Multicenter phase I/II trial of the safety of allogeneic endothelial cell implants after the creation of arteriovenous access for hemodialysis use: The V-HEALTH study

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Objectives: **Vascular access dysfunction is the major cause of morbidity in patients on hemodialysis to treat end stage renal disease. Preclinical studies have demonstrated that the perivascular placement of implants containing allogeneic aortic endothelial cells (Vascugel) reduces thrombosis, inflammation, stenosis and increases lumen diameter in porcine models of arteriovenous fistulae (AVF) and arteriovenous grafts (AVG). We conducted a phase I/II clinical study to investigate the safety of Vascugel placement around the surgical anastomotic sites of newly constructed dialysis accesses.**

Methods: **From July 2006 to August 2006, eight patients (4 AVG, 4 AVF) were treated with two Vascugel sponges at the venous anastomosis in the open–label phase I trial. From January 2007 to August 2007 57 patients (30 AVG and 27 AVF) were randomized in a 2:1 fashion to receive either Vascugel or control matrices (placebo) at surgery. The phase II AVG patients had sponges placed at both the venous and arterial anastomoses. All patients were followed for 24 weeks. The primary objective of the study was to demonstrate the safety (incidence of infection, intervention, and thrombosis) of Vascugel compared with placebo within 30 days post-surgery. Secondary endpoints included assessments of patency, lumen diameter, and immunologic sensitization to human leukocyte antigens (HLA) determined by measurement of panel reactive antibodies (PRA).**

Results: **There was no difference in early complication rates between the Vascugel and placebo groups at 4 weeks (10.9% vs 21.1%, respectively). There were no statistically significant differences in primary or assisted primary patency between the intent to treat groups at 24 weeks. Vascugel treated AVG had a primary patency rate of 38% and an assisted primary patency rate of 72% (vs 23% and 58%, respectively, for placebo). Vascugel treated AVF had a primary patency rate of 60% at 24 weeks and an assisted primary patency rate of 96% (vs 62% and 88%, respectively, for placebo). A greater than 30% increase in PRA was detected in 9 of the 46 (19.5%) Vascugel treated patients and one of the 19 (5.2%) placebo patients ($P = .26$) and was not associated with any evidence of local or systemic complications.**

Conclusions: **Targeted local therapy with perivascular, allogeneic endothelial cells is a safe and novel therapeutic approach that may be ideally suited to control the response to injury at surgical anastomoses. Larger randomized trials are needed to determine if Vascugel can prolong AVG or AVF patency. (J Vasc Surg 2009;50:1359-68.)**

Vascular access failure is a major complication in patients receiving hemodialysis to treat end stage renal disease (ESRD).[1,2] Graft thrombosis is the cause of 80% of all vascular access dysfunction in expanded polytetrafluoroethylene (ePTFE) hemodialysis grafts, and in over 90% of thrombosed arteriovenous grafts (AVG) the underlying pathology is stenosis caused by venous neointimal hyperplasia at either the venous anastomotic site or in the proximal vein.[3] Although arteriovenous fistulae (AVF) is the preferred form of permanent dialysis access, it has significant limitations secondary to both early and late failures.[4] Concomitant with efforts to increase the prevalence of AVF in the hemodialysis population, there is a growing incidence of nonmaturation, often resulting in prolonged use of indwelling catheters.[5] Failure to mature of AVF is a complex issue related to many factors such as vessel size, surgical technique, and the development of accessory veins.[6] However, both early and late fistula failure are characterized by vascular stenosis that often occurs within the first few centimeters of the anastomosis in approximately 20% to 40% of cases.[7-9]

The vascular endothelium is central to an understanding of vascular biology and critical to vascular repair after

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Competition of interest: Dr Nugent is a cofounder and has company ownership in Pervasis Therapeutics. Drs Conte, Lawson, and Roy-Chaudhury are on Pervasis' advisory board and have been paid honorariums for corporate speaking and speakers' bureau participation. Mr Gaccione has been paid consultant fees by Pervasis Therapeutics.

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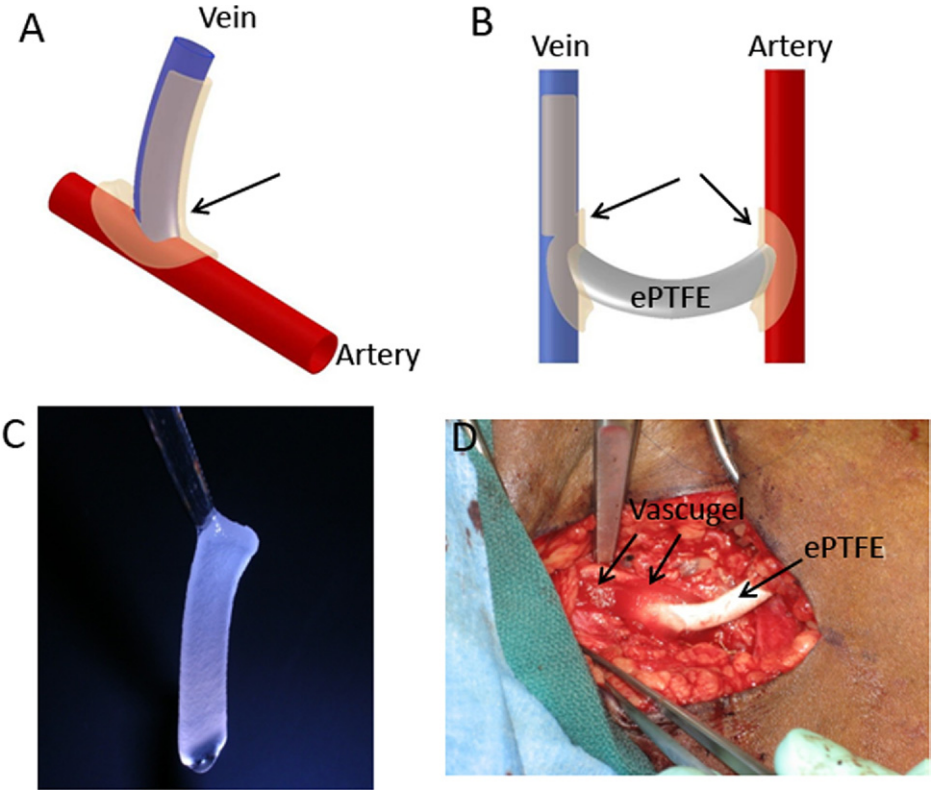

