Ziltivekimab for treatment of anemia of inflammation in patients on hemodialysis

Supplementary Appendix

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List of Investigators

A list of the Investigator names, in alphabetical order according to last name, and their respective site addresses is below.

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Methods

Inclusion Criteria

Number	Inclusion Criterion Details
1	Age > 18 years at the time of signing of the ICF.
2	Receiving chronic hemodialysis for ≥3 months prior to Screening via an arteriovenous fistula or arteriovenous graft. Note: Every effort was made to recruit patients who had a well-functioning arteriovenous fistula or graft to minimize the chance of requiring a central venous catheter during the Treatment Period.
3	The patient agreed to comply with the contraception and reproduction restrictions of the study. Women of childbearing potential must have been using a method of contraception that was "highly effective" (i.e., < 1% failure rate) OR Postmenopausal women must have had no menstrual bleeding for ≥1 year before initial dosing and either have been over the age of 60 years or have had an elevated plasma FSH level (i.e., > 40 mIU/mL) at Screening; AND All female patients of childbearing potential must have had a documented negative pregnancy test result at Screening. Patients with elevated β-HCG levels believed to be due to end-stage renal disease may have been enrolled if documented to not be pregnant.
4	The patient was able to give written informed consent and has signed a consent form approved by the Investigator's Institutional Review Board or Independent Ethics Committee.
5	At least one documented spKt/V > 1.2 within 8 weeks prior to the Full Screening Period.
6	"Genotype positive" (genotype GG or AG, as defined by the Central Lab via genetic testing developed by the Sponsor) at Screening.
7	Two serum IL-6 levels ≥ 4 pg/mL measured ≥1 week apart during the Screening Period.
8	Receiving one of the following IV or SC ESA drugs continuously prescribed for a minimum of 8 weeks prior to Full Screening: epoetin alfa, darbepoetin alfa, or methoxypolyethylene glycol-epoetin beta. • Epoetin alfa must not have had more than one dose missed or held during Week -3 weeks prior to Randomization. • Darbepoetin alfa must not have had any doses missed or held during the 4 weeks prior to Randomization. • Methoxypolyethylene glycol-epoetin beta must not have had any doses missed or held during the 6 weeks prior to Randomization.

Number	Inclusion Criterion Details
9	Stable ESA doses prior to randomization, defined as follows: ***For ESAs dosed weekly or more frequently:*** No change in the total weekly dose during Week -1 compared with Week -2. ***For ESAs dosed less often than weekly:*** Any dose given during Full Screening Week -2 and Week -1 was unchanged from the immediately preceding dose. Note: If a dose was given on both Week -2 and Week -1, then both doses were the same. (These 2 doses could be different than earlier doses).
10	Had been receiving IV or dialysate iron regularly and continuously (e.g., with each dialysis or each week) during the 3 weeks prior to randomization OR had received no IV or dialysate iron during this time frame and use of IV or dialysate iron was not anticipated through Study Week 24. If parenteral iron had been administered during the 3 weeks prior to randomization, the regimen had been stable (same elemental iron dose, same frequency, and same iron product) with no more than 2 missed or held doses if dosed with each dialysis and no missed or held doses (if dosed weekly or less frequently) during this time frame.
11	If receiving oral ferric citrate, the patient had been prescribed it for ≥2 weeks prior to Full Screening and the dose was not anticipated to be changed through Study Week 24.
12	At least 2 ferritin values during Screening > 300 ng/mL.
13	At least 2 TSAT values during Screening between 15% and 50% (inclusive).
14	ESA resistance index (confirmed by the Sponsor) during the 2 weeks prior to randomization: **For patients receiving epoetin alfa:** $\frac{\text{Total weekly dose of epoetin alfa (units)}}{(\text{hemoglobin [g/dL]} * \text{target dry body weight [kg]})} > 8 \text{ units/kg per g/dL}$ **For patients receiving darbepoetin alfa:** $\frac{\text{Total weekly dose of darbepoetin alfa (mcg)} * 300}{(\text{hemoglobin [g/dL]} * \text{target dry body weight [kg]})} > 8 \text{ units/kg per g/dL}$ **For patients receiving methoxy polyethylene glycol-epoetin beta:** $\frac{\text{Weekly dose of methoxy polyethylene glycolepotetin beta (mcg)} * 300}{(\text{hemoglobin [g/dL]} * \text{target dry body weight [kg]})} > 8 \text{ units/kg per g/dL}$
15	During the 4 weeks prior to randomization, the mean of 3 consecutive hemoglobin measurements between ≥ 8.5 and ≤ 11.0 g/dL, with the difference between the first and last measurements being < 1.2 g/dL. Note: Qualifying hemoglobin measurements were drawn 2 or more days apart pre-dialysis, with no more than 2 measurements within a calendar week. There were ≥9 days between the first and last of the 3 qualifying hemoglobin values.

