Supplementary Material

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1. Inclusion and exclusion criteria

HV study

Inclusion criteria

(1) Males between the ages of 20 to less than 40 years old at the time of the acquisition of informed consent

(2) Subjects with a body-mass index (BMI) of 18.5 to less than 25.0 at the time of the screening tests

(3) Subjects judged by the principal investigator or a subinvestigator to be appropriate for participation in the study based on the results of the screening tests and the tests conducted prior to the investigational drug treatment

(4) Subjects who are capable of receiving an explanation of this study prior to participation in the study, of understanding the details, and of providing written informed consent themselves

Exclusion criteria

(1) Subjects not judged to be healthy subjects because they have some sort of disease according to a medical judgment by the principal investigator or subinvestigator

(2) Subjects with a medical history that is believed to make them inappropriate for participation in this study, such as liver disturbance, renal disturbance, cardiovascular disease, hematological disease, endocrinological disease, metabolic disease, lung

disease, digestive tract disease, skin disease, nerve disease, urological disease, immunological disease, psychiatric disease, or a disease that may cause abnormalities in the blood vessels of the fundus, etc.

(3) Subjects who meet any of the following criteria in the screening tests or the tests conducted on Day -1 and prior to the investigational drug treatment

· Red blood cell count: 535×10^4 /μL or more

· Hemoglobin: 16.2 g/dL or more

· Hematocrit: 47.5% or more

· Reticulocyte ratio: Outside of the reference value range

(4) Subjects who meet any of the following criteria in the screening tests

· Serum EPO concentration: Outside of the reference value range

· Ferritin: 30 ng/mL or less, or 262 ng/mL or more

(5) Subjects who meet any of the following criteria in the vital signs in the screening tests and the tests conducted prior to the investigational drug treatment

· Blood pressure: Systolic blood pressure of 140 mmHg or more, or diastolic blood pressure of 90 mmHg or more

· Pulse rate: Less than 40 bpm, or 100 bpm or more

· Body temperature: 37.5°C or more

(6) Subjects with a history of drug allergies or allergies to food

(7) Subjects with major allergic factors (such as asthma that requires treatment, etc.)

(8) Subjects who have used a pharmaceutical product (including over-the-counter drugs) within 1 week prior to the investigational drug treatment

(9) Subjects who cannot comply with a rule prohibiting the ingestion of alcohol during the period from 2 days prior to the screening tests and up to the time of screening, and during the period from 2 days prior to hospital admission and up to Day 8

(10) Subjects who cannot comply with a rule prohibiting smoking during the period from the time of hospital admission and up to Day 8

(11) Subjects who cannot comply with a rule requiring the use of the appropriate contraceptive methods (contraception on the part of both the subject and his partner: use of condoms by the subject; use of oral birth control, an intrauterine device or a contraceptive diaphragm by the subject's partner) during the period from the time of hospital admission and up to the completion of the study

(12) Subjects from whom it would be difficult to collect blood samples from a peripheral vein

(13) Subjects who have undergone the collection of blood samples that corresponds to any of the following

- Blood component donation during the period from 2 weeks prior to the implementation of the screening tests and up to the time of hospital admission
- Collection of blood samples (such as blood donation) exceeding 200 mL during the period from 4 weeks prior to the implementation of the screening tests and up to the time of hospital admission
- Collection of blood samples (such as blood donation) exceeding 400 mL during the period from 12 weeks prior to the implementation of the screening tests and up to the time of hospital admission

(14) Subjects who were treated with another investigational drug within 16 weeks prior to the investigational drug treatment

(15) Subjects who are dependent on alcohol or drugs, or with a history of alcohol or drug dependence

(16) Subjects who had a positive result on a urine drug test (benzodiazepines, cocaine, stimulants, cannabis, barbiturates, opioids, phencyclidines, tricyclic antidepressants) at screening test.

(17) Subjects who had a positive result for any hepatitis B virus surface (HBs) antigen, hepatitis C virus (HCV) antibody, or human immunodeficiency virus (HIV) antigen/antibody or positive serologic test for syphilis at screening test.

(18) Other subjects who were judged to be inappropriate for enrollment in the study by the principal investigator or sub-investigator.

CKD study

Inclusion criteria

<Criteria for both chronic kidney disease (CKD) patients not undergoing dialysis (i.e., ND patients) and CKD patients undergoing hemodialysis (i.e., HD patients) >

(1) Age: between ≥ 20 years and < 75 years at the time of providing written informed consent for participation in the study.

