TYPE 1 DIABETES: PATHOPHYSIOLOGY AND PREVENTION

ISPAD WILEY

Human plasma-derived alpha$_1$-proteinase inhibitor in patients with new-onset type 1 diabetes mellitus: A randomized, placebo-controlled proof-of-concept study

William H. Lagarde[1] | Kecia L. Courtney[2] | Barry Reiner[3] | Kimberly Steinmann[4] | Eva Tsalikian[5] | Steven M. Willi[6]

[1]Division of Pediatric Endocrinology, Department of Pediatrics, WakeMed Children's Hospital, Raleigh, North Carolina, USA

[2]Clinical Development, Grifols Therapeutics Inc. Research Triangle Park, North Carolina, USA

[3]Barry J Reiner, MD, LLC, Baltimore, Maryland, USA

[4]Clinical Development, Grifols Therapeutics Inc. Research Triangle Park, North Carolina, USA

[5]Pediatric Endocrinology and Diabetes, Stead Family Department of Pediatrics, University of Iowa Carver College of Medicine, Iowa City, Iowa, USA

[6]Department of Endocrinology and Diabetes, Children's Hospital of Philadelphia, Department of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania, USA

Correspondence
Steven M. Willi, Department of Endocrinology and Diabetes, Children's Hospital of Philadelphia, Department of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, 3500 Civic Center Blvd; Room 12-179, Philadelphia, PA 19104.
Email: willi@email.chop.edu

Present address
Kecia L. Courtney, Biogen, Research Triangle Park, North CarolinaUSA

Funding information
Grifols Biotherapeutics Inc, Clinical Development, Durham, NC, USA

Abstract

Background: While circulating levels of alpha$_1$-proteinase inhibitor (alpha$_1$-PI) are typically normal, antiprotease activity appears to be compromised in patients with Type 1 diabetes mellitus (T1DM). Because alpha$_1$-PI [human] (alpha$_1$-PI[h]) therapy can inhibit pro-inflammatory mediators associated with β-cell destruction and reduced insulin production, it has been proposed for T1DM disease prevention. The aim of this study was to evaluate safety, tolerability, and efficacy of intravenous (IV) alpha$_1$-PI[h] in preserving C-peptide production in newly diagnosed T1DM patients.

Participants: Seventy-six participants (aged 6–35 years) were randomized at 25 centers within 3 months of T1DM diagnosis.

Methods: A Phase II, multicenter, partially blinded, placebo-controlled, proof-of-concept study evaluating four dosing regimens of alpha$_1$-PI[h] (NCT02093221, GTI1302): weekly IV infusions of either 90 or 180 mg/kg, each for either 13 or 26 weeks. Safety and efficacy were monitored over 52 weeks with an efficacy evaluation planned at 104 weeks. The primary efficacy endpoint was change from baseline in the 2-h area-under-the-curve C-peptide level from a mixed-meal tolerance test at

52 weeks. A battery of laboratory tests, including inflammatory biomarkers, constituted exploratory efficacy variables.

Results: Infusions were well tolerated with no new safety signals. All groups exhibited highly variable declines in the primary outcome measure at 52 weeks with no statistically significant difference from placebo. Interleukin-6 (IL-6) was reduced from baseline in all alpha$_1$-PI treatment groups but not the placebo group.

Conclusion: Pharmacologic therapy with alpha$_1$-PI[h] is safe, well tolerated, and able to reduce IL-6 levels; however, due to variability in the efficacy endpoint, its effects on preservation of C-peptide production were inconclusive.

KEYWORDS

alpha 1-proteinase inhibitor, C-peptide, Interleukin-6, prolastin, Type 1 diabetes

1 | INTRODUCTION

Type 1 diabetes mellitus (T1DM) is the clinical manifestation of an autoimmune disease that results in the loss of pancreatic β-cells with a corresponding decrease in endogenous insulin production.[1] Daily exogenous insulin injections are required to maintain glycemic control and reduce the risk of long-term complications, such as nephropathy, neuropathy, and retinopathy.[2] Despite improvements in monitoring and treatment, the number of patients achieving American Diabetes Association (ADA) treatment goals continues to be suboptimal,[3] and the search for alternative treatment paradigms continues.

Even modest residual β-cell function is associated with improved glycemic control and reduced long-term complications.[4] Hence, research efforts to identify disease-modifying treatments aim to replace, regenerate, or protect β-cell function and insulin secretion.[5] To date, no strategy has achieved this outcome with satisfactory results.[4]

Alpha$_1$-proteinase inhibitor (alpha$_1$-PI) is a serine proteinase inhibitor that has recently become a research focus for diabetes due to its anti-inflammatory activities.[6-8] Patients with T1DM have been found to have lower circulating alpha$_1$-PI concentrations or reduced anti-inflammatory alpha$_1$-PI activity compared to those without diabetes.[9-11] Encouraging results from preclinical studies with alpha$_1$-PI have demonstrated its ability to attenuate cell-mediated autoimmunity, restore euglycemia, and prevent overt T1DM in nonobese diabetic mice.[12-14] Furthermore, alpha$_1$-PI prolongs graft survival and euglycemia in nonobese diabetic mice receiving islet cell transplantation.[12,15-20]

The efficacy of therapy with alpha$_1$-PI [human] (alpha$_1$-PI[h]) in T1DM has not yet been examined in a placebo-controlled clinical trial. In nonplacebo-controlled clinical studies, alpha$_1$-PI[h] was well tolerated in patients with T1DM without significant safety findings.[21-23] Efficacy studies have suggested increased C-peptide secretion (a marker for insulin release) in response to a meal stimulus and reduced expression of genes involved with nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation and apoptosis pathways.[22,23]

The present placebo-controlled, Phase II, proof-of-concept study was designed to examine the safety and tolerability of intravenous (IV) alpha$_1$-PI[h] in patients with newly diagnosed T1DM and evaluate its effect on insulin release over 2 years. In addition, steady-state pharmacokinetic parameters were assessed in a subgroup of participants.

2 | METHODS

2.1 | Study design

This was a multicenter, randomized, partial-blinded, five-arm, placebo-controlled study to evaluate the safety and efficacy of four dosing regimens of IV alpha$_1$-PI[h] in children (6–11 years old) and teens/adults (12–35 years old) with new onset T1DM in the United States (Clinicaltrials.gov identifier: NCT02093221, protocol GTI1302).

The study was designed to last a maximum of 105 weeks, consisting of a ≤7-day screening period and a 13- or 26-week treatment period followed by 39- or 26-week safety and efficacy follow-up period (total of 52 weeks), and an efficacy follow-up extension period of up to an additional 52 weeks to evaluate the durability of treatment effect (Figure 1). Pharmacokinetics (PK) of IV alpha$_1$-PI at steady state was assessed in 15 participants at Weeks 13 and 15 participants at Week 26.

