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Application of human type I pancreatic elastase (PRT-201) to the venous anastomosis of arteriovenous grafts in patients with chronic kidney disease

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ABSTRACT:
Purpose: **To explore the safety and efficacy of PRT-201 applied to the outflow vein of a newly created arteriovenous graft (AVG).**
Methods: **Randomized, double-blind, placebo-controlled, single-dose escalation study of PRT-201 (0.01 to 9 mg) applied to the graft-vein anastomosis and adjacent outflow vein immediately after AVG placement. The primary outcome measure was safety. The efficacy measures were intraoperative increases in outflow vein diameter and blood flow rate, primary unassisted patency, and secondary patency by dose groups (placebo, low, medium, high and All PRT-201).**
Results: **A total of 89 patients were treated (28 placebo and 61 PRT-201). There were no significant differences in the proportion of placebo and PRT-201 patients reporting adverse events. Intraoperative outflow vein diameter increased 5% (p=0.14) in the placebo group compared with 13% (p=0.01), 15% (p=0.07) and 12% (p<0.001), in the low, medium and high groups, respectively. The comparison between the high and placebo groups was marginally statistically significant (p=0.06). The intraoperative blood flow did not change in the placebo group, and increased in the low, medium and high groups by 19% (p=0.34), 36% (p=0.09) and 46% (p=0.02), respectively. The low group had the longest primary unassisted and secondary patency and the fewest procedures to restore or maintain patency; however, the differences between groups were not statistically significant.**
Conclusions: **PRT-201 was well tolerated and increased AVG intraoperative outflow vein diameter and blood flow. Low dose tended to increase secondary patency and decrease the rate of procedures to restore or maintain patency. Larger studies with these doses will be necessary to confirm these results.**

Key words: **Arteriovenous shunt, Graft occlusion, Pancreatic elastase, PRT-201, Renal dialysis, Vascular patency**

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INTRODUCTION

Reliable access to large volumes of rapidly flowing blood is essential for hemodialysis. Unfortunately, hemodialysis vascular access is not reliable and access failure is one of the most common causes of hospitalization for hemodialysis patients (1). Two general types of permanent vascular access sites are created in order to enable hemodialysis: an arteriovenous fistula (AVF) and an arteriovenous graft (AVG). AVG surgery has a higher short-term success but is less desirable because of a higher long-term complication rate (1-3). The majority of AVG complications are secondary to an accumulation of intimal hyperplasia in the vein wall at the connection of the graft to

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the vein, and in the adjacent outflow vein segment (4). Smooth muscle cell migration following vascular injury is central to this process (4). Normally quiescent cells in the media and adventitia proliferate following injury and migrate to the intima where they secrete extracellular matrix leading to progressive stenosis of the vein lumen, reduced blood flow and eventual thrombosis (5, 6).

PRT-201 is a recombinant human type I pancreatic elastase under development to improve vascular access outcomes in patients with chronic kidney disease. In animal models, PRT-201 doses that fragment the majority of elastin in blood vessels result in persistent vasodilation (7, 8). Enlarging vessel lumen area by this mechanism could increase the survival of grafts by lengthening the time to critical lumen stenosis due to the progressive accumulation of intimal hyperplasia. Lower PRT-201 doses that result in partial fragmentation in the adventitia may decrease intimal hyperplasia formation by inhibiting the migration of adventitial myofibroblasts to the intima due to the chemotactic properties of elastin fragments within the adventitia (8-12).

This phase 1-2 clinical trial was designed to explore the safety of PRT-201 administered over a wide range of doses to the AVG venous anastomosis and adjacent outflow vein. Other important objectives were to look for other evidence of pharmacological activity (e.g., diameter and blood flow increase), to pilot the clinically relevant endpoints including primary unassisted and secondary patency that could be used in a subsequent phase 2 study, and to determine if higher or lower doses are more likely to improve AVG outcomes.

METHODS

Trial design

This was a phase 1-2, randomized, double-blind, placebo-controlled study of a single application of PRT-201. Treatment was given in dose cohorts of six patients, with four patients receiving PRT-201 at the same dose and two patients receiving placebo. Eight dose levels (0.01-9 mg) were studied. A total of 15 cohorts were treated allowing more than 1 cohort per dose level. Dosing started with 0.1 mg and ascended to 9 mg. Doses of 0.01 and 0.03 mg were added by protocol amendment and the last six cohorts were enrolled to these two doses. The main study ended when the last patient treated was followed for a minimum of 6 months. Patients completing the main study with a patent AVG (i.e., the AVG had not lost secondary patency) entered into a registry for the collection of additional information on the AVG. The registry ended when the final patient treated in the main study was followed for 6 months in the registry and therefore for a total of 12 months in the main study plus registry.