Fig 1. Schematic and images of sponge placement. **A,** Diagram of placement of two 1 × 4 × 0.3 cm sponges (arrows) adjacent to venous anastomosis and outflow segment in AVF patients. **B,** Diagram of placement of two 1 × 4 × 0.3 cm sponges adjacent to venous anastomosis and outflow segment (phase I and II) and one 1 × 4 × 0.3 cm sponge adjacent to arterial anastomosis (phase II) in AVG patients. **C,** Image of a 1 × 4 × 0.3 cm sponge used in the clinical trials. **D,** Image of placement around venous anastomosis and outflow vein in an AVG subject.

injury. The endothelium regulates local biology by providing structural boundaries to the circulating blood while also producing and supplying compounds that have the capacity to regulate vascular physiology, such as heparan sulfate (HS) and transforming growth factor-β_1 (TGF-β_1) among many others.[10-13] Upon damage to the vessels during the placement of a new AV access, the endothelium is disrupted and underlying smooth muscle cells (SMC) are injured. Biomechanical and hemodynamic forces such as wall tension and shear stress are important modulators of the injury response. These events lead to inflammation as well as proliferation and migration of SMC, resulting in lesion formation, constrictive remodeling, and lumen loss.[14] Due to the complexity of the mechanisms of AV access failure, it is unlikely that a single factor aimed at any one process will successfully treat the causes of failure. In fact, at the present time, despite the enormity of this problem, there are no effective pharmacologic measures for the prevention or treatment of hemodialysis access failure.[15,16]

The use of endothelial cells (EC) as a cell-based therapy to modify vascular injury has been under active investigation for some time, primarily in the form of methods to improve luminal endothelialization of prosthetic grafts and damaged native vessels.[17,18] However, the ability of the endothelium to regulate vessel wall biology may not be limited to a luminal location, and recent studies have suggested that perivascular endothelial cell implants influence the vessel response to injury across a spectrum of preclinical models.[19-23] In the present study, we investigated the safety and feasibility of adventitial allogeneic endothelial implants in a clinical setting of AV access placement.

METHODS

Study design. A single protocol was developed which encompassed a dual study approach, treatment of AVG and AVF. The Vascular intimal Hyperplasia: Extending Arterial and venous patency, Limiting vascular Trauma, and inhibiting Hyperplasia while re-establishing vascular health (V-HEALTH) protocol was a two-stage (phase I and II) design within each of the surgical treatment options. An initial limited phase I safety and feasibility trial of Vascugel enrolled four patients in each group, AVF and AVG (total of eight patients at three participating sites), with each patient receiving two implants placed adjacent to the venous anastomosis (Fig 1). The phase I patients were followed for 30 days, and their data were reviewed by an independent data monitoring committee (DMC, Appendix, online only), which recommended proceeding to the

phase II portion of the study. The phase II study was a randomized, double-blind, study in patients undergoing creation of AVG or AVF. Patients were randomized 2:1 to receive either Vascugel or control matrices (placebo) immediately prior to surgery, using a computer-generated permuted block randomization. The proposed sample size of 30 patients in each of the AVG and AVF groups (20 Vascugel and 10 placebo) was considered appropriate for an exploratory phase I/II study to collect necessary information regarding the safety and activity of the investigational product for this indication.

Study patients. The institutional review board at each participating site approved the protocol and all study patients provided written informed consent. Individuals undergoing placement of new upper extremity fistula or grafts were eligible for enrollment in V-HEALTH if they were currently receiving maintenance therapy of ESRD with hemodialysis. Major exclusion criteria included patients on an active transplant list, more than one prior access in the target limb, chronic, systemic immunosuppressive therapy, CBC hematocrit <24%, platelet count $<100 \times 10^3/\mu L$, white blood cell count $< 4000/\mu L$ or $> 12{,}000\ \mu L$, bleeding disorder or hypercoagulable condition, hepatitis B, hepatitis C, HIV/AIDS, two times lab control value of albumin, ALT (SGPT), alkaline phosphatase, AST/(SGOT) or total bilirubin, allergy to bovine or porcine products, allergy to collagen/gelatin products, panel reactive antibodies (PRA) >30%, pregnant, and current IV drug use. Patients also underwent preoperative vein mapping to identify blood vessels in the upper extremities that were suitable for AVG or AVF creation.

Investigational product. Vascugel is being developed as a novel cell therapy product for use following vascular access surgery and is composed of allogeneic aortic EC cultured in a gelatin (Gelfoam; Pfizer, New York, NY) matrix. The human EC, isolated from the aorta of single cadaver donors, are obtained from Lonza (Walkersville, Md) and tested extensively for endothelial cell purity; biological function (assays for secretion of HS, TGF-β_1 and fibroblast growth factor, uptake of acetylated LDL as well the ability to inhibit cultured SMC proliferation), the presence of bacteria, fungi, known human pathogens, and other adventitious agents according to FDA proposed rules.[24,25] The cells are cryopreserved for later expansion and formulation in gelatin sponges. Vascugel was supplied to the clinical sites as sponges having dimensions of $1.0 \times 4.0 \times 0.3$ cm. Prior to shipment to the clinic, in vitro cohorts of Vascugel sponges were assayed for cell number, viability, and secreted levels of HS and TGF-β_1. Each sponge contained approximately 1.23×10^6 human aortic EC (≥90% viability) secreting levels of $0.69 \pm 0.05\ \mu g/mL/d$ HS and 566 ± 29 pg/mL/d TGF- β_1. Placebo sponges were packaged identically and were of the same shape and size, but lacked EC.

