Human type I pancreatic elastase treatment of arteriovenous fistulas in patients with chronic kidney disease

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Objective: **This study explored the safety and efficacy of recombinant type I pancreatic elastase (PRT-201) topically applied once to the external surface of an arteriovenous fistula.**
Methods: **This was a randomized, double-blind, placebo-controlled trial. Adults with kidney disease undergoing creation of a radiocephalic fistula (RCF) or brachiocephalic fistula were randomized to treatment with placebo (n = 51), PRT-201 at 10 μg (n = 51), or PRT-201 at 30 μg (n = 49). The primary efficacy measure was unassisted primary patency (PP) over 1 year. Secondary efficacy measures were secondary patency (SP), unassisted maturation by ultrasound interrogation, use for hemodialysis, and hemodynamically significant lumen stenosis.**
Results: **Median PP was 224 days for placebo and >365 days for the PRT-201 groups. At 1 year, 45%, 54%, and 53% of placebo, 10-μg, and 30-μg patients retained PP. The risk of PP loss was nonsignificantly reduced for 10 μg (hazard ratio [HR], 0.69; *P* = .19) and 30 μg (HR, 0.67; *P* = .17) vs placebo. In the subset (44% of patients) with a RCF, the median PP was 125 days for placebo and >365 days for the PRT-201 groups. At 1 year, 31%, 50%, and 63% of placebo, 10-μg, and 30-μg RCFs retained PP. The risk of RCF PP loss was nonsignificantly reduced by 10 μg (HR, 0.59; *P* = .18) and significantly reduced by 30 μg (HR, 0.37; *P* = .02) vs placebo. At 1 year, 77%, 81%, and 83% of placebo, 10-μg, and 30-μg patients retained SP. The risk of SP loss was nonsignificantly reduced for 10 μg (HR, 0.79; *P* = .61) and 30 μg (HR, 0.76; *P* = .55) vs placebo. In the subset with RCFs, 65%, 82%, and 90% of placebo, 10-μg, and 30-μg patients retained SP at 1 year. The risk of RCF SP loss was nonsignificantly reduced for 10 μg (HR, 0.45; *P* = .19) and 30 μg (HR, 0.27; *P* = .08) vs placebo. At month 3, 67%, 87% (*P* = .03), and 92% (*P* < .01) of the placebo, 10-μg, and 30-μg group fistulas had unassisted maturation by ultrasound interrogation. At month 3 in the subset with an RCF, 47%, 74% (*P* = .17), and 93% (*P* < .01) of placebo, 10-μg, and 30-μg group fistulas had unassisted maturation by ultrasound interrogation. Adverse event reports were not meaningfully different between groups.**
Conclusions: **PRT-201 appeared safe. The primary efficacy end point was not met. However, both PRT-201 doses were associated with improved unassisted maturation. The 30-μg dose was associated with increased PP in the subset with RCF. (J Vasc Surg 2014;60:454-61.)**

An arteriovenous fistula (AVF) is the most desirable form of vascular access for hemodialysis, resulting in the highest patency rate and the lowest complication rate. Unfortunately, 40% to 60% of AVFs will lose unassisted primary patency (PP) due to thrombosis or a procedure to restore or maintain patency within the first year after surgical creation, in part due to intimal hyperplasia induced by vascular injury at the time of surgery.[1-10] Patency loss is commonly managed with procedures such as thrombectomy and percutaneous transluminal angioplasty, but ~50% of AVFs that undergo intervention require a repeat procedure ≤12 months.[11-13] Secondary patency (SP) loss (abandonment) occurs in ~25% of AVFs within the first year after surgical creation, resulting

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in the need for surgical creation of a new permanent access.[4,7,8]

Porcine pancreatic elastase applied to the adventitia of arteries and veins after vessel injury has been shown to result in partial fragmentation of elastin and decreased neointimal hyperplasia by inhibiting cell migration to the intima, possibly due to the chemotactic properties of elastin fragments within the adventitia.[14,15] PRT-201 is a recombinant type I pancreatic elastase (molecular weight ~26 kD) that is expressed in human skin but not the pancreas.[16] PRT-201 is produced in the yeast *Komagataella phaffii* (formerly *Pichia pastoris*).

PRT-201 and other pancreatic elastases are inactivated by antiproteases present in blood, so to be effective, PRT-201 must be applied to the outside of blood vessels.[17] In animal pharmacology and toxicology studies, PRT-201 fragmented and removed elastin fibers from blood vessels in a time-dependent and concentration-dependent manner. In a phase 1 clinical trial, PRT-201 was applied topically to the external adventitial surface of newly created AVFs. Doses (3, 10, and 33 μg) that fragment elastin fibers in the vein adventitia were associated with prolonged AVF PP, fewer angioplasty procedures, and less AVF lumen stenosis.[18] These benefits were not observed at higher doses (100 μg to 9 mg) that fragment elastin fibers in the vein adventitia, media, and intima.