The protocol, informed consent form and all amendments were reviewed and approved by each center's Institutional Review Board. This study was conducted in accordance with the ethical principles originating from the Declaration of Helsinki and current Good Clinical Practice Guidelines and in compliance with the Code of Federal Regulations (CFR), 21 CFR 312. This trial was preregistered at www.clinicaltrials.gov (Identifier NCT01001351).

Participants

Patients were at least 18 years of age with chronic kidney disease and were either receiving maintenance hemodialysis or were expected to initiate maintenance hemodialysis within 3 months. Exclusion criteria were alpha 1-antitrypsin deficiency and suspected ipsilateral outflow vein or central vein lumen stenosis or occlusion.

Interventions

Immediately after creation of the AVG, 2.5 mL of PRT-201 or placebo solution was delivered by the surgeon as a series of drops over 10 minutes to the exposed graft-vein anastomosis and the adjacent outflow vein. Drug application was followed by lavage of the surgical site with saline for 1 minute.

Outcomes

Digital photographs and intraoperative blood flow measurements of the AVG outflow vein were obtained before and after the administration of PRT-201 or placebo to assess changes in vein diameter and AVG blood flow rate. From the digital photographs, a central blinded reader (Canfield Scientific, Fairfield, NJ) calculated mean pre- and posttreatment outflow vein diameters from measurements at 1 mm intervals along the visible vein. AVG blood flow rate was measured using a handheld flow probe and flowmeter (Transonic Systems, Ithaca, NY) which utilized a transit-time ultrasound technique (13).

Patients returned at 1 week for a safety assessment and again at 4 weeks for a full physical examination, electrocardiogram, safety laboratory tests, and duplex ultrasonography of the AVG. At 3 and 6 months the patients returned for examinations of the AVG and duplex ultrasonography. All adverse events were recorded for 6 months. Adverse events pertaining to the extremity with the study AVG were recorded for up to 12 months, unless the AVG was abandoned or study participation was discontinued. In the registry, patients were contacted by study staff every 3 months to obtain information on whether the patient was on hemodialysis, was using the AVG for hemodialysis and had any procedures performed on the AVG.

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The primary efficacy endpoint was the proportion of patients with a 25% or greater intraoperative increase in the diameter of the AVG outflow vein. The secondary efficacy endpoints included the change and percent change in the intraoperative diameter of the AVG outflow vein, the change and percent change in the intraoperative blood flow volume of the AVG outflow vein, the proportion of patients with a 25% or greater intraoperative increase in the AVG blood flow and primary unassisted patency and secondary patency. Loss of primary unassisted patency was defined as the first occurrence of either access thrombosis or an intervention to restore or maintain patency. Secondary patency loss was defined as abandonment of the AVG for vascular access.

Duplex ultrasound examinations were performed at 4 weeks and 3 and 6 months using a standardized imaging protocol. All ultrasound examinations were reviewed by an experienced ultrasound expert (VasCore, Massachusetts General Hospital, Boston, MA). The operator measured the graft blood flow volume in triplicate at two locations. The first location was in the midpoint of the arterial limb and the second was the midpoint of the venous limb. The presence of hemodynamically significant lumen stenosis was assessed, and was defined as lumen stenosis of 50% or greater and a peak systolic velocity ratio of >2:1.

The investigator, clinical staff, patients and the central readers of the photographs and ultrasounds remained blinded to study treatment; the research pharmacist at each site was unblinded. PRT-201 was supplied as 5 mg vials that were reconstituted with 0.5 mL of water then diluted as necessary with phosphate-buffered saline (PBS) containing 0.01% polysorbate 80. PRT-201 and placebo (PBS) were both identical appearing clear, nonviscous liquids that frothed slightly if shaken.

