	157406
16	(b) (4) (b) (4) Test	157408

Reviewer Comments:

In study no. 2, 8 baboons were implanted with the HAV as an AV graft in the arm. Occlusion and histological evaluation of animal B12 led the applicant to conduct a series of in vitro tests to optimize the HAV decellularization manufacturing process, with the hypothesis that residual cellular remnants may have contributed to the inflammatory responses observed in these baboons. The specific manufacturing changes made to the decellularization process for subsequent studies and used for the intended clinical product included (b) (4)

The performance of the finalized HAV graft was

evaluated in study nos. 3 and 4 using the baboon AV model and study no. 5 using the porcine hindlimb ischemia model. It is somewhat unclear if the optimized HAV decellularization manufacturing process represented a significant improvement in HAV graft performance as compared to the prior HAV used in study no. 2, considering the surgical revisions and/or complications (i.e., thrombosis, loss of patency, infection) that still presented in baboon study no. 3 and 4.

Overview of Toxicology Studies

Study #2

Report Number		B-005-RPT
Date Report Signed		08-Aug-2012
Title		Implantation of the Humacyte Vascular Graft in a Baboon (<i>Papio sp.</i>) Arteriovenous Graft from the Axillary Artery to the Brachial Vein: Evaluation of Graft Function, Toxicology and Immunological Response
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		<p><u>Primary Objectives</u></p> <ol style="list-style-type: none"> 1. To evaluate the performance of the Humacyte vascular graft in maintaining patency and providing physiological function 2. To assess local and systemic toxicity associated with the graft <p><u>Secondary Objectives</u></p> <ol style="list-style-type: none"> 1. To characterize host response and remodeling 2. To determine immunological response to the graft 3. To determine the ability of the graft to be accessed during the implantation period
Study Animals	Strain/Breed	Baboon
	Species	(b) (4)
	Age	Unspecified
	Body Weight	21.2 – 28.4 kg
	#/sex/group	3/M (B8, B10, B11) for the 12-week duration 5/M (B5, B6, B7, B9, B12) for the 24-week duration
	Total #	8
Test Article(s)		HAV
Control Article(s)		<ol style="list-style-type: none"> 1. The positive control was a homogenate of micronized HAV graft suspended in (b) (4) 2. The negative control was (b) (4) <p>Note: Each control article was packaged in a 1-mL syringe, and the volume administered was 0.1 mL. For animal B5, the intradermal (ID) injection was 0.2 mL for both injections at Week 4, as noted in the protocol deviation.</p>
Route of Administration		<ol style="list-style-type: none"> 1. Surgical implantation of the HAV 2. ID injection of HAV extract or (b) (4) vehicle control

Study Groups and Description of the Implant Procedure	<p>Adult male baboons (b) (4) were implanted with the HAV that was 6 mm in diameter and 13 – 15 cm in length as an AV graft in the upper arm. Placement of the grafts was from the axillary artery to the brachial vein with end-to-side anastomoses in each baboon. The grafts were placed directly under the skin to facilitate venipuncture access throughout the study. Besides intravenous (IV) heparin administration during implantation and daily aspirin, no other anticoagulant or immunosuppressive medications were administered. The implant durations per study protocol were 12 weeks for animals B8, B10, and B11 and 24 weeks for B5, B6, B7, B9, and B12. While the animals were anesthetized for the HAV implant, they received 2 ID injections of the skin challenge material: a micronized HAV (positive control) as well as (b) (4) (negative control). The skin challenge was additionally repeated at Week 4 and wheal formation was assessed 2 – 3 days after each ID administration.</p> <p>Note: Baboons B7 and B12 were explanted early at Week 12 to 13 due to occlusion of the HAV.</p>
Dosing Regimen	Single implantation: two ID injections of HAV extract or (b) (4) control
Randomization	No
Description of Masking	No
Scheduled Sacrifice Time Points	Baboons were euthanized at 12 weeks and 24 weeks.

Animal Model Selection:

Baboons were chosen based on their phylogenetic similarity to humans, which the applicant hypothesizes may reduce the likelihood of mounting an immune response to the human proteins that compose the HAV. Additionally, adult male baboons (20 – 40 kg) are physically large enough to support implantation and anastomosis of a 6-mm diameter graft into a clinically relevant anatomic setting (upper arm). However, baboons are relatively small compared to an adult human, and while the vasculature in the upper arm is of adequate size to implant a 6-mm diameter graft, it is notably smaller than the vasculature in humans. Finally, by implanting the HAV into the upper arm of the animal, non-invasive evaluation of blood flow through the graft could be assessed throughout the study via ultrasound (US). Superficial placement of the graft in the arm also allowed for direct puncture of the graft for angiography assessment of narrowing, obstruction, or dilatation of the graft and adjacent vasculature.

Key Evaluations and Assessments:

In-life assessments:

- US measurement of the treatment arm was conducted pre-operatively and post-operatively on Weeks 2, 4, 12 and 24.
- Angiogram measurement and graft puncture of the treatment arm was conducted post-operatively on Weeks 4, 12, and 24.
- Clinical observations were performed once daily beginning on Day 1.
- Body weights were recorded pre-operatively, on Day 0, and on post-operatively on Weeks 2, 4, 12, and 24.

- Blood was collected on Day 0 and post-operative Weeks 4, 12, and 24 for hematology, clinical chemistry, serum for immunoglobulin G (IgG) titer and panel reactive antibody (PRA), and T-cell isolation.
- Allergic sensitivity evaluation was conducted 2 - 3 days after each ID injection (i.e., on Day 0 and Week 4).

Necropsy: Baboons were euthanized at post-operative Week 12 and 24 and underwent gross macroscopic examination and organs weights were collected. Microscopic histopathological evaluation was performed on selected tissues (i.e., HAV graft and connected vasculature, heart, spleen, liver, kidney, brain, lung, and lymph node).