We report the findings of a phase 2 clinical study that compared the safety and efficacy of PRT-201 at 10 μg and 30 μg vs placebo topically administered at the time of AVF creation.

METHODS

Trial design. This was a randomized, double-blind, placebo-controlled, study of a single application of PRT-201. The protocol, informed consent form, and all amendments were reviewed and approved by each center's Institutional Review Board. A full list of participating centers can be found in the Appendix (online only). This study was conducted in accordance with the ethical principles originating from the Declaration of Helsinki and current Good Clinical Practices and in compliance with the Code of Federal Regulations (21 CFR 312). This trial was preregistered at www.clinicaltrials.gov (Identifier NCT01305824).

Participants. Patients were at least 18 years old with chronic kidney disease and were receiving maintenance hemodialysis or expecting to initiate maintenance hemodialysis ≤6 months and undergoing the creation of a radiocephalic fistula (RCF) or brachiocephalic fistula (BCF).

Interventions. Immediately after creation of the AVF, a 2.5-mL PRT-201 solution or placebo was topically delivered as a series of drops over 10 minutes to the exposed inflow artery, anastomosis, and outflow vein. Drug application was followed by lavage of the wound with saline for 1 minute. PRT-201 was supplied as a lyophilized powder in 5-mg vials that was reconstituted with phosphate-buffered saline with .01% polysorbate 80 and diluted to a final concentration of 4 or 12 μg/mL by an unblinded research pharmacist. PRT-201 and placebo (phosphate-buffered saline) were identical in appearance; both are clear nonviscous liquids that froth slightly if shaken.

Outcomes. Safety assessments and evaluation of the AVF were performed at 2 and 6 weeks, and at 3, 6, 9, and 12 months after study drug administration. Safety evaluations included ascertainment of adverse events, physical examinations, duplex ultrasound imaging, and laboratory studies. The primary efficacy end point was PP. The secondary efficacy end points included SP, unassisted maturation by ultrasound interrogation, use for hemodialysis, and hemodynamically significant lumen stenosis. A loss of PP was defined as the first occurrence of access thrombosis or a procedure performed to restore or maintain AVF patency (thrombectomy, thrombolysis, percutaneous transluminal angioplasty, stent placement, or surgical revision). A loss of SP was defined as AVF abandonment (ie, decision to place a new permanent access).

Duplex ultrasound imaging was performed at 6 weeks and 3 months using a standard protocol. All ultrasound examinations were reviewed by an independent and experienced core laboratory (VasCore, Boston, Mass) masked to treatment assignment and outcome. The core laboratory measured outflow vein lumen diameter and blood flow rate to assess maturation and the presence of hemodynamically significant stenosis in the AVF circuit.

Successful maturation was defined using the National Kidney Foundation (NKF) Kidney Disease Outcomes Quality Initiative (KDOQI) criteria (average cephalic vein lumen diameter ≥6 mm and an outflow vein blood flow rate ≥600 mL/min)[9] and also separately using the criteria published by Robbin et al[19] (average cephalic vein lumen diameter ≥4 mm and an outflow vein blood flow rate ≥500 mL/min). Unassisted maturation was defined as maturation with no prior procedure to restore or maintain patency.

Hemodynamically significant stenosis was defined as a peak systolic velocity (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. The PSV ratio was determined by dividing the PSV within the stenotic segment by the PSV in an adjacent proximal normal segment. Stenosis was categorized as being within or outside of the treatment zone, which included the anastomosis, the adjacent 2 cm of inflow artery, and the adjacent 5 cm of outflow vein. Results of the core laboratory interpretations were not shared with the study team or treating clinicians.

Statistical methods. The study planned for 150 to be randomly and equally allocated into one of three groups. It was assumed that the proportion with PP at the end of the study would be 50% in the placebo group and 80% in each of the PRT-201-treated groups, corresponding to a hazard ratio (HR) of 0.32. A two-sided, log-rank test at the $\alpha = .05$ level would have >80% power with 50 patients per group to detect this treatment effect.

The full analysis set was defined as all patients who received any amount of study agent. This was the primary analysis set for all analyses of efficacy and safety. Patients were analyzed according to the actual study drug received. PP time was estimated by the 25th, 50th (median), and