2.2 | Participants

Procedures were approved by the Institutional Review Board/Ethics Committee of each participating research center. The study was conducted in accordance with the Declaration of Helsinki, including written informed consent from each subject or guardian.

Enrolled participants were between 6 and 35 years of age and received a diagnosis of T1DM according to ADA criteria[24] within 3 months prior to randomization. The first 25 subjects enrolled (circa 5 participants per treatment arm) were between 12 and 35 years of age. Enrollment was opened to children aged 6–11 years after demonstration of acceptable safety/tolerability (see Section 2.5). Other major inclusion criteria included current use of injected insulin therapy and at least one positive result on testing for antibodies against islet
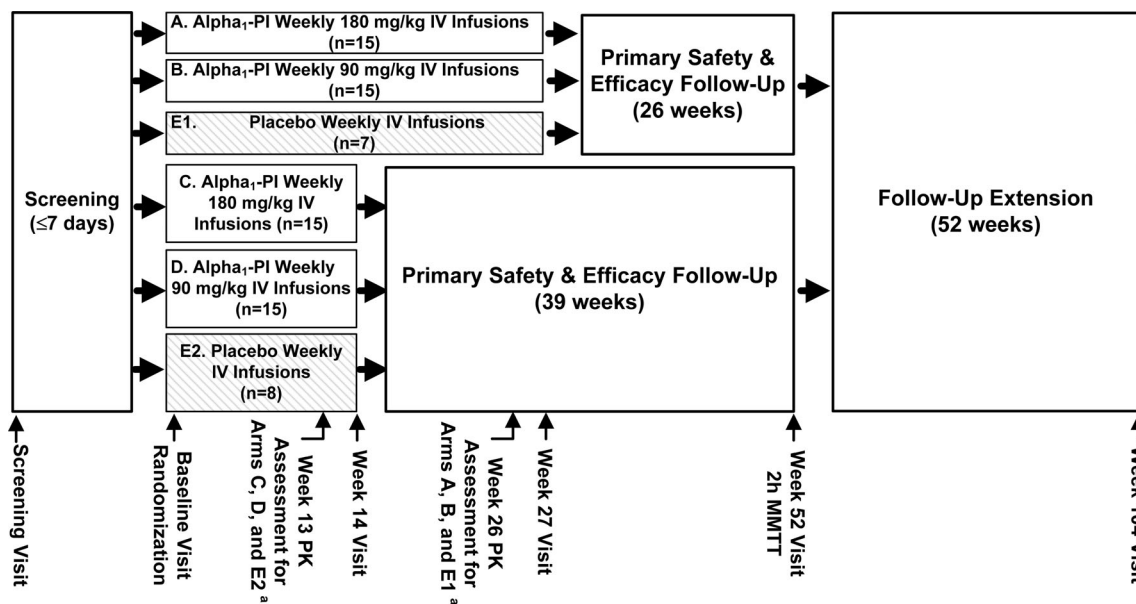
FIGURE 1 Study design. [a]Blood samples for total serum alpha1-PI concentration were collected from approximately 30 patients, consisting of at least three teens/adults (ages 12–35 years old) and at least three children (ages 6–11 years old) from each treatment arm, to evaluate the steady state PK of IV-administered alpha1-PI. Alpha1-PI, alpha1-proteinase inhibitor (Prolastin-C); PK, pharmacokinetics

cell antigen 512, insulinoma-associated protein 2 (IA-2), glutamic acid decarboxylase (GAD), or insulin (unless having received insulin for >7 days). If not currently on insulin therapy, then positive results for ≥2 of the above listed antibodies were required. Body mass index (BMI) was required to be ≤28 kg/m^2 for adults (aged ≥20 years) or ≤90% for children and teens.

Main exclusion criteria included any medical condition that might confound results or pose a risk to participants, including diabetic retinopathy, neuropathy, nephropathy, or known thrombophilia. Use of medications that could confound results was not permitted, including exenatide or any antidiabetic agents other than insulin within 1 month before screening, and chronic use of systemic steroids (inhaled steroids were acceptable) above a stable dose equivalent to 5 mg/day prednisone (e.g., 10 mg every 2 days) within the 4 weeks before randomization. A full listing of inclusion/exclusion criteria is provided in Supplementary List 1.

Participants were randomized into one of five treatment arms, based on a computer-generated 1:1:1:1:1 randomization schedule issued to an unblinded pharmacist or designee. Randomization was stratified by age group (children versus teen/adults). Within the 26-week treatment groups and the 13-week treatment groups, treatment was fully blinded and placebo-controlled. For blinding purposes, the placebo arm was split into the two durations (26 and 13 weeks) of weekly infusions.

PK of IV alpha$_1$-PI[h] were assessed in 30 participants, consisting of at least 3 teens/adults and 3 children from each treatment arm.

2.3 | Treatments

All randomized participants received standard-of-care treatment for their diabetes as defined by the ADA. Alpha$_1$-PI[h] (commercially available as PROLASTIN-C, Grifols Therapeutics LLC, NC) is a sterile, stable, lyophilized preparation of purified human alpha$_1$-PI indicated for chronic augmentation and maintenance therapy in adults with clinical evidence of emphysema due to severe hereditary deficiency of alpha$_1$-PI.[25] Reconstituted alpha$_1$-PI[h] contains no preservatives, has a pH of 6.6–7.4, and is normally administered as indicated as a weekly IV infusion of 60 mg/kg. Weekly doses in the present study were 90 mg/kg, based on a previous study in patients with T1DM,[23] and a higher exploratory dose of 180 mg/kg, based on anticipated blood concentrations required to suppress humoral and cellular immune response markers as observed in ex vivo studies with fresh blood from T1DM patients.[23] The placebo used in this study was 0.9% sodium chloride for injection (United States Pharmacopeia).

The study pharmacist, or designee, was the only unblinded study personnel at each center. All participants received the same total infusion volume for all treatments with no visible differences in the external aspects of treatments (infusion bags were covered with a nontransparent sleeve and IV tubing was opaque).

2.4 | Measurements

The primary efficacy endpoint was the change from baseline in the 2-h area under the curve (AUC) for C-peptide after a mixed-meal tolerance test (MMTT) at 52 weeks after randomization. C-peptide was chosen as the primary measure of efficacy because it provides a sensitive and clinically validated assessment of β-cell function in patients with Type 1 diabetes, even in the presence of exogenous insulin therapy.[26] Blood draws for serum C-peptide (and glucose levels) were collected during each MMTT. The measurements of C-peptide and glucose in serum samples were performed in a central laboratory.