Key Results:

In-life assessments: Functional assessments of the vascular graft included graft puncture, angiogram, and US throughout the study. Of the 8 grafts, 5 remained patent throughout the planned study period, which included 3 of the planned 6-month implants and two of the planned 3-month implants. In the angiograms, there were no instances of graft dilatation or aneurysm observed. Three baboons (B7, B11, and B12) were found with occlusions with no other adverse clinical signs. Clinical observations were limited to findings such as post-operative swelling and redness, as well as surgical incision perturbation by a few animals. However, there were no apparent indications of systemic toxicity elicited by graft placement. There were no apparent graft-related effects on body weight changes, clinical hematology or serum chemistry, and organ-specific functional tests. No increases in PRA were observed in the baboons, suggesting a lack of anti-human antibody production against the implanted HAV.

Necropsy:

HAV Systemic Effects: No gross or histologic findings were noted in major organ systems of animals examined at termination other than in 2 animals (B8 and B11) that displayed swollen and/or inflamed lymph nodes and animal B11 had a persistent, localized infection in the HAV graft arm due to the animal opening the surgical incision, which may have contributed to the inflamed lymph nodes. Histological examination showed minimal to mild lymphoid hyperplasia, characterized by an increase in the number of lymphocytes in the cortex and paracortex and an increased number of germinal centers, which occurred in 3 out of the 5 baboons in the Week 12 implant cases. In animal B7 and B8, an increased presence of mild lymphoid hyperplasia and in animal B11 minimal lymphoid hyperplasia of the proximal axillary draining lymph node was observed; however, other lymph nodes in these animals did not present with any histopathological changes. The lymph tissue of the three animals explanted at Week 24 was considered normal.

HAV explant characterization: Macroscopic examination of the HAV and associated vasculature at explant indicated that the grafts were intact and were not dilated, constricted or aneurysmal except in animals B11 and B12. Enlargement of the outflow brachial vein was observed, which is a common response associated with increased blood flow into the vein. Suture retention and/or burst pressure of the implant graft was evaluated before implantation and when explanted. Suture retention involved gradually increasing the weight to determine the force needed to have a

polypropylene (b) (4) suture pulled axially through a (b) (4) length of the HAV. Burst pressure testing using hydrostatic pressure was used to determine the pressure at which a (b) (4) length of graft burst. Suture retention strength of the explants remained similar to or slightly greater than those of the HAV prior to implantation. The burst pressure measurements showed a similar trend as the suture retention measurements. The graft had increased suture retention and burst pressure post-explant suggesting they gained mechanical strength in vivo possibly due to host remodeling of the HAV with associated infiltration of host cells and ECM protein deposition.

Analysis of the ECM post-explant showed increased collagen type I, GAGs, and elastin in the explanted HAV at 6 months as compared to the pre-implant HAV. Collagen type I and III, and fibronectin within the graft wall displayed a more organized structure with increased circumferential alignment for the 6-month explant. GAGs were minimal in HAVs prior to implantation, and elastin was absent, whereas for the explant timepoints an overall increase in GAG content and elastin deposition into the scaffold wall was observed. Scarring was not observed. Host tissue growth that resembled adventitia was evident around the exterior of the grafts.

In the majority of animals at termination, histological examination of these grafts revealed minimal to mild infiltration of host cells into the HAV graft wall. The infiltrate consisted predominantly of elongated cells within the ECM that stained positive for smooth muscle actin. The presence of an endothelial layer on the luminal graft surface was evident in most sections. Infiltrating immune cells such as neutrophils, macrophages, plasma cells, eosinophils, B-cells, T-cells, and multinucleated FBGCs were observed in smaller quantities as typically associated with implantation surgeries. Cellular infiltration into the graft was more pronounced near the arterial and venous anastomoses in comparison to the mid-graft region and reflected a focal, multi-focal or diffuse distribution of cells, which involved approximately 10 – 30% of the HAV graft wall thickness. Infiltrating cells were more prominent at the graft exterior wall than the graft interior and cellular infiltration increased with longer residence time of the HAV graft in vivo. Angiogenesis and capillary deposition in the graft ECM was observed in some of the 6-month HAV explant samples. Trace amounts of pannus ingrowth were present in sections of the HAV graft lumen. The tissue that formed around the graft perimeter consisted of loose, fibrous connective tissue infiltrated with blood vessels and smooth muscle actin positive cells. Alizarin-stained sections showed no evidence of calcification.

In animals B11 and B12, the grafts lost patency and were partially resorbed. Macroscopic examination of the grafts at explant showed that significant portions of the graft from the mid-graft down through the venous anastomosis were no longer evident and had been resorbed by the host tissue. This level of resorption indicated that the grafts had likely been occluded an extended amount of time prior to explant. Histological examination of these grafts showed both smooth muscle actin positive cells and a severe inflammatory reaction dominated by lymphocytes and macrophages in the areas where the graft was resorbed as well as where the graft was still intact near the arterial anastomosis. Animal B11 opened the surgical incision by Day 2, leading to contamination of the wound with the graft being grossly visible along part of the HAV length. After incision re-closure, this animal had persistent fluid collection and redness around the incision site that lasted beyond Week 4, indicative of a persistent and localized infection.

HAV immune responses: Animal B12 was found to have a measurable PRA of 16% prior to implantation but values did not increase during the study, implying that the animal had pre-existing immunogenicity to a subset of human antigens. In vitro tests for T-cell proliferation and IgG binding upon exposure to the HAV were developed and run for all baboons. T-cells isolated from animals post-implantation had low proliferation rates of 3 – 17% when exposed to the HAV and was similar to exposure to the ePTFE negative control. Additionally, increases in IgG titer, arbitrarily defined as > 25-fold increase over t = 0 titer, were observed in all study animals regardless of functional outcome. In addition to the implantation of the HAV, a positive control article consisting of the micronized extract of the HAV graft material (0.25 ng/mL) as well as the (b) (4) negative control were ID injected (0.1 mL per injection) into the upper hip of the animals on Day 0 to monitor for the immune responses such as delayed-type hypersensitivity (DTH). No induration was noted after 48 – 72 hours post-injection.

