SYNOPSIS MD2014.01

Sponsor name	Prothya Biosolutions Netherlands B.V. Plesmanlaan 125 1066 CX Amsterdam The Netherlands
Name of Investigational Product:	Human Apotransferrin
Study Title:	Efficacy and Safety of human apotransferrin in patients with β-thalassemia intermedia
Protocol identification:	MD2014.01 EudraCT no. 2014-001936-12
Study Site:	Dept. of Haematology Amsterdam UMC Location AMC Amsterdam The Netherlands
Principle Investigator:	B.J. Biemond, PhD, MD Dept. of Haematology Amsterdam UMC Location AMC Amsterdam The Netherlands
Study Period:	11-Jun-2019 (FPI) – 11-Oct-2021 (LPO) Prematurely stopped: 31 March 2022
	The study was prematurely terminated on 31 March 2022, because no clinical effect was observed and no study medication was available anymore.
Study Phase:	II
Objectives	Primary Objective: to determine the effect of apotransferrin administration in patients with β-thalassemia intermedia on erythropoiesis as reflected by haemoglobin level or transfusion dependency. Secondary Objectives: 1. To evaluate the effect of apotransferrin administration on the pharmacokinetics of transferrin 2. To assess the effect of apotransferrin administration on the iron metabolism as reflected by serum iron, NTBI and LPI levels, hepcidin, ferritin, soluble Transferrin Receptor (sTfR) and transferrin saturation. 3. To determine the effect of apotransferrin administration on oxidative stress as assessed by plasma levels of advanced glycation end products (AGEs) and the lipid peroxidation product malondialdehyde (MDA). 4. To determine the effect on markers of erythropoiesis like reticulocyte count, erythropoietin levels, red blood cell indices and spleen size. 5. To assess safety of apotransferrin transfusion in patients with β-thalassemia intermedia
Methodology:	Prospective, open-label, non-controlled, single-centre
Number of Subjects	Planned: 12 Actual: 8

SYNOPSIS MD2014.01

Inclusion criteria	• Non-transfusion-dependent β-thalassemia intermedia, defined as patients with microcytic anaemia in combination with an elevated HbA2 (>2.5%) and a haemoglobin of <6.2 mmol/L, or transfusion-dependent β-thalassemia treated with a regular transfusion schedule.
	• Age above 17 years.
	• Adequate renal and hepatic function tests as indicated by the following laboratory values:
	  • Serum creatinine ≤1.0 mg/dL (≤ 88.7 µmol/L); if serum creatinine >1.0 mg/dL (>88.7 µmol/L), then the glomerular filtration rate (GFR) must be >60 mL/min/1.73 m^2 as calculated by the Modification of Diet in Renal Disease equation where the predicted GFR (mL/min/1.73 m^2) = 186 x (Serum Creatinine in mg/dL) - 1.154 x (age in years) - 0.203 x (0.742 if patient is female) x (1.212 if patient is black)
	  • Aspartate aminotransferase (ASAT)/ alanine aminotransferase (ALAT) ≤2.5 × ULN
	  • Alkaline phosphatase (AP) ≤ 2.5 × ULN
	• WHO performance 0, 1 or 2.
	• Signed informed consent.

Exclusion criteria:	• Known with allergic reactions against human plasma or plasma products. • Concurrent severe and/or uncontrolled medical condition (e.g. uncontrolled diabetes, infection, hypertension, pulmonary disease). • Cardiac dysfunction as defined by myocardial infarction within the last 6 months of study entry, unstable angina, or unstable cardiac arrhythmias. • Pregnant or lactating females. • Known with IgA deficiency with anti-IgA antibodies
Medication/Dosage	340 mg/kg human apotransferrin every two weeks (protocol v.4.0 and 5.0) A loading dose of human apotransferrin of 170 mg/kg on day -1 followed by a maintenance dose of 170 mg/kg once every 2 weeks from day 0 onwards (protocol v.2.0 and v.3.0)
Batch Numbers study medication:	16C10H710x 19F18H710x
Study Duration	Planned: 16 weeks for NTDβTI and 20 weeks for TDβTI patients + 1-3 weeks follow-up till transferrin is back to baseline
Criteria for Evaluation: **<u>Efficacy</u>**	Haematological response defined as: 1. Change from baseline of haemoglobin level, expressed as mmol/L and percentage in NTDβ1TI and TDβ1TI patients (before transfusion) 2. Change from baseline of number of RBC units transfused/week in TDβ1TI patients. Secondary endpoints:

	1. Number of patients with an increase in haemoglobin of > 0.9 mmol/L (1.5 g/dL) in NTDβTI and TDβTI patients (before blood transfusion) compared to baseline 2. Number of patients with a reduction of at least 50% of the number of RBC units transfused/week compared to baseline in TDβTI patients. 3. Increase iron metabolism as reflected by serum iron, hepcidin, NTBI levels and iron saturation. 4. Oxidative stress as assessed by plasma levels of advanced glycation end products (AGEs) and MDA 5. The effect on markers of erythropoiesis like reticulocyte count, erythropoietin levels, red cell indices and spleen size
Pharmacokinetics	Transferrin levels prior to and 5 min, 2 h, and 1, 4, 7, and 14 days after infusion of apotransferrin at week 4. Moreover, transferrin trough level prior to each infusion and 2 weeks after the last infusion. The following pharmacokinetic parameters were to be assessed: After infusion week 4 (or later as applicable): transferrin trough levels (C_{trough}), C_{min}, C_{max}, t_{max}, $t_{1/2term}$, λ_z, area under the curve (AUC_τ), $C_{avg,ss}$ Before every infusion: C_{0h} (before 1st infusion) and C_{trough}
Safety	Safety was monitored by measuring vital signs (blood pressure, pulse rate, body temperature) and by recording all adverse events during and after infusion for the duration of the study. All adverse events (number, type) were analysed regarding causality, seriousness, outcome and expectedness.