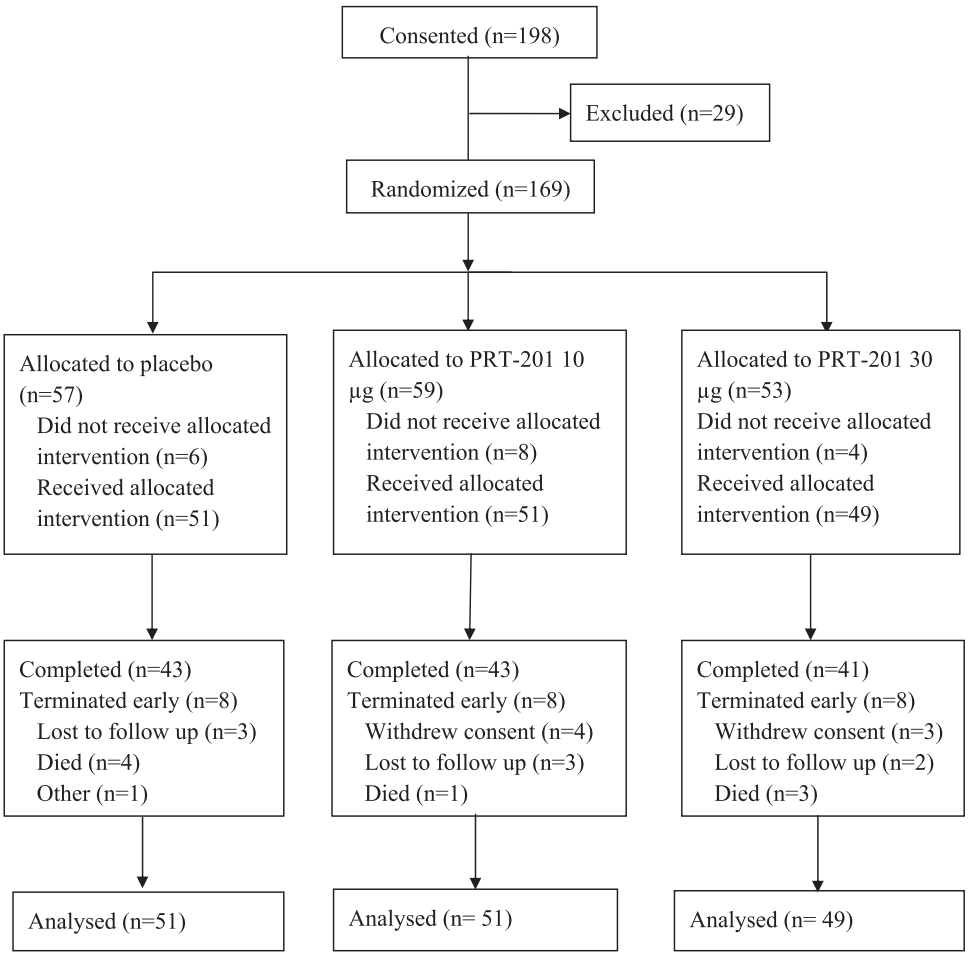

Fig 1. Patient flow through the study. *PRT-201,* Recombinant type I pancreatic elastase.

75th percentiles calculated by using the Kaplan-Meier life-test methods to estimate the survival functions. A log-rank test was used to test the equality of the survival curves between each PRT-201 dose vs placebo. A similar analysis was performed for SP. A proportional hazard model was used to explore important baseline covariates of age, sex, race, medical history (diabetes, vascular disease, hypertension, hypercholesterolemia, ischemic heart disease, peripheral artery disease, cardiovascular disease, renal transplant), dialysis status (predialysis or receiving dialysis), AVF type (RCF or BCF), type of anesthesia (general, local, nerve block), exposed vein and arteriotomy lengths (longer or shorter than median), and method of dilation (hydrostatic or mechanical) to assess their effect on PP.

The numbers and percentages of patients achieving assisted and unassisted maturation, assisted and unassisted use of the AVF for hemodialysis, requiring procedures to restore or maintain patency, and with hemodynamically significant stenosis were summarized. Each PRT-201 group vs placebo was tested using a Pearson's χ^2 test or the Fisher exact test (cell size <5) as appropriate. A rate of procedures to restore or maintain patency per person-year at risk was calculated as the total number of procedures per person-year on study. A Wilcoxon rank sum test was used to compare treatment groups.

Several additional analyses were performed after database lock. These included Kaplan-Meier analyses and Cox proportional hazards modeling of time to loss of PP in a subgroup omitting procedures directed at cephalic arch or central vein stenosis and Kaplan-Meier analyses of time to PP and SP loss, and procedure rates in the subgroups with RCF and BCF.

RESULTS

Participants. The study randomized 169 patients, and 151 were treated at 23 centers (range, 1-25 treated per center). The reasons for nontreatment were an access procedure other than RCF or BCF (11 patients), withdrawn consent (2 patients), enrollment closed (4 patients), and ineligible medical history (recent renal cell carcinoma, 1 patient). Of the 151 patients who were treated, 127 (84%) completed the study, and 24 (16%) discontinued before completion (Fig 1).