Study procedures. Study patients underwent planned creation of surgical AV access using standard surgical and anesthetic techniques per practice of the local treating physicians. Sponges were placed at the conclusion of the procedure after all bleeding at the sites has been controlled and immediately before surgical closure. For all patients, implant administration consisted of two sponges placed adjacent to the venous anastomosis and outflow vein. Phase II AVG patients received a third sponge adjacent to the arterial anastomosis, for a total of three implanted sponges. Patients received implant application at the time of access placement and no repeat applications occurred during this study. All phase II study patients, investigators, and other members of the study team were blinded to treatment assignment. The use of medications such as antibiotics, heparin, and antithrombotics was at the discretion of the treating physician and not specified in the protocol.

Following surgery, patients were seen and examined at 2, 4, 12, and 24 weeks to evaluate healing, access related complications, adverse reactions, hospitalizations, patency, and maturation. PRA were measured from serum samples obtained at screening, 2, 4, 12, and 24 weeks. Serum samples were screened for PRA using the LABScreen (One Lambda, Inc, Canoga Park, Calif) Luminex platform. The LABScreen Luminex method detects human leukocyte antigen (HLA) antibodies using flow cytometric technology. A number of exploratory analyses were also performed to evaluate vein remodeling in AVF by color-flow duplex ultrasound at 2, 4, 12, and 24 weeks and lumen diameter in AVG and AVF by protocol-mandated angiography at 12 and 24 weeks for AVG and 24 weeks for AVF (Appendix, online only). After the 24-week evaluation, patients were offered the option of entering an extension study, where safety and efficacy will continue to be assessed every 24 weeks for approximately 2.5 years (144 weeks).

Safety monitoring and study outcomes. The primary outcome of the trial was safety, assessed by the incidence of infection (local or systemic), need for revision, and development of thrombosis within 30 days after surgery. The medical monitor was responsible for reviewing the safety data for each patient enrolled in the study on an ongoing basis, and a Pharmacovigilance group (Appendix, online only) forwarded all serious adverse event (SAE) reports to the DMC. Secondary outcomes were loss of primary and assisted primary patency.[26] In an attempt to focus the patency analysis to within the treatment zone, "anastomotic patency" was assessed by a blinded adjudication committee (Appendix, online only), which consisted of an interventional radiologist, a vascular surgeon, and a nephrologist. This committee used operative notes, radiology reports, and primary review of angiograms to determine if an access failure could be specifically determined to be related or unrelated to the treated anastomotic zone. If the occlusion or causative lesion was determined to be in the treatment area, or if no clear evidence existed to assign a culprit lesion, then the determination was made that an event occurred. If a specific causative lesion could be assigned outside of the treatment zone, then the determination was that an event did not occur. Additional secondary outcomes were measurements of immunological sensitization, defined as an increase in HLA antibodies using PRA testing after surgery >30% compared with screening levels;