Reviewer comments:

- 1. Only male baboons were used due to their larger size and blood vessel diameter as compared to females. The HAV is approximately 6-mm in diameter and implanted in baboons with blood vessels estimated to be 2- to 3-mm in diameter for the original end-to-side anastomosis. The applicant indicates that this mismatch in blood vessel diameter may have contributed non-laminar blood flow, which can lead to thrombosis and blood vessel occlusion and is a significant limitation of this animal model. The porcine ischemia model was a closer anatomical match for blood vessel diameter and reflects greater similarity to humans based on size.*
- 2. The HAV implants remained patent and retained overall physiological function in the majority of baboons. No indications of systemic toxicity were noted in any of the animals. Inflammation at the incision site of a few animals appeared to be the result of perturbation and incision re-opening. From an immunological perspective, the HAV was well-tolerated by the baboons. Lastly, the graft was successfully accessed and appeared to maintain its structural integrity in vivo.*

Study #3

Report Number	(b) (4) 2011-B-009-V-RPT
Date Report Signed	31-JUL-2012
Title	Implantation of the Humacyte Vascular Graft in a Baboon (<i>Papio sp.</i>) Arteriovenous Graft from the Axillary Artery to the Brachial Vein: A One Month Study to Evaluate a New Decellularization Process on Graft Function and Inflammatory Response
GLP Status	No
Testing Facility	(b) (4)
Objective(s)	To evaluate the impact of a new decellularization process change on the performance of the HAV in: 1) maintaining mechanical integrity; 2) resisting aneurysmal dilatation; 3) assessing local inflammation associated with the graft; 4) assessing patency and physiological function.

Study Animals	Strain/Breed	Baboon
	Species	(b) (4)
	Age	Unspecified
	Body Weight	31.8 kg
	#/sex/group	Single male
	Total #	1
Test Article(s)		HAV
Control Article(s)		Not applicable
Route of Administration		1. Surgical implantation of the HAV 2. ID injection of HAV extract or (b) (4) vehicle control
Description of the Implant Procedure		One adult male baboon was implanted with an HAV, with the 6 mm diameter graft placed in the right arm between the axillary artery near the armpit and brachial vein near the elbow with end-to-side anastomosis. The graft was placed directly under the skin to facilitate graft access. Other than IV heparin during the implantation and daily aspirin orally, no other anticoagulant or immunosuppressive medications were administered. While anesthetized for HAV implantation, the animal also received 2 ID injections of skin challenge solution consisting of a micronized HAV extract (positive control) and a (b) (4) negative control. The skin challenge was also repeated 1 day prior to graft explant.
Dosing Regimen		Single implantation: two ID injections of HAV extract or (b) (4) control
Randomization		No
Description of Masking		No
Scheduled Sacrifice Time Points		Day 27 post-implantation

Key Evaluations and Assessments:

In-life assessments:

- US was performed pre-operatively, on Day 12 (Week 2), and Day 27 (Week 4).
- Clinical observations were performed once daily beginning on Day 1.
- Body weights were recorded on Days 0, 12, 13, 19, 26, and 27.
- Blood was collected on Day 0 and before necropsy at Week 4 for analysis of hematology, clinical chemistry, and serum for IgG and PRA.
- An angiogram of the graft was performed at Week 4.

Necropsy: Following euthanasia, organ weights were obtained, and gross examination was conducted. Microscopic evaluations were performed on the HAV and adjacent vasculature as well as selected tissues including the heart, spleen, liver, kidney, brain, lung, and lymph nodes.

Key Results:

In-life assessments: No morbidity or mortality occurred during the study. However, on Day 12, US imaging showed an occluded graft. On Day 13, a Fogarty catheter was used to perform a thrombectomy. Following a failed attempt to remove the blood clots that formed in the anastomosis, a second surgery was conducted revising the venous anastomosis from an end-to-side anastomosis to an end-to-end anastomosis to the brachial vein, which contributed to the graft length being shortened from 11.9 cm to 10.5 cm. The HAV showed favorable blood flow after surgical intervention and remained patent for the remainder for the study. Functional assessment of the graft included graft puncture, angiogram, and ultrasonography demonstrating

that the graft functioned as intended with the exception of the occlusion on Day 12. The graft was otherwise well-tolerated, and the baboon showed no clinical signs of toxicity. Body weight decreased throughout the study most likely due to fasting before the surgical procedures and the administration of general anesthesia and ketamine for restraint during the handling procedures. There was a failure of the incision to adequately heal, possibly due to the animal attempting to open the wound incision.

Clinical Pathology: Clinical chemistry and hematological findings showed no evidence of systemic toxicity. Hemoglobin, hematocrit, and bilirubin levels were within normal ranges at explant. ID injection of the (b) (4) control showed no evidence of reaction. The site of the homogenized HAV ID injection at Week 4 was bruised, red, and slightly indurated at the time of injection, but was not more indurated, harder, and redder when observed and palpated 24 hours post-injection. PRA values did not increase during the study indicating HAV implantation did not stimulate baboon anti-human antibody production.

Necropsy: Gross observation of the major organs (i.e., heart, liver, kidney, brain, and lung) at necropsy indicated that there were no notable abnormalities or changes attributable to the HAV. Although gross evaluation of the axillary lymph node indicated swelling, microscopic evaluation revealed findings that were considered incidental, or typical spontaneous findings in the baboons. The HAV suture retention results were the same pre-implant and post-explant, indicating that the graft maintained mechanical strength during the implantation period. Macroscopic examination of the HAV and adjacent vasculature at explant showed that the graft was intact and not dilated, constricted, or aneurysmal, and no scarring was observed. Host tissue growth that resembled adventitia was evident around the exterior of the HAV. The vast majority of the cells infiltrating the wall of the HAV were smooth muscle actin positive and an endothelial cell layer on the lumen of the HAV was also evident in most sections of the graft. Histologic evaluation of the graft showed a mild inflammatory response.

