Outcomes of Desidustat Treatment in people with Anemia and Chronic Kidney Disease: A Phase 2 Study

Table of Contents for Supplemental Material

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Supplemental Table 1: Inclusion and exclusion criteria

Inclusion Criteria:	1. Male or female patient ≥18 to ≤65 years of age with anemia of CKD stage I – IV
	2. Men who agreed to use adequate contraception when sexually active or women without childbearing potential.
	3. Estimated GFR ≥15 mL/min/1.73 m^2
	4. Patients had not on dialysis and had not expected to begin dialysis during the treatment period of the study
	5. Screening Hb concentration ≥ 6.5g/dL to ≤ 11 g/dL (Mean value if two times Hb evaluation for enrollment)
	6. No iron, folate or Vitamin B12 deficiency. (Serum ferritin levels ≥100 μg/L and < 1000 μg/L or transferrin saturation ≥ 20%)
	7. Body weight ≥ 45 kg
	8. Given written informed consent, which included compliance with protocol
Exclusion Criteria	1. Any erythropoietin treatment within 4 weeks prior to participating in the study
	2. Intravenous iron within 10 days prior to participating in the study
	3. Red blood cell transfusion within 8 weeks prior to participating in the study
	4. History of previous or concurrent cancer
	5. Currently active clinically significant cardiovascular disease such as uncontrolled arrhythmia, congestive heart failure, any class 3 or 4 cardiac disease as defined by the New York Heart Association Functional Classification or history of myocardial infarction prior to first dose with study drug
	6. Serologic status reflected active hepatitis B or C infection or HIV infection
	7. Active infection at initiation of study
	8. History of renal transplant
	9. Major surgery within 90 days of the first day of study drug dosing, and minor surgery within 30 days of the first day of study drug dosing
	10. Unable to swallow tablets or disease significantly affected gastrointestinal function and/or inhibited small intestine absorption such as; mal-absorption syndrome, resection of the small bowel or poorly controlled inflammatory bowel disease affected the small intestine
	11. History of uncontrolled autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura (ITP) or thalassemia

12. Presence or a history of bleeding disorders or clinical conditions (e.g. gastrointestinal bleeding or constitutional disorders) that might increase risk of life-threatening bleeding

13. History of stroke or intracranial hemorrhage within 6 months prior to enrollment

14. History of allergic reactions attributed to compounds of similar chemical or biologic composition to ZYAN1 or to any erythropoiesis-stimulating agent

15. Required or was receiving anticoagulation with warfarin or equivalent vitamin K antagonists or other medications within 28 days of the first dose of study drug that in the investigator's opinion, could compromise patient safety

16. Pregnant and breastfeeding women

17. Current life-threatening illness, medical condition or organ system dysfunction which, in the Investigator's opinion, could compromise the patient's safety

18. Abnormal baseline laboratory investigations as follows:
    - WBC count $\leq 4 \times 10^3$/uL
    - Platelets count $\leq 100 \times 10^3$/uL
    - Bilirubin ≥ 1.5 mg/dL
    - ALT and/or AST ≥ 2.5 times of the ULN

19. Other laboratory abnormalities that, in the opinion of the investigator, would be compromised the patient's safety or interfere with data interpretation

20. Presence of other systemic disorders or diseases (e.g., respiratory, gastrointestinal, endocrine, immunological, dermatological, neurological, psychiatric disease or any other body system involvement) which, in the Investigator's opinion, could be compromised the patient's safety

21. History of significant alcoholism or drug abuse within the past 1 year. History or presence of significant smoking (more than 10 cigarettes per day) or consumption of tobacco/nicotine products (more than 10 times per day)

22. History of difficulty with donating blood

23. History or presence of any clinically significant electrocardiogram (ECG) abnormalities during screening

24. Participants who had participated in any drug research study other than the present trial within past 3 months

25. Participants who had donated one unit (350 ml) of blood in the past 3 months or history of whole blood transfusion in last 120 days prior to entry in the study

26. Type I DM

27. History of chronic inflammatory disease (RA, Celiac disease, UC, crohns disease, SLE)

28. In case of Type II DM patients, HbA1c >7.5 gm% and patients was stabilized on anti-hyperglycemic drugs (except Insulin) less than 03 months

29. In case of hypertensive patients, systolic and diastolic BP were greater than 140 and 90 mm of hg respectively and patients was stabilized on antihypertensive drugs less than 03 months

30. Female volunteers with following criteria were to be recruited:

    - History of pregnancy or lactation in the past 3 months
    - Fertile female volunteers not protected against pregnancy by adequate long-term anti-fertility measures
    - History of less than 1 year of menopause and not used adequate long-term anti-fertility measures
    - Used hormone replacement therapy
    - Unable to give assurance for protection against pregnancy for 3 months after the participation in this trial
    - Positive urine pregnancy test on the day of check-in
    - Positive serum β-hCG level at the screening visit

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Supplemental Table 2: List of reagents/methods used other than Desidustat or its metabolites