(2) Serum concentration of erythropoietin (EPO): < 50 mIU/mL at screening test 1, 2, or 3.

(3) Transferrin saturation (TSAT) ≥ 20% or ferritin ≥ 100 ng/mL at screening test 1.

(4) Patients meeting any of the following criteria.

1) Patients who has not used erythropoiesis-stimulating agent (ESA) ≥ eight weeks before from screening test 1.

2) Patients who has used ESA, other than epoetin beta pegol, ≥ four weeks before from screening test 1 and has met all of the following criteria A) to C).

A) The total ESA dosage for each week could be changed within a range of 50%, compared to the total ESA dosage for one week before screening test 1, for four weeks or more before screening test 1.

B) Acceptable to discontinue ESA the day following screening test 1 to Follow-up 2.

C) The fluctuating range of Hb concentration between screening tests 1 and 2 is within ± 0.5 g/dL per week (the same criteria applied between screening tests 2 and 3).

(5) Patients who receive an explanation about the study before participating in the study and can understand the contents and are willing and able to provide written consent.

<Criteria for ND patients>

(6) CKD patients who never received dialysis and do not need to receive dialysis during the study period.

(7) Patients with an Hb concentration at screening test 1 (ESA present at screening test 2) ≥ 10.0 g/dL to < 13.0 g/dL.

(8) Patients with an estimated glomerular filtration rate (eGFR) at screening test 1 ≥ 15 mL/min/1.73m^2 to < 45 mL/min/1.73m^2 (Using the calculation formula published by the Japanese Society of Nephrology).

<Criteria for HD patients>

(9) Patients who received hemodialysis (including diafiltration) three times per week ≥ 12 weeks from acquisition consent.

(10) Patients with an Hb concentration at screening test 1 (ESA present at screening test 2) ≥ 10.0 g/dL to < 12.0 g/dL.

Exclusion criteria

<Criteria for both ND and HD patients>

(1) Patients with anemia other than that caused by CKD.

(2) Patients who have severe infection, systemic hematopathy (e.g. myelodysplastic syndrome, hemoglobinopathy), peptic ulcer or clear hemorrhagic lesion such as gastrointestinal hemorrhage.

(3) Patients with immune disorder with severe inflammation.

(4) Patients with uncontrolled secondary hyperparathyroidism (e.g. intact PTH ≥ 500 pg/mL).

(5) Patients who already had or will have a kidney transplantation.

(6) Patients who have a complication such as proliferative retinopathy, macular edema, or macular degeneration which requires treatment. Or, patients who had a

complication such as proliferative retinopathy, macular edema, or macular degeneration which required treatment within 12 months before screening test 1.

(7) Patients with congestive heart failure (New York Heart Association classification ≥ Class III).

(8) Patients with a medical history of thrombotic disease in the six months before screening test 1 (e.g. heart infarction, brain infarction, transient ischemic attack or thrombophlebitis).

(9) Patients with malignant tumors or with a past history of malignant tumors in the five years before screening test 1 (however, patients could be included if no treatment had been given, recurrence had not been observed, and recurrence was not observed during the study period).

(10) Patients with uncontrolled blood pressure; SBP > 170 mmHg or DBP > 100 mmHg at screening test 1 (ESA present, screening tests 1 and 2), (HD patient, evaluated before dialysis).

(11) Patients with clinical significant hepatopathy (e.g. AST or ALT ≥ 2.5 times the upper limit of the reference value or total bilirubin ≥ twice the upper limit of the reference value).

(12) Patients with a past history of drug allergy or food allergy.

(13) Patients with a significant allergic disposition (e.g. asthma requiring treatment).

(14) Patients scheduled to undergo surgery during the study period.

(15) Patients who were administered other study drugs in the 12 weeks before screening test 1.

(16) Patients who have received TP0463518.

(17) Patients who are unable to stop drinking alcohol from two days before admission until the completion of Follow-up 2.

(18) Patients who are unable to stop smoking from admission until the completion of Follow-up 2.

(19) Patients who have received an erythrocyte transfusion in the 16 weeks before screening test 1.

(20) Patients who may possibly become pregnant (patients who have not been permanently sterilized or entered menopause [amenorrhea ≥ 12 months]).

(21) Patients who cannot comply with appropriate contraceptive methods (contraception by both the subject and the partner of the subject; use of oral contraceptives, intrauterine contraceptive devices, or pessaries for females, and use of condoms for males) from the acquisition of consent to the completion of the study.

(22) Patients who have unstable psychiatric disease that could possibly affect the acquisition of consent and evaluation of the study.