Statistical methods

All patients who received any amount of PRT-201 or placebo were included in the analyses of safety and efficacy. The PRT-201 patients were grouped into low (0.01 and 0.03 mg), medium (0.1, 0.3, 1.0 mg) and high (3.0, 6.0, 9.0 mg) groups to facilitate analysis of dose response using larger group sizes. All statistical tests were two-sided at the 5% significance level. A Fisher's exact test was used to test the difference in response between placebo and the low, medium, high and all PRT-201 groups. A Cochran-Armitage test for trend was performed to examine a dose–response relationship. P values for within-group change and percentage change in vein diameter and AVG blood flow rate were from paired *t*-tests. P values for treatment group comparisons were from ANOVA or ANCOVA. P values for comparison of survival curves were from log-rank tests. A number of additional analyses were specified after database lock, including the number and percentage of patients with procedures to restore or maintain AVG patency, the procedure rate per person year at risk, the number of procedure days per person year at risk, repeated time-to-event analysis (including all thrombotic events, procedures to restore or maintain patency and secondary patency loss events) and analyses of multiple endpoints by graft configuration (loop vs. non-loop). A proportional hazard model was used to analyze the data from the exploratory analysis modeling repeated time-to-event outcomes.

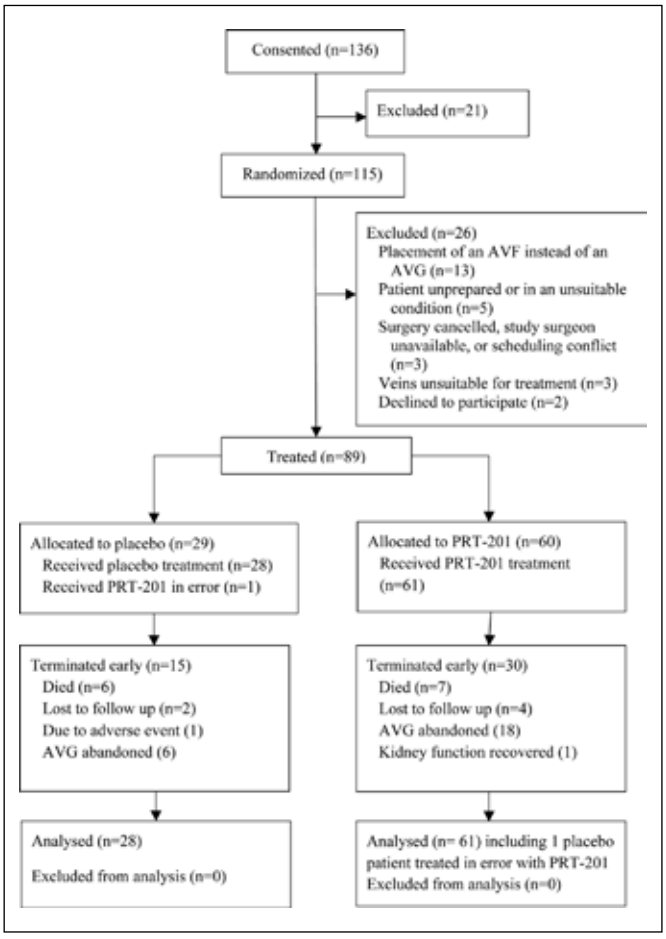

Fig. 1 - *Patient flow through the study.*

RESULTS

Participants

One hundred and fifteen patients were randomized to the study. Twenty-six randomized patients were not treated. Figure 1 summarizes patient flow through the study. Of the 89 patients who were treated, 44 completed the study. Forty of the 44 who completed the study entered the long-term registry study.

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Baseline data

A total of 28 patients received placebo and 61 patients received PRT-201, including 12 patients each in the 0.01 mg and 0.03 mg groups; 4 patients each in the 0.1 mg, 0.3 mg, 1.0 mg and 3.0 mg groups; 5 patients in the 6.0 mg group and 16 patients in the 9.0 mg group. One patient was randomized to placebo but was treated with PRT-201 6.0 mg. This patient was analyzed "as treated." Table I summarizes baseline characteristics and relevant surgical details by treatment group.