Section	Parameter	Reagents/Methods	Instrument
Hematology	Complete Blood Count	Fully Automated 5 part Differentiation	Fully Automated Hematology Analyzer Advia 2120 from Siemens
	Retic Count	Laser optical, Oxazine staining	
	Blood Group	Tube agglutination	Not Applicable
	ESR	Westergreen	Westergreen
Biochemistry / Immuoassay	HbA1c	Tina-Quant	Cobas 6000 from Roche Diagnostics
	AST	IFCC without pyridoxal phosphate activation	Cobas 6000 from Roche Diagnostics
	ALT	IFCC without pyridoxal phosphate activation	Cobas 6000 from Roche Diagnostics
	ALP	IFCC- p-nitrophenyl phosphate	Cobas 6000 from Roche Diagnostics
	Total Bilirubin	Colorimetric - 3,5 dichlorophenyl diazonium	Cobas 6000 from Roche Diagnostics
	Total Protein	Biuret-colorimetric	Cobas 6000 from Roche Diagnostics
	Albumin	Bromcresol green -Colorimetric	Cobas 6000 from Roche Diagnostics
	Globulin	Calculated from total protein and albumin	Cobas 6000 from Roche Diagnostics
	GGT	Enzymatic Colorimetric	Cobas 6000 from Roche Diagnostics
	Amylase	IFCC G7 p-nitrophenol	Cobas 6000 from Roche Diagnostics
	Lipase	Enzymatic-Colorimetric	Cobas 6000 from Roche Diagnostics
	Urea	Urease,Kinetic	Cobas 6000 from Roche Diagnostics
	Creatinine	Jaffe, Kinetic	Cobas 6000 from Roche Diagnostics
	Electrolytes	ion-selective electrodes -Indirect	Cobas 6000 from Roche Diagnostics
	Total Cholesterol	Cholesterol Esterase Oxidse Peroxidase Enzymatic-Colorimetric Assay	Cobas 6000 from Roche Diagnostics
	Triglyceride	Glycerol phosphate oxidase Enzymatic-Colorimetric Assay	Cobas 6000 from Roche Diagnostics
	HDL-C	Cholesterol oxidase, Peroxidase Enzymatic colorimetric Assay	Cobas 6000 from Roche Diagnostics
	LDL-C	Cholesterol oxidase, Peroxidase Enzymatic colorimetric Assay	Cobas 6000 from Roche Diagnostics
	CRP	Particle Enhanced Immuno-Turbidimetric Assay	Cobas 6000 from Roche Diagnostics
	Iron	FerroZine method without deproteinization Colorimetric assay	Cobas 6000 from Roche Diagnostics
	TIBC	Calculated	--
	Ferritin	Electrochemiluminescence immunoassay based on the principle of Sandwich Assay	Cobas 6000 from Roche Diagnostics
	Transferrin Saturation	Calculated	--

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	RBS	Hexokinase	Cobas 6000 from Roche Diagnostics
	Hepcidin	Hepcidin 25 (bioactive) HS ELISA Kit from DRG Inc.is a solid Phase enzyme-linked immunosorbent assay based on the principle of competitive binding.	Fully Automated ELISA system, EVOLIS TWIN PLUS from Bio-Rad
	Vitamin B12	Electrochemiluminescence immunoassay based on the principle of Competition Assay	Cobas 6000 from Roche Diagnostics
	Folic Acid	Electrochemiluminescence immunoassay based on the principle of Competition Assay	
	β-hcg	Electrochemiluminescence immunoassay based on the principle of Sandwich Assay	
	EPO	Immulite 1000 EPO is a solid-phase, enzyme-labeled chemiluminescence immunometric asay on the principle of Sandwich Assay	Immulite 1000 from Siemens
	eGFR	Calculated	--
	HIV	Electrochemiluminescence immunoassay based on the principle of Sandwich Assay	Cobas 6000 from Roche Diagnostics
	HbsAg	Electrochemiluminescence immunoassay based on the principle of Sandwich Assay	
	HCV	Electrochemiluminescence immunoassay based on the principle of Sandwich Assay	
	Microalbumin	Calculated from Urine Alb. and Urine Creatinine	Cobas 6000 from Roche Diagnostics
Urinalysis	pH	This test is based on pH indicator method.	Cobas u411 from Roche Diagnostics
	Protein	The test is based on the principle of the protein error of a pH indicator.	
	Bilirubin	The test is based on the coupling of bilirubin with a diazonium salt.	
	Ketones	Ths test is based on the principle of Legal's test.	
	Specific Gravity	This test is based on ion concentration detection of the urine.	
	Glucose	The glucose determination is based on the specific glucose-oxidase/peroxidase reaction (GOD/POD method).	
	Erythrocyte	Peroxidase method.	
	Urobilinogen	Diazonium salt method.	
	Nitrite	The test is based on the principle of the Griess test.	
	Leukocyte	Leukocyte esterase method.	
	Red Blood Cells	Microscopic Method	--
	Pus Cells		
	Epithelial Cells		
	Others		

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Supplemental Table 3: Definition of populations

Population	Definition
Safety	All randomised patients who received at least one dose of the study medication.
Modified intent-to-treat	All randomized patients who received at least one dose of trial medication and had at least one post-baseline measurement. The missing post-baseline data were imputed using the last observation carried forward.
Per-protocol	All patients in the mITT population who met the eligibility criteria, completed the study in compliance with the protocol and had no major protocol deviation.

mITT, modified intent-to-treat; PP, per-protocol.

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Supplemental Table 4: Proportion of responders at end-of-treatment (week 6)

Treatment	mITT n (%)	PP n (%)
Desidustat 100 mg (N = 29)	19 (65.52)	16 (61.54)
Desidustat 150 mg (N = 28)	21 (75.00)	20 (83.33)
Desidustat 200 mg (N = 29)	24 (82.76)	18 (81.82)
Placebo (N = 30)	7 (23.33)	3 (11.54)

mITT, modified intent-to-treat; PP, per-protocol.