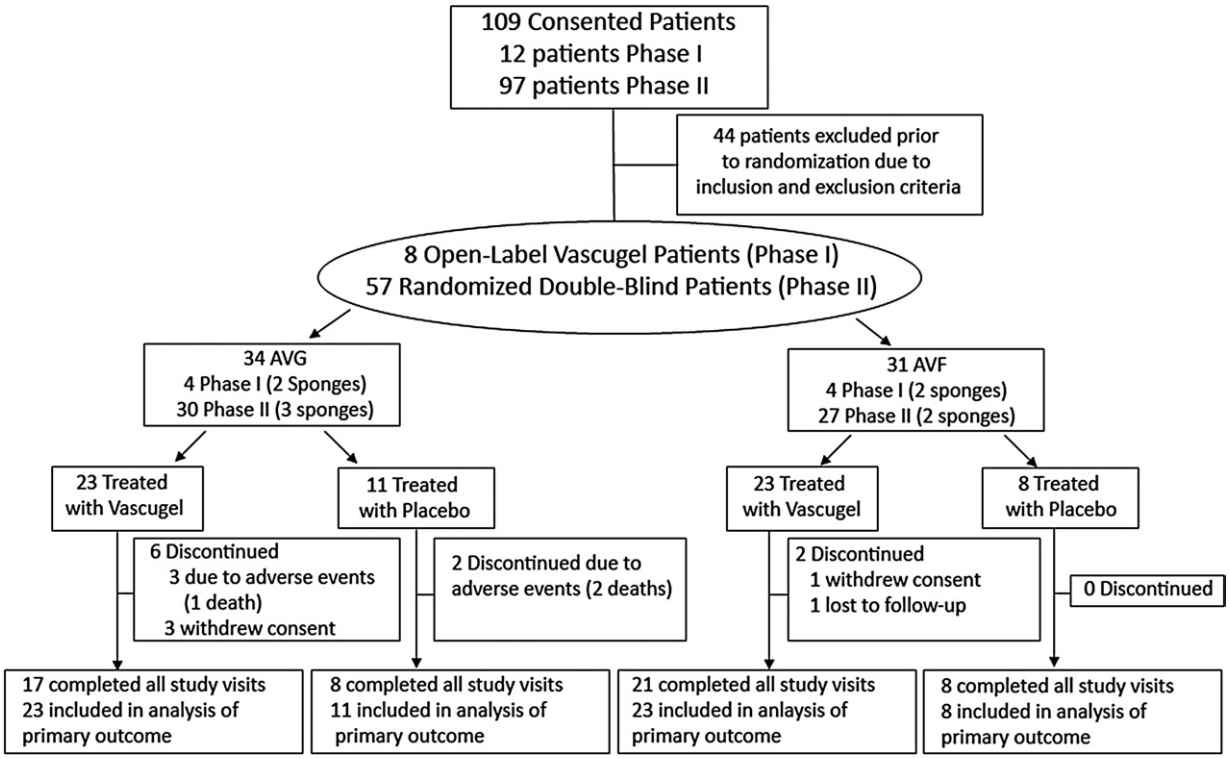

Fig 2. Phase I and phase II combined participant flow. Secondary outcome analysis for the AVG population was performed on 19 Vascugel patients in the ITT group and 14 in the mITT group; 11 placebo patients in the ITT group and eight in the mITT group. Two of the 23 Vascugel (8.7%) and two of the 11 placebo (18%) AVG were never used for dialysis during the 24-week follow-up period. Secondary outcome analysis for the AVF population was performed on 23 Vascugel patients in the ITT group and 12 in the mITT group; eight placebo patients in the ITT group and six in the mITT group. Three of the 23 Vascugel (13%) and one of the eight placebo (12.5%) AVF were never used for dialysis during the 24-week follow-up period.

and time to first access use calculated as the time from the access placement to the first successful hemodialysis use.

Statistical methods. The primary and secondary safety outcome analyses were performed on all enrolled patients (safety population). The secondary efficacy outcome analyses were performed on the intent-to-treat population (ITT), which included all randomized patients who were confirmed to have received Vascugel or placebo, excluding the four AVG patients enrolled in the phase I portion of the study because they received a different dose of product. Secondary efficacy outcomes were also analyzed for the modified intent-to-treat population (mITT), which was prespecified in the protocol and defined in accordance with The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines as the subset of ITT patients who had successful hemodialysis initiation within the first 2 months of creation of the AVG or within the first 3 months of creation of the AVF. The primary endpoint of infection (local wound or systemic), need for revision, and development of thrombosis within 30 days, the secondary efficacy endpoint of positive lumen diameter changes at the anastomotic sites for graft patients, and the secondary safety endpoint of PRA >30% , were analyzed as discrete outcomes. The two treatment groups were compared using Fisher exact test. The secondary safety endpoint (PRA) and the angiogram change analyses at each location compared the two treatment groups using a Wilcoxon rank sum test.

Secondary endpoint patency rates were described in a continuous fashion, from the day of access placement until the day of intervention or abandonment (an event) or the subject's last day on study (censored). Overall duration of patency was analyzed using the Kaplan-Meier product limit method. Results were summarized descriptively using Q25, median (95% CI), and Q75. Time to first use was calculated as the time from the access placement to the first successful hemodialysis use and analyzed in the same fashion as for patency durations. For all secondary time-to-event endpoints inferential comparisons were made using the log-rank statistic.

RESULTS

Patient population. A total of 109 patients were screened with eight enrolled in the phase I portion of the study and 57 randomized, 38 to receive Vascugel and 19 to receive placebo (Fig 2), in the phase II portion of the study.

Table I. Baseline characteristics of the participants[a]

Characteristic	*AVG*		*AVF*	
	Vascugel N = 23	*Placebo N = 11*	*Vascugel N = 23*	*Placebo N = 8*
Age, years	59.9 ± 15.7	59.5 ± 19.2	53.2 ± 17.9	57.8 ± 18.1
Male, %	52.2	63.6	56.5	62.5
Black, %	73.9	72.7	34.8	25.0
Cardiovascular disease, %	100	100	100	100
Diabetes mellitus, %	69.5	63.6	52.2	50
Body mass index, (kg/m^2)	29.7 ± 7.9	27.9 ± 4.2	26.3 ± 6.7	28.7 ± 4.1
Systolic blood pressure (mm Hg)	145.2 ± 27.4	143.5 ± 36.7	127.8 ± 21.2	144.4 ± 24.5
Diastolic blood pressure (mm Hg)	79.2 ± 11.1	78.8 ± 19.2	76.6 ± 14.2	76.9 ± 18.1
Prior AV access in index arm[b]				
Prior AVG (%)	4 (17.4)	0	1 (4.3)	0
Prior AVF (%)	12 (52.2)	5 (45.5)	5 (21.7)	1 (12.5)
No prior access	7 (30)	6 (55)	18 (78)	7 (88)
Antithrombotic use, %	91.3	81.8	78.3	62.5
Antiplatelet use, %	73.9	63.6	60.9	62.5
Anticoagulant use, %	47.8	45.5	52.2	50.0
Statin use, %	39.1	36.4	56.5	37.5
Hemoglobin, g/dL	12.7 ± 2.1	12.2 ± 2.5	13.3 ± 1.9	13.9 ± 1.7
% Panel reactive antibodies	1.6 ± 4.2	4.0 ± 7.3	0.43 ± 1.2	0.88 ± 2.5
Hemodialysis initiated before study access creation, %	100	100	100	100
Study access[c]				
Forearm, %	1 (4.3)	1 (9.1)	2 (8.7)	1 (12.5)
Upper arm, %	22 (95.7)	10 (90.9)	21 (91.3)	7 (87.5)

AVG, Arteriovenous graft; *AVF,* arteriovenous fistulae.

[a]No statistically significant differences observed in baseline characteristics between groups.

[b]One Vascugel AVF subject had two previous accesses, one AVG and one AVF.

[c]Study patients underwent planned creation of surgical AVG or AVF per standard practice and guidelines of the local treating physicians.