Number	Inclusion Criterion Details
16	Investigator anticipated no change to ESA, parenteral, or oral iron products, doses, or dosing regimen during Study Weeks 1 through 4 and that following the protocol guidance for ESA and iron dose changes was clinically appropriate for the patient.
17	Serum Vitamin B-12 and folate levels above the laboratory lower normal range during Full Screening.

Exclusion Criteria

Number	Exclusion Criterion Details
1	Any indwelling vascular catheter (including that used for dialysis) or anticipated of use of an indwelling catheter anytime during the study (including the Initial Screening and Full Screening Periods). Patients for whom a central venous catheter is used within 2 weeks prior to Step 2 of the Initial Screening Period (blood draw for IL-6) were also considered screening failures.
2	Use of systemic immunosuppressive drugs during the Full Screening Period or anticipated use of such drugs any time during the study. Note: Use of otic, ophthalmic, inhaled, and topical corticosteroids or local corticosteroid injections were not exclusionary. Oral prednisone up to 5 mg per day (or equivalent) was permitted.
3	Clinical evidence or suspicion of active or smoldering infection (e.g., diabetic foot ulcer) or use of systemic antibiotics, systemic antivirals, or systemic antifungals within 2 weeks prior to Step 2 of the Initial Screening Period (blood draw for IL-6), during the Initial Screening Period, or the Full Screening Period. "Systemic" was defined as oral or intravenous drugs that were absorbed into the circulation.
4	Hospitalization or outpatient procedures within 2 weeks prior to Step 2 of the Initial Screening Period (blood draw for IL-6), during the Initial Screening Period, or the Full Screening Period unless approved by the Medical Monitor. Note: The Medical Monitor was contacted for approval and determination of timing of IL-6 measurements if enrolling patients with hospitalizations or outpatient procedures during this time frame was considered.
5	Inability to undergo an MRI scan (e.g., weight over the limits for MRI machine, claustrophobia that could not be managed, certain metallic indwelling foreign bodies). Requirement for MRI could exempted by the medical monitor due to practical considerations. In such situations, patients could be enrolled even if they were unable to undergo MRI. [This exclusion was necessary because MRI was conducted during the trial; those results are not reported in this paper.]
6	Positive tuberculosis blood test at Screening
7	Evidence of HIV-1 or HIV-2 infection by serology at Screening.
8	Hepatitis B or C by serology (i.e. Hepatitis B Surface Antigen or Hepatitis C antibody positive) at Screening.
9	AST or ALT > 2.5x ULN at Screening.

Number	Exclusion Criterion Details
10	History of liver cirrhosis or home oxygen use (other than nocturnal-only oxygen for those with sleep apnea).
11	History of gastrointestinal ulceration or active diverticulitis in the 1 year prior to Initial Screening.
12	Absolute neutrophil count < 2.0 x 10^9/L at Initial and Full Screening.
13	Platelet count < 100 x 10^9/L at Initial and Full Screening.
14	Expected to receive any investigational drug or any of the exclusionary drugs listed in Appendix B of the protocol during the Treatment Period or Safety Follow-Up Period.
15	Received an investigational drug within 30 days prior to the start of the Full Screening Period.
16	Known allergy to the Study Drug or any of its ingredients.
17	Currently breastfeeding
18	Any condition that could interfere with, or for which the treatment might interfere with, the conduct of the study or interpretation of the study results, or that would in the opinion of the Investigator increase the risk of the patient's participation in the study. This would include but was not limited to alcoholism, drug dependency or abuse, psychiatric disease, epilepsy, anemia attributable to a primary hematologic disease (e.g., sickle cell anemia), or any unexplained blackouts.
19	Actively treated or active malignancy (other than non-melanoma skin cancers or cervical carcinoma in situ considered cured at the time of Full Screening) during the 1 year prior to Full Screening.
20	Myocardial infarction during the 2 months prior to Full Screening or during Screening.
21	Known or suspected occult or active bleeding other than that related to the hemodialysis procedure.
22	Received a red blood cell or whole blood transfusion within 2 months prior to Full Screening or anticipated to receive a blood transfusion at any time during the study.
23	Had inflammatory bowel disease that had been clinically active during the 3 months prior to Full Screening or bone marrow or organ transplant. Note: Patients with a previously explanted kidney transplant were eligible as were those who had an implanted kidney transplant, but had not received immunosuppression for ≥6 months prior to Full Screening.
24	Anticipated to receive an organ transplant during the time frame of the study.
25	iPTH at Screening > 2500 pg/mL.