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Supplemental Table 5: Change from baseline to week 6 in mean efficacy assessments - mITT population

Safety Parameters	Analysis	Desidustat 100 mg (N=29)	Desidustat 150 mg (N=28)	Desidustat 200 mg (N=29)	Placebo (N=30)
Iron (µg/dL)	Baseline	61.90 ± 26.90	60.17 ± 40.73	59.87 ± 27.23	70.36 ± 29.86
	Change	5.63 ± 28.13	-0.20 ± 31.98	0.28 ± 60.86	4.34 ± 25.61
TIBC (µg/dL)	Baseline	274.54 ± 74.68	267.29 ± 69.34	291.56 ± 75.90	256.74 ± 63.61
	Change	30.30 ± 74.52	53.93 ± 68.85	70.60 ± 78.03	-5.47 ± 54.07
Transferrin (%)	Baseline	25.07 ± 13.78	24.75 ± 20.06	22.34 ± 12.44	28.87 ± 12.32
	Change	-0.93 ± 11.10	-4.78 ± 14.52	-5.67 ± 19.00	2.17 ± 10.99
CRP (mg/dL)	Baseline	0.54 ± 0.80	1.51 ± 2.35	0.91 ± 1.97	0.56 ± 0.72
	Change	0.06 ± 0.75	-0.69 ± 2.13	0.22 ± 0.91	-0.22 ± 0.85
LDL (mg/dL)	Baseline	105.72 ± 39.41	91.10 ± 43.34	89.66 ± 32.20	96.00 ± 42.93
	Change	-18.46 ± 32.53	-18.15 ± 29.52	-26.74 ± 20.81	-1.17 ± 25.87
TG (mg/dL)	Baseline	164.79 ± 97.14	134.40 ± 57.66	198.49 ± 120.50	142.94 ± 68.75
	Change	-23.63 ± 76.14	29.29 ± 62.59	-6.51 ± 131.65	15.04 ± 67.53
EPO (mg/dL)	Baseline	13.47 ± 9.08	12.62 ± 7.51	10.97 ± 4.50	13.36 ± 11.29
	Change	16.57 ± 53.72	28.13 ± 38.67	54.50 ± 113.37	0.49 ± 9.37
Hepcidin (ng/dL)	Baseline	86.57 ± 84.18	124.79 ± 113.64	80.78 ± 87.77	96.14 ± 93.30
	Change	-59.73 ± 78.96	-91.36 ± 106.86	-59.24 ± 94.95	-33.54 ± 75.38

CRP, C-reactive protein; EPO, erythropoietin; LDL, Low-density lipoprotein; mITT, modified intent-to-treat; n, number of patients; SD, standard deviation TIBC, total iron binding capacity; TG, triglyceride. Data are presented in mean ± SD.

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Supplemental Table 6: Change from baseline to week 6 in mean efficacy assessments - PP population

Safety Parameters	Analysis	Desidustat 100 mg (N=26)	Desidustat 150 mg (N=24)	Desidustat 200 mg (N=22)	Placebo (N=26)
Iron (µg/dL)	Baseline	59.84 ± 26.68	57.95 ± 39.89	64.87 ± 20.67	66.40 ± 22.52
	Change	5.86 ± 28.42	0.57 ± 33.79	3.39 ± 65.27	5.24 ± 26.91
TIBC (µg/dL)	Baseline	281.30 ± 72.59	270.65 ± 71.20	287.82 ± 80.89	252.33 ± 61.81
	Change	31.29 ± 76.84	56.61 ± 72.53	85.95 ± 72.59	-2.31 ± 46.58
Transferrin (%)	Baseline	23.38 ± 12.41	24.21 ± 20.99	24.77 ± 11.46	27.73 ± 10.47
	Change	-0.96 ± 11.45	-4.75 ± 15.29	-6.09 ± 20.22	2.54 ± 11.35
CRP (mg/dL)	Baseline	0.54 ± 0.83	1.62 ± 2.51	0.39 ± 0.50	0.61 ± 0.76
	Change	0.06 ± 0.78	-0.89 ± 2.18	0.09 ± 0.65	-0.23 ± 0.90
LDL (mg/dL)	Baseline	106.46 ± 39.46	87.50 ± 42.65	93.03 ± 33.54	99.43 ± 41.68
	Change	-19.28 ± 33.72	-16.15 ± 23.78	-27.85 ± 22.02	-3.53 ± 23.83
TG (mg/dL)	Baseline	167.51 ± 101.90	124.00 ± 45.97	192.21 ± 128.10	141.92 ± 70.03
	Change	-23.38 ± 78.83	31.55 ± 60.24	0.28 ± 139.85	17.23 ± 67.81
EPO (mg/dL)	Baseline	13.65 ± 9.42	13.58 ± 7.70	10.69 ± 4.66	13.49 ± 11.65
	Change	7.29 ± 14.52	26.07 ± 38.47	32.02 ± 54.18	1.32 ± 9.31
Hepcidin (ng/dL)	Baseline	88.17 ± 87.51	139.09 ± 116.72	93.81 ± 95.87	104.38 ± 97.21
	Change	-60.39 ± 80.45	-107.92 ± 106.70	-77.59 ± 92.64	-37.67 ± 78.67

CRP, C-reactive protein; EPO, erythropoietin; LDL, Low-density lipoprotein; N, number of patients; PP, per-protocol; SD, standard deviation TIBC, total iron binding capacity; TG, triglyceride. Data are presented in mean ± SD.