Baseline data. Table I summarizes baseline characteristics by treatment group. There were no significant

Table I. Baseline characteristics by treatment group

Variable	*Placebo (n = 51)*	*PRT-201*	
		10 μg (n = 51)	*30 μg (n = 49)*
Male, %	63	55	55
White, %	63	78	74
Age, mean ± SD, years	59 ± 15	59 ± 18	59 ± 15
≥65 years, %	35	45	31
BMI,[a] mean ± SD, kg/m^2	31 ± 8	31 ± 8	35 ± 8
RCF, %	47	45	41
IHD, %	49	59	57
PAD, %	29	20	22
CVD, %	18	22	22
Predialysis, %	57	55	71
CKD due to DM, %	39	43	55
CKD due to HTN, %	35	28	22
Duration CKD, mean ± SD, months	44 ± 44	54 ± 66	60 ± 75
Tobacco free, %	55	49	41
Local anesthesia, %	53	57	43
Nerve block, %	26	26	35
Fluid dilation, %	80	69	76
Mechanical dilation, %	37	35	31
Running sutures, %	94	94	96
Non-absorbable sutures, %	92	92	96
Exposed vein length, mean ± SD, cm	3.4 ± 1.0	3.5 ± .8	3.5 ± 1.0
Arteriotomy length mean ± SD, cm	0.9 ± 0.3	0.9 ± 0.3	0.8 ± 0.3

BMI, Body mass index; *CKD,* chronic kidney disease; *CVD,* cerebrovascular disease; *DM,* diabetes mellitus; *HTN,* hypertension; *IHD,* ischemic heart disease; *PAD,* peripheral artery disease; *PRT-201,* recombinant type I pancreatic elastase; *RCF,* radiocephalic fistula; *SD,* standard deviation.
[a]$P < .05$.

differences in baseline characteristics or surgical practices across randomized treatment groups, with the exception of body mass index, which was higher in the 30-μg group.

Safety. 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 creation. There were no statistically significant differences between the PRT-201 treatment groups and placebo. There were no meaningful dose-related increases in adverse events, which, as expected, were most frequently related to the surgery and were mainly mild, intermittent, and resolved ≤2 weeks. Of the 15 reported cases of steal syndrome, one in the 30-μg group was secondary to an arteriovenous graft and not related to the study AVF. Nine of the remaining 14 were graded as mild and required no treatment. Five were graded as moderate, and two of these were managed without intervention. The remaining three received banding of the outflow vein, ligation of the distal radial artery, and balloon angioplasty in one case each.

No significant findings were discovered during physical examinations or clinical laboratory testing, including chemistry, hematology, and coagulation panels. Pretreatment anti-PRT-201 antibodies were detected in four patients. Post-treatment anti-PRT-201 antibodies were detected in one placebo patient, three 10-μg patients, and two 30-μg patients. One of the patients in the 10-μg group was previously positive at baseline. The highest titer in any positive test was 1:2. Retests were performed at 6 months in one placebo patient, two 10-μg patients, and one 30-μg patient who had detectable post-treatment anti-PRT-201 antibodies, and all tests were negative.

Table II. Number and proportion of patients with commonly reported adverse events[a]

Event	*Placebo (n = 51), No. (%)*	*PRT-201*	
		10 μg (n = 51), No. (%)	*30 μg (n = 49), No. (%)*
Any adverse event	42 (82)	39 (77)	43 (88)
AVF thrombosis	13 (26)	8 (16)	7 (14)
Steal syndrome	7 (14)	2 (4)	6 (12)
Hypoesthesia	7 (14)	6 (12)	6 (12)
AVF incision pain	5 (10)	9 (18)	9 (18)
AVF site complication	5 (10)	4 (8)	4 (8)
Peripheral edema	5 (10)	0 (0)	2 (4)
Nausea	5 (10)	1 (2)	2 (4)
Arterial stenosis	4 (8)	5 (10)	0 (0)
Paresthesia	1 (2)	1 (2)	5 (10)
Erythema	1 (2)	1 (2)	5 (10)

AVF, Arteriovenous fistula; *PRT-201,* recombinant type I pancreatic elastase.
[a]Treatment of emergency adverse events occurring in at least 5% of placebo or the combined PRT-201 treatment groups.

Patency. Fig 2, *A* displays the Kaplan-Meier analysis of PP. The median PP time was 224 days in the placebo group and >365 days in the PRT-201 groups. At 1 year, 45%, 54%, and 53% of placebo, 10-μg, and 30-μg patients retained PP. The risk of PP loss was not significantly reduced vs placebo for 10 μg (HR, 0.69; 95% confidence interval [CI], 0.39-1.22; $P = .19$) or 30 μg (HR, 0.67; 95% CI, 0.38-1.19; $P = .17$).

In the placebo group, initial loss of patency was due to thrombosis (occlusion) in 12 patients and a procedure to restore or maintain patency in 15. In the 10-μg group, the initial loss of patency was due to thrombosis in seven patients and a procedure to restore or maintain patency in 14. In the 30-μg group, the initial loss of patency was due to thrombosis in seven patients and a procedure to restore or maintain patency in 13. Referral to an interventionalist resulting in a procedure to restore or maintain patency was due to clinical problems with the AVF or findings identified by routine AVF surveillance.