Baseline characteristics in the two treatment groups were similar (Table I). Perioperative antibiotics were given in 52.2% of Vascugel patients and in 52.6% of placebo patients. Heparin was administered during the surgical procedure in 36.9% of Vascugel patients and in 26.3% of placebo patients. The number of patients who died, were lost to follow-up, or withdrew consent during the course of the trial was eight (17.4%) in the Vascugel group and two (10.5%) in the placebo group. The target of 2:1 randomization was achieved in the AVG group with a total of 23 patients in the Vascugel group and 11 patients in the placebo group. Randomization in the AVF group was 3:1 with 23 patients in the Vascugel group and eight patients in the placebo group. The imbalance in randomization between study arms occurred secondary to several patients undergoing AVG placement based on intraoperative findings, after having been initially assigned to the planned AVF group.

Primary safety outcome. Comparison of the Vascugel group and the placebo group in the safety population as well as in each AVG and AVF safety subpopulation showed no statistical difference between the two groups in the incidence of local wound infection, access intervention or the incidence of acute thrombosis within 30 days postsurgery (Table II). The Vascugel group demonstrated an excellent safety profile. Separate analysis of wound infection, access intervention and incidence of acute thrombosis in the AVG ITT population that received three Vascugel sponges showed no increase in early complications at 4 weeks due to the addition of the third sponge (10.5% for Vascugel vs 36.4% for placebo). A summary of adverse events by system organ class is presented in Table III. The majority of these events were unrelated to treatment as determined by the investigator at each site; 8.7% of Vascugel patients and 26.3% of placebo patients experienced events that were considered possibly related. Events considered possibly related in the placebo group included cellulitis, AVG thrombosis, and venous stenosis. Events considered possibly related in the Vascugel group included AVG thrombosis and venous stenosis. None of the events was considered probably or definitely related.

SAEs were recorded from the time of consent for all patients enrolled in the study who received Vascugel or placebo. The majority of events were expected as typical comorbidities in the ESRD patient population. The majority of these events were unrelated to treatment as determined by the investigator at each site as well as the study medical monitor; 4.3% patients in the Vascugel group and none of the patients in the placebo group experienced events that were considered possibly related. Events considered possibly related were AVG thrombosis. Overall, 38 patients (58% of the patients enrolled) reported a total of 73 SAEs. There was no statistically significant difference between groups in the number of SAEs reported.

Secondary efficacy outcomes - AVG cohort. Treatment with Vascugel did not significantly prolong unassisted or

Table II. Primary endpoint summary at 30 days: Safety population

Assessment	*Safety Population*		*AVG*		*AVF*	
	Vascugel N = 46	*Placebo N = 19*	*Vascugel N = 23*	*Placebo N = 11*	*Vascugel N = 23*	*Placebo N = 8*
Local wound infection,[a] access intervention or Thrombosis[b]	5 (10.9%)[c]	4 (21.1%)	3 (13%)[d]	4 (36.4%)	2 (8.7%)[c]	0
• Local wound infection[f]	2 (4.3%)	2 (10.5%)	1 (4.3%)	2 (18.2%)	1 (4.3%)	0
• Access intervention[f]	2 (4.3%)	1 (5.3%)	1 (4.3%)	1 (9.1%)	1 (4.3%)	0
• Thrombosis[f]	2 (4.3%)	2 (10.5%)	2 (8.7%)	2 (18.2%)	0	0

AVG, Arteriovenous graft; *AVF,* arteriovenous fistulae.

[a]Local infection was defined as wound dehiscence with access exposure; graft removal for confirmed graft infection; purulent drainage with positive gram stain or cultures; or as four of the five following criteria: (1) need for intravenous antibiotics, (2) fever (>100.5), (3) increased WBC count (>12,000/μL), (4) significant erythema, or (5) wound dehiscence without access exposure.

[b]Total number of subjects who experienced any event (%).

[c]$P = .430$.

[d]$P = .178$.

[e]$P > .999$.

[f]Individual subjects who experienced the specific event (%).

Table III. Adverse event by system organ class

System organ class	*Vascugel[a] n = 46*	*Placebo[a] n = 19*
Blood and lymphatic system disorders	4 (8.7%)	2 (10.5%)
Cardiac disorders	9 (19.6%)	4 (21.1%)
Gastrointestinal disorders	13 (28.3%)	5 (26.3%)
General disorders/administration site conditions	14 (30.4%)	7 (36.8%)
Infections (systemic and access)	18 (39.1%)	9 (47.4%)
Procedural and access complications	20 (43.5%)	8 (42.1%)
Abnormal lab values	3 (6.5%)	2 (10.5%)
Musculoskeletal/connective tissue disorders	7 (15.2%)	3 (15.8%)
Nervous system disorders	5 (10.9%)	2 (10.5%)
Renal and urinary disorders	2 (4.3%)	0
Respiratory and thoracic disorders	9 (19.6%)	3 (15.8%)
Skin and subcutaneous tissue disorders	9 (19.6%)	2 (10.5%)
Vascular disorders	16 (34.8%)	9 (47.4%)

[a]Number of subjects (%).

assisted primary AVG patency compared to placebo in the ITT or mITT populations (Fig 3). At 24 weeks, the primary patency rate for ITT patients who received Vascugel was 38% vs 23% for placebo. Similarly, primary patency in the mITT group was 49% vs 25% for Vascugel and placebo, respectively. Vascugel assisted primary patency rates were 72% for ITT and 78% for mITT, vs 58% and 50%, respectively, for the placebo group. Adjudication of graft events to determine anastomotic patency did not result in significant differences in patency rates (data not shown), due to the difficulty in determining the presence and/or location of the causative lesion in the grafts, especially after interventional procedures performed to treat thrombosed grafts. There was no statistically significant difference in time to first AVG use between the Vascugel and placebo groups.