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Supplemental Table 7: Pharmacokinetic parameters after first and last dose

PK Parameters **Single dose**	Desidustat 100 mg (N = 11)	Desidustat 150 mg (N = 11)	Desidustat 200 mg (N = 11)
T_{max} (hr)*	2.00 (1.30 – 6.00)	2.00 (1.97 – 10.00)	2.01 (1.97 – 5.98)
C_{max} (ng/mL)	10968.46 ± 4482.80	12476.46 ± 6147.36	19614.86 ± 7808.68
$AUC_{0-t}{}^{\#}$ (hr*ng/mL)	96399.10 ± 33703.69	126389.41 ± 36419.38	176668.05 ± 75934.02
$AUC_{0-\infty}$ (hr*ng/mL)	163278.33 ± 170449.61	153391.44 ± 24421.08	202971.49 ± 92013.96
Elimination rate constant (1/hr)	0.09 ± 0.04	0.11 ± 0.02	0.10 ± 0.04
Half-life (hr)	13.79 ± 16.61	6.56 ± 0.97	7.72 ± 2.55
Volume of distribution (L)	14.39 ± 8.71	9.44 ± 1.44	12.35 ± 4.84
Clearance (L/hr)	0.96 ± 0.36	1.01 ± 0.16	1.21 ± 0.57
Multiple dose (steady state)			
$T_{max,ss}$ (hr)*	2.00 (1.30 – 8.00)	2.00 (1.97 – 6.00)	2.00 (2.00 – 6.00)
$C_{max,ss}$ (ng/mL)	9665.98 ± 4375.04	12737.08 ± 4153.92	17664.95 ± 8108.99
AUC_{0-t} (hr*ng/mL)	79280.37 ± 38544.25	117853.38 ± 34581.51	154308.55 ± 72569.01
Elimination rate constant (1/hr)	0.09 ± 0.04	0.12 ± 0.03	0.09 ± 0.03
Half-life (hr)	8.48 ± 3.13	6.20 ± 1.53	8.70 ± 3.69
Volume of distribution (L)	12.21 ± 3.71	11.00 ± 2.50	13.90 ± 6.00
Clearance (L/hr)	1.08` ± 0.36	1.29 ± 0.38	1.16 ± 0.39
C_{min} (ng/mL)	198.98 ± 214.89	150.07 ± 261.59	452.49 ± 516.73
Fluctuation Index	502.94 ± 193.50	487.43 ± 158.89	497.00 ± 132.73
Accumulation Index (%)	1.03 ± 0.03	1.01 ± 0.01	1.03 ± 0.05

(*) T_{max} and $T_{max,ss}$ is presented as Median (Range), (#) t, 24 hrs. PK, pharmacokinetic.
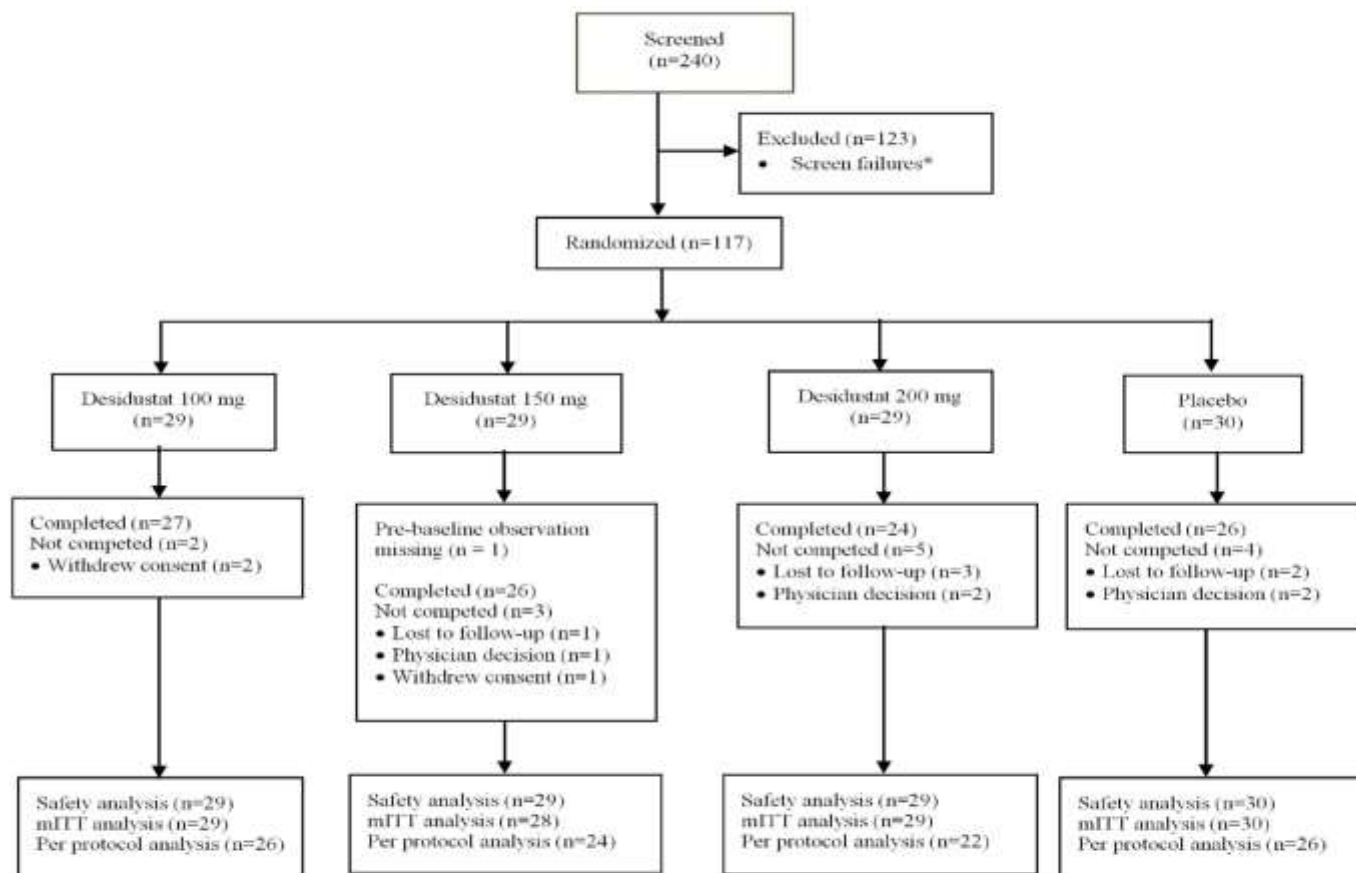
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Supplemental Table 8: Potassium (mmol/L) levels during treatment duration

