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A multi-center, dose-escalation study of human type I pancreatic elastase (PRT-201) administered after arteriovenous fistula creation

Eric K. Peden[1], David B. Leeser[2], Bradley S. Dixon[3], Mahmoud T. El-Khatib[4], Prabir Roy-Chaudhury[4], Jeffrey H. Lawson[5], Matthew T. Menard[6], Laura M. Dember[7], Marc H. Glickman[8], Pamela N. Gustafson[9], Andrew T. Blair[9], Marianne Magill[10], F. Nicholas Franano[9], Steven K. Burke[9]

[1] Department of Cardiovascular Surgery, The Methodist Hospital, Houston, TX - USA
[2] Department of Transplantation, New York-Presbyterian Hospital-Weill Cornell Medical Center, New York, NY - USA
[3] Department of Medicine, University of Iowa, Iowa City, IA - USA
[4] Department of Medicine, University of Cincinnati, Cincinnati, OH - USA
[5] Department of Surgery, Duke University, Durham, NC - USA
[6] Department of Surgery, Brigham and Women's Hospital, Boston, MA - USA
[7] Renal-Electrolyte and Hypertension Division, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA - USA
[8] Eastern Virginia Medical School, Norfolk, VA - USA
[9] Research and Development, Proteon Therapeutics, Waltham, MA - USA
[10] Natick, MA - USA

ABSTRACT

Purpose: **To explore the safety and efficacy of PRT-201.**

Methods: **Randomized, double-blind, placebo-controlled, single-dose escalation study of PRT-201 (0.0033 to 9 mg) applied after arteriovenous fistula (AVF) creation. Participants were followed for one year. The primary outcome measure was safety. Efficacy measures were the proportion with intra-operative increases in AVF outflow vein diameter or blood flow ≥25% (primary), changes in outflow vein diameter and blood flow, AVF maturation and lumen stenosis by ultrasound criteria and AVF patency.**

Results: **The adverse events in the PRT-201 group (n=45) were similar to those in the placebo group (n=21). There were no differences in the proportion with ≥25% increase in vein diameter or blood flow, successful maturation or lumen stenosis. There was no statistically significant difference in primary patency between the dose groups (placebo n=21, Low Dose n=16, Medium Dose n=17 and High Dose n=12). In a subgroup analysis that excluded three participants with early surgical failures, the hazard ratio (HR) for primary patency loss of Low Dose compared with placebo was 0.38 (95% CI 0.10-1.41, P=0.15). In a Cox model, Low Dose (HR 0.27, 95% CI 0.04-0.79, P=0.09), white race (HR 0.17, 95% CI 0.03-0.79, P=0.02), and age <65 years (HR 0.25, CI 0.05-1.15, P=0.08) were associated (P<0.10) with a decreased risk of primary patency loss.**

Conclusions: **PRT-201 was not different from placebo for safety or efficacy measures. There was a suggestion for improved AVF primary patency with Low Dose PRT-201 that is now being studied in a larger clinical trial.**

Key words: Arteriovenous fistula, Clinical trial, Pancreatic elastase, PRT-201, Renal dialysis

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INTRODUCTION

A functional arteriovenous fistula (AVF) is the most desirable form of vascular access for maintenance hemodialysis because it has the longest patency and fewest complications (1,2). Unfortunately, up to 60% of newly created AVFs do not mature adequately for use and require repeated surgical and endovascular interventions (1,3-7). During this time, a central venous catheter with its inherent risks is frequently used if hemodialysis is required (2). Usable AVFs are at risk of patency loss most often because of neointimal hyperplasia leading to stenosis, typically at the arteriovenous anastomosis and adjacent outflow vein (8). Patency loss occurs in 40% to 50% of AVFs within one year of creation and is often addressed with invasive procedures such as thrombectomy, thrombolysis, and balloon and patch angioplasty (9-12). Despite a high rate of initial success, interventions to restore and maintain patency are characterized by poor post-intervention patency and complications (2,13).