Fig 2, *B* displays the Kaplan-Meier analysis of PP in patients with a RCF. Median patency was 125 days in the placebo group and >365 days in the PRT-201 groups. At 1 year, 31%, 50%, and 63% of placebo, 10-μg, and 30-μg patients retained PP. The risk of PP loss was nonsignificantly reduced by 10 μg (HR, 0.59; 95% CI, 0.28-1.28; $P = .18$) and significantly reduced by 30 μg (HR, 0.37; 95% CI, 0.15-.91; $P = .02$) vs placebo. Fig 2, *C* displays the Kaplan-Meier analysis of PP in patients with a BCF. At 1 year, 57%, 58%, and 46% of placebo, 10-μg, and 30-μg

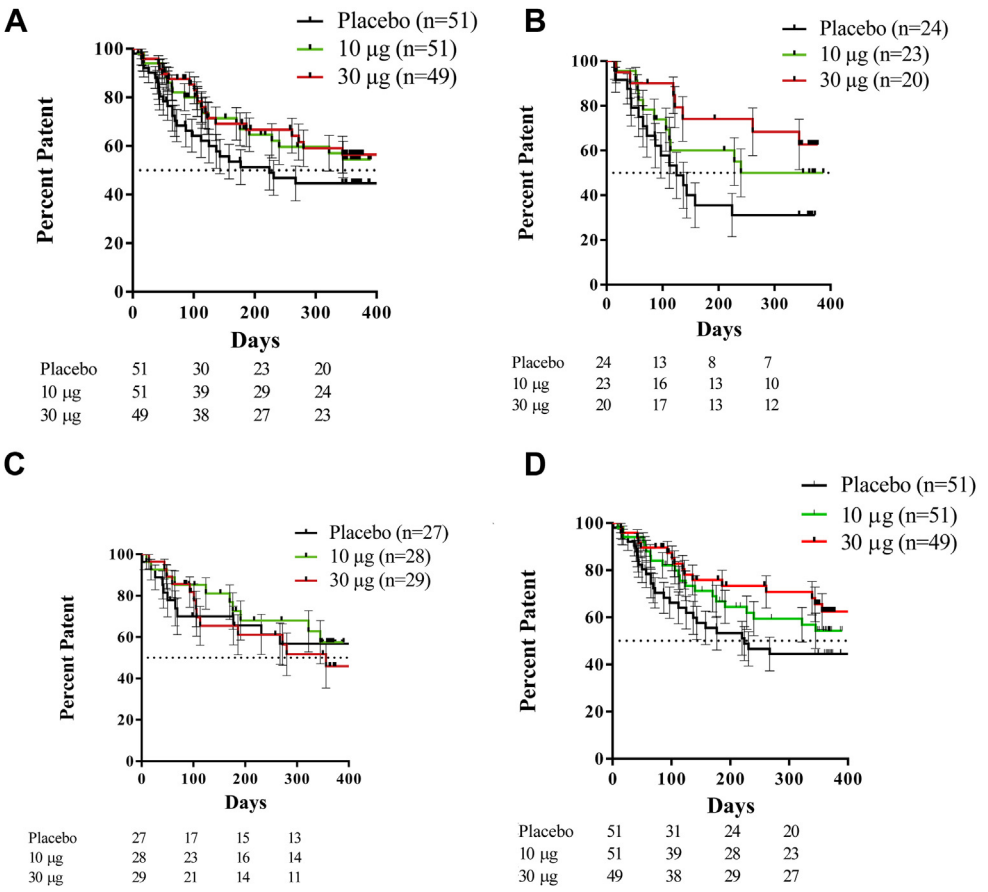

Fig 2. Kaplan-Meier plots (*error bars* show the standard error) of unassisted primary patency (PP) in **(A)** all patients, the subsets with **(B)** radiocephalic fistulas (RCFs) and **(C)** brachiocephalic fistulas (BCFs), and **(D)** all patients excluding procedures directed at the central veins or cephalic arch.

patients retained PP. Risk of PP loss was not different vs placebo for 10 µg (HR, 0.86; 95% CI, 0.36-2.02; $P = .72$) or 30 µg (HR, 1.1; 95% CI, 0.49-2.46; $P = .82$). If procedures directed at the cephalic arch or central vein stenosis were excluded, 68% of 30-µg patients retained PP at 1 year (HR, 0.74; 95% CI, 0.31-1.78; $P = .46$).

Cox proportional hazard modeling was performed with a number of demographic and intraoperative characteristics. For the comparison of the 10-µg and placebo groups, predialysis status at baseline, BCF, shorter exposed vein length, shorter arteriotomy length, and white race were associated with a decreased risk of PP loss. After adjusting for baseline differences in these characteristics, the HR for PP loss for the 10-µg group vs placebo was 0.76 (95% CI, 0.43-1.36; $P = .35$). For the comparison of the 30-µg and placebo groups, predialysis status at baseline, use of topical papaverine at the time of surgery, and shorter exposed vein length were associated with a decreased risk of PP loss. The use of general anesthesia was associated with an increased risk of PP loss. After adjusting for baseline differences in these characteristics, the HR for unassisted patency loss for the 30-µg group vs the placebo group was 0.59 (95% CI, 0.32-1.10; $P = .098$).