Quantitative angiography data (methods in Appendix, online only) showed no significant differences between groups in lumen diameter changes from 12-24 weeks (data not shown). There was no observable difference in minimal lumen diameter (MLD).

Secondary efficacy outcomes - AVF cohort. Treatment with Vascugel did not significantly prolong unassisted or assisted primary fistula patency compared with placebo in the ITT or mITT populations (Fig 4). At 24 weeks, the primary patency rates for ITT patients who received Vascugel were 60% vs 62% for placebo. Primary patency in the mITT group was 75% vs 65%, for Vascugel and placebo, respectively. Assisted primary patency rates for ITT were 96% vs 88% for Vascugel and placebo, respectively. Assisted primary patency in the mITT population was 100% in both treatment groups. Blinded adjudication of fistula events was more straightforward and resulted in a primary anastomotic patency in the ITT Vascugel group of 73% at 24 weeks compared with 58% in the placebo group and 92% vs 64% for Vascugel vs placebo in the mITT group (Fig 4). Decisions to adjudicate an event as unrelated to the anastomotic treatment area included cases of angioplasty of central venous stenosis outside of the treatment area and ligation of collateral venous branches without stenosis. There was no statistically significant difference in time to first AVF use between the Vascugel and placebo groups. Angiography was performed only at 24 weeks in AVF patients and analysis showed no significant differences between groups (data not shown). By ultrasound, positive changes in venous lumen diameter were observed over time in both groups (5.57 ± 1.64 mm and 5.71 ± 1.11 mm at 2 weeks, 5.93 ± 1.89 mm and 5.31 ± 1.24 mm at 4 weeks, 6.92 ± 2.47 mm and 5.86 ± 1.92 mm at 12 weeks, 7.01 ± 2.66 mm and 7.41 ± 2.844 mm at 24 weeks for Vascugel and placebo, respectively).

Secondary safety outcome. A secondary safety endpoint of the study was to measure the development of PRA over time. PRA testing determines the presence of anti-HLA antibodies in serum samples and is reported as a percentage. No statistically significant increases were observed in class II anti-HLA antibodies (data not shown). At

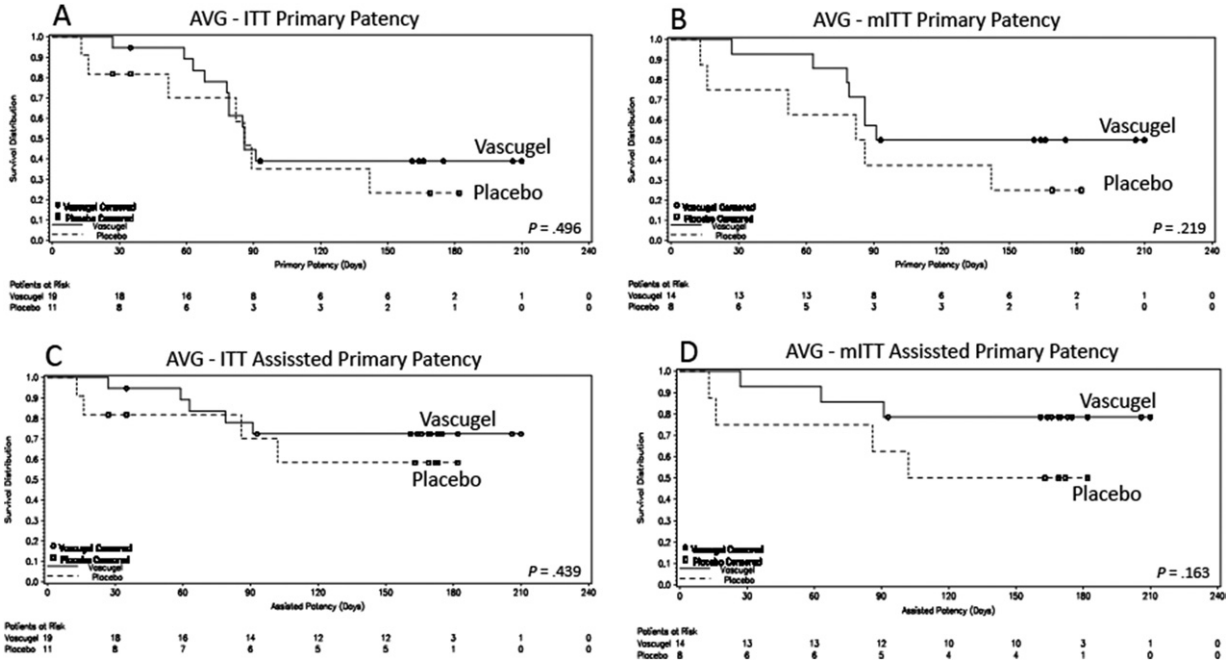

Fig 3. AVG secondary outcome of patency analyzed using the Kaplan Meier product limit method. **A,** ITT primary patency; **B,** mITT primary patency; **C,** ITT assisted primary patency; **D,** mITT assisted primary patency. Comparison of the two treatment groups was made using the log-rank test.