Summary – Conclusions

Subject disposition: Of the 8 included subjects, 5 were included in the intent-to-treat (ITT) and safety (SAF) analysis sets. Three subjects were excluded from the ITT and SAF analysis sets, as they were randomised under protocol version 3.0 and received a lower dose of apotransferrin. These subjects were included in the protocol V3 analysis set. Two out of the 3 subjects of the V3 population, were included in the study for a second time. In the ITT/SAF population, 2 subjects were TDβTI and 3 subjects were NTDβTI. In the protocol V3 population, 1 subject was TDβTI and 2 subjects were NTDβTI.

PHARMACOKINETIC RESULTS

After administration of a single IV dose of 170 mg/kg or 340 mg/kg apotransferrin at steady-state conditions, mean trough concentrations increased after the first apotransferrin administration and starting from Week 2 remained stable during the study. Mean trough transferrin concentrations increased with dose, and were above the before infusion concentrations at baseline for the 340 mg/kg dose, while limited accumulation was observed after 170 mg/kg apotransferrin administration. The mean C_{max} and $C_{avg,ss}$ transferrin increased with dose. The fluctuation index appeared to be comparable for both 170 mg/kg or 340 mg/kg apotransferrin. Baseline-corrected $t_{1/2term}$ was 104.9 hours (4.37 days) after administration of a single IV dose of 340 mg/kg apotransferrin. The observed PK results for the 340 mg/kg administration match the previously simulated PK results for 340 mg/kg administration based on the 170 mg/kg administration.

EFFICACY RESULTS

No clear effect of apotransferrin administration on erythropoiesis as reflected by haemoglobin level or transfusion dependency was observed in patients with β-thalassemia intermedia.
The change from baseline (i.e., last value before first apotransferrin dose for NTDβTI patients, and mean value from blood transfusion history within 20 weeks before first apotransferrin dose

for TDβ1TI patients) was low (or negative) at most time points for all subjects. For 2 subjects in the TDβTI group (n=3), an increase from baseline of more than 0.9 mmol/L was observed: on one occasion for one subject in the protocol V3 population (170 mg/kg dose group) and twice for one subject in the ITT population (340 mg/kg dose group). These increases were not sustainable.

Despite the clear suppression of NTBI levels directly post-infusion of apotransferrin, no sustained effect of apotransferrin administration on the iron metabolism as reflected by serum iron, NTBI, hepcidin, ferritin, sTfR, and transferrin saturation levels was observed in any of the patients with β-thalassemia intermedia. Oxidative stress data, erythroferrone and LPI results were not available, as it was decided not to determine these parameters given the non-sustained effect of apotransferrin observed on NTBI levels. No clear effect of apotransferrin administration on markers of was observed in any of the patients with β-thalassemia intermedia.

SAFETY RESULTS

For the safety population (n=5), a total of 15 AEs were reported in 5 subjects during the study, all of which emerged during the treatment period. Of the 15 TEAEs, 3 TEAEs reported by 2 (40.0%) subjects were considered possibly related to the study treatment: 1 event of fatigue reported by 1 (20.0%) subject, and 1 event of dizziness and 1 event of peripheral coldness reported by 1 (20.0%) subject. Other TEAEs were considered not or unlikely related to study drug administration.

For the protocol V3 population (n=3), a total of 21 AEs were reported in 3 subjects during the study, all of which emerged during the treatment period. Of the 21 TEAEs, 5 TEAEs reported by 3 (100.0%) subjects were considered at least possibly related to the study treatment: 1 event of fatigue and 1 event of muscle spasms reported by 1 (33.3%) subject, 1 event of pyrexia and 1 event of dizziness reported by 1 (33.3%) subject, and 1 event of oral dysaesthesia reported by 1 (33.3%) subject.

No SAEs or deaths were reported in the study and none of the TEAEs led to study treatment permanent discontinuation or study discontinuation.

For the haematology or biochemistry parameters and vital signs, no clear trend was observed in the absolute values, nor in the changes from baseline over time.

CONCLUSION:

No clear effect of apotransferrin administration on erythropoiesis or transfusion dependency was observed in patients with β-thalassemia intermedia. Also no sustained effect of apotransferrin on NTBI levels was observed, despite a clear suppression of NTBI levels directly post-infusion. In addition, no sustained changes were shown in other parameters related to iron metabolism or markers of erythropoiesis.

Overall, it can be concluded that repeated intravenous administration of apotransferrin was tolerated well with only a few non-serious AEs possibly related to the study medication, all of mild or moderate intensity.

Date of report: 10 March 2023