PRT-201 is a recombinant human type I pancreatic elastase normally expressed in the skin that preferentially cleaves the peptide bonds of hydrophobic amino acid sequences abundant in elastin (14-16). Elastin fibers form a three-dimensional network in blood vessel walls that controls resting vessel diameter and imparts elasticity (17). Because PRT-201 is inactivated by antiproteases in blood it is applied to the outside of blood vessels and acts locally rather than systemically (18). In animal models of AVF, type I pancreatic elastase treatment of blood vessels led to fragmentation of elastin fibers, vessel dilation, increased blood flow, inhibition of intimal hyperplasia formation and improved access patency (19-22). If replicated in patients, these effects could improve the maturation and patency of AVFs, reduce the need for interventional procedures, and increase the number of patients using an AVF for vascular access for hemodialysis instead of a catheter or arteriovenous graft. This first study in humans was designed to explore the safety and pharmacologic effects of PRT-201 and explore efficacy endpoints for future clinical trials.

MATERIALS AND METHODS

Trial design

This was a sequentially adaptive, phase 1-2, randomized, double-blind, placebo-controlled, dose-escalation study of a single application of PRT-201. Treatment was administered in dose cohorts of six (11 cohorts total), with four receiving PRT-201 and two receiving placebo. Nine dose levels (0.0033-9 mg) were studied with 0.0033 mg as the starting dose. Under supervision of a Data Monitoring Committee (DMC), the dose was escalated employing an adaptive clinical trial design focused on acute pharmacologic activity and safety in the two weeks following surgery.

The protocol, informed consent form, and all amendments were reviewed and approved by each center's Institutional Review Board (IRB). This study was performed in accordance with the ethical principles originating from the Declaration of Helsinki and current Good Clinical Practice (GCP) Guidelines and in compliance with the Code of Federal Regulations (CFR), 21 CFR 312. This trial was pre-registered at www.clinicaltrials.gov (Identifier NCT00679991).

Participants

Patients at least 18 years of age with chronic kidney disease, who were either receiving maintenance hemodialysis or were expected to commence maintenance hemodialysis within six months and required the creation of an AVF, were enrolled. Exclusion criteria were alpha 1-antitrypsin deficiency, cephalic vein lumen diameter by ultrasound < 2.0 mm with a tourniquet, lack of continuity of the cephalic vein with the subclavian vein, central venous stenosis or occlusion, and treatment with any investigational agent within 30 days or investigational antibody therapy within 90 days of signing the informed consent document.

Interventions

Immediately after creation of a radiocephalic or brachiocephalic AVF, 2.5 mL of PRT-201 or placebo solution was delivered by the surgeon as a series of drops over 10 minutes to the surgically exposed inflow artery, anastomosis and outflow vein. Study drug application was followed by lavage of the AVF and wound with saline for one minute. Digital photographs of the AVF with diameter standards and intra-operative blood flow measurements of the AVF outflow vein were obtained before and after study drug administration. A central reader (Canfield Scientific, Fairfield, NJ) masked to treatment assignment calculated mean pre- and post-treatment outflow vein diameters at 1 mm intervals along the visible vein using the digital photographs. AVF blood flow was measured using a handheld flow probe (Transonic Systems, Ithaca, NY) using a transit-time ultrasound technique (23).

Outcomes

Assessments were performed at 1, 2 and 6 weeks, and 3, 6, 9 and 12 months. Safety assessments included ascertainment of adverse events (AEs), physical examinations, duplex Doppler ultrasounds, vital signs, electrocardiograms (ECGs), chest radiographs and laboratory studies including chemistry, hematology and coagulation panels and testing for anti-PRT-201 antibodies using a validated assay. Special attention was given to local findings including AVF lumen stenosis, vascular steal, excessive arm swelling, aneurysm, pseudoaneurysm, hematoma, seroma, infection and wound healing. The primary efficacy endpoint was the proportion with ≥25% intra-operative increase in AVF outflow vein diameter or blood flow immediately after treatment. The pre-defined secondary efficacy endpoints included the change and percentage change in intra-operative AVF outflow vein diameter and blood flow immediately following PRT-201 administration; the proportion with AVF patency by physical examination; AVF unassisted primary and secondary patency; the proportion with hemodynamically significant lumen stenosis (HSS) in the AVF circuit; AVF maturation by duplex Doppler ultrasound criteria; AVF use for hemodialysis; and time to initial cannulation of the newly created AVF. Loss of unassisted primary patency was defined as AVF thrombosis, the occurrence of a procedure to maintain or restore AVF patency (e.g. balloon or patch angioplasty), or two consecutive post-surgery visits with lack of a bruit audible by stethoscope throughout systole and diastole 8 cm