Visit	Desidustat 100 mg (N=29)	Desidustat 150 mg (N=28)	Desidustat 200 mg (N=29)	Placebo (N=30)
Screening	4.78 ± 0.61	4.54 ± 0.74	4.56 ± 0.76	5.04 ± 0.87
Week 2	4.72 ± 0.63	4.55 ± 0.70	4.48 ± 0.94	4.82 ± 0.86
Week 6	4.71 ± 0.74	4.62 ± 0.75	4.61 ± 0.88	4.80 ± 0.96

Note: Values given as mean ± standard deviation.

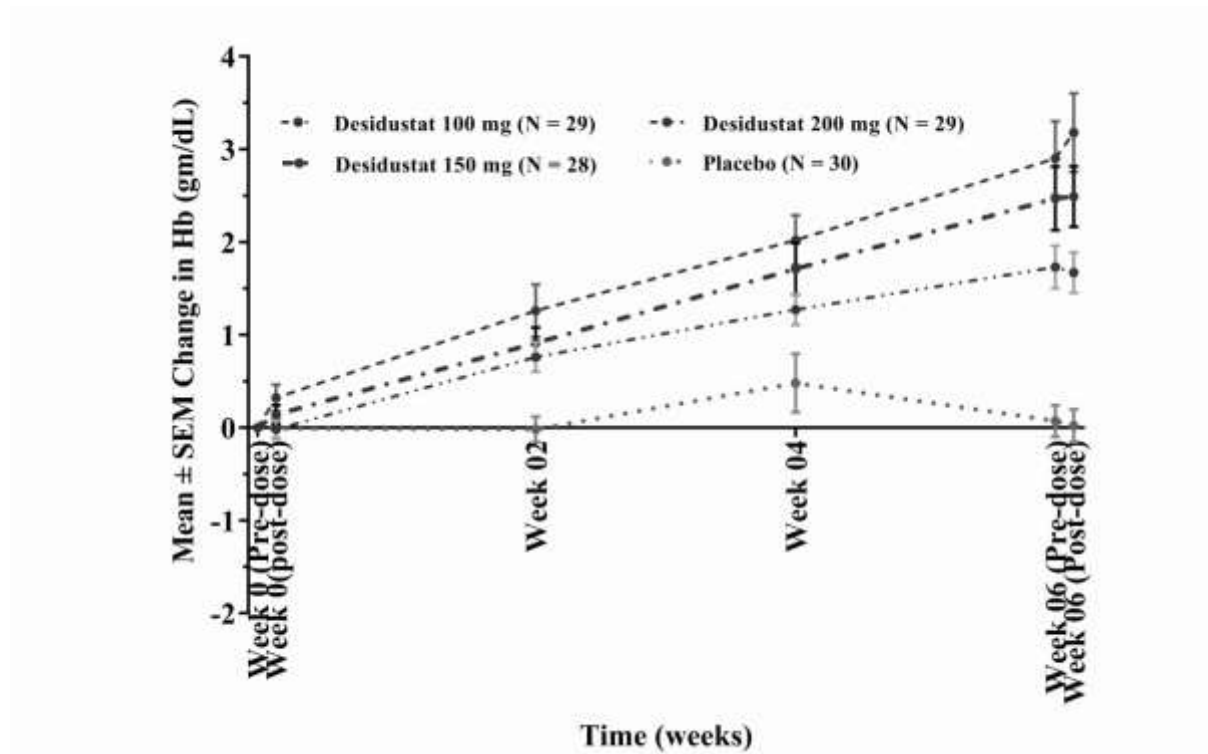**Outcomes of Desidustat Treatment in people with Anemia and Chronic Kidney Disease: A Phase 2 Study**


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                              Screened
                              (n=240)
                                 │
                                 ├──────────────►  Excluded (n=123)
                                 │                   • Screen failures*
                                 ▼
                          Randomized (n=117)
                                 │
        ┌────────────────┬───────┴────────┬─────────────────┐
        ▼                ▼                ▼                 ▼
  Desidustat 100 mg  Desidustat 150 mg  Desidustat 200 mg  Placebo
     (n=29)             (n=29)             (n=29)           (n=30)
        │                 │                 │                 │
        ▼                 ▼                 ▼                 ▼
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| Desidustat 100 mg	Desidustat 150 mg	Desidustat 200 mg	Placebo
Completed (n=27) Not competed (n=2) • Withdrew consent (n=2)	Pre-baseline observation missing (n = 1) Completed (n=26) Not competed (n=3) • Lost to follow-up (n=1) • Physician decision (n=1) • Withdrew consent (n=1)	Completed (n=24) Not competed (n=5) • Lost to follow-up (n=3) • Physician decision (n=2)	Completed (n=26) Not competed (n=4) • Lost to follow-up (n=2) • Physician decision (n=2)
Safety analysis (n=29) mITT analysis (n=29) Per protocol analysis (n=26)	Safety analysis (n=29) mITT analysis (n=28) Per protocol analysis (n=24)	Safety analysis (n=29) mITT analysis (n=29) Per protocol analysis (n=22)	Safety analysis (n=30) mITT analysis (n=30) Per protocol analysis (n=26)