(23) Patients with a history of or current drug or alcohol dependence/abuse.

(24) Patients who had a positive result on a urine drug test (benzodiazepines, cocaine, stimulants, cannabis, barbiturates, opioids, phencyclidines, tricyclic antidepressants) at screening test 1.

(25) Patients who had a positive result for any HBs HCV antibody, or HIV antigen/antibody or positive serologic test for syphilis at screening test 1.

(26) Other patients who were judged to be inappropriate for enrollment in the study by the principal investigator or sub-investigator.

2. Methods for determining concentration of TP0463518 in plasma and urine

Plasma was obtained by centrifugation of blood samples (3000 rpm, 4°C, 5 min). Obtained plasma and urine samples were stored at ≤−20°C until analysis.

Plasma and urine samples were subjected to solid phase extraction using OASIS HLB cartridge (30 mg/1 cc; Waters, MA, USA), with the eluate injected into a high performance liquid chromatography-tandem mass spectrometry (LC/MS/MS) system comprising Nexera X2 system (Shimadzu, Kyoto, Japan) and TripleQuad^{TM} 6500 mass spectrometer (AB SCIEX, CA, USA). Data were collected and processed using Analyst 1.6.2 software (AB SCIEX, CA, USA). TP0463518 and the internal standard (IS; deuterium-labeled TP0463518) were analyzed using an Atlantis T3 column (4.6 mm i.d. × 50 mm, 3 μm particle size; Waters, MA, USA) with water/formic acid (1000:0.5, v/v) solution and acetonitrile as the mobile phase under a gradient condition. The mass spectrometer was operated in a negative ionization mode, with the selected reaction monitoring transitions as follows: *m/z* 430 → *m/z* 100 for TP0463518 and *m/z* 434 → *m/z* 100 for IS. Assays were validated and performed in a contracted laboratory (Sumika Chemical Analysis Service, Ltd., Osaka, Japan).

3. Methods for EPO glycosylation pattern analysis

Complete details of the method have been described previously.[1] Briefly, the EPO glycosylation pattern was determined using the EPO WGA MAIIA Isoform Distribution Kit (MAIIA Diagnostics, Sweden). Peripheral and umbilical cord plasma purchased from BIOPREDIC International and STEMCELL Technologies Inc., respectively, were used as controls for mainly kidney-derived EPO and mainly liver-derived EPO, respectively. Percentage of migrated isoform (PMI) is calculated as the ratio of EPO migration in a low versus high *N*-acetylglucosamine-containing solution for a given sample.

1. Lönnberg M, Andrén M, Birgegård G, Drevin M, Garle M, Carlsson J: Rapid detection of erythropoiesis-stimulating agents in urine and serum. *Anal Biochem* 420: 101–114, 2012

4. Table S1. Demographic and baseline characteristics

Healthy volunteer study

Group	**Placebo**	**3 mg**	**6 mg**	**11 mg**	**20 mg**	**36 mg**
	(n = 10)	**(n = 6)**	**(n = 6)**	**(n = 6)**	**(n = 6)**	**(n = 6)**
Age (years)	26.4 ± 6.1	26.5 ± 6.0	23.2 ± 3.4	30.7 ± 8.2	23.0 ± 3.3	22.8 ± 3.4
Body weight (kg)	60.57 ± 4.37	61.68 ± 5.23	61.47 ± 3.73	62.70 ± 9.39	65.00 ± 6.68	62.97 ± 8.48
BMI	20.26 ± 1.70	20.53 ± 1.86	21.25 ± 2.17	21.15 ± 1.88	21.68 ± 2.47	21.03 ± 1.89
eGFR(mL/min/1.73 m^2)	93.58 ± 8.35	88.85 ± 14.29	90.50 ± 8.55	89.22 ± 11.02	86.32 ± 8.73	99.63 ± 9.61
Hb (g/dL)	14.84 ± 0.56	14.78 ± 0.87	14.33 ± 0.63	14.72 ± 0.64	15.23 ± 0.43	14.88 ± 0.68
EPO (mIU/mL)	9.48 ± 3.62	8.87 ± 4.48	9.60 ± 2.80	10.98 ± 3.42	7.97 ± 2.37	8.17 ± 3.83