Study #4

Report Number		(b) (4) 2011-B-10-V-RPT
Date Report Signed		31-JUL-2012
Title		Implantation of the Humacyte Vascular Graft in a Baboon (<i>Papio sp.</i>), Arteriovenous Graft from the Axillary Artery to the Brachial Vein: Evaluation of Graft Function, Toxicology, and Immunological Response
GLP Status		Yes
Testing Facility		(b) (4)
Objective(s)		<ol style="list-style-type: none"> 1. To evaluate the performance of the HAV in providing physiologic function and patency retention 2. To assess local and systemic toxicity associated with the HAV including: 1) characterization of cellular host response and remodeling of the HAV; 2) assessment of the immunological response to the HAV; 3) evaluation of the HAV to withstand venipuncture access
Study Animals	Strain/Breed	Baboon
	Species	(b) (4)
	Age	Unspecified

	Body Weight	28.3 to 30.4 g
	#/sex/group	No group specification, all males
	Total #	3
Test Article(s)		HAV
Control Article(s)		Not applicable
Route of Administration		1. Surgical implantation of the HAV 2. ID injection of HAV extract or (b) (4) control
Description of the Implant Procedure		Adult male baboons were implanted with a HAV, with the graft initially placed in the arm between the axillary artery and the brachial vein. Additional surgical modification was made at implant resulting in graft positioning between the axillary artery and cephalic vein. The cephalic vein was used due to the smaller diameter of the brachial vein as determined during implantation. While the animals were anesthetized, they also received 2 ID injections of skin challenge solution consisting of a micronized HAV extract (positive control) as well as (b) (4) (negative control). The skin challenge was also repeated at Week 4. Each ID injection was evaluated for wheal formation 48 to 72 hours after injection. Other than IV heparin during implantation and daily aspirin orally, no other anticoagulant or immunosuppressive medications were administered.
Dosing Regimen		Single implantation: two ID injections of HAV extract or (b) (4) control
Randomization		No
Description of Masking		No
Scheduled Sacrifice Time Points		Week 24

Key Evaluations and Assessments:

In-life assessments:

- US monitoring was scheduled pre-operatively, and at weeks 0, 2, 4, 12, and 24 in order to assess graft patency.
- Clinical observations were performed once daily beginning on Day 1.
- Body weights were recorded at every sedation.
- Blood was collected on Day 0 and during Weeks 4, 12, and 24 for analysis of hematology, clinical chemistry, serum for IgG and PRA, and T-cell isolation.
- An angiogram of the graft was performed during Weeks 4, 12, and 24.

Necropsy: Following euthanasia, tissues and organs were examined grossly and collected, and organ weights were obtained. Microscopic evaluations were performed on selected tissues including the HAV, adjacent vasculature, heart, spleen, liver, kidney, brain, lung, and lymph node.

Key Results:

In-life assessments: No morbidity or mortality occurred during the study and the baboons either gained weight or were within 0.5 kg of their pre-study weight and showed no clinical signs of toxicity. Clinical chemistry and hematological findings also showed no evidence of systemic toxicity. Hemoglobin, hematocrit, bilirubin, partial thromboplastin time (PTT), and activated partial thromboplastin time (APTT) levels were within normal ranges throughout the study, indicating that the HAV graft material was non-hemolytic. There was no increase in PRA levels

during the study indicating that HAV implantation did not induce anti-human antibody production.

Post-surgical vein spasm and low initial flow rates were noted in animals B15 and B16, and these 2 HAV grafts were found to be acutely thrombosed during the Week 2 US imaging. Analysis of seroma fluid collected in the implant arm of these two animals confirmed that the fluid was low in cellularity and blood. The venous anastomosis of both grafts appeared to be spasmed and fibrotic following reoperation. The 2 HAV grafts were surgically revised at the venous anastomosis (revision to the brachial vein, end-to-end anastomosis) along with clot removal with a Fogarty balloon catheter. The animals and the grafts tolerated the surgical revisions and interventional procedures and the grafts remained patent until the planned explant.

Animal B14 had persistent inflammation and evidence of wound infection for the first 2 weeks after implantation that necessitated extended antibiotic treatment. An aneurysm was detected following US imaging at Week 12 and was subsequently resected and replaced with a portion of ePTFE graft. The animal was sacrificed at Week 14 due to a thrombosis in the ePTFE portion of the AV graft.

Necropsy: Gross and histopathological observation of the major organs (i.e., heart, liver, kidney, brain, and lung) indicated no significant abnormalities attributable to the HAV. Macroscopic examination of all grafts and associated vasculature at explant indicated that the grafts were intact and were not dilated, constricted, or aneurysmal other than in animal B14. Host tissue growth that resembled adventitia was evident around the exterior of the grafts.

In animal B14, the cellular host response to the graft was moderate to severe and involved inflammatory immune cell accumulation and there was also evidence of graft resorption throughout the aneurysmal tissue at Week 14. Histological examination of the non-aneurysmal portion of the graft at Week 14 explant showed cellular infiltrates, consisting predominantly of lymphocytes, macrophages, and multinucleated FBGCs that penetrated the majority of the graft. Immunostaining showed a high number of smooth muscle actin positive cells and macrophages and a moderate number of B cells and T cells. In animals B15 and B16, minimal to mild infiltration of host cells into the HAV graft wall was observed. The infiltrate had elongated cells within the HAV ECM that morphologically appeared similar to SMCs as well as a number of macrophages. The tissue that formed around the graft perimeter consisted of fibrous connective tissue infiltrated with smooth muscle actin positive cells. Alizarin-stained sections showed no evidence of calcification. Immunostaining showed that B cells were rarely observed, whereas T cells and macrophages were identified, although in low numbers.