*eGFR or creatinine out of limit as per eligibility (n = 27); Hb out of limit as per eligibility (n = 15); Low iron, folate or vitamin B12 (n = 4); HbA1c out of limit as per eligibility (n = 13); Significant X-Ray, ECG or ECHO finding (n = 3); Out of limit WBC, platelet, bilirubin, ALT or AST (n = 5); Significant higher EPO value (n = 1); HBsAg, HCV positive (n = 4); Not reported (n = 4); withdrew consent (n = 15); Other reasons (n = 29); Eligible but recruitment completed (n = 3).

Supplementary Figure 1: Patient disposition

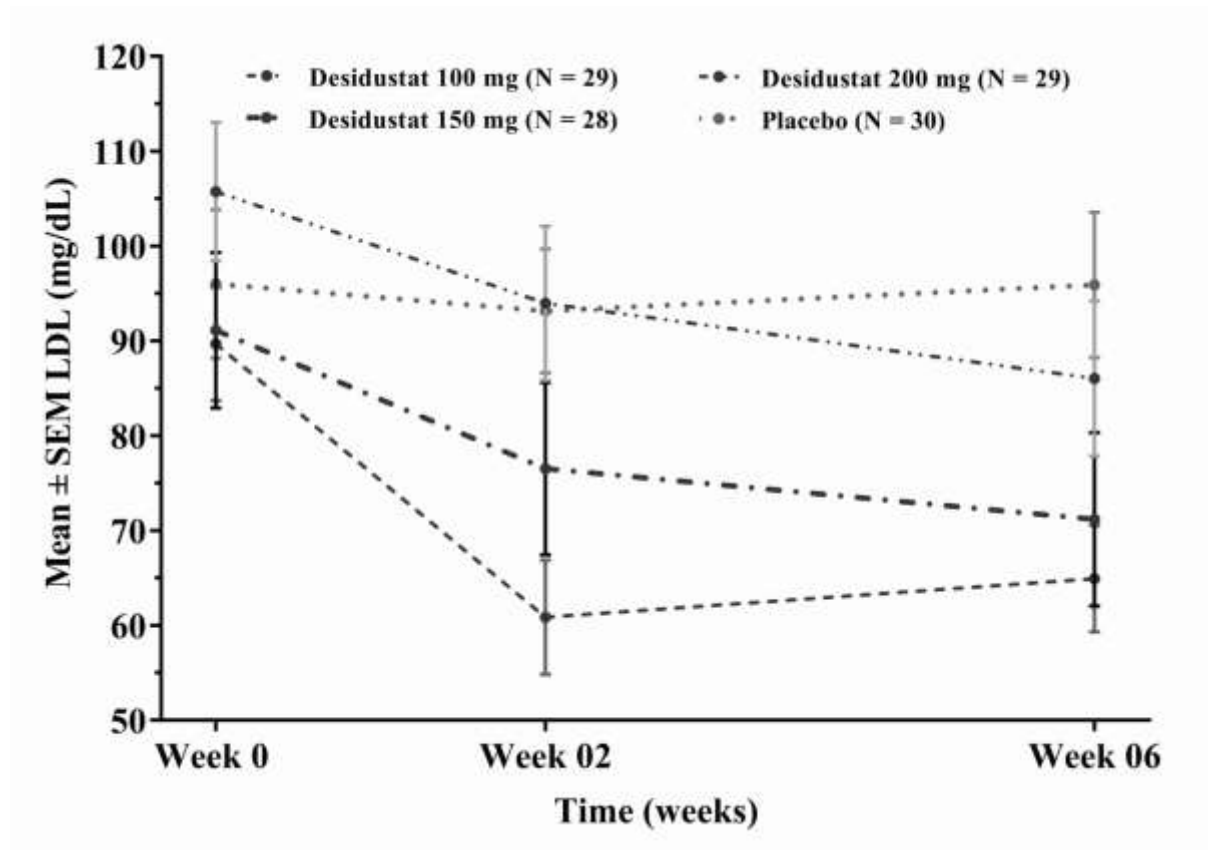**Outcomes of Desidustat Treatment in people with Anemia and Chronic Kidney Disease: A Phase 2 Study**