Safety

Adverse events were consistent with the medical conditions experienced by patients with chronic kidney disease undergoing AVG surgery, with the most common events summarized in Table II. Few patients had adverse events considered related to study drug, and the percentages in the placebo group and PRT-201-treated group were similar (Tab. III). One patient with a brachial artery to brachial vein loop graft treated with 0.01 mg underwent multiple balloon angioplasties and stent placements across the arterial and venous anastomoses. At one of these procedures at month 11, angiography detected a fistula between the arterial and venous anastomoses, which was closed with a covered stent. One patient with an axillary artery to axillary vein loop graft treated with 9 mg developed a pseudoaneurysm at month 4 secondary to a 3 mm disruption of the arterial anastomosis that was repaired surgically. Both events were considered possibly related to study treatment.

There were no significant findings related to physical examination, vital signs, chest radiographs, electrocardiograms, or clinical laboratory testing including chemistry, hematology and coagulation panels. There was no

TABLE I - BASELINE CHARACTERISTICS BY TREATMENT GROUP

	Placebo n=28	**Low n=24**	**Medium n=12**	**High n=25**	**All PRT-201 n=61**
Male (%)	61	46	67	40	48
African-American (%)	71	67	58	48	57
Age (mean years ± SD)	60±13	57±11	54±12	56±13	56±12
BMI (mean kg/m2 ± SD)	28±8	30±8	31±8	31±11	31±9
CKD 2 DM (%)	32	42	42	60	49
CKD 2 HTN (%)	54	38	50	20	33
Tobacco free (%)	46	46	50	44	46
Aspirin (%)	29	37	50	44	43
Clopidogrel (%)	4	12	42	28	25
Exposed vein length (mean cm ± SD)	4.1±1.0	4.3±1.4	3.7±0.9	4.1±0.9	4.1±1.2
GorePropaten (%)	54	67	33	64	66
Loop configuration (%)	39	38	58	60	51
Upper arm (%)	82	88	67	68	75
Brachial artery (%)	79	71	92	84	80
Axillary artery (%)	14	21	8	12	15
Other artery (%)	7	8	0	4	5
Axillary vein (%)	39	46	42	52	48
Basilic vein (%)	18	29	33	28	30
Brachial vein (%)	25	12	25	20	16
Cephalic vein (%)	18	12	0	0	5

BMI = body mass index; CKD = chronic kidney disease; DM = diabetes mellitus; HTN = hypertension.

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TABLE II - NUMBER AND PROPORTION (%) OF PATIENTS WITH COMMON ADVERSE EVENTS†

N (%)	Placebo n=28	Low n=24	Medium n=12	High n=25	All PRT-201 n=61
Any adverse event	27 (96)	24 (100)	11 (92)	22 (88)	57 (93)
AVG thrombosis	13 (46)	10 (42)	6 (50)	10 (40)	26 (43)
Venous stenosis‡	9 (32)	10 (42)	5 (42)	10 (40)	25 (41)
Procedural pain	8 (29)	7 (29)	2 (17)	10 (40)	19 (31)
Postprocedural edema	7 (25)	3 (13)	4 (33)	8 (32)	15 (25)
Peripheral edema	3 (11)	1 (4)	1 (8)	1 (4)	3 (5)
Sepsis	3 (11)	0 (0)	0 (0)	1 (4)	1 (2)
Hypoesthesia	1 (4)	4 (17)	3 (25)	1 (4)	8 (13)

AVG = arteriovenous graft.
†Treatment emergent adverse events occurring in ≥10% of placebo or the all PRT-201 groups.
‡Arterial and venous stenosis reported as an adverse event, not stenosis detected by ultrasound.

TABLE III - NUMBER AND PROPORTION (%) OF PATIENTS WITH ADVERSE EVENTS CONSIDERED POSSIBLY, PROBABLY OR DEFINITELY RELATED TO STUDY TREATMENT IN THE OPINION OF THE PRINCIPAL INVESTIGATOR

N (%)	Placebo n=28	Low n=24	Medium n=12	High n=25	All PRT-201 n=61
Any adverse event	4 (14)	3 (8)	2 (17)	3 (12)	8 (13)
AVG thrombosis	1 (4)	2 (8)	1 (8)	1 (4)	4 (7)
Postprocedural edema	1 (4)	0 (0)	1 (8)	0 (0)	1 (2)
Hypoesthesia	0 (0)	0 (0)	1 (8)	0 (0)	1 (2)
Neuropathy peripheral	0 (0)	0 (0)	0 (0)	1 (4)	1 (2)
Blister	0 (0)	0 (0)	0 (0)	1 (4)	1 (2)
Erythema	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)
Arterial stenosis	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)
Arteriovenous fistula	0 (0)	1 (4)	0 (0)	0 (0)	1 (2)
Arterial pseudoaneurysm	0 (0)	0 (0)	0 (0)	1 (4)	1 (2)
Venous stenosis	2 (7)	0 (0)	0 (0)	0 (0)	0 (0)

AVG = arteriovenous graft.

evidence that PRT-201 was immunogenic based on serum testing for anti PRT-201 antibodies.