downstream from the anastomosis. Secondary patency loss was defined as abandonment of the access site (e.g. creation of a new permanent vascular access). Duplex Doppler ultrasounds were performed at 6 weeks and 3 and 6 months using a standard protocol. All ultrasounds were reviewed by a central reader (VasCore, Boston, MA) masked to treatment assignment. The reader measured outflow vein lumen diameter and blood flow to establish AVF maturation and also assessed for the presence of HSS in the AVF circuit. Outflow vein diameter was measured in duplicate at three locations and the six individual diameter values were averaged. Blood flow was measured three times in the AVF outflow vein 5 cm from the arteriovenous anastomosis and the three values were averaged. Successful AVF maturation was defined using the National Kidney Foundation (NKF) criteria of average cephalic vein lumen diameter ≥6 mm and an outflow vein blood flow ≥600 mL/min (2) and also using the criteria published by Robbin and colleagues of average cephalic vein lumen diameter ≥4 mm and an outflow vein blood flow ≥500 mL/min (6). Doppler ultrasound was used to identify HSS by documenting an increase in the peak systolic velocity (PSV) ratio. The PSV ratio was determined by dividing the PSV within the stenotic segment by the PSV in an adjacent upstream normal segment. A PSV ratio ≥2 in the inflow artery or outflow vein or a PSV ratio ≥3 with a minimum PSV of 400 cm/s at the anastomosis were considered hemodynamically significant (24). Stenosis was categorized as being within or outside the treatment zone which included the anastomosis, the adjacent 2 cm of inflow artery and the adjacent 5 cm of outflow vein. UItrasound readings results from the central reader were not shared with the study team or treating clinicians.

The cohort size of six (four active and two placebo) was chosen based on pharmacology studies in groups of four animals that demonstrated statistically significant increases in AVF outflow vein diameter and blood flow following PRT-201 treatment. The randomization sequence was computer generated in blocks of six without stratification. Randomization occurred after the screening assessments had been conducted and before the scheduled surgery. If a participant was randomized but not treated, the randomized treatment was reassigned to a replacement participant after the remaining participants in the cohort were randomized. The investigator, clinical staff, participants and the central readers of the photographs and ultrasounds remained blinded to study treatment. PRT-201 was supplied as 5 mg vials that were reconstituted at the clinical site by an unblinded research pharmacist with 0.5 mL of water then diluted as necessary with phosphate buffered saline containing 0.01% polysorbate 80 (PBS). PRT-201 and placebo (PBS) were both clear non-viscous liquids that frothed slightly if shaken.

Statistical methods

All participants who received any PRT-201 or placebo were included in the safety and efficacy analyses. The primary analysis of the safety and efficacy outcomes was performed when the last patient had completed the 6-week post-treatment visit. In addition, an analysis of safety and secondary efficacy outcomes was performed when the last patient had completed the 6-month and 12-month visits.

The PRT-201 participants were grouped into Low Dose (0.0033, 0.01, 0.033 mg), Medium Dose (0.1, 0.33, 1.0 mg), and High Dose (3.0, 6.0, 9.0 mg) groups to facilitate analysis of dose-response using larger group sizes. During data review it was noted that there were three early surgical failures that appeared unrelated to the study treatment. A post hoc exploratory subgroup was created (All Treated Minus Surgical Failures) that excluded early surgical failures defined as loss of secondary patency within two weeks of the AVF surgery. This subgroup had not been pre-specified in the analysis plan.

Within group changes in vein diameter and AVF blood flow were compared with paired *t* tests, treatment group comparisons of change and percentage change were made using ANOVA or ANCOVA, and comparison of survival curves used log rank tests or Cox proportional hazards models. Adverse events were compared using Fisher's exact tests. All statistical tests were two-sided at the 5% statistical significance level and were for exploratory purposes only.