Secondary efficacy variables included change from baseline in MMTT-stimulated C-peptide 2-h AUC at Weeks 14, 27, 39, 69, 87, and 104; glycosylated hemoglobin (HbA1c) levels at 14, 27, 39, 52, 69, 87, and 104 weeks after randomization; mean daily exogenous insulin dose (units/kg/day) requirements from the 3–7 days prior to each visit; mean daily glucose levels prior to meals and at bedtime from the 3–7 days prior to each visit; and the number of patient-reported severe hypoglycemic episodes from baseline through Week 104. Severe hypoglycemia was defined according the ADA Workgroup on Hypoglycemia definition as an event requiring assistance of another person to actively administer carbohydrates, glucagon, or other resuscitative actions.[27] Insulin use and blood glucose levels, along with meal times, were recorded daily by glucometer or insulin pump. A composite efficacy endpoint of insulin dose-adjusted A1C (IDAA1C), defined as A1C (percent) + (4 × insulin dose [units per kilogram per 24 h]), was also analyzed as a proxy for remission.[28]

Secondary PK variables included $AUC_{0-7 \text{ days}}$, $AUC_{0-21 \text{ days}}$, AUC_{0-inf}, mean trough serum total alpha$_1$-PI, maximum serum alpha$_1$-PI concentration (C_{max}), time to reach C_{max} (T_{max}), terminal half-life ($t_{1/2}$), clearance volume (CL), and volume of distribution (V_d). Participants who underwent PK assessment had Week 26 or Week 13 infusions and serial blood sampling up to 8-h postinfusion and at additional time points through Day 21 (Supplementary List 2).

Speculative and exploratory efficacy variables (Supplementary List 3) included biomarkers at baseline and Weeks 14, 27, 39, 52, 69, 87, and 104. Biomarkers included humoral and cellular immune response markers (including interleukin [IL]-1β, IL-6, and tumor necrosis factor [TNF]-α), and apoptosis biomarkers (Caspase 1, Caspase 3).

Primary safety variables included treatment-emergent adverse events (AEs), adverse drug reactions (all AEs that have a definite, probable, possible or doubtful/unlikely causal relationship), serious AEs (SAEs), discontinuations due to an AE, temporally associated AEs (during/within 72 h of investigational product infusion), arterial or venous thrombotic and thromboembolic AEs, clinical laboratory parameters (hematology, chemistry, urinalysis), and vital signs (heart rate, blood pressure, respiratory rate, and temperature).

2.5 | Statistical analyses

Clinical considerations were used to estimate 15 subjects per treatment arm (total enrollment of 75 participants) in this study. No formal calculations of statistical power were made, as this was a proof-of-concept study and not designed to be fully powered, but to observe potential trends. The 26-week and 13-week placebo groups were combined for all analyses.

Primary efficacy analyses compared each active treatment arm to placebo by using analysis of covariance (ANCOVA) with treatment as a fixed factor and baseline value of MMTT-stimulated C-peptide 2-h AUC and age as covariates. No adjustments for multiplicity were considered. Treatment effects were analyzed by Pearson Chi-square test in a two-way frequency table.

Efficacy analyses were based on all participants randomized (intent-to-treat [ITT] population) and all randomized participants without any major protocol deviations relevant to the analysis (i.e., per-protocol [PP] population). If any randomized participants dropped out early with missing data for MMTT-stimulated C-peptide 2-h AUC at Week 52, a last observation carried forward (LOCF) method was used. For the longitudinal measurements of MMTT-simulated C-peptide 2-h AUC, the treatment effects were explored by using the mixed-effect model repeated measures. Safety analyses were based on all randomized participants who received any amount of investigational product (safety population).

Analysis of PK parameters was based on the PK population, which included a subset of participants who were selected for steady state PK evaluation, had received investigational product and had sufficient and valid serum concentration data to facilitate calculation of PK parameters.

Safety and tolerability data were reviewed prior to expanding enrollment to subjects aged 6–11 years. Unblinded interim analyses of efficacy, biomarker, PK, and safety data were to be conducted after all participants reached steady state at Week 5 of dosing, after all participants finished study treatment, and after all participants completed the Week 52 visit.

3 | RESULTS

3.1 | Participant characteristics

Of 76 participants enrolled/randomized (ITT population) at 25 centers, all but one participant received study treatment (safety population; Figure 2). In total, four participants discontinued prior to completing treatment and an additional six discontinued prior to Week 52. A list of sites involved in the study is shown in Supplementary List 4.

The study was terminated by the study sponsor at the planned unblinded interim analysis of efficacy after all participants completed the Week 52 visit due to inconclusive primary efficacy results. Fewer than half of the randomized participants completed the Week 104 follow-up visit due to premature study termination. For all treatment groups, treatment compliance, infusion compliance, and overall compliance were good, ranging from 80% to 120%.

Participant demographics and other baseline characteristics were generally well balanced across the treatment groups (Table 1). Mean age of participants was similar among treatment groups (16.8 years overall). Distribution of participants across age categories was also similar across treatment groups, though the range of BMI within each treatment group was wide because of the wide range of ages. Consistent with the demographics for T1DM, most participants (96.1%) identified as white (93.4% as exclusively white; 2.6% as multiple race), which was similarly represented across the groups. Most patients (88.2%) were ethnically not Hispanic or Latino.