Efficacy

Table IV summarizes intraoperative measurements of vein diameter and AVG blood flow by treatment group. Technical confounders limited the number of patients with evaluable photographic data. In approximately half the patients, the graft was sewn on top of the vein, obscuring the vein in the photographic images. Among patients with interpretable pre- and posttreatment photographs, 5 of 38 (13%) patients who received PRT-201 and 0 of 8 placebo patients met the response criterion (>25% increase in vein diameter).

Pre- and posttreatment blood flow data were available in 81 of the 89 patients. The proportion of patients with a 25% or greater increase in blood flow volume was greater among PRT-201-treated patients (33%) than placebo-treated patients (15%). The difference between placebo and the high group (9 of 21 patients, 43%) was marginally statistically significant (p=0.052). The test for the trend of

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TABLE IV - ARTERIOVENOUS GRAFT OUTFLOW VEIN DIAMETER AND BLOOD FLOW RATE IMMEDIATELY PRE- AND POSTTREATMENT

	Placebo	Low	Medium	High	All PRT-201
Vein diameter (mean mm ± SD and median)					
N	8	13	9	16	38
Pre	6.1±1.9	5.3±1.3	6.1±1.3	5.8±2.4	5.7±1.8
Post	6.5±2.2	5.9±1.4	6.8±1.1	6.4±2.5	6.3±1.9
Change					
mean ± SD	0.36±0.53	0.62±0.83	0.73±1.05	0.62±0.57	0.65±0.77
median	0.06	0.21	0.44	0.58	0.43
% change					
mean ± SD	4.8±8.0	12.8±15.9*	14.5±21.0	11.9±8.5‡	12.9±14.4‡
median	0.83	3.8	10.8	12.5	11.5
Blood flow rate (mean mL/min ± SD and median)					
N	26	23	11	21	55
Pre	715±608	550±458	697±762	510±330	564±488
Post	648±486	546±415	812±816	566±264	607±480
Change					
mean ± SD	-66±281	-4±269	115±273	56±249	43±262
median	-33	-66	22	56	6
% change					
mean ± SD	-1±48	19±96	36±80	46±85*	33±88
median	-14	-14	12	22	2

*Within group p<0.05.
‡Within group p<0.001.

TABLE V - DUPLEX DOPPLER DETERMINED AVG BLOOD FLOW RATE

	Placebo n=28	Low n=24	Medium n=12	High n=25	All PRT-201 n=61
AVG blood flow rate (mean mL/min ± SD)					
4 wks	n=20 1226±618	n=20 1120±456	n=11 1463±859	n=15 1220±392	n=46 1235±564
3 mos	n=15 1456±575	n=13 1104±274	n=8 1136±434	n=11 1071±378	n=32 1101±344
6 mos	n=12 1199±414	n=11 1207±471	n=5 1126±726	n=12 1063±374	n=28 1131±471

AVG = arteriovenous graft.

increasing response with increasing dose (placebo-low-medium-high) was statistically significant (p=0.04). The increases in vein diameter and blood flow occurred primarily in the subgroup of patients with loop grafts (n=41, 47%). In patients with loop grafts, vein diameter changed by –2±1% (p=0.17) for the placebo group and 17±16% (p<0.0001, p=0.053 vs. placebo) for the all PRT-201 group, and blood flow increased 6±60% (p=0.77) for the placebo group and 68±111% (p<0.01, p=0.12 vs. placebo) for the all PRT-201 group. Table V summarizes AVG blood flow rate. By 4 weeks postsurgery, the average AVG blood flow by duplex ultrasonography was approximately 1200 mL/min in both the placebo and all PRT-201 groups. In those patients on hemodialysis at study entry, the AVGs began to be used at 4 weeks postsurgery and the proportion in use increased over time. The three most common reasons for nonuse were that the AVG surgery site had not healed sufficiently for use, the study site had a standard