RESULTS

Participants

A total of 97 participants were consented and 66 were treated. Figure 1 summarizes patient flow through the study. The reasons for non-treatment were scheduling conflicts (n=16), principally that the patient's surgery occurred during time-out periods for DMC reviews between cohorts, failure to meet eligibility criteria (n=13), and withdrawn consent (n=2). A single patient randomized to placebo was treated with PRT-201 in error. This patient was included in the PRT-201 group for all analyses. Of the 66 participants who received study drug, 59 (89%) completed the study and 7 (11%) discontinued early. The reasons for discontinuation were kidney transplantation (n=4), loss to follow up (n=2) and death because of cancer (n=1).

Baseline data

Table I summarizes baseline characteristics including surgical details by treatment group. There were no statisti-

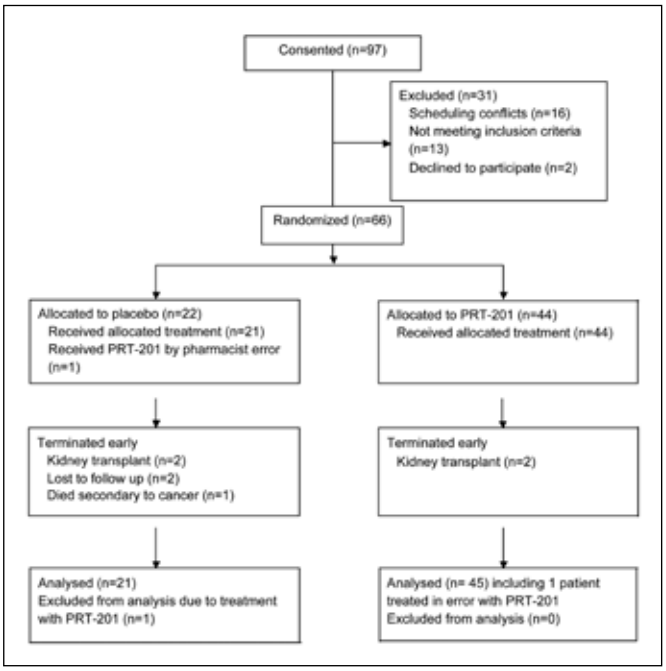

Fig. 1 - *Participant flow throughout the study.*

cally significant differences between the placebo and the all PRT-201 groups for any of the characteristics summarized in the table with the exception of the percent predialysis (P=0.04). In 67% of cases, saline was used to dilate the cephalic vein prior to creation of the AVF and assess for the presence of vein stenosis. A mechanical dilator was used in 44% of cases to dilate the cephalic veins prior to AVF creation. Saline was used alone in 23 participants, a mechanical dilator alone in eight, saline and a mechanical dilator in 20 and neither in 15. Sutures for the anastomosis were most often non-absorbable (83%); most anastomotic sutures were placed in a running fashion (71%). The mean arteriotomy length was 0.76 cm (range 0.4-1.2 cm).

Safety

Three participants, all of whom were from the Low Dose group, had early AVF failure. One patient had acute AVF thrombosis on the day of surgery. This patient experienced early thrombosis of two other AVFs, one created before and one created after participation in this trial. Based on clinical history and the presence of anti-heparin antibodies, this patient was later given the diagnosis of heparin-induced thrombocytopenia, a known risk factor for thrombosis following heparin exposure. The patient had received intra-operative heparin with all three AVF creation surgeries. A second patient had bleeding at the AV anastomosis one week after AVF creation and required surgical revision. Histology of the resected vein showed intact elastin staining, suggesting that the pharmacologic removal of elastin was not the cause of the bleeding. A third patient was found to have a non-patent AVF at the