All participants had endogenous levels of alpha$_1$-PI considered to be normal, ranging from 0.9 to 2.3 g/L. No clinically meaningful differences in mean baseline alpha$_1$-PI concentrations, stimulated
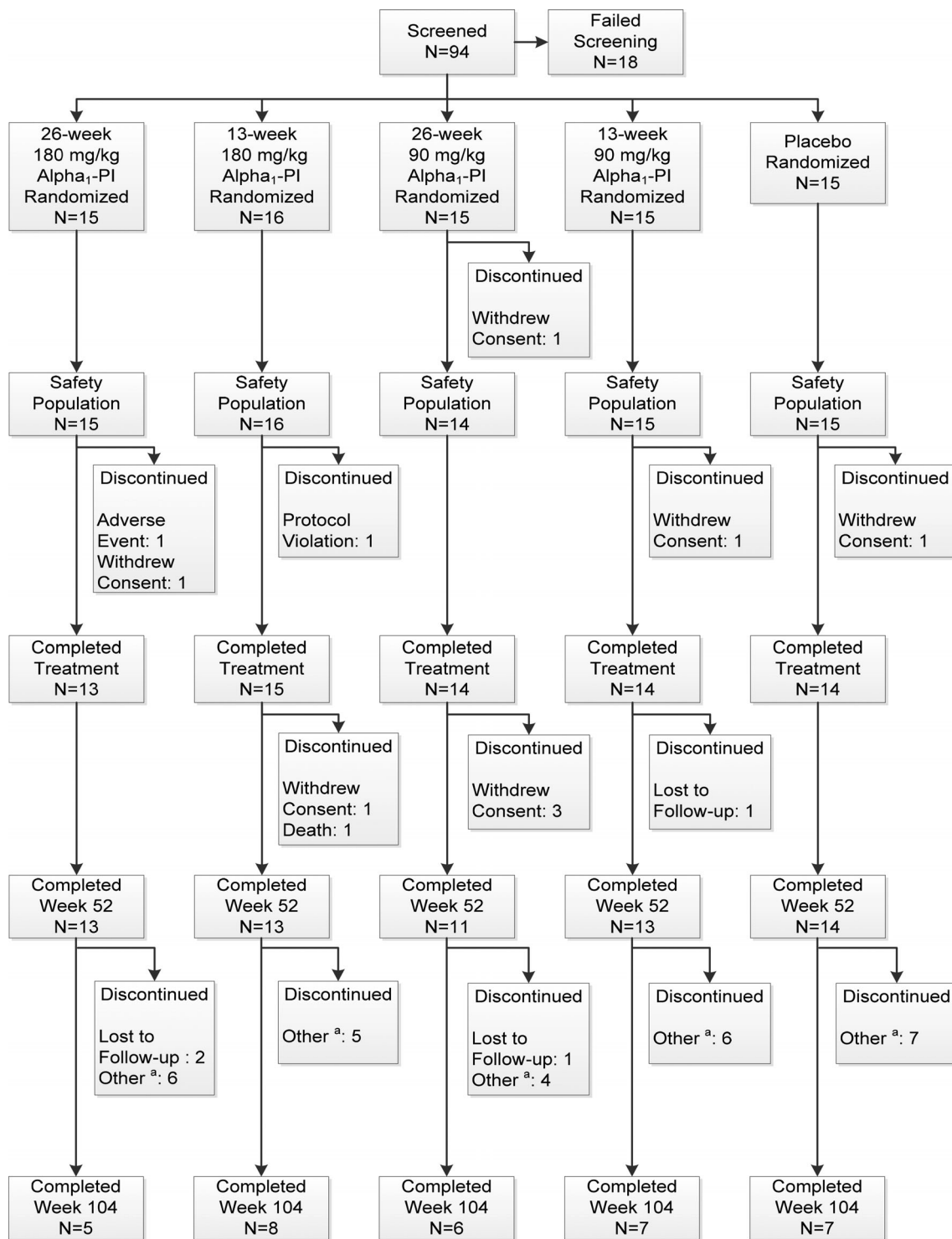
FIGURE 2 Patient disposition. [a]Other = study terminated by sponsor because planned follow-up to determine the duration of drug response was unnecessary owing to no apparent response at Week 52. Alpha1-PI, alpha1-proteinase inhibitor (Prolastin-C)

C-peptide levels, or HbA1c values were noted among treatment groups (Table 1). Overall, the mean time since diagnosis of T1DM was approximately 2 months and over 35% of participants had a family history of T1DM. GAD antibodies were present in 85.5% of participants at baseline, and no major differences were noted among treatment groups in the number or type of antibodies present.

3.2 | Efficacy

3.2.1 | Primary outcome variable

At Week 52, all treatment groups displayed a numerical decline in MMTT-stimulated C-peptide 2-h AUC from baseline (Figure 3);

TABLE 1 Demographics and baseline characteristics (ITT population)

	26-week 180 mg/kg Alpha$_1$-PI[h] n = 15	13-week 180 mg/kg Alpha$_1$-PI[h] n = 16	26-week 90 mg/kg Alpha$_1$-PI[h] n = 15	13-week 90 mg/kg Alpha$_1$-PI[h] n = 15	Placebo n = 15	Total N = 76
Male, n (%)	9 (60.0)	8 (50.0)	6 (40.0)	11 (73.3)	11 (73.3)	45 (59.2)
Age (years), mean (SD)	17.9 (7.0)	15.7 (6.1)	16.9 (6.6)	16.7 (5.5)	16.7 (4.1)	16.8 (5.8)
Age category (years), n (%)						
6–11	2 (13.3)	3 (18.8)	3 (20.0)	3 (20.0)	2 (13.3)	13 (17.1)
12–17	7 (46.7)	7 (43.8)	7 (46.7)	7 (46.7)	6 (40.0)	34 (44.7)
18–35	6 (40.0)	6 (37.5)	5 (33.3)	5 (33.3)	7 (46.7)	29 (38.2)
Ethnicity, n (%)						
Hispanic or Latino	3 (20.0)	1 (6.3)	3 (20.0)	2 (13.3)	0	9 (11.8)
Not Hispanic or Latino	12 (80.0)	15 (93.8)	12 (80.0)	13 (86.7)	15 (100.0)	67 (88.2)
Race, n (%)[a]						
White	15 (100.0)	15 (93.8)	15 (100.0)	13 (86.7)	15 (100.0)	73 (96.1)
Black or African American	0	2 (12.5)	0	2 (13.3)	0	4 (5.3)
Asian	1 (6.7)	0	0	0	0	1 (1.3)
Height (cm), mean (SD)	163.0 (11.7)	160.5 (16.7)	163.1 (14.4)	168.7 (15.7)	171.1 (20.2)	165.2 (16.1)
Weight (kg), mean (SD)	55.9 (15.1)	52.0 (15.4)	59.5 (23.2)	60.5 (15.5)	66.5 (19.8)	58.8 (18.2)
BMI (kg/m^2), mean (SD)	20.8 (3.9)	19.7 (3.1)	21.6 (4.9)	201.0 (3.3)	22.1 (3.1)	21.0 (3.7)
BMI Z score, mean (SD)						
<19 years old	−0.29 (1.13)	−0.45 (0.92)	0.12 (0.85)	0.25 (1.10)	0.31 (0.95)	−0.02 (1.23)
≥19 years old	0.06 (0.73)	−0.38 (0.70)	0.48 (0.05)	−0.39 (0.90)	0.31 (0.48)	0.02 (0.72)
Alpha$_1$-PI (g/L), mean (SD)[b]	1.31 (0.33)	1.38 (0.34)	1.36 (0.21)	1.20 (0.16)	1.22 (0.17)	1.29 (0.25)
C-peptide (nmol/L), mean (SD)[c]	0.22 (0.12)	0.22 (0.09)	0.21 (0.08)	0.26 (0.11)	0.23 (0.10)	n/a
MMTT-stimulated C-peptide 2 h AUC (min × nmol/L), mean (SD)	63.47 (35.69)	65.48 (34.19)	72.75 (36.64)	58.21 (25.34)	62.46 (27.75)	n/a
HbA1c (%), mean (SD)	8.27 (2.08)	8.89 (2.25)	8.05 (1.83)	9.12 (1.791)	8.74 (2.34)	n/a
Time since diagnosis (months), mean (SD)	2.08 (0.74)	2.01 (0.82)	1.97 (0.78)	1.86 (0.70)	1.85 (0.87)	1.96 (0.77)
Family history of T1DM—yes, n (%)	4 (26.7)	6 (37.5)	4 (26.7)	6 (40.0)	7 (46.7)	27 (35.5)
Antibodies present, n (%)						
IA-2	9 (60.0)	8 (50.0)	9 (60.0)	7 (46.7)	9 (60.0)	42 (55.3)
GAD antibodies	13 (86.7)	13 (81.3)	11 (73.3)	14 (93.3)	14 (93.3)	65 (85.5)
Anti-insulin antibodies	4 (26.7)	4 (25.0)	5 (33.3)	3 (20.0)	4 (26.7)	20 (26.3)
Concomitant medications, n (%)						
Any	8 (53.3)	14 (87.5)	14 (100.0)	14 (93.3)	12 (80.0)	62 (81.6)
Corticosteroids	1 (6.7)	1 (6.3)	3 (21.4)	0	0	5 (6.6)