HAV immune responses: T cells isolated from baboons post-implant at all time points, had unremarkable proliferation rates of 1% to 7% when re-exposed to the HAV in vitro and were similar to the 1% to 5% proliferation rate when exposed to ePTFE as the non-specific negative control. Increases in IgG titer, arbitrarily defined as a >25-fold increase over the baseline titer, were observed in all study animals regardless of the functional outcome. No induration (i.e., bump or welt formation) was noted for any ID injection at any timepoint.

Reviewer Comments:

1. *Implantation of the HAV into 3 baboons was performed for up to 16, 21, or 23 weeks. Two animals had thrombus formation which required surgical revision and one animal had an infection and aneurysm which led to an early termination. The other adverse events in this study were largely due to the surgical procedures of HAV implantation and angiography and no other systemic toxicities were observed.*
2. *Blood clots and loss of patency are a common adverse finding with the use of synthetic grafts, including ePTFE, in the clinic and can be resolved by explanting the graft if necessary. The applicant indicates that the mismatch between the 6 mm diameter HAV and baboon blood vessel diameter (2- to 3-mm) may have contributed to the findings of thrombosis and subsequent occlusion and loss of patency.*

Study #5

Report Number		RAD-STY-H091
Date Report Signed		30-Nov-2015
Title		Comparison of Humacyte Acellular Vessels (HAVs) to Polytetrafluoroethylene (PTFE) Grafts Implanted in a Porcine Model of Hind Limb Ischemia/Reperfusion Injury
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		<p><u>Primary Objectives</u></p> <ol style="list-style-type: none"> 1. To evaluate graft patency via duplex ultrasonography 2. To assess the resultant hind limb function (e.g., gait) after repair of a common iliac artery injury with a commercially available ePTFE graft versus the HAV in a normovolemic vascular injury model after 0- or 6-hours of ischemia <p><u>Secondary Objectives</u></p> <ol style="list-style-type: none"> 1. To quantify systemic biomarkers of inflammation and ischemia within animals before and after implantation 2. To histologically evaluate the HAV and ePTFE grafts at the time of euthanasia
Study Animals	Strain/Breed	(b) (4) Cross Female Pigs
	Species	(b) (4)
	Age	Adult, not specified
	Body Weight	60 to 90 kg
	#/sex/group	<ol style="list-style-type: none"> 1. 2 / F / Method development – ePTFE 2. 2 / F / Method development – HAV 3. 9 / F / 0 hours ischemia – ePTFE 4. 9 / F / 6 hours ischemia – ePTFE 5. 9 / F / 0 hours ischemia – HAV 6. 9 / F / 6 hours ischemia – HAV <p>Note: Only female animals were used due to the need for intraoperative and post-operative bladder catheterization to obtain urine samples for measurement of myoglobin concentration.</p>
	Total #	44
Test Article(s)		HAV

Control Article(s)	ePTFE graft (b) (4) manufactured by (b) (4). (b) (4) ePTFE grafts had a standard wall thickness of 0.64 mm, and internal diameter of 6 mm, and were ~3 cm long when implanted				
Route of Administration	Surgical implantation				
Description of the Injury Model and Implant Procedure	The surgical procedure involved first exposing, ligating, and excising a 3 cm segment of the right common iliac artery. Blood flow to the right hind limb was re-established either immediately (0-hour ischemia) or 6 hours later (6-hour ischemia) by surgically implanting either an HAV or ePTFE interposition graft (each 6 mm internal diameter, 3 cm long) within the excised region of the right common iliac artery. On the day of surgery, before anesthetic induction, the animal was assessed for gait using a modified Tarlov hind limb function score.				
Study Groups	Table 1. Experimental Group Assignment and Animal Identification				
	Study Phase	ePTFE		HAV	
		0 hour	6 hours	0 hour	6 hours
	Method Development				
		5974	-	-	5965
		6002	-	-	6004
	Experimental Study				
		6242	6243	6252	6207
		6210	6190	6206	6213
		6212	6211	6241	6251 ^d
		6356	6312	6274	6273
		6364	6313	6297 ^c	6279
		6368	6316 ^b	6353	6298
		6370	6318	6354	6299
		6371 ^a	6351	6369	6300
		6373	6352	6372	6375
		6414			6376 ^c
					6389
	Abbreviations: HAV = Humacyte Acellular Vessel; ePTFE = polytetrafluoroethylene. ^a Pig 6371 was removed from study because a lap sponge was left within the animal and euthanized on post-operative day (POD) 14. This pig was replaced with Pig 6414. ^b Pig 6316 was euthanized post-operatively due to the non-graft related technical error where the graft was too long when implanted and kinked and attempted repair was unsuccessful and the pig was subsequently removed from the study. This pig was not replaced by another animal. ^c Pig 6297 was euthanized post-operatively due to surgical complications unrelated to graft function and this pig was removed from study. This pig was not replaced with another animal. ^d Pig 6251 was removed from study because of accidental nerve damage during surgery and subsequent leg paralysis (graft was patent) and euthanized on POD 7. This pig was replaced with Pig 6375. ^e Pig 6376 was removed from study because it received an incorrect medication regimen and was euthanized on POD 1. This pig was replaced with Pig 6389. Note: 1 additional animal (number 6280) died before treatment assignment and graft implantation.				
	Dosing Regimen	Single HAV or ePTFE implantation			
Randomization	Yes				

Description of Masking	No
Scheduled Sacrifice Time Points	Female pigs were euthanized on POD 28

Animal Model Selection:

The porcine model was used due to its anatomical similarity between humans and swine with regards to vasculature, muscle mass, and size. Specifically, the porcine iliac artery is of similar size to most human femoral arteries, an artery commonly injured following lower limb trauma, and appropriately sized for implantation of the 6 mm diameter HAV. Only female pigs were used due to the need for intraoperative and post-operative bladder catheterization and to obtain urine samples for measurement of myoglobin concentration.