In an analysis of PP loss that excluded corrective procedures directed at the cephalic arch or central vein stenosis, the HR for PP loss was 0.69 for the 10-µg group (95% CI, 0.39-1.23; $P = .20$) and 0.52 for the 30-µg group (95% CI, 0.28-0.97; $P = .04$) vs placebo (Fig 2, *D*).

At 1 year, 77%, 81%, and 83% of placebo, 10-µg, and 30-µg patients retained SP. The risk of SP loss was not significantly reduced vs placebo for 10 µg (HR, 0.79; 95% CI, 0.33-1.92; $P = .61$) or 30 µg (HR, 0.76; 95% CI, 0.31-1.89; $P = .55$). In the subset with RCFs, 65%, 82%, and 90% of placebo, 10-µg, and 30-µg patients retained SP at 1 year. The risk of SP loss was not significantly different vs placebo for 10 µg (HR, 0.45; 95% CI, 0.14-1.51; $P = .19$) or 30 µg (HR, 0.27; 95% CI, 0.06-1.29; $P = .08$). For patients with BCFs, 88%, 79%, and 78% of placebo, 10-µg, and 30-µg patients retained SP at 1 year. The risk of SP loss was not significantly different vs placebo for 10 µg (HR, 1.71; 95% CI, 0.41-7.17; $P = .46$) or 30 µg (HR, 2.01; 95% CI, 0.50-8.04; $P = .32$).

Rate of procedures to restore or maintain patency per patient per year. At least one procedure to restore or maintain AVF patency was required in 41% of placebo patients, 36% of 10-µg patients, and 27% of 30-µg patients.

Table III. Overall rate of procedures to restore or maintain arteriovenous fistula (*AVF*) patency per patient per year on study and rate excluding procedures directed at cephalic arch (*CA*) or central vein (*CV*) stenosis

Variable	*Placebo (n = 51), mean ± SD*	*PRT-201 10 μg (n = 50), mean ± SD*	P[a]	*PRT-201 30 μg (n = 48), mean ± SD*	P[a]
All AVF	0.9 ± 1.2	0.8 ± 1.5	.53	0.4 ± 0.7	.07
All AVF excluding CA/CV	0.8 ± 1.2	0.7 ± 1.5	.44	0.2 ± 0.5	<.01
	(n = 24)	*(n = 23)*		*(n = 20)*	
RCF	1.0 ± 1.2	0.8 ± 1.3	.63	0.3 ± 0.6	.06
RCF excluding CA/CV	1.0 ± 1.2	0.8 ± 1.3	.63	0.3 ± 0.6	.06
	(n = 27)	*(n = 27)*		*(n = 28)*	
BCF	0.7 ± 1.2	0.7 ± 1.6	.72	0.4 ± 0.7	.50
BCF excluding CA/CV	0.7 ± 1.2	0.7 ± 1.6	.54	0.1 ± 0.4	.07

BCF, Brachiocephalic fistula; *PRT-201,* recombinant type I pancreatic elastase; *RCF,* radiocephalic fistula; *SD,* standard deviation.
[a]*P* value vs placebo.

Table III summarizes the rates of procedures to restore or maintain patency per patient per year. The data were also examined excluding procedures directed at the cephalic arch or a central vein stenosis, which occurred in 11 patients, 10 of whom had BCFs.

Unassisted maturation. Table IV summarizes the vein lumen diameter and blood flow data in all AVFs and in the subsets of RCFs and BCFs. Table V summarizes the proportion of patients with unassisted maturation at week 6 and month 3 by the NKF-KDOQI and Robbin et al criteria. There were no statistically significant differences in achievement of unassisted maturation between placebo and either active treatment group by either definition at week 6.

The proportion with unassisted maturation at month 3 by NKF-KDOQI criteria and by the Robbin et al criteria was significantly greater in the 30-μg group than in the placebo group. The proportion of patients with unassisted maturation at month 3 by the Robbin et al criteria was significantly greater in the 10-μg and 30-μg groups than in the placebo group. The percentages with assisted maturation at month 3 were 54%, 67%, and 70% by NKF-KDOQI criteria and 80%, 92%, and 92% by the Robbin et al criteria in the placebo, 10-μg, and 30-μg groups, respectively.

Among patients with RCFs, the proportion with unassisted maturation at week 6 according to the Robbin et al criteria was significantly greater in the 30-μg group (75%) than in the placebo group (36%; $P = .01$). The proportion of patients with unassisted maturation at month 3 was greater by NKF-KDOQI criteria and significantly greater by Robbin et al criteria in the 30-μg group than in the placebo group. The percentages with assisted maturation at month 3 were 29%, 42%, and 57% by NKF-KDOQI criteria and 59%, 84%, and 93% by the Robbin et al criteria in the placebo, 10-μg, and 30-μg groups, respectively, and there were no significant differences between groups.