TABLE I - BASELINE CHARACTERISTICS BY TREATMENT GROUP

	Placebo N=21	Low Dose N=16	Medium Dose N=17	High Dose N=12	All PRT-201 N=45
Male (%)	71	75	71	75	73
White (%)	43	50	35	50	44
Age (mean ± SD)	52 ± 17	53 ± 16	65 ± 11	51 ± 15	57 ± 15
≥65 years (%)	24	31	53	25	38
BMI (mean ± SD)	31 ± 7	26 ± 5	31 ± 7	32 ± 7	30 ± 7
RC AVF (%)	62	44	47	75	53
IHD (%)	33	19	35	17	24
PAD (%)	5	0	18	8	9
CVD (%)	10	6	18	8	11
Predialysis (%)	29	38	71	58	56
CKD 2º DM (%)	38	19	41	42	33
CKD 2º HTN (%)	38	31	35	33	33
Tobacco free (%)	33	44	41	42	42
Nerve block (%)	48	44	47	42	44
Fluid dilation (%)	81	63	65	50	60
Mechanical dilation (%)	43	38	41	58	44
Running sutures (%)	67	69	82	67	73
Non-absorbable sutures (%)	86	81	88	75	82
Exposed vein (cm ± SD)	3.1 ± 0.8	3.3 ±0.8	3.4 ± 1.1	3.1 ± 0.9	3.3 ± 0.9
Arteriotomy (cm ± SD)	0.76±0.21	0.59 ± 0.27	0.81 ± 0.23	0.92 ± 0.30	0.76 ± 0.29

BMI, body mass index; CVD, cerebrovascular disease; CKD, chronic kidney disease; DM, diabetes mellitus; HTN, hypertension; IHD, ischemic heart disease; PAD, peripheral artery disease; RC AVF, radiocephalic arteriovenous fistula.

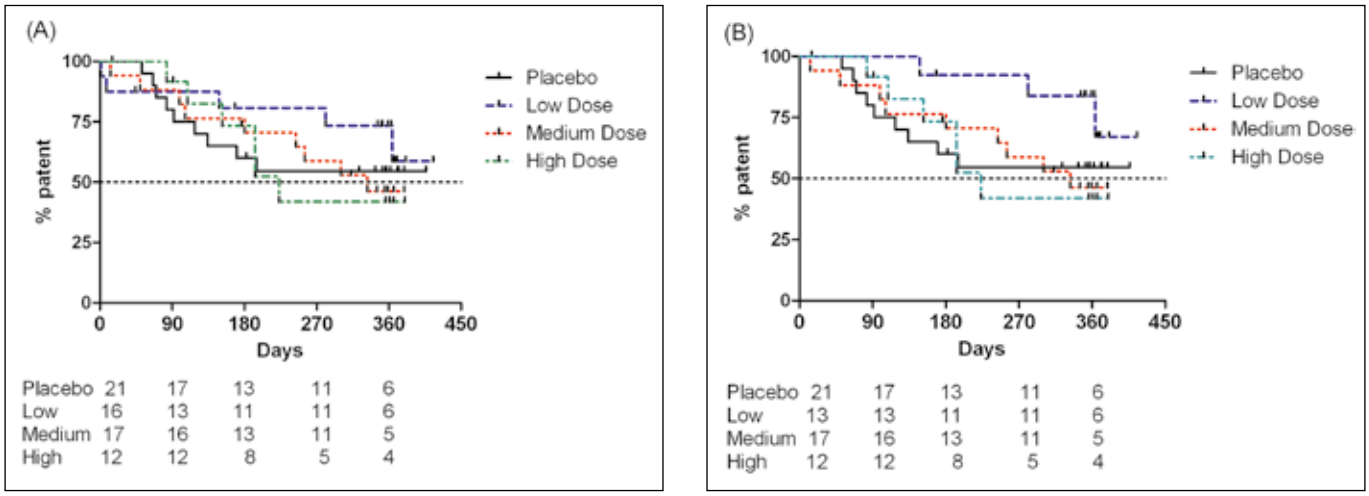

Fig. 2 - *Kaplan-Meier plots of AVF unassisted primary patency for the All Treated population (A) and the All Treated Minus Surgical Failures population (B) that excludes three participants with early surgical failures.*

one-week visit.

Adverse events, the most common of which are summarized in Table II, were consistent with the medical conditions experienced by patients with chronic kidney disease undergoing AVF surgery. There were no statistically significant differences in the proportion of placebo and PRT-201 participants reporting adverse events. There were no meaningful dose-related increases in adverse events. There were no clinically significant differences between the placebo group and the all PRT-201 group related to physical examinations, vital signs, chest radiographs, ECGs or clinical laboratory testing including chemistry, hematology and coagulation panels. Immunogenicity testing identified anti-PRT-201 antibodies following treatment in a single patient treated with 6 mg of PRT-201. This patient did not demonstrate any skin-related adverse events or physical findings.