Key Evaluations and Assessments:

In-life assessment: Study assessments were performed pre-operatively (before anesthetic induction), at 30 minutes after graft implantation, and on POD 1, 2, 7, 14, 21, and 28, and included evaluation of blood flow and hind limb function as well as biochemical tests. Animals were observed daily by the veterinary technicians for any signs of disease or post-surgical infection or complications. Clinical assessment of limb locomotor function was evaluated according to a modified Tarlov score and were based on a 4-point scale that correlated gait observations of animals in an open field (i.e., 0 = paralyzed gait; 1 = able to sit; 2 = stands but unable to bear weight on affected limb; 3 = stands and walks but with gait or posture abnormality; 4 = no gait or posture abnormality) (Gabriel E. Burkhardt, 2010). Implanted HAV or ePTFE graft patency was assessed by serial duplex US measurements of blood flow at the right common femoral artery (downstream of implant), distal and proximal native iliac artery, and midgraft (middle of HAV or ePTFE implant) locations. HAV and ePTFE grafts were considered patent when recorded peak systolic blood flow velocities measured at these locations were > 45 cm/s. Hind limb function was evaluated using a modified Tarlov gait measurement scoring system.

Sample collection: Blood samples were collected for analysis of arterial blood gases and selected hematology and clinical chemistry tests including pH, lactate, creatine kinase (CK), LDH, and myoglobin (serum and urine) for evidence of ischemia and/or reperfusion injury. Remote organ dysfunction was evaluated by aspartate aminotransferase (AST) levels and systemic markers of inflammation were evaluated by measuring circulating interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) levels.

Necropsy: Animals were humanely euthanized if graft patency was lost (i.e., < 45 cm/s blood velocity) or at the conclusion of the 4-week study period on POD 28. After the animal was euthanized, all HAV and ePTFE grafts were explanted with surrounding tissue and vasculature for subsequent histological analysis.

Key Results:

In-life assessment: Twelve animals were euthanized, and one died unexpectedly before scheduled euthanasia. Based on the defined threshold for the loss of patency (Marie Gerhard-Herman, 2006) during US recording (peak systolic velocity < 45 cm/s), 6 animals were

euthanized early: 3 from the 0-hour ischemia ePTFE group, 2 from the 6-hour ischemia ePTFE group, and 1 from the 0-hour ischemia HAV group. Of the 6 other animals euthanized prior to the end of study, 5 were euthanized for technical or surgical errors and excluded from the study and 1 was euthanized due to loss of weight and lack of appetite (i.e., failure to thrive) on POD 14 but graft patency was observed at the time of death. Kaplan-Meier plots of HAV or ePTFE patency for the 0- or 6-hour ischemia groups did not show significant differences in percent patency between the HAV and ePTFE grafts. The mean velocity values at each of the 5 US measurement locations did not show significant differences between the HAV and ePTFE groups. Results from gait measurement scoring as well as biochemical analyses confirmed that the ischemia and/or reperfusion injury was more severe after a 6-hour ligation of the iliac artery when compared to the 0-hour ischemia groups regardless of the graft material. The patency rate within the 0-hour ischemia group was greater for the HAV than the ePTFE grafts at both POD 14 mid-study (HAV: 7/8 = 87.5%; ePTFE: 6/9 = 66.7%) and POD 28 (HAV: 6/7 = 85.7%; ePTFE: 6/9 = 66.7%). Patency rates for the 6-hour ischemia groups were slightly greater than the 0-hour ischemia groups at both POD 14 (HAV: 9/9 = 100%; ePTFE: 8/8 = 100%) and POD 28 (HAV: 8/8 = 100%; PTFE: 6/8 = 75%). There was no significant difference in the Tarlov scores between HAV and ePTFE grafts in either the 0- or 6-hour ischemia group at any of the timepoints measured and functional recovery as measured by the Tarlov scores at POD 14 to POD 28 was not statistically significant.

Clinical Pathology: CK, LDH, AST, and urine myoglobin values were often significantly elevated in pigs that received 6-hour ischemia as compared to the 0-hour ischemia and were indicative of the extent of ischemic tissue damage. Evaluation of the clinical pathology/biomarkers revealed individual animal variability; however, there were no apparent differences between animals receiving the HAV or ePTFE graft material for the same ischemia interval group. Systemic indicators of muscle damage (serum and urine myoglobin levels) and tissue ischemia were observed following surgical manipulation through approximately POD 2; however, there was no indication of distinct non-recoverable organ damage that would suggest host incompatibility.

Necropsy: Histological analysis at explant showed that both the HAV and ePTFE grafts typically had some native intimal hyperplasia and host reactivity. Luminal occlusion was scored as 0%, < 25%, 25 – 50%, 50 – 75%, or > 75% of the lumen space occupied by thrombus and/or pannus ingrowth. HAV or ePTFE grafts that lost patency during the study showed greater amounts of luminal occlusion that included the presence of thrombus with some pannus ingrowth observed in non-patent samples from pigs 6318 and 6352 in the ePTFE 6-hour ischemia group. Conversely, animals with HAV or ePTFE grafts that remained patent through POD 28 had less luminal occlusion and was mainly the result of pannus ingrowth. Both HAV and ePTFE grafts showed evidence of pannus ingrowth which was more pronounced at the anastomosis site.

Microscopic examination of surrounding native tissue

Multinucleated FBGCs were typically observed in response to the presence of a foreign material and were indicative of an inflammatory response. As the HAV consists of human collagen and additional ECM proteins, its implantation into the pig represents a xenogeneic transplantation response which likely contributed to an inflammatory reaction to the HAV. Likewise, ePTFE is a synthetic polymer which induces an inflammatory reaction upon implantation into this pig

model. It was noted that fewer FBGCs were observed in the mid-graft region than at the anastomotic regions. The frequency and distribution of the FBGCs did not appear to correlate to the ischemic interval time or loss of patency in samples examined, but no FBGCs were identified in samples explanted 7 days or less after implantation. Both the HAV and ePTFE grafts had pannus ingrowth at the anastomoses. Intimal hyperplasia was also often observed in mid-graft sections of both the HAV and ePTFE explants.