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TABLE VI - RATES OF PROCEDURES TO RESTORE OR MAINTAIN AVG PATENCY

	Placebo n=28	Low n=24	Medium n=12	High n=25	All PRT-201 n=61
All procedures to restore or maintain patency (mean number per patient per year ± SD)					
Procedure days	2.3±3.3	1.5±1.9	2.1±1.9	2.1±2.7	1.9±2.2
Procedures	4.4±6.1	2.5±4.0	3.5±3.3	4.0±6.0	3.3±4.8
Thrombectomy or thrombolysis procedures (mean number per patient per year ± SD)					
Procedure days	1.9±2.8	0.7±1.2	1.3±1.5	1.3±2.7	1.1±2.0
Procedures	2.0±2.9	0.7±2.0	1.5±1.9	1.3±2.7	1.1±2.0

AVG = arteriovenous graft.

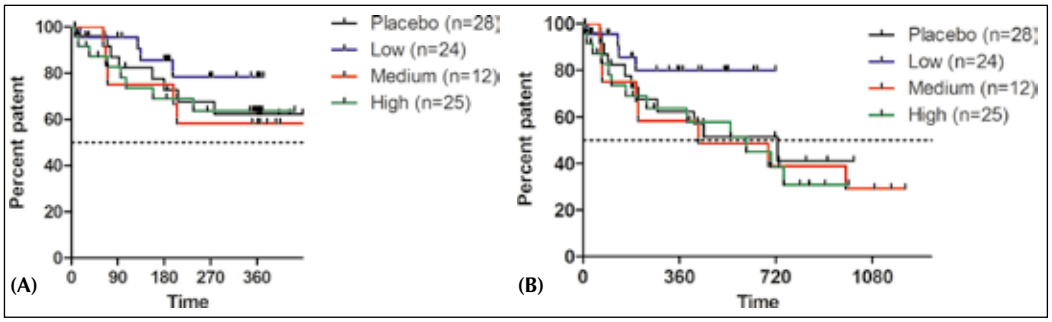

Fig. 2 - *Kaplan–Meier plot of secondary patency in main **(A)** and the main study combined with the registry **(B)**.*

protocol allowing use only after a certain time period following surgery or the AVG was no longer patent.

The proportions of patients with ultrasound criteria for hemodynamically significant stenosis at 4 weeks were 11%, 13%, 8% and 20% in the placebo, low, medium and high groups. The stenosis location was almost exclusively at the venous anastomosis, with the exception of one stenosis in the outflow vein in each of the placebo and the all PRT-201 groups. The proportions of patients with hemodynamically significant stenosis remained low at 3 and 6 months.

The majority of patients in each group lost primary unassisted patency. There were no significant differences between treatment groups for time to primary unassisted patency loss. Median primary unassisted patency days were 105, 173, 120 and 64 days in the placebo, low, medium and high groups, respectively. At 1 year 19% of patients in each group retained primary unassisted patency. In approximately 50% of each group, the initial primary unassisted patency loss events were associated with AVG thrombosis. In approximately 80% of each group, the initial primary unassisted patency loss event was treated with angioplasty, usually at the graft-vein anastomosis.

Table VI summarizes the rates of procedure days and procedures to restore or maintain patency and thrombectomy/thrombolysis procedures per patient per year at risk. Although the lowest rates were observed in the low group, there were no statistically significant differences between groups.

Figure 2A displays the time to loss of secondary patency by treatment group. There were no statistically significant differences between groups. The comparison of low vs. placebo yielded a p-value of 0.28. Among all patients, 21 of 25 secondary patency loss events were preceded by a thrombotic event. In the remaining four patients, three lost secondary patency due to infection and one lost secondary patency due to the AVG being too deep to cannulate. Figure 2B displays the time to loss of secondary patency by treatment group using combined data from the main study and registry. There was an apparent prolongation of secondary patency (hazard ratio 0.41) in the low group although this was not statistically significant (p=0.12). At the end of 2 years 51%, 80%, 39% and 39% of placebo, low, medium and high group patients, respectively, retained secondary patency. An analysis of time-to-multiple patency loss events, including thrombosis, procedures to restore or maintain patency and secondary patency loss events, yielded a hazard ratio of 0.70 in the low group (p=0.13) versus placebo.