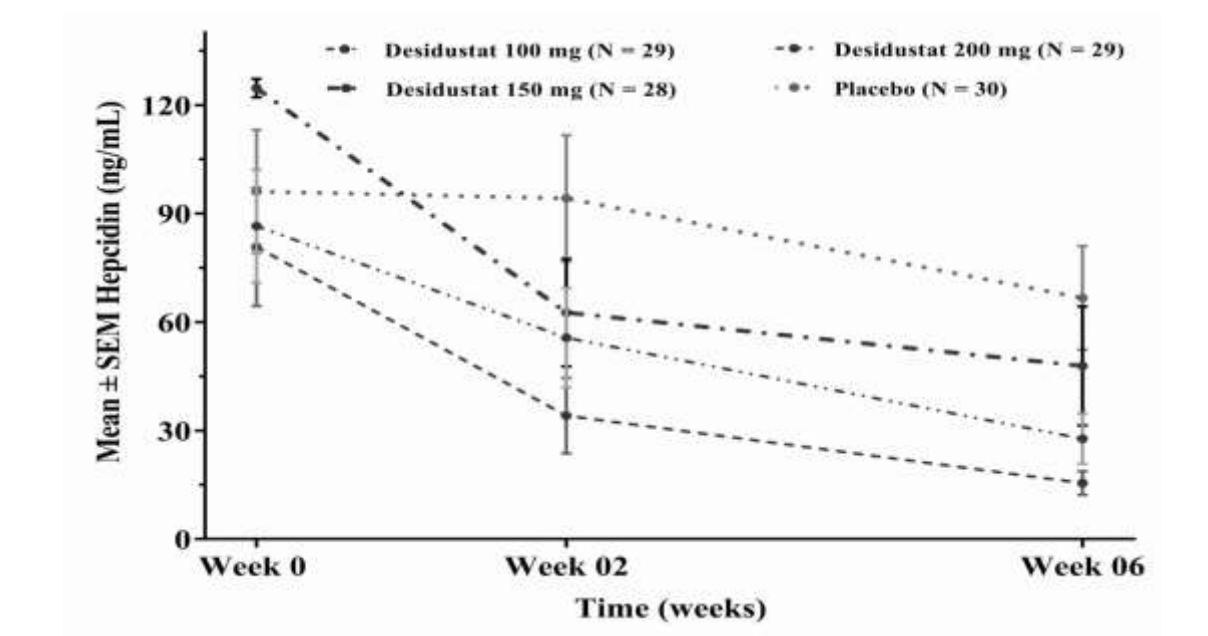**Supplemental Figure 2: Change in hemoglobin at week 6**
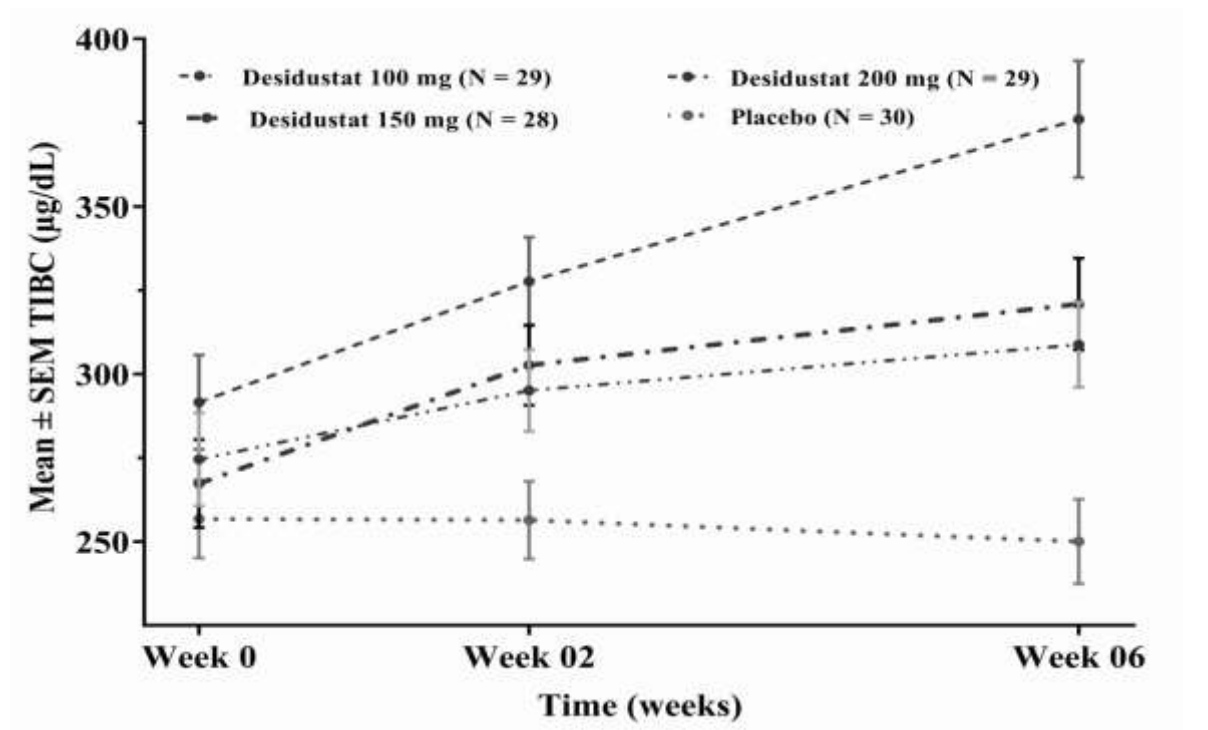
Supplemental Figure 3a: Change in concentration of TIBC