Table IV. Duplex Doppler-determined arteriovenous fistula (*AVF*) outflow vein lumen diameter and blood flow rate

		PRT-201	
Fistula type	*Placebo*	*10 μg*	*30 μg*
All patients, No.	51	51	49
Vein lumen diameter, mm			
Week 6			
No.	42	43	44
Mean ± SD	6.1 ± 1.6	6.2 ± 1.7	6.7 ± 1.8
Month 3			
No.	38	37	37
Mean ± SD	6.7 ± 1.9	7.1 ± 2.2	7.3 ± 1.9
AVF blood flow rate, mL/min			
Week 6			
No.	41	39	40
Mean ± SD	1048 ± 546	991 ± 678	974 ± 648
Month 3			
No.	35	36	35
Mean ± SD	1084 ± 709	1203 ± 897	1181 ± 819
RCF, No.	24	23	20
Vein lumen diameter, mm			
Week 6			
No.	19	22	20
Mean ± SD	5.4 ± 1.6	5.2 ± 1.1	6.0 ± 1.8
Month 3			
No.	17	19	14
Mean ± SD	5.6 ± 2.0	6.1 ± 1.4	6.7 ± 1.6
AVF blood flow rate, mL/min			
Week 6			
No.	18	18	17
Mean ± SD	618 ± 376	684 ± 404	738 ± 260
Month 3			
No.	17	19	13
Mean ± SD	646 ± 530	790 ± 622	1157 ± 970
BCF, No.	27	28	29
Vein lumen diameter, mm			
Week 6			
No.	23	21	24
Mean ± SD	6.6 ± 1.3	7.2 ± 1.6	7.2 ± 1.6
Month 3			
No.	21	18	23
Mean ± SD	7.6 ± 1.3	8.2 ± 2.4	7.6 ± 2.0
AVF blood flow rate, mL/min			
Week 6			
No.	23	21	23
Mean ± SD	1384 ± 403	1254 ± 761	1149 ± 788
Month 3			
No.	18	17	22
Mean ± SD	1498 ± 606	1665 ± 946	1195 ± 741

BCF, Brachiocephalic fistula; *PRT-201,* recombinant type I pancreatic elastase; *RCF,* radiocephalic fistula; *SD,* standard deviation.

Use for hemodialysis. During the course of follow-up, 70% of patients received hemodialysis, and the percentages of those patients with unassisted usability were 53%, 49%, and 69% for the placebo, 10-μg, and 30-μg groups. The percentages of patients with assisted usability were 76%, 74%, and 83% for the placebo, 10-μg, and 30-μg groups. None of the differences among groups were

Table V. Unassisted maturation—percentage

Fistula type	*Placebo*	*PRT-201 10 μg*	P[a]	*PRT-201 30 μg*	P[a]
Week 6					
All types					
No.	47	46		46	
NKF-KDOQI,[9] %	43	41	.90	50	.47
Robbin et al,[19] %	60	67	.43	76	.09
RCF					
No.	22	22		20	
NKF-KDOQI,[9] %	23	18	.48	45	.08
Robbin et al,[19] %	36	50	.17	75	.01
BCF					
No.	25	24		26	
NKF-KDOQI,[9] %	60	62	1.0	54	.78
Robbin et al,[19]%	80	83	1.0	77	1.0
Month 3					
All types					
No.	39	39		37	
NKF-KDOQI,[9] %	46	64	.11	70	.03
Robbin et al,[19] %	67	87	.03	92	<.01
RCF					
No.	17	19		14	
NKF-KDOQI,[9] %	24	37	.48	57	.08
Robbin et al,[19] %	47	74	.17	93	<.01
BCF					
No.	22	20		23	
NKF-KDOQI,[9] %	64	90	.07	78	.34
Robbin et al,[19] %	82	100	.11	91	.41

BCF, Brachiocephalic fistula; *NKF-KDOQI,* National Kidney Foundation Kidney Disease Outcomes Quality Initiative; *PRT-201,* recombinant type I pancreatic elastase; *RCF,* radiocephalic fistula.
[a]*P* value vs placebo.

significant, with the exception of unassisted usability in the subset of patients with a BCF, in which there was a significantly ($P = .03$) higher percentage of patients with unassisted usability in the 30-μg group (82%) vs the placebo group (50%).

Hemodynamically significant lumen stenosis. At week 6, hemodynamically significant stenosis in the treatment zone was observed in 51%, 30%, and 39% of the placebo, 10-μg, and 30-μg groups ($P = .048$ for 10 μg vs placebo). At month 3, hemodynamically significant stenosis in the treatment zone was observed in 40%, 41%, and 35% of the placebo, 10-μg, and 30-μg groups. There were no differences in hemodynamically significant stenosis among groups at month 3.

DISCUSSION

PRT-201 appeared safe and was associated with a 33% reduction in the HR for loss of PP in the 30-μg group, but this was not statistically significant. The study was powered to detect a 70% reduction in the HR in the overall population but was not powered to detect differences in the subset of patients by AVF type. Treatment with PRT-201 was associated with a significant dose-dependent improvement in unassisted maturation by duplex ultrasound imaging, and patients treated with 30-μg also exhibited a trend for fewer procedures to restore or maintain patency. SP was well maintained but not different between the groups.