Abbreviations: Alpha$_1$-PI[h], alpha$_1$-proteinase inhibitor [human] (Prolastin-C); AUC, area under the curve; BMI, body mass index; GAD, glutamic acid decarboxylase; HbA1c, glycosylated hemoglobin; IA-2, insulinoma-associated protein 2; ITT, intent-to-treat; MMTT, mixed-meal tolerance test; n/a, not available; T1DM, Type 1 diabetes mellitus.

[a]Multiple race was counted multiple times to each detailed race.

[b]One patient in the 13-week high dose Alpha$_1$-PI treatment group not assessed; n = 15.

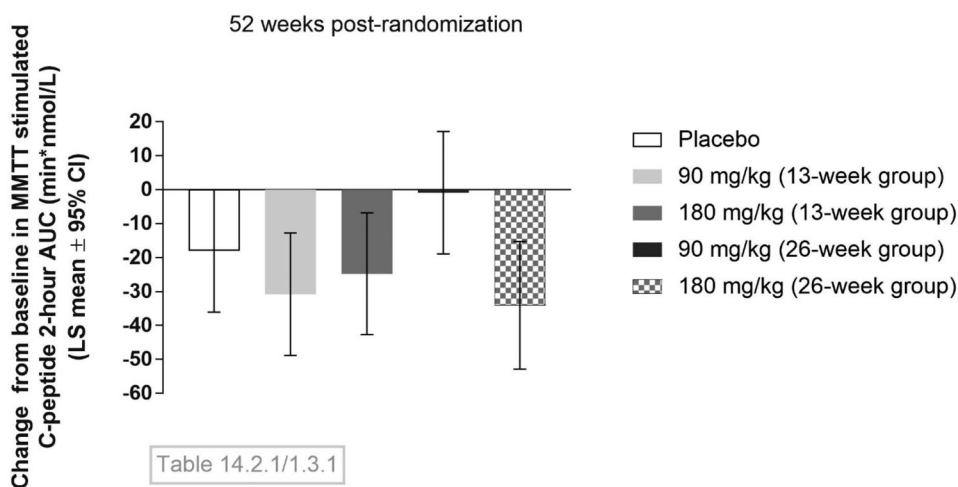[c]Calculated as average of −10 min (pre-drink) and 0 min.

FIGURE 3 Change from baseline in MMTT-stimulated C-peptide 2-hour AUC at Week 52: Analysis using ANCOVA with baseline AUC and age as covariates using LOCF method (ITT population). ANCOVA, analysis of covariance; AUC, area under the curve; CI, confidence interval; ITT, intent-to-treat; LOCF, last observation carried forward; LS, least square; MMTT, mixed-meal tolerance test

however, there was considerable variability. No statistically significant change was observed for alpha$_1$-PI[h] treatment groups relative to placebo at Week 52 using the ANCOVA for the ITT population with LOCF. Results were similar with the PP population (data not shown). C-peptide levels were below the 0.2 pmol/ml threshold for measurement in four participants, though post hoc analysis excluding these participants suggested this did not affect the trial outcome (data not shown).

3.2.2 | Secondary variables

No statistically significant changes in any of the secondary variables were observed compared to placebo. Mean change from baseline values for the 2-h AUC of MMTT-stimulated C-peptide levels decreased steadily in all treatment groups between Weeks 14 and 104, except for the 26-week 90 mg/kg dose group, which exhibited increasing values through Week 27, that declined thereafter (Supplementary Figure 1). At all time points, the placebo group showed the greatest numerical mean decrease in HbA1c levels from a baseline value that was similar to the other groups (Table 1). Otherwise, the recorded HbA1c levels, mean daily insulin dose, or mean daily glucose level were unremarkable (Table S1). Similarly, post hoc analysis of IDAA1C classified as ≤9 ("partial remission") or >9 showed no clear pattern (Table S2).[29]

Severe hypoglycemic episodes during the study (baseline to Week 104) were infrequent, affecting only five participants), and no alpha$_1$-PI[h] treatment group had a statistically significant difference in their incidence relative to placebo. Four participants reported one episode (one in the 26-week, high-dose group; two in the 13-week, high-dose group; and one in the placebo group) and one participant in the 26-week, low-dose group reported three episodes.

3.2.3 | Exploratory variables

IL-6 was numerically decreased in all treatment groups at Weeks 39 and 52 except in the placebo treatment group (Figure 4). All

alpha$_1$-PI[h] treatment groups had mean decreases in IL-1β that were numerically greater than decreases in the placebo treatment group at Week 52; however, all treatment groups (including placebo) showed variability in IL-1β over the course of the study and no trend was observed (Table S3).

No consistent differences were noted between groups for the rest of exploratory efficacy variables (Supplementary List 3).