Host cell infiltration into HAV and ePTFE graft wall

Microscopic examination showed that a mixed population, consisting of inflammatory and non-inflammatory cells infiltrated the HAV and ePTFE graft walls over time. The inflammatory populations were polymorphonuclear, basophilic and monocytic blood cells that were more predominant on the outside wall of the HAV graft. The non-inflammatory cell population consisted of cells containing nuclei with a more elongated morphology that infiltrated well into the HAV graft interior. Microscopically the cells were morphologically similar to a myofibroblast cell type that had previously been observed in HAV explant samples obtained from NHPs. The percentage of the HAV or ePTFE graft wall occupied by host cells that migrated from the exterior native tissue or from the lumen was scored as 0%, < 25%, 25 - 50%, 50 - 75%, or > 75%. With the exception of the HAV explanted one day after implantation (animal #6372), all HAV anastomotic and mid-graft histology sections showed some degree (< 25% and 25 – 50%) of host cell infiltration, typically from the exterior surrounding tissues. As anticipated, the HAV sections with longer implant durations showed more cellular infiltration compared to those that lost patency and were explanted prior to POD 28. The ePTFE graft also showed some host cell infiltration into some of the wider interstices of the ePTFE graft. Most of the cellular infiltration was found within < 25 or 25 – 50% of the PTFE wall volume; however, unlike those observed in the HAV, higher amounts of cellular infiltration (25 – 50% and 50 – 75%) were observed in 2 ePTFE grafts (i.e., animal #6318 and #6352) that lost patency 21 days post-implantation.

Host endothelial cell coverage on the lumen of HAVs and ePTFE grafts

Endothelial cell coverage as determined by cell morphology and location with hematoxylin and eosin (H&E) staining was scored as 0%, < 25%, 25 – 50%, 50 – 75%, or > 75% of luminal circumference covered. No endothelial cells were observed on the lumen of the ePTFE grafts in any of the samples examined. A subset of the HAV sections did show a varying degree (most < 25%) of luminal coverage by endothelial cells.

Note: Two animals were euthanized post-operatively due to technical errors unrelated to HAV or ePTFE graft performance. In addition, one animal (Pig #6241) from the 0-hour ischemia HAV group died unexpectedly on POD 28 after Tarlov scoring and blood sample collection but before US measurements. While the health and gait of the animal was normal, the status of the graft patency at POD 28 for this animal is unknown. Due to the lack of US data for this animal, it was removed from the percent patency calculations. Another animal (Pig #6389) from the 6-hour ischemia HAV group had a patent graft but was euthanized on POD 14 due to failure to thrive since the animal was not eating and had experienced significant weight loss.

Reviewer Comments:

- 1. The sponsor utilized the porcine model due to the similarity in size between humans and pigs with regards to the native blood vasculature and muscle mass. The porcine iliac artery is similar in size to human femoral arteries, an artery commonly injured following lower limb trauma, and appropriately sized for implantation of the 6 mm diameter HAV or ePTFE grafts. Prior studies using this porcine model and technique have demonstrated the ability to induce and measure the extent of ischemic damage as well as recovery of limb function following injury. This nonclinical study compared the short-term (28-day) patency of the HAV to a synthetic ePTFE vascular graft in an established porcine model of hind limb ischemia and reperfusion injury. This animal model has previously been used to evaluate reperfusion via temporary vascular shunts and vessel reconstruction via patch angioplasty (Gabriel E. Burkhardt, 2010). Normal blood velocity is expected to remain at an average of 100 cm/s and a minimal drop to 45 cm/s was considered a negative result due to the intervention and predictive of loss of patency (DF Bandyk, 1985).*
- 2. In study no. 5, the applicant evaluated a 3-cm long HAV in pigs whereas clinical subjects could be implanted with a HAV up to 40-cm long, which represents a considerable difference in HAV length in the pig model as compared to its clinical application that could impact graft function in clinical subjects. Likewise, the location of the proximal and distal anastomosis sites in the pig model (common iliac artery) were different from the intended sites in clinical subjects (i.e., common femoral artery, superficial femoral artery, or popliteal artery, etc.).*
- 3. The flow dynamics and pressure exerted on the HAV in the common iliac position in swine should resemble that of an interposition vascular bypass on the human superficial femoral artery when systolic blood pressure is maintained within a normal range (i.e., 100 – 150 mmHg). The anatomic site selection for this animal study was based on established arterial trauma models in swine models, and the size of the animals were selected for their similarities in vascular size and muscle mass to humans. The common iliac artery of a female sus scrofa swine, weighing approximately 75 kg, is similar to that of the human superficial femoral artery (6 – 8 mm). Due to differences in pig vasculature and the desire to avoid anastomotic mismatch in the diameter of the HAV with the native pig arteries, the common iliac artery in the pigs was selected to implant the 6-mm diameter HAV and ePTFE grafts. In order to implant the grafts without a diameter mismatch in the common iliac artery, placement prior to its bifurcation was needed and the length of the grafts was limited to ~3 cm because turbulence and blood flow disruption tends to increase due to diameter mismatch between the native artery and the HAV or ePTFE graft.*
- 4. Although the graft length in the porcine study was limited to 3 cm, in the arterial setting, pressure and flow rates in the artery and the graft were not significantly different based on the graft length. In general, a graft that is longer may be more prone to thrombosis and clinical data with the longer graft is likely more relevant to assess this risk.*
- 5. Assessment of resistance to mechanical failure in the arterial setting was evaluated in both small and long segments of the HAV. In the AV application, the HAV only experiences arterial pressure near the arterial anastomosis. In the arterial reconstruction application, the HAV experiences arterial pressure at both anastomoses and throughout*

its length. In the applicant's porcine ischemia study, there were no observed instances of mechanical failure in any of the HAVs.