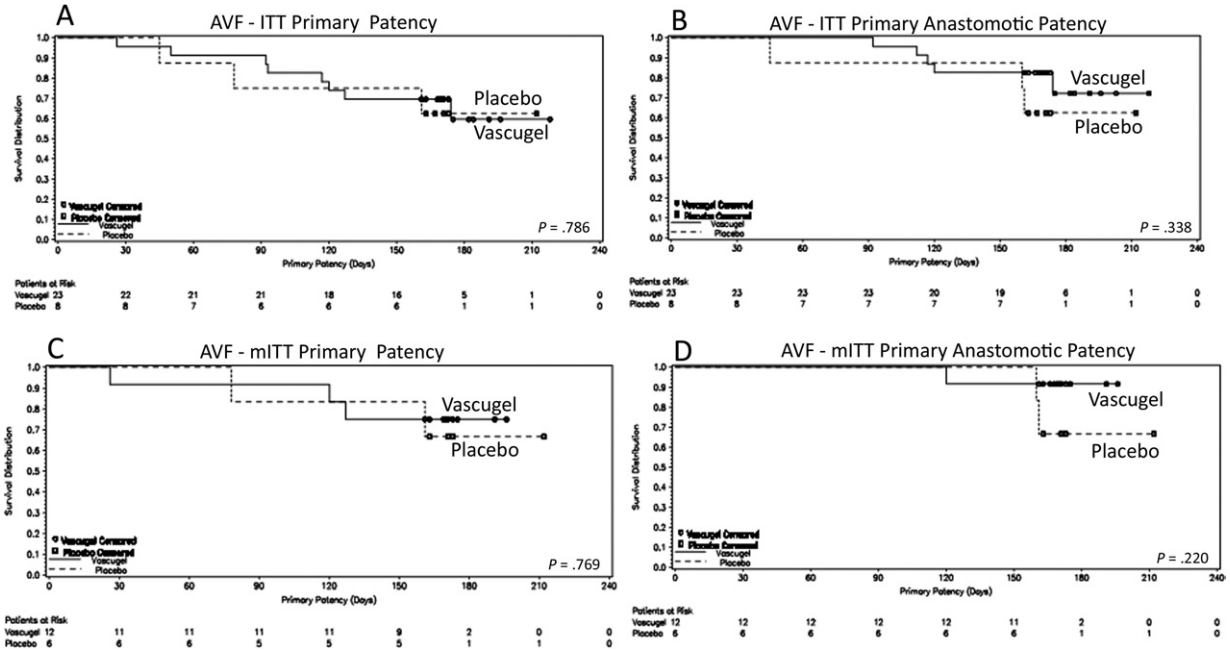

Fig 4. AVF secondary outcome of patency analyzed using the Kaplan Meier product limit method. **A**, ITT primary patency; **B**, ITT primary anastomotic patency; **C,** mITT primary patency; **D,** mITT primary anastomotic patency. Comparison of the two treatment groups was made using the log-rank test.

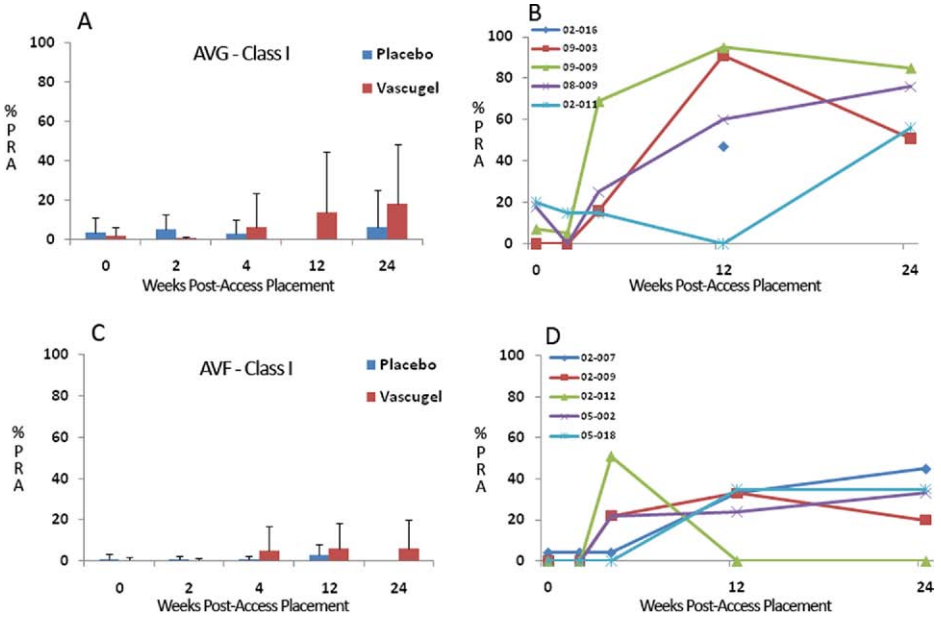

Fig 5. Bar graph of increase in % PRA at 2, 4, 12, and 24 weeks. **A,** At 2 weeks, more AVG placebo patients had an elevation in class I anti-HLA antibodies compared with Vascugel patients. No statistically significant differences were observed at any of the other time points. **B,** Time course of PRA response in four AVG Vascugel patients (02-016, 08-009, 09-003, and 09-009) and one AVG placebo subject (02-011). Only baseline and 12-week PRA were obtained for 02-016 and therefore the complete time course could not be assessed. **C,** No statistically significant differences were observed between AVF placebo and Vascugel patients at any of the time points. **D,** Time course of PRA response in five Vascugel patients. Increases of PRA ≥30% in either AVG or AVF patients did not reach statistical significance when the two treatment groups were compared ($P = .26$). The presence of HLA antibodies in serum samples was determined using the LABScreen Luminex platform which utilizes a panel of color-coded micro-beads coated with purified class I or class II HLA antigens and preoptimized reagents for the detection of class I or class II HLA antibodies in human sera and the LABScan 100 flow analyzer for data acquisition and analysis.

2 weeks, more AVG placebo patients had an elevation in class I antibodies compared with Vascugel patients ($P < .005$, Fig 5). No statistically significant differences were observed at any of the other time points or in AVF patients. An increase >30% compared with baseline levels in class I anti-HLA antibodies was observed in four Vascugel AVG patients, in one placebo AVG subject, and in five AVF Vascugel patients and none of the placebo AVF patients ($P = .26$, Fig 5). In all of the Vascugel patients who experienced PRA elevations, one or more of the antibodies were specific to the donor EC HLA antigens. Statistical analysis revealed no relationship between an increase in PRA and either access patency or the incidence of adverse events at 24 weeks. Larger trials and longer time points are needed to confirm this observation.