Primary unassisted patency and secondary patency were examined by graft configuration (loop vs. nonloop). There were no significant differences between treatment groups for time to primary unassisted patency loss in either

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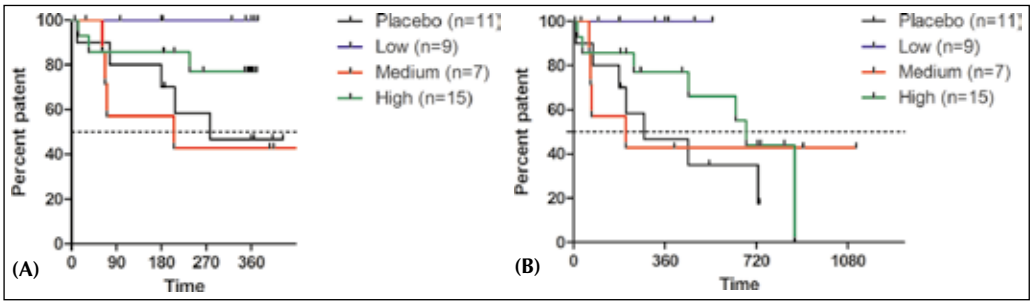

Fig. 3 - *Kaplan–Meier plots of secondary patency in the subset of patients with loop grafts in the main study **(A)** and the main study combined with the registry **(B)**.*

subgroup. Figure 3 displays the time to loss of secondary patency in the subset with loop graft configurations in the main study (Fig. 3A) and in the main study combined with the registry (Fig. 3B).

DISCUSSION

Among patients with interpretable pre- and post-treatment photographs, more PRT-201-treated patients met the vein diameter response criterion but the results were not statistically significant, possibly due to the small number of patients that could be analyzed. Measurement of outflow vein blood flow using a handheld flow probe proved more readily interpretable and more PRT-201-treated patients met the flow response criteria. There was also a significant increase in AVG blood flow, with a significant dose–response relationship supporting a pharmacological effect. The greatest increase in flow was observed in the subset of patients with loop grafts. In the loop graft configuration the arterial anastomosis is exposed to study drug and this may have contributed to the flow increase. Type I elastase has been shown to cause arterial smooth muscle cell relaxation (14). The observed acute increase in blood flow did not translate into relative increases in blood flow by ultrasound at 4 weeks or 3 and 6 months.

The proportions of patients with hemodynamically significant stenosis by duplex ultrasound were low at 4 weeks and 3 and 6 months. These data may be confounded by many patients having interventions such as balloon angioplasty prior to the ultrasound examinations.

The primary unassisted patency rates of the grafts in our study were similar to those of the Dialysis Access Consortium AVG trial, in which approximately 80% of patients lost primary unassisted patency in the course of a year (2). The rates of access-related procedures to restore or maintain patency tended to be lower in the low group but the difference was not significantly different. The low group had a lower proportion with secondary patency loss and those events occurred later in time. This is perhaps a consequence of needing fewer procedures, in particular fewer thrombectomies/thrombolysis procedures, to restore or maintain patency. Thrombotic events are the leading cause of AVG abandonment (1). The analysis that considered both primary and secondary patency loss events showed a 30% reduction in the risk of patency loss in the low group. This analysis approach may have advantages in this disease because the nature of AVG failure involves multiple patency loss events leading, ultimately, to secondary patency loss. Analysis by graft configurations suggested that PRT-201 had a greater effect in the subset of patients with loop grafts.

With regard to safety (the primary outcome measure), there were no signals that PRT-201 resulted in increases in systemic toxicity. The method of administration, a single, 10-minute application to the adventitia of the AVG venous anastomosis and adjacent outflow vein, minimizes the potential for systemic exposure. Any absorbed PRT-201 is likely to be immediately inactivated by antiproteases present in the blood (15). Local toxicity at the site of administration was observed at high doses (e.g., 50 mg) in the nonclinical animal studies. A similar percentage of patients in the placebo and PRT-201-treated groups reported adverse events at the site of administration and these adverse events were not dose-related. Two patients had delayed complications related to the graft-vessel anastomosis. One followed a number of endovascular procedures and the other was spontaneous. PRT-201 has been studied in seven nonclinical animal studies in dogs and swine where a single dose of PRT-201 ranging from 0.3 to 20 mg was applied to the venous anastomosis and outflow vein immediately after AVG creation, as was done in the clinical trial. There were no observed cases of leaking at the graft-artery or graft-vein anastomoses or pseudoaneurysms (data on file at Proteon). Larger studies will be required to further define the safety profile of PRT-201 in the treatment AVGs.