In the subgroup of patients who received a RCF, 30-μg was associated with a 63% reduction in the HR for loss of PP and a trend for improved SP. There was also a dose-dependent improvement in unassisted maturation assessed by duplex ultrasound imaging.

The KDOQI Guidelines favor placement of RCFs, which preserve vascular territory and have lower rates of arm swelling, steal syndrome, and cephalic arch stenosis.[9] However, RCFs often fail to mature, and recent literature indicates that up to 70% lose PP ≤1 year after surgical creation.[6,7,9] PRT-201 may have shown greater benefit in RCF because stenosis occurs predominantly in the perianastomotic area, which is within the treatment zone of PRT-201.[20,21] BCFs suffer from stenosis that often manifests in other locations.[20,21] RCFs are also more likely to suffer SP loss.[7] For patients receiving hemodialysis, AVF abandonment requires placement of a catheter for dialysis, typically followed by surgical placement of a new AVF or arteriovenous graft.

A weakness of the current study is the small number of patients per dose group and in the subsets by fistula type. However, the results were consistent across a number of related end points and suggest that the 30-μg dose is more effective than the 10-μg dose and that the greatest benefits of PRT-201 treatment are observed in the subset with RCFs. The secondary analyses were considered exploratory and were not corrected for multiple testing.

CONCLUSIONS

The results of the current study suggest that PRT-201 applied to the periadventitial surface of an AVF at the time of surgical creation is safe and may result in improved maturation and PP in RCFs. These results will need to be confirmed in larger, appropriately powered studies.

Pamela Gustafson, Francesca Lindow, Missy Magill, and Daniel Gottlieb contributed to study design, protocol development, study operations, data analysis, and report writing. Drs James Kaufman, Michael Gaziano, and Dirk Hentschel were members of the Safety Review Committee. The work of many subinvestigators, study coordinators, research pharmacists, and administrators at the investigative sites made this study possible.

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Conception and design: SB, BD, LD, MJ, EP
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Data collection: RH, EP, TO, BB, BD, AS, SJ, MJ, SB
Writing the article: RH, SB
Critical revision of the article: EP, TO, BB, BD, AS, SJ, MJ
Final approval of the article: RH, SB
Statistical analysis: SB
Obtained funding: SB
Overall responsibility: SB

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INVITED COMMENTARY

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The purpose of this randomized, double-blind, placebo-controlled clinical trial was to test the hypothesis that topically applied human pancreatic elastase would assist maturation and extend the patency and usefulness of dialysis-access arteriovenous fistulas (AVFs). The authors and entrepreneurs are to be congratulated for their novel and ambitious approach to the difficult problem of hemodialysis access, as well as for their revealing and aspirational trial. Dependable maturation of AVFs remains a significant clinical challenge, and to date, there are no available chemical strategies designed to improve outflow vein remodeling. Should this strategy ultimately prove efficacious, it would represent a significant advance in a challenging field of medicine.

Unfortunately, the investigators overestimated the potential benefit of their therapy, because their prespecified primary end point of a 30% absolute improvement in 1-year primary patency was not achieved. The negative trial results will certainly present regulatory challenges, and further study will undoubtedly be required to secure approval. However, because the primary patency of the control, low-dose, and high-dose groups was 45%, 54%, and 53%, respectively, a positive effect of the drug appeared to be demonstrated. Thus, the study was not a failure; it was simply underpowered to demonstrate the 10% effect size of the experimental intervention.

Certainly, a simple maneuver, such as intraoperative elastase application, would be clinically useful and widely applied if it reliably provided a 10% increase in patency. Moreover, there were positive trends favoring the experimental groups in all metrics, most notably for primary patency in radiocephalic AVFs and in "maturation-by-3-months" in all groups. Improvements in the number and rapidity by which radiocephalic AVFs matured into useful conduits would be welcome, indeed.

The concept that topically applied human pancreatic elastase can assist the maturation of outflow veins in dialysis access AVFs has been demonstrated. This bold trial should be viewed as a mechanistic and clinical success and this development group should muster the will and financial resources to continue their important line of research.

APPENDIX (online only). Participating centers

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118	Vincent Scavo, MD	Indiana/Ohio Heart, Fort Wayne, Ind
119	Eric Ladenheim, MD	Ladenheim Dialysis Access Centers
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122	Barry Browne, MD	California Institute Renal Research, San Diego, Calif
123	Andrew Duda, MD	Sparrow Clinical Research Institute, Lansing, Mich
124	Timothy O'Connor, MD	Renal Care Associates, Peoria, Ill
125	Robert Hye, MD	Kaiser Permanente, San Diego, Calif
126	Andres Schanzer, MD	University of Massachusetts Medical Center, Worcester, Mass
127	Larry Scher, MD	Montefiore Medical Center, Bronx, NY
128	Michael Conte, MD	University of California, San Francisco, San Francisco, Calif
130	Michael Moritz, MD	Lehigh Valley Hospital, Allentown, Pa
131	Earl Schuman, MD	Legacy Oregon Surgical, Portland, Ore
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