Study #6

Study Objective: To evaluate the local effects of a test article in direct contact with living skeletal muscle of the rabbit for one week.

Study design: This study was conducted in accordance with the (b) (4)

Briefly, 5 sections of the HAV and the control material (i.e., USP high density polyethylene RS) were surgically implanted (3 mm x 10 mm) into the left and right paravertebral muscles of 3 healthy adult male and female (b) (4) rabbits weighing greater than 2.5 kg at the beginning of the study. The rabbits were monitored clinically during the 1-week exposure period and then animals were sacrificed and underwent gross and histopathological evaluation.

Results: All rabbits survived to the scheduled study endpoint and no abnormal clinical signs were noted for any of the animals during the study. The average of the HAV site scores (12.8) minus the average of the control site scores (9.8) equals the irritant ranking score (3.0) and the HAV was considered to be a slight irritant as compared to the USP high density polyethylene RS.

Reviewer comment:

The HAV and control article implant sites contained the same tissue components including chronic-active foreign body inflammation (i.e., macrophages, eosinophils, lymphocytes, and multinucleated FBGCs) with early fibrosis, when implanted in the muscle of rabbits for one week. There was a minimal amount of tissue ingrowth into the HAV, since the HAV was retained in the implant sites during tissue processing

Study #7

Study Objective: To evaluate the local effects of a test article in direct contact with living skeletal muscle tissue of rabbits.

Study design: This study was similarly conducted in accordance with (b) (4) and (b) (4) as referenced in Study #6 but was for a longer duration (i.e., 4 weeks).

Results: All rabbits (n = 3) survived to the scheduled study endpoint and no abnormal clinical signs were noted for any of the animals during the study. The average of HAV site scores (15.8) minus the average of the control site score (7.5) equals the irritant ranking score (8.3). Based on the observations of the study pathologist an irritant ranking score of 8.3 the test article was considered a slight irritant as compared to USP high density polyethylene RS.

Reviewer comment:

Tissue reaction to the HAV consisted of chronic-active foreign body inflammation and minimal amounts of fibrosis with mild infiltration of the HAV. The tissue reaction to the control material consisted of a minimal to mild amount of fibrosis with a small amount of chronic-active foreign body inflammation. The control material was removed from implant sites prior to tissue processing; however, the control material does not have ingrowth as far as the tissue reaction (per pathologist's experience with this control material). The applicant has indicated that tissue ingrowth into the HAV is an anticipated and positive finding. However, even if the tissue ingrowth measurements were removed from the HAV implant site table, the HAV would still be considered a slight irritant, when compared to the control article.

Developmental and Reproductive Toxicology Studies:

No nonclinical studies have been conducted to assess the potential of the HAV for effects on reproduction, fertility, fetal/embryonic development, or pre-/post-natal development. The HAV is primarily comprised of human ECM proteins (see Section 3.2.P.1 for detailed description and composition of HAV) that are endogenously expressed in humans and therefore, are not expected to impact developmental and reproductive toxicity.

Genotoxicity Studies:

The HAV is primarily comprised of human ECM proteins (see Section 3.2.P.1 for detailed description of the HAV composition) that are endogenously expressed in humans and therefore, and not anticipated to be genotoxic. In vivo genotoxicity studies have not been conducted of the HAV. However, the applicant did conduct two in vitro assessments to demonstrate that the HAV is non-mutagenic (study no. 17) and non-genotoxic (study no. 18).

Study Number	Study Title / Publication Citation	Report Number
17	Bacterial Mutagenicity Test – (b) (4) Assay (Using (b) (4) Strains and (b) (4) Strain)	157407
18	Human Acellular Vessel (HAV): (b) (4) Test in Human Peripheral Blood Lymphocytes	9602400

Carcinogenicity/Tumorigenicity Studies:

There have been no studies conducted to date that assess the potential for carcinogenicity in animals. The HAV is primarily composed of human ECM proteins (see Section 3.2.P.1 for detailed description of the HAV composition) that are endogenously expressed in humans and therefore, are not anticipated to be carcinogenic.

Study #17

Study objective: To evaluate the mutagenic potential of the test article (or its metabolites) by measuring its ability to induce mutations at selected loci of several strains of bacteria in the presence or absence of microsomal enzymes by a method compliant with the requirements specified in ISO 10993-3:2003 (Joyce McCann, 1975).

Results: The test article did not induce substantial increases in reversion rates of the type that are associated with mutagenesis. Furthermore, no substantial test article toxicity was noted that may have interfered with the ability of the system to detect mutagens. As none of the tested strains showed an increase in reversion rates when treated with the test article, the test article was considered non-mutagenic.

Study #18

Study objective: To determine the potential genotoxicity of HAV using an in vitro mammalian chromosome aberration test in human peripheral blood lymphocytes.

Results: The greatest concentration achieved (i.e., 1000 and 500 µg/mL for methanol and ethanol extracts, respectively) were selected for chromosome aberration evaluation. The HAV did not cause any statistically significant increases in the proportion of aberrant metaphases at any experimental timepoint. Likewise, the proportion of aberrant metaphases for all negative controls were comparable to the test article groups which were within the laboratory negative historical control range. No substantial increases in the incidence of chromatid or chromosome gaps or polyploidy were observed at any experimental timepoint. The positive control caused statistically significant increases in the proportion of aberrant metaphases in each phase of the study.

Reviewer Comments:

The applicant's in vitro assessments indicate that the HAV is non-mutagenic.

APPLICANT'S PROPOSED LABEL

Information in Section 13.2 (Animal Toxicology and Pharmacology) was removed because it was not necessary for the safe use of the product.

CONCLUSION OF NONCLINICAL STUDIES

Review of the nonclinical studies did not identify any safety concerns that could not be addressed in the product label. The nonclinical data support approval of the BLA.

KEY WORDS/TERMS

Human Acellular Vessel, Polytetrafluoroethylene, Extracellular matrix, collagen, patency

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