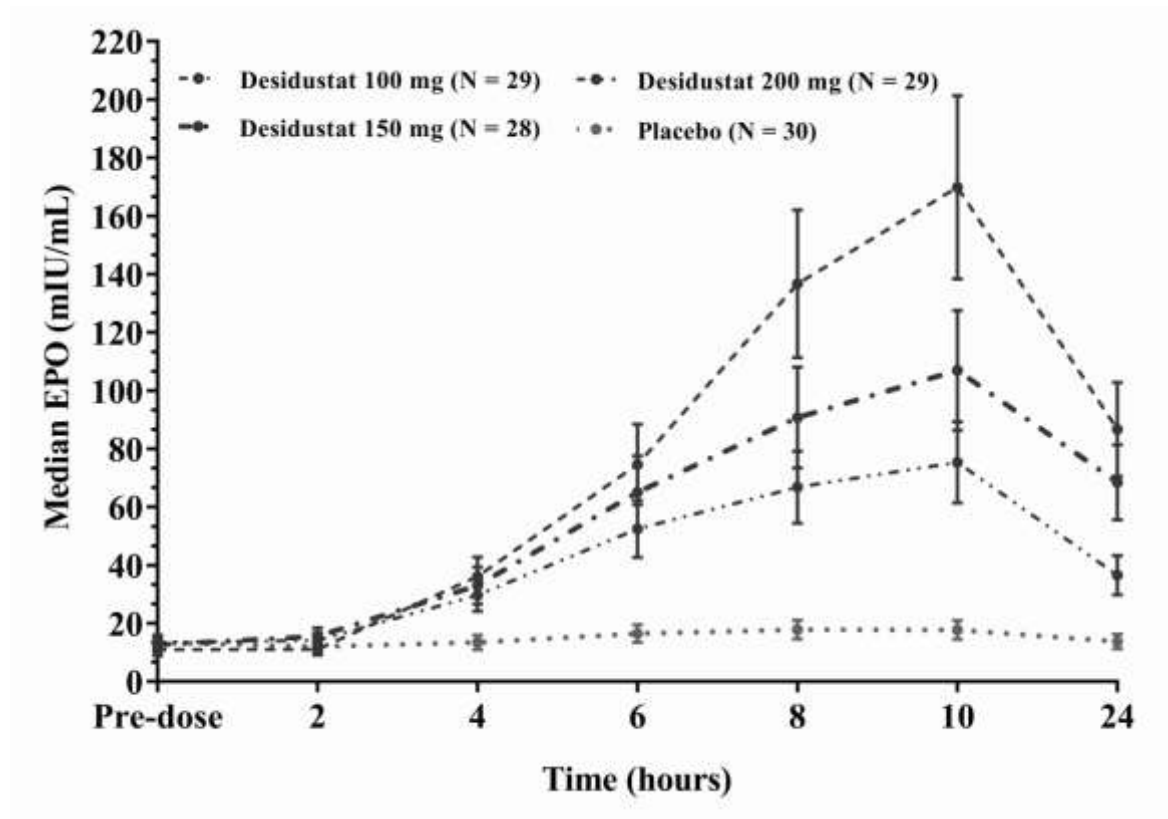**Outcomes of Desidustat Treatment in people with Anemia and Chronic Kidney Disease: A Phase 2 Study**

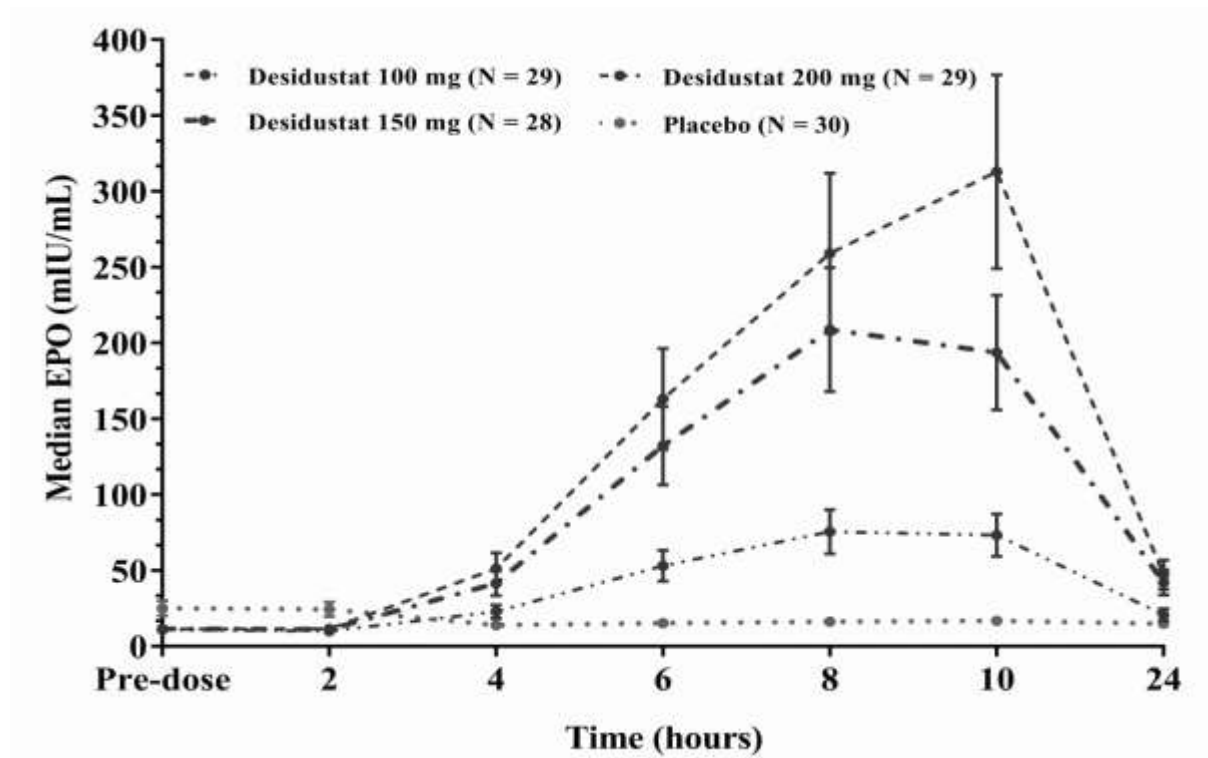**Supplemental Figure 3b: Change in concentration of LDL**

Supplemental Figure 3c: Change in concentration of hepcidin

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Supplemental Figure 4a: Erythropoietin levels after first dose at week 0

Supplemental Figure 4b: Erythropoietin levels after last dose at week 6