In summary, PRT-201 represents a novel approach to possibly prevent the high rate of failure of hemodialysis access. PRT-201 may improve secondary patency and reduce the number of procedures needed to restore or maintain patency in AVGs. The PRT-201 doses associated with this benefit have also been shown to prolong primary patency and reduce the number of procedures needed to restore

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or maintain patency in AVFs (10, 16). However, additional studies will be necessary to confirm these results.

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REFERENCES

1. NKF-KDOQI. Clinical practice guidelines for vascular Access. Am J Kidney Dis. 2006;48:S177-S247.
2. Dixon BS, Beck GJ, Vazquez MA, et al; DAC Study Group. Effect of dipyridamole plus aspirin on hemodialysis graft patency. N Engl J Med. 2009;360(21):2191-2201.
3. Maya ID, Oser R, Saddekni S, Barker J, Allon M. Vascular access stenosis: comparison of arteriovenous grafts and fistulas. Am J Kidney Dis. 2004;44(5):859-865.
4. Roy-Chaudhury P, Sukhatme VP, Cheung AK. Hemodialysis vascular access dysfunction: a cellular and molecular viewpoint. J Am Soc Nephrol. 2006;17(4):1112-1127.
5. Lee T, Roy-Chaudhury P. Advances and new frontiers in the pathophysiology of venous neointimal hyperplasia and dialysis access stenosis. Adv Chronic Kidney Dis. 2009; 16(5):329-338.
6. White JJ, Ram SJ, Jones SA, Schwab SJ, Paulson WD. Influence of luminal diameters on flow surveillance of hemodialysis grafts: insights from a mathematical model. Clin J Am Soc Nephrol. 2006;1(5):972-978.
7. Burke SK, Franano FN, Mendenhall H, et al. Recombinant human elastase (PRT-201) dilates outflow veins in a rabbit mode of arteriovenous fistula. J Am Soc Nephrol. 2008;18:34A.
8. Franano FN, Hance KA, Henry C, Bland K, Burke S. PRT-201 dilates outflow veins and improves maturation rates in a rabbit model of AVF. Nephrol Dial Transplant. 2007;22:vi155-vi156.
9. Burke SK, Wong MD, Madonald K, et al. Measurement of elastin content of human cephalic vein following treatment with PRT-201, a recombinant human type I pancreatic elastase. J Am Soc Nephrol. 2012;23:967A.
10. Peden EK, Leeser DB, Dixon BS, et al. A multi-center, dose-escalation study of human type I pancreatic elastase (PRT-201) administered after arteriovenous fistula creation. J Vasc Access. 2013;14(2):143-151.
11. Amabile PG, Wong H, Uy M, et al. *In vivo* vascular engineering of vein grafts: directed migration of smooth muscle cells by perivascular release of elastase limits neointimal proliferation. J Vasc Interv Radiol. 2002;13(7): 709-715.
12. Wong AH, Waugh JM, Amabile PG, Yuksel E, Dake MD. *In vivo* vascular engineering: directed migration of smooth muscle cells to limit neointima. Tissue Eng. 2002;8(2): 189-199.
13. Beldi G, Bosshard A, Hess OM, Althaus U, Walpoth BH. Transit time flow measurement: experimental validation and comparison of three different systems. Ann Thorac Surg. 2000;70(1):212-217.
14. Chew DK, Orshal JM, Khalil RA. Elastase promotes aortic dilation by inhibiting Ca2+ influx into vascular smooth muscle. J Cardiovasc Pharmacol. 2004;43(4):504-513.
15. Qamar AA, Burke SK, Lafleur JD, et al. The ability of serum from alpha 1-antitrypsin-deficient patients to inhibit PRT-201, a recombinant human type I pancreatic elastase. Biotechnol Appl Biochem. 2012;59(1):22-28.
16. Hye RJ, Peden EK, O'Connor TP, et al. Human type I pancreatic elastase treatment of arteriovenous fistulae in patients with chronic kidney disease. J Vasc Surg. 2014; in press.

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