DISCUSSION

The V-HEALTH trial represents the first use of a novel allogeneic endothelial implant in AV access patients. The primary outcome of the trial, safety, was met. There was no difference in the incidence of early complications for the Vascugel group compared with placebo. There was no evidence of local or systemic safety concerns for the Vascugel group based on analysis of reported adverse events and SAEs. The adverse events observed were expected as typical vascular access related complications or comorbidities associated with the ESRD patient population. The present study was not powered to detect efficacy of Vascugel, and thus, larger trials will be necessary to detect statistical differences in patency between treatment groups. A subset of V-HEALTH patients entered into an extension study where patency and access survival will continue to be assessed. Results from the extension study will be the subject of a follow-up report. The results of the present study support further clinical investigation of Vascugel in the setting of dialysis access surgery to explore efficacy, optimal treatment dose, and potential consequences of repeat administration.

A major challenge in caring for patients undergoing hemodialysis for kidney failure is maintaining a functioning vascular access. The current therapy for a failing vascular access is either surgical revision or percutaneous interventions.[27] Although the exact causes of AVF and AVG stenosis and thrombosis are not completely understood, they are likely encompassed within the paradigm of the vessel wall injury response. Loss of endothelial integrity followed by platelet activation, inflammation, and the migration and proliferation of fibroblasts and SMC characterize this gen-

eralized response.[14] Dramatic changes in biomechanical stresses also occur as a result of the high-flow environment. Preclinical studies have shown that when placed outside a blood vessel, at the adventitia, EC decrease thrombosis, stenosis and negative remodeling[21,28] and provide the scientific basis for the clinical development of Vascugel as a locally applied perivascular therapy to improve healing at sites of vascular interventions. Both Vascugel and Gelfoam degrade in vivo between 4 to 6 weeks, however, animal data have demonstrated beneficial effects of the perivascular EC several months after degradation.[23,29]

The use of allogeneic EC has multiple benefits on the manufacturing process, product release and quality assurance. The use of qualified cell banks from single donors is amenable to strict viral as well as functional testing prior to formulation of the final product. Patients with cardiovascular disease and diabetes (100% and 59% of the V-HEALTH population, respectively) are thought to have dysfunctional endothelium and circulating auto-antibodies making use of autologous EC from these patients less desirable.[30-33] However, the use of allogeneic EC in patients that may undergo kidney transplant required that we monitor for immunosensitization. An elevation in PRA over the course of the study, defined as a 30% increase over baseline, was observed in 19.5% of the Vascugel group (9/46) and in 5.2% of the placebo group (1/19). The majority of elevations observed in the Vascugel patients were class I and donor specific. There was no relationship between the presence of PRA, access patency, or adverse events. Outside of the context of this trial, sensitization is not an uncommon clinical finding. Sensitization occurs in most people through exposure to a foreign alloantigen, which can occur through pregnancy, blood transfusions or prior transplantation of solid organs or bone marrow.[34-36] The implications of PRA increases or sensitization for potential kidney transplant candidates are twofold: an increase in time on the transplant wait list until a suitable donor is found[37] and if transplanted, an increase in risk of antibody mediated rejection may occur,[38,39] which can be ameliorated with programs such as paired kidney exchange, immunosuppression, or desensitization protocols.[40] The V-HEALTH study was conducted in dialysis patients who were not under consideration for transplantation at the time of enrollment and therefore the results of transplantation in V-HEALTH patients are unknown at this time. Additional PRA data will continue to be collected in the extension study.

The present study had several limitations. It was designed as a small safety and feasibility study of a novel cellular implant and therefore, not powered to detect therapeutic benefit. The treatment of both AVG and AVF allowed for patients to be enrolled and not lost due to last minute decisions made concerning the type of access and for safety to be assessed in both AVF and AVG. However, this approach made efficacy analysis more difficult. In addition, a number of interventions that affected AVG primary patency were triggered by the 12-week protocol-mandated angiogram (30% of the Vascugel and 12% of the placebo group). This observation is reflected in 24-week AVG primary patency rates in the control group that are somewhat lower than those reported in the literature.[41,42] Design of future trials in which patency is a primary endpoint should not specify an angiogram unless indicated by clinical events. Targeted local therapy with tissue engineered endothelial cell implants is a novel therapeutic approach that is safe and may be ideally suited to control the response to injury and inhibit clinical vascular dysfunction. Larger, randomized trials will need to be performed to determine if there is a benefit of Vascugel on AV access patency, maturation and survival.

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Final approval of the article: MC, HN, PG, IG, PR-C, JL
Statistical analysis: PG, HN
Obtained funding: HN, MC, P R-C, JL
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APPENDIX, online only

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METHODS

Ultrasound and Angiography Lumen Diameter Measurements All lumen diameter measurements were performed by a blinded interventional radiologist provided by a core Imaging Lab (RadPharm). Venous lumen diameters for AVF patients were obtained by US at 1 cm, 3 cm and 5 cm from the venous anastomosis. Diameters at each location were averaged to obtain one venous measurement per patient at each time point. Venous and arterial lumen diameters were obtained by angiography at the anastomosis and up to 5 cm from the anastomosis at 0.5 cm increments. Graft lumen diameters were obtained up to 5 cm into the graft from either the venous or arterial anastomosis in 0.5 cm increments. Minimum lumen diameter was calculated for each patient by taking the smallest measured diameter over the 5 cm length of vessel. The frequency of positive venous diameter changes was determined by determining the distribution of patients with either positive or negative changes at each of the distances measured (ie, at the anastomosis, at the 10 distances measured between 0.5 and 5.0 cm into the venous outflow segment).