Efficacy

There was no difference between groups for the primary efficacy outcome measure, the proportion with a ≥25% increase in the diameter of the AVF outflow vein or blood flow immediately after treatment. None of the participants in the PRT-201 or placebo groups had an intra-operative increase in AVF outflow diameter of ≥25%. An increase in intra-operative AVF blood flow of ≥25% was observed in 42% of PRT-201 and 52% of placebo participants. Table III summarizes the secondary efficacy outcome measures change and percentage change in intra-operative AVF outflow vein diameter and blood flow immediately following treatment. The increases in vein diameter and blood flow were statistically significant for the PRT-201 group when compared with the pretreatment values. The

TABLE II - NUMBER AND PROPORTION OF PARTICIPANTS WITH COMMON ADVERSE EVENTS[1]

N (%)	Placebo N=21	Low Dose N=16	Medium Dose N=17	High Dose N=12	All PRT-201 N=45
Any adverse event	18 (86)	15 (94)	16 (94)	11 (92)	42 (93)
Procedural pain	4 (19)	5 (31)	6 (35)	4 (33)	15 (33)
Venous stenosis[2]	6 (29)	3 (19)	5 (29)	6 (50)	14 (31)
Ecchymosis	4 (19)	4(25)	4(24)	1 (8)	9 (20)
AVF thrombosis	3 (14)	4 (25)	3 (18)	1 (8)	8 (18)
Arthralgia	0 (0)	5 (31)	1 (6)	0 (0)	6 (13)
Hypoaesthesia	4 (19)	3(19)	0 (0)	3 (25)	6 (13)
Procedural complications	0 (0)	2 (12)	1(6)	2 (17)	5 (11)
Hematoma	2 (10)	2 (12)	2 (12)	1 (8)	5 (11)
Steal syndrome	5 (24)	2 (12)	1 (6)	1 (8)	4 (9)

[1]Adverse events occurring in at least 10% of placebo or the combined PRT-201 treatment groups. [2] Venous stenosis reported as an adverse event, not stenosis detected by US.

corresponding increases in the placebo group were not statistically significant with the exception of percentage change in blood flow. The intra-operative increases in AVF outflow vein diameter and blood flow seen in the PRT-201 groups were not statistically different from those seen in the placebo group. An analysis of the intra-operative AVF outflow vein diameter data did not demonstrate an effect of saline or mechanical dilation on the immediate change in vein diameter following PRT-201 treatment: saline dilation (n=13, +4.2%), mechanical dilation (n=6, +8.0%), saline and mechanical dilation (n=14, +8.0%), and no saline or mechanical dilation (n=12, +2.6%). As shown in Table IV, AVF outflow vein lumen diameter and blood flow increased in the weeks and months following surgery. There were no significant differences between the PRT-201 and placebo groups at any of the time points.

There was no difference between the PRT-201 and placebo groups in the proportion of participants with AVF maturation. For the PRT-201 group, 45% and 76% matured by the NKF criteria and the Robbin et al criteria, respectively, compared with 50% and 83%, respectively, for the placebo group.

An HSS was identified in 54% of the PRT-201 group and 58% of placebo group from the six-week ultrasound. The stenosis occurred in the treatment zone in 88% of cases. The Low Dose group had the lowest percentage of participants with HSS at six weeks (38%). Median times to HSS were 59, 209, 49 and 43 days for the placebo, Low Dose, Medium Dose and High Dose groups, respectively. None of these differences were statistically significant.

Approximately half the participants underwent angiography on the study AVF as ordered by a treating physician to assess access dysfunction (PRT-201 44%, placebo 52%). Balloon angioplasty of the vascular access was performed in 33% of participants in the PRT-201 group compared with 43% in the placebo group. Fewer participants in the Low Dose group required angiography (31%) or angioplasty of the access circuit (19%) compared with the all PRT-201 and placebo groups. None of these differences were statistically significant.