3.3 | PK subgroup analysis

Alpha$_1$-PI PK exhibited dose proportionality for adjusted AUC$_{0-7 days}$, adjusted AUC$_{0-21 days}$, adjusted AUC$_{0-inf}$, adjusted C$_{max}$, CL, and mean trough levels (Table S4). AUC$_{0-7 days}$, AUC$_{0-21 days}$, and mean trough levels were notably higher in all the alpha$_1$-PI[h] treatment groups compared to placebo (data not shown). No meaningful differences among treatment groups were noted for $t_{1/2}$ values. Alpha$_1$-PI concentrations were maintained during dosing and returned toward baseline after the treatment was stopped at 13 or 26 weeks (Figure 5).

3.4 | Safety

Assessment of AEs showed no consistent treatment-related pattern for any event. The most common AEs included headache, nasopharyngitis, upper respiratory tract infection, and vomiting (Table S5). Considering the small number of participants in the study and the events reported, the frequencies of AEs overall were similar among treatment groups.

No thrombotic or thromboembolic events were reported. Most AEs were either mild or moderate in severity with three being considered as severe. One participant (26-week high-dose group) discontinued treatment due to a nonserious, moderate AE (migraine), which was considered possibly related to investigational product.

SAEs were reported for three participants, all in the 13-week high-dose group, (one patient each: suicide, spontaneous abortion,

FIGURE 4 Change from baseline in interleukin (IL)-6: intent-to-treat (ITT) population. A. 13-week groups. B. 26-week groups. Placebo group is common to both groups
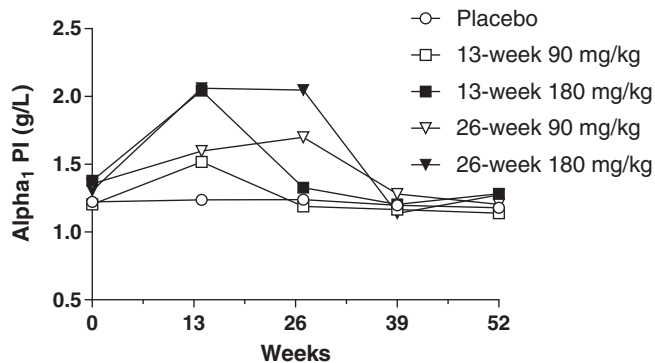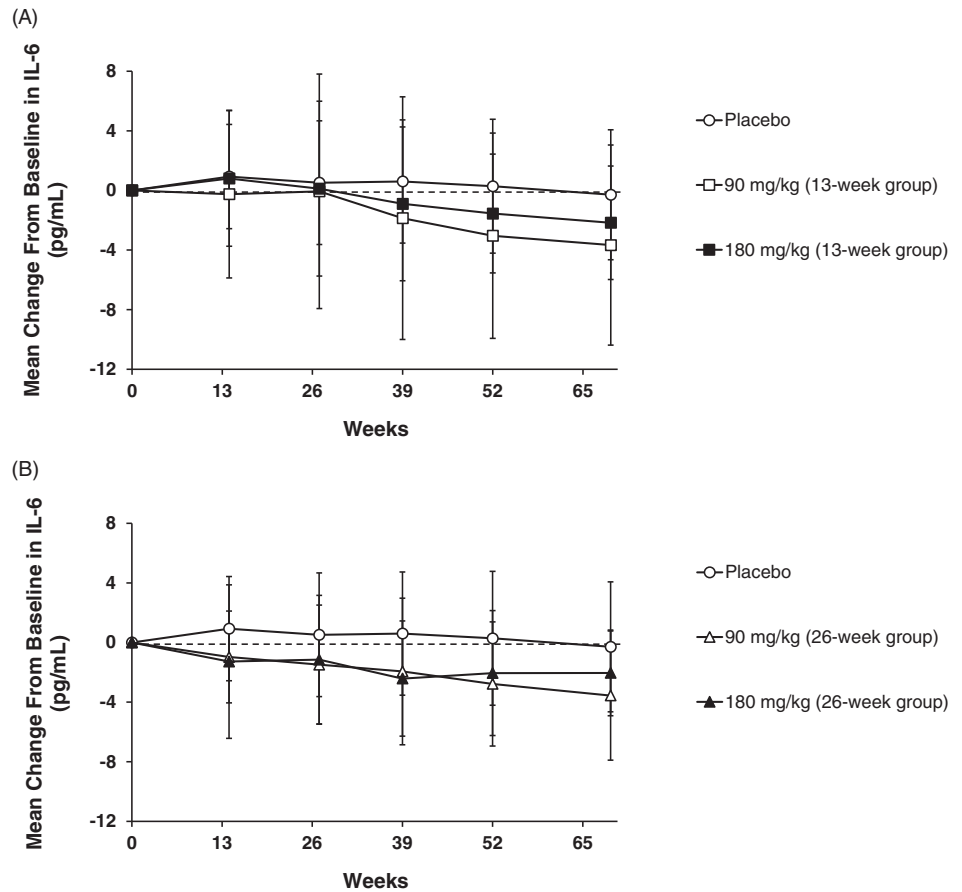

FIGURE 5 Alpha1-PI concentrations over 52 weeks. Alpha1-PI, alpha1-proteinase inhibitor (Prolastin-C)

and hyperglycemia) with causality assessed as unrelated to the investigational product.

No meaningful differences among treatment groups were noted in the frequencies of possibly related AEs. No AEs were reported to have a definite relationship to investigational product. Two possibly related AEs (noncardiac chest pain and dyspnea, occurring in the same participant in the 13-week low-dose alpha$_1$-PI[h] treatment group) were reported as having a probable causal relationship to investigational product. Changes from baseline values in hematology, clinical chemistry, urinalysis parameters, and vital signs were small in all

treatment groups, with no clinically meaningful differences between treatment groups.

4 | DISCUSSION

We examined the hypothesis that administration of IV alpha$_1$-PI[h] in T1DM patients with normal alpha$_1$-PI concentrations would preserve β cell function over 1 year compared to placebo-treated patients. Preclinical studies of TIDM showed alpha$_1$-PI induced immune tolerance, facilitated the expansion of insulin-secreting transplanted islets, and preserved β cells in nonobese diabetic mice with new onset, overt diabetes.[12-14] Administering alpha$_1$-PI prolonged islet allograft survival after islet transplantation in mice and murine cellular immunity appeared to be modulated.[16-19] Furthermore, alpha$_1$-PI attenuated the production of inflammatory mediators involved in cell aging (such as IL-6 and IL-8) in drosophila and human cell lines.[30]

The current placebo-controlled, proof-of-concept clinical study demonstrated no improvement in β-cell function among T1DM patients with normal alpha$_1$-PI concentrations for the dose levels of alpha$_1$-PI[h] tested. Efficacy was assessed from residual C-peptide production in response to stimulation (primary), with subordinate efficacy measurements from concentrations of HbA1c, daily glucose, daily insulin requirements, and insulin dose adjusted HbA1c. Alpha$_1$-PI [h] treatment was associated with numerical decreases in IL-6 in all

treatment groups except for placebo. Exploratory measurements of other inflammatory markers were unremarkable.