Chronic kidney disease study

Group		**ND**			**HD**	
		1 mg	**6 mg**	**11 mg**	**1 mg**	**11 mg**
		(n = 3)	**(n = 7)**	**(n = 10)**	**(n = 4)**	**(n = 5)**
Sex[a]	Male	2	3	8	4	4
	Female	1	4	2	0	1
Age (years)		69.0 ± 4.4	66.9 ± 3.9	65.8 ± 6.2	64.5 ± 2.6	63.4 ± 9.8
Body weight (kg)		58.53 ± 11.40	61.60 ± 13.40	60.90 ± 8.74	69.03 ± 7.29	58.74 ± 9.74
BMI		25.10 ± 7.11	23.30 ± 3.14	23.49 ± 2.59	24.60 ± 1.72	22.34 ± 3.54
Time on dialysis (years)		-	-	-	4.63 ± 2.24	5.60 ± 3.84
eGFR (mL/min/1.73 m^2)[b]		40.60 ± 8.78	31.61 ± 9.20	31.39 ± 11.42	5.85 ± 1.55	7.36 ± 2.70

Hb (g/dL)	11.97 ± 0.50	11.31 ± 0.44	11.05 ± 0.75	11.28 ± 1.18	11.32 ± 1.17
EPO (mIU/mL)	10.30 ± 0.72	9.57 ± 2.41	10.46 ± 4.64	14.33 ± 16.65	6.64 ± 2.24

Data are presented as mean ± SD.

[a]Number of subjects.

[b]eGFR values in HD patients were for reference purposes only.

BMI, body mass index; eGFR, estimated glomerular filtration rate; EPO, erythropoietin; Hb, hemoglobin; HD, CKD patients undergoing hemodialysis; HV, healthy volunteers; ND, CKD patients not undergoing dialysis; SD, standard deviation.

5. Table S2. Pharmacokinetic parameters of TP0463518

		n	C_{max} (ng/mL)	T_{max}[a] (h)	$AUC_{0-\infty}$ (h*ng/mL)	$t_{1/2}$ (h)	CL/F (L/h)	Fe_{0-24h} (%)
HV	3 mg	6	160 ± 22.7	1.25 (1.00–4.00)	712 ± 169	6.40 ± 0.591	4.43 ± 1.13	80.7 ± 3.84
	6 mg	6	326 ± 112	1.50 (1.00–6.00)	1630 ± 541	5.91 ± 1.00	4.02 ± 1.26	78.0 ± 7.82
	11 mg	6	526 ± 169	2.00 (1.00–3.00)	2690 ± 1050	7.16 ± 1.88	4.67 ± 1.80	67.6 ± 8.67
	11 mg (Fed)	6	356 ± 93.8	2.50 (2.00–4.00)	1990 ± 473	7.07 ± 2.04	5.80 ± 1.37	65.6 ± 8.75
	20 mg	6	941 ± 136	1.50 (1.00–3.00)	4570 ± 984	6.91 ± 2.24	4.55 ± 0.975	70.3 ± 3.82
	36 mg	6	1710 ± 336	1.75 (1.00–2.00)	6980 ± 1280	7.41 ± 1.89	5.31 ± 1.01	73.8 ± 12.2
ND	1 mg	3	47.2 ± 3.85	4.00 (4.00–6.00)	655 ± 61.4	12.7 ± 2.35	1.54 ± 0.137	51.4 ± 14.1
	6 mg	7	272 ± 68.0	4.00 (3.00–12.0)	3950 ± 970	12.8 ± 4.69	1.63 ± 0.537	55.7 ± 11.2

	11 mg	10	513 ± 139	3.99 (3.00–12.0)	6450 ± 1800	12.6 ± 4.12	1.85 ± 0.609	50.9 ± 9.00
HD[b]	1 mg	4	28.4 ± 6.36	4.99 (3.00–8.07)	1130 ± 322	27.6 ± 5.14	0.966 ± 0.378	0.153 ± 0.193
	11 mg	5	441 ± 195	5.97 (3.95–8.08)	18100 ± 15700	25.8 ± 8.87	0.975 ± 0.675	0.593 ± 1.11

Data are presented as mean ± SD.

[a]Median (Min–Max)

[b]In HD patients, $AUC_{0-\infty}$, $t_{1/2}$, and CL/F were calculated, assuming that hemodialysis had no effect on drug elimination.

$AUC_{0-\infty}$, area under the concentration-time curve extrapolated to infinity; CL/F, apparent clearance; C_{max}, the maximum concentration; EPO, erythropoietin; Fe_{0-24h}, the urinary excretion rate of TP0463518 from time 0 to 24 h; HD, CKD patients undergoing hemodialysis; HV, healthy volunteers; ND, CKD patients not undergoing dialysis; SD, standard deviation; $t_{1/2}$, elimination half-life; T_{max}, time to maximum concentration.