In survival analyses there was no significant difference in AVF unassisted primary patency between the dose

TABLE III - VEIN DIAMETER AND AVF BLOOD FLOW IMMEDIATELY PRE- AND POST-TREATMENT

	Placebo N=21	Low Dose N=16	Medium Dose N=17	High Dose N=12	All PRT-201 N=45
Vein diameter (mm or % ± SD)					
Pre	5.1 ± 0.9	5.2 ± 1.1	4.5 ± 1.0	4.7 ± 0.8	4.8 ± 1.1
Post	5.2 ± 1.1	5.5 ± 1.2	4.7 ± 1.0	4.9 ± 0.8	5.0 ± 1.0
Change	0.12 ± 0.3	0.30 ± 0.39*	0.23 ± 0.29†	0.14 ± 0.18	0.24 ± 0.31‡
% change	2.1 ± 7.1	6.1 ± 7.0†	5.7 ± 6.6†	3.2 ± 4.2	5.3 ± 6.3‡
Blood flow (mL/min or % ± SD)					
Pre	243 ±145	264 ± 180	333 ±164	320 ±237	304 ±19
Post	296 ±142	365 ±321	355 ±156	376 ±26	364 ±248
Change	50 ± 132	102 ±187*	33 ± 91	56 ± 60†	65 ± 130†
% change	41 ± 76*	43 ± 68*	17 ± 30*	19 ± 23*	27 ± 47‡

*P<0.05, †P<0.01, and ‡P<0.001. Tests of statistical significance for within group change from immediately before to immediately after treatment from paired *t* tests.

TABLE IV - DUPLEX DOPPLER DETERMINED AVF OUTFLOW VEIN LUMEN DIAMETER AND BLOOD FLOW

	Placebo N=21	Low Dose N=16	Medium Dose N=17	High Dose N=12	All PRT-201 N=45
Vein lumen diameter (mm ± SD)					
6 weeks	6.6 ± 1.8	7.2 ± 2.2	5.9 ± 1.1	6.1 ± 1.0	6.4 ± 1.6
3 months	7.2 ± 1.6	8.0 ± 2.9	6.3 ± 1.8	6.9 ± 1.7	7.0 ± 2.3
6 months	8.0 ± 2.4	9.4 ± 3.7	6.2 ± 1.6	6.9 ± 1.4	7.5 ± 2.8
AVF blood flow (mL/min ± SD)					
6 weeks	1063 ± 687	1362 ± 1245	1047 ± 544	1120 ± 802	1174 ± 889
3 months	1538 ± 1246	1540 ±1434	1126 ± 707	1173 ±775	1277 ±1014
6 months	1669 ± 1435	1302 ± 647	1002 ± 732	1118 ± 654	1141 ± 670

groups or in the Low Dose group compared with the placebo group (Fig. 2, panel A). The HR of the Low Dose group compared with placebo was 0.79 (95% confidence interval [CI] 0.28-2.24, P=0.66). In the All Treated Minus Surgical Failures subgroup that excluded the three early surgical failures, there was no statistically significant difference in AVF unassisted primary patency between the dose groups or in the Low Dose group compared with the placebo group (Fig. 2, panel B). The HR of the Low Dose group compared with placebo was 0.38 (95% CI 0.10-1.41, P=0.15). An exploratory analysis using a Cox proportional hazard model was performed using the Low Dose and placebo groups from the All Treated Minus Surgical Failures subgroup with sex, age (<65 or 65 years), race (white or non-white), diabetes (as the cause of CKD or not), dialysis status (on maintenance hemodialysis or not), AVF type (radiocephalic or brachiocephalic), and atherosclerotic vascular disease (any cerebrovascular, cardiovascular and peripheral vascular or none) as covariates. In the final model that only included covariates associated with the outcome (P<0.10), treatment with Low Dose (HR 0.27, 95% CI 0.04-0.79, P=0.09), white race (HR 0.17, 95% CI 0.03-0.79, P=0.02), and age <65 years (HR 0.25, CI 0.05-1.15, P=0.08) were associated with decreased risk of unassisted primary patency loss.