This study is the first placebo-controlled trial of alpha$_1$-PI[h] use in T1DM. In a small uncontrolled clinical study in recently diagnosed T1DM children and adolescents (n = 24), alpha$_1$-PI at doses of up to 80 mg/kg over 28 weeks was associated with reduced HbA1c from 8.4% to 7.1% and peak C-peptide of ≥0.2 pmol/mL in more patients than compared with historical controls.[22] Another uncontrolled study in recently diagnosed adults and children with T1DM (n = 16), suggested alpha$_1$-PI[h] administered for 6 weeks at up to 90 mg/kg may have reduced C-peptide secretion in some patients.[23] In fresh blood from T1DM patients not included in the latter trial, alpha$_1$-PI added ex vivo inhibited secretion of IL-6 and IL-8 and reduced the expression of the genes involved with NF-κB activation and apoptosis pathways.[23] Over 50% inhibition of these processes was seen when alpha$_1$-PI concentrations were >2 g/l.[23]

These results suggest that alpha$_1$-PI[h] doses higher than 90 mg/kg may be needed to suppress TNF-α and NF-κB pathways.[22,23] In the present study, mean alpha$_1$-PI concentrations of >2 g/l were observed in the 180 mg/kg dose group during the dosing period, but this concentration was not sustained throughout the study period; hence, even higher doses may be required. Importantly, weekly infusions had demonstrable dose–response effects on alpha$_1$-PI, with dose proportionality for several PK parameters. Treatment increased concentrations of alpha$_1$-PI in subjects with normal baseline concentrations, thereby demonstrating the ability of weekly alpha$_1$-PI [h] infusions to boost alpha$_1$-PI levels above the physiologic range.

Mean IL-6 concentrations decreased in all alpha$_1$-PI[h] treatment groups at weeks 39 and 52, whereas increases were seen in the placebo group at these timepoints. Although the observed differences were not statistically significant, these data point to an anti-inflammatory effect of Alpha$_1$-PI[h] in T1DM, an important observation as increased IL-6 levels are implicated in the pathogenesis of T1DM.[31] Future studies of alpha$_1$-PI[h] treatment of inflammatory conditions like T1DM should continue to explicate these potential anti-inflammatory effects.

Weekly alpha$_1$-PI[h] infusions as high as 180 mg/kg for up to 26 weeks were well tolerated in this study, with no new safety signals. Alpha$_1$-PI[h] products licensed for alpha$_1$-PI deficiency have an established long-term safety profile, with doses as high as 250 mg/kg administered every 4 weeks for up to 1 year.[32-34] Experience from other clinical studies in T1DM have also suggested alpha$_1$-PI [h] products are well tolerated in patients with diabetes without any significant additional safety findings.[21-23] Taken together, tolerance and safety are unlikely to be a barrier to future studies investigating doses higher than 180 mg/kg which may be necessary to fully evaluate efficacy.

Study limitations include the small sample size of this proof-of-concept study, which prohibited an adequate power level to establish statistical significance or demonstrate efficacy based on primary or secondary endpoints. In particular, the observed variability in the primary outcome measure (stimulated C-peptide production) was too large to discern any differences. However, this degree of variability is representative of trials in this field.[35] Continued research that leads to the development of a less capricious measure would be helpful for future studies. Undoubtedly, the transient partial remission period ("honeymoon phase") reported to occur within 3–12 months after starting insulin treatment[28,36] may have contributed to the variability of C-peptide levels. The influence of this variability might have been reduced by block randomization within age strata, in light of the established relationship between age at presentation and the rate of loss in β-cell function and C-peptide production.[37] However, such complex randomization schemes would be inappropriate for a proof of concept study such as this. Extending the study beyond 12 months might also help to reduce the confounding influence of this variability in the natural history of C-peptide production. The study of patients at high risk for the development of T1D (i.e., individuals with "Phase 2" Type 1 diabetes) constitutes another sound strategy for assessing the therapeutic potential of this compound.

In summary, weekly infusions of alpha$_1$-PI[h] at up to 180 mg/kg for 26 weeks was well tolerated; however, this Phase II, proof-of-concept study was inconclusive regarding the clinical benefit of alpha$_1$-PI[h] supplementation in T1DM because of the small number of subjects evaluated. Larger trials with consideration to reducing within-group variability could better address this question.

AUTHOR CONTRIBUTIONS

All authors had access to the study data and contributed equally to the development of this article. All authors have read and approved the final manuscript.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/pedi.13162.

ORCID

Steven M. Willi 🔗 https://orcid.org/0000-0002-4910-2425

REFERENCES

1. Atkinson MA, Maclaren NK. The pathogenesis of insulin-dependent diabetes mellitus. *N Engl J Med.* 1994;331(21):1428-1436.

2. Maahs DM, West NA, Lawrence JM, Mayer-Davis EJ. Epidemiology of type 1 diabetes. *Endocrinol Metab Clin North Am*. 2010;39(3):481-497.

3. Cheung BM, Ong KL, Cherny SS, Sham PC, Tso AW, Lam KS. Diabetes prevalence and therapeutic target achievement in the United States, 1999 to 2006. *Am J Med*. 2009;122(5):443-453.

4. Steffes MW, Sibley S, Jackson M, Thomas W. Beta-cell function and the development of diabetes-related complications in the diabetes control and complications trial. *Diabetes Care*. 2003;26(3):832-836.

5. Tooley JE, Waldron-Lynch F, Herold KC. New and future immunomodulatory therapy in type 1 diabetes. *Trends Mol Med*. 2012;18(3):173-181.

6. Bergin DA, Hurley K, McElvaney NG, Reeves EP. Alpha-1 antitrypsin: a potent anti-inflammatory and potential novel therapeutic agent. *Arch Immunol Ther Exp (Warsz)*. 2012;60(2):81-97.

7. Breit SN, Wakefield D, Robinson JP, Luckhurst E, Clark P, Penny R. The role of alpha 1-antitrypsin deficiency in the pathogenesis of immune disorders. *Clin Immunol Immunopathol*. 1985;35(3):363-380.

8. Churg A, Dai J, Zay K, et al. Alpha-1-antitrypsin and a broad spectrum metalloprotease inhibitor, RS113456, have similar acute anti-inflammatory effects. *Lab Invest*. 2001;81(8):1119-1131.