Randomization

Within a cohort, the first two patients were randomized in a 1:1 ratio to ziltivekimab or placebo. The remaining patients in the cohort were randomized ≥48 hours later (after monitoring adverse events in the first 2 patients) in a 7:1 ratio of ziltivekimab to placebo, i.e., two total placebo patients and 8 total ziltivekimab patients per cohort. Randomization by cohort was performed so that we could proceed in a step-wise manner, cohort by cohort, monitoring safety in each cohort, prior to moving on to a cohort using a higher dose. To assure that at least one active patient and one placebo patient were dosed as the first two patients in a cohort, the first two patients were randomized 1:1 active to placebo. The remaining patients in each 10-patient cohort were randomixed 7:1 active to placebo to ensure a complete cohort randomization ratio of 8:2 active to placebo. Data for patients given the same ziltivekimab dose (or placebo) from different cohorts were pooled for data analysis.

Laboratory analyses

Serum chemistry analytes (sodium, potassium, chloride, bicarbonate, alanine transaminase, aspartate transaminase, blood urea nitrogen, creatinine, bilirubin, alkaline phosphatase, albumin, glucose, calcium, phosphate, total cholesterol, low-density lipoprotein [LDL] cholesterol, high-density lipoprotein [HDL] cholesterol and triglycerides [TG]) were measured using a Beckman Coulter analyzer and reagents (Beckman Coulter, Inc., Brea, CA). Hematology analyses (hemoglobin, hematocrit, reticulocyte count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution width, red blood cells, white blood cells, platelets, neutrophils, monocytes, eosinophils, basophils, lymphocytes, and reticulocyte hemoglobin content (CHr) were conducted using an Advia 2120i

Hematology System with Advia reagents (Siemens Healthineers, Siemens Healthcare Diagnostics, Inc., Tarrytown, NY).

HsCRP and SAA were measured on a Siemens BNII System using a CardioPhase hsCRP kit and N Latex SAA kit, respectively (Siemens Healthineers, Siemens Healthcare Diagnostics, Inc., Tarrytown, NY). Ferritin was measured on a Roche E170, E601 immunoassay analyzer with a Roche Ferritin reagent kit (Roche, Indianapolis, IN); TSAT was measured on an Olympus Beckman Coulter Chemistry analyzer with the transferrin reagent R1+R2 kit (Beckman Coulter, Inc., Brea, CA); fibrinogen was measured using a Siemens BNII system with a Siemens, N Antisera to Human Fibrinogen kit (Siemens, Tarrytown, NY).

IL-6 and hepcidin were measured using a Tecan Sunrise™ reader (Tecan Trading AG, Switzerland) with a Human-IL-6 Quantikine® ELISA kit and a Human Hepcidin Quantikine® ELISA kit (USA R&D Systems, Inc., Minneapolis, MN), respectively. Anti-drug antibodies to ziltivekimab were measured using a validated sensitive electroluminescence method (Intertek, San Diego, CA).

Genomic DNA was extracted from whole blood using a commercial miniprep kit (QIAmp DNA blood mini kit, QIAGEN, Germantown, MD). The *TMPRSS6* gene was amplified by polymerase chain reaction and genotype was determined by Sanger sequencing.

Instructions for ESA and parenteral iron dose adjustments

Weeks 1 – 4

- ESA doses are not to be adjusted or held during Weeks 1 through 4 unless one or more confirmed hemoglobin values (i.e., at least 2 consecutive measurements) or the patient's clinical status necessitates such due to a clear and present safety risk
- Parenteral iron doses are not to be adjusted or held during Weeks 1 through 4
- Patients not receiving parenteral iron products during Screening must not to be started on these during Weeks 1 through 4
- The Medical Monitor must be contacted prior to any contemplated ESA or parenteral iron dose changes

Weeks 5 – 24

- Changes to ESA dosing must be based on hemoglobin values that have been confirmed (i.e., at least 2 consecutive measurements)
- Do not increase the dose more frequently than once every 4 weeks. The recommended dose increase at any given time is 25%
- Decreases in dose may occur more frequently than once every 4 weeks. The recommended minimum duration between dose reductions is 3 weeks
- If the hemoglobin rises rapidly (e.g., more than 1 g/dL in any 2-week period), reduce the dose by 25%. A greater reduction may be made, if rise in hemoglobin is unacceptably rapid
- The Medical Monitor must be contacted prior to any contemplated ESA dose changes that depart from the above protocol requirements and recommendations unless clinical urgency precludes this. If the Medical Monitor is not contacted in advance, please send a notification within 24 hours that an off-protocol ESA dose change was made

- Patients not receiving parenteral iron products must not be started on these during Weeks 5 through 24
- Parenteral iron doses are not to be adjusted or held during Weeks 5 through 24, unless approved by the Medical Monitor