Eleven participants lost secondary patency during the 12 months of the study and there were no differences between treatment groups for time to secondary patency loss. Of the 35 participants who were receiving maintenance hemodialysis at study entry, very few were using the study AVF for hemodialysis by six weeks (12%) although this increased at three months (41%) and six months (66%). There were no differences between treatment groups in the proportion who ultimately used the study AVF for hemodialysis or in time to cannulation. Mean times to cannulation in those participants who used the AVF for hemodialysis were 87 days (range 23-190) in the PRT-201 group and 82 days (range 41-254) in the placebo group.

DISCUSSION

This clinical trial is the first use of PRT-201 in humans. The trial was designed to assess safety and pharmacologic activity and to explore potential efficacy endpoints for future trials. Although limited by the small number of treated participants, there was no suggestion of safety concerns with the use of PRT-201 over a broad range of doses. PRT-201 treatment resulted in statistically significant but modest increases in intra-operative AVF outflow vein diameter and blood flow. This modest outflow vein dilation seen after PRT-201 treatment in humans contrasts with the greater dilation observed in preclinical studies in animals (19). This difference may be because of the higher pressure in the venous limb of more peripheral AVFs in the human arm compared to the lower pressure in more central veins used in experimental AVFs (25). Human veins are close to maximum dilation at this pressure and therefore removal of elastin would promote only modest additional acute dilation.

Approximately half the participants had mature fistulas at six weeks using the NKF ultrasound criteria; although more were mature when applying the criteria of Robbin et al. The rates of AVF suitability for hemodialysis are similar to those reported from the large NIH sponsored Dialysis Access Consortium study (7). The rate of loss of unassisted primary patency was also high and consistent with the published literature (9-12). The most common cause of patency loss is reported to be stenosis because of neointimal hyperplasia formation, usually at the fistula anastomosis or the first few centimeters of outflow vein (8). Most participants in the current study developed stenosis within six weeks of AVF creation primarily in the treatment area. The initial treatment for the AVF stenosis was most commonly balloon angioplasty which has a high rate of initial success but has poor post-intervention patency often leading to repeated interventions (26). Suppressing neointimal hyperplasia and stenosis formation could reduce the need for such interventions.

Although this study was not powered to reveal efficacy, there was a suggestion that low doses of PRT-201 may be associated with increased time to development of HSS, fewer angioplasty procedures and improved AVF unassisted primary patency. All of the doses administered in this study were pharmacologically active and the low doses would be expected to cause partial elastin fragmentation (19-21,27). In published work in an animal vein bypass graft model, adventitial application of a porcine homologue of PRT-201 in doses that partially fragmented elastin was associated with a decrease in venous neointimal hyperplasia (28). This same effect was observed in an animal model of femoral artery balloon angioplasty (29). Elastin fragmentation in the outer aspect of a vein or artery may generate chemotactic elastin fragments that reduce subsequent neointimal hyperplasia formation by recruiting myofibroblasts, the cells that create neointimal hyperplasia, to the adventitia instead of the intima. In pharmacology studies, PRT-101, a recombinant porcine homologue of PRT-201, was associated with a reduction in venous neointimal hyperplasia in a rabbit AVF model (22).

The major limitation of this study is the small number of participants treated in each PRT-201 dose group. This was appropriate given that this was a first-in-man, dose-escalation study. However, small numbers of participants can result in baseline imbalances between treatment groups that can confound the results. The small number also limits the statistical power and generalizability. Although the results were not statistically significant, we were encouraged by the trends in the Low Dose group and designed and enrolled a larger trial (www.clinicaltrials.gov, identifier NCT01305824) the results of which will be reported in 2013.

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Conflict of interest: Gustafson and Burke are employed by Proteon. Franano has ownership in Proteon and serves on the Board of Directors.

Meeting presentations: The results of this study were presented by Peden at Controversies in Dialysis Access on October 11, 2011, by Dixon at The American Society of Nephrology Meeting on November 12, 2011, by Leeser at The VEITH Symposium, November 16, 2011 and by Peden at St. George's Vascular Access Course, April 16, 2012.

Institutional Review Board (IRB)/Ethics Committee approval was obtained.

Address for correspondence:
Steven K. Burke
Proteon Therapeutics, Inc.
200 West Street
Waltham, MA 02451, USA
sburke@proteontherapeutics.com

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