9. Hashemi M, Naderi M, Rashidi H, Ghavami S. Impaired activity of serum alpha-1-antitrypsin in diabetes mellitus. *Diabetes Res Clin Pract*. 2007;75(2):246-248.

10. Lisowska-Myjak B, Pachecka J, Kaczynska B, Miszkurka G, Kadziela K. Serum protease inhibitor concentrations and total antitrypsin activity in diabetic and non-diabetic children during adolescence. *Acta Diabetol*. 2006;43(4):88-92.

11. Sandler M, Gemperli BM, Hanekom C, Kuhn SH. Serum alpha 1-protease inhibitor in diabetes mellitus: reduced concentration and impaired activity. *Diabetes Res Clin Pract*. 1988;5(4):249-255.

12. Koulmanda M, Bhasin M, Hoffman L, et al. Curative and beta cell regenerative effects of alpha1-antitrypsin treatment in autoimmune diabetic NOD mice. *Proc Natl Acad Sci USA*. 2008;105(42):16242-16247.

13. Lu Y, Tang M, Wasserfall C, et al. Alpha1-antitrypsin gene therapy modulates cellular immunity and efficiently prevents type 1 diabetes in nonobese diabetic mice. *Hum Gene Ther*. 2006;17(6):625-634.

14. Song S, Goudy K, Campbell-Thompson M, et al. Recombinant adeno-associated virus-mediated alpha-1 antitrypsin gene therapy prevents type I diabetes in NOD mice. *Gene Ther*. 2004;11(2):181-186.

15. Koulmanda M, Bhasin M, Fan Z, et al. Alpha 1-antitrypsin reduces inflammation and enhances mouse pancreatic islet transplant survival. *Proc Natl Acad Sci U S A*. 2012;109(38):15443-15448.

16. Lewis EC, Mizrahi M, Toledano M, et al. Alpha1-antitrypsin monotherapy induces immune tolerance during islet allograft transplantation in mice. *Proc Natl Acad Sci USA*. 2008;105(42):16236-16241.

17. Lewis EC, Shapiro L, Bowers OJ, Dinarello CA. Alpha1-antitrypsin monotherapy prolongs islet allograft survival in mice. *Proc Natl Acad Sci USA*. 2005;102(34):12153-12158.

18. Molano RD, Pileggi A, Song S, et al. Prolonged islet allograft survival by alpha-1 antitrypsin: the role of humoral immunity. *Transplant Proc*. 2008;40(2):455-456.

19. Pileggi A, Molano RD, Song S, et al. Alpha-1 antitrypsin treatment of spontaneously diabetic nonobese diabetic mice receiving islet allografts. *Transplant Proc*. 2008;40(2):457-458.

20. Shahaf G, Moser H, Ozeri E, Mizrahi M, Abecassis A, Lewis EC. Alpha-1-antitrypsin gene delivery reduces inflammation, increases T-regulatory cell population size and prevents islet allograft rejection. *Mol Med (Cambridge, Mass)*. 2011;17(9-10):1000-1011.

21. Gottlieb PA, Alkanani AK, Michels AW, et al. Alpha1-antitrypsin therapy downregulates toll-like receptor-induced IL-1beta responses in monocytes and myeloid dendritic cells and may improve islet function in recently diagnosed patients with type 1 diabetes. *J Clin Endocrinol Metab*. 2014;99(8):E1418-E1426.

22. Rachmiel M, Strauss P, Dror N, et al. Alpha-1 antitrypsin therapy is safe and well tolerated in children and adolescents with recent onset type 1 diabetes mellitus. *Pediatr Diabetes*. 2016;17(5):351-359.

23. Weir GC, Ehlers MR, Harris KM, et al Alpha-1 antitrypsin treatment of new-onset type 1 diabetes: an open-label, phase I clinical trial (RETAIN) to assess safety and pharmacokinetics.

24. American Diabetes Association. Standards of medical care in diabetes—2013. *Diabetes Care*. 2013;36(suppl 1):S11-S66.

25. Grifols Therapeutics Inc. *Full Prescribing Information for Prolastin-C Liquid*. https://www.prolastin.com.

26. Palmer JP, Fleming GA, Greenbaum CJ, et al. C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve beta-cell function: report of an ADA workshop, 21–22 October 2001. *Diabetes*. 2004;53(1):250-264.

27. Workgroup on Hypoglycemia ADA. Defining and reporting hypoglycemia in diabetes: a report from the American Diabetes Association workgroup on hypoglycemia. *Diabetes Care*. 2005;28(5):1245-1249.

28. Mortensen HB, Hougaard P, Swift P, et al. New definition for the partial remission period in children and adolescents with type 1 diabetes. *Diabetes Care*. 2009;32(8):1384-1390.

29. Weir GC, Koulamnda M. Control of inflammation with alpha1-antitrypsin: a potential treatment for islet transplantation and new-onset type 1 diabetes. *Curr Diab Rep*. 2009;9(2):100-102.

30. Yuan Y, DiCiaccio B, Li Y, et al. Anti-inflammaging effects of human alpha-1 antitrypsin. *Aging Cell*. 2018;17(1):e12694.

31. Chen YL, Qiao YC, Pan YH, et al. Correlation between serum interleukin-6 level and type 1 diabetes mellitus: a systematic review and meta-analysis. *Cytokine*. 2017;94:14-20.

32. Hubbard RC, Sellers S, Czerski D, Stephens L, Crystal RG. Biochemical efficacy and safety of monthly augmentation therapy for alpha 1-antitrypsin deficiency. *JAMA*. 1988;260(9):1259-1264.

33. Barker AF, Siemsen F, Pasley D, D'Silva R, Buist AS. Replacement therapy for hereditary alpha1-antitrypsin deficiency. A program for long-term administration. *Chest*. 1994;105(5):1406-1410.

34. Barker AF, Iwata-Morgan I, Oveson L, Roussel R. Pharmacokinetic study of alpha1-antitrypsin infusion in alpha1-antitrypsin deficiency. *Chest*. 1997;112(3):607-613.

35. Greenbaum CJ, Beam CA, Boulware D, et al. Fall in C-peptide during first 2 years from diagnosis: evidence of at least two distinct phases from composite type 1 diabetes TrialNet data. *Diabetes*. 2012;61(8):2066-2073.

36. Nagl K, Hermann JM, Plamper M, et al. Factors contributing to partial remission in type 1 diabetes: analysis based on the insulin dose-adjusted HbA1c in 3657 children and adolescents from Germany and Austria. *Pediatr Diabetes*. 2017;18(6):428-434.

37. Sherry NA, Tsai EB, Herold KC. Natural history of β-cell function in type 1 diabetes. *Diabetes*. 2005;54(suppl 2):S32-S39.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.