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Multicenter, phase 1 study of etavopivat (FT-4202) treatment for up to 12 weeks in patients with sickle cell disease

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Abstract:
Etavopivat is an investigational, once-daily, oral, selective erythrocyte pyruvate kinase (PKR) activator. A multicenter, randomized, placebo-controlled, double-blind, 3-part, phase 1 study (https://clinicaltrials.gov/study/NCT03815695) was conducted to characterize the safety and clinical activity of etavopivat. Thirty-six patients with sickle cell disease (SCD) were enrolled into 4 cohorts: one single-dose; two multiple ascending doses; one open-label [OL]. In the OL cohort, 15 patients (median age 33.0 [range, 17–55] years received 400-mg etavopivat once daily for 12 weeks; 14 completed treatment. Consistent with the mechanism of PKR activation, increases in ATP and decreases in 2,3 diphosphoglycerate were observed and sustained over 12 weeks' treatment. This translated clinically to an increase in hemoglobin (mean maximal increase 1.6 [range, 0.8–2.8] g/dL), with >1 g/dL increase in 11 (73%) patients during treatment. Additionally, oxygen tension at which hemoglobin is 50% saturated was reduced (P=.0007) with concomitant shift in point-of-sickling (P=.0034) to lower oxygen tension in oxygen-gradient ektacytometry. Hemolysis markers (absolute reticulocyte count, indirect bilirubin, lactate dehydrogenase) decreased from baseline, along with matrix metalloproteinase-9 and erythropoietin. In the OL cohort, adverse events (AEs) were mostly grade 1/2, consistent with underlying SCD; 5 patients had serious AEs. Vaso-occlusive pain episode was the most common treatment-emergent AE (n=7) in the OL cohort. In this first study of etavopivat in SCD, 400 mg once daily for 12 weeks was well-tolerated, resulting in rapid and sustained increases in hemoglobin, improved RBC physiology, and decreased hemolysis.

Conflict of interest: COI declared - see note

COI notes: E.W., S.F., J.G., I.O., P.S., and M.R. were employees of and held stock in Forma Therapeutics, Inc., at the time of this study. E.W., S.F., I.O., P.S., and M.R. are currently employees of Novo Nordisk. P.F.K is a consultant to Forma Therapeutics, Inc., and was an employee when the study was performed, and holds stock in Forma Therapeutic, Inc. I.O. is a former principal investigator at Levine Cancer Institute/Atrium health and formerly held consultancies with Forma therapeutics, Novo Nordisk, Agios, GBT, Novartis, Cheisi, Acceleron and Emmaus, and served on speaker's bureau for Novartis, GBT; she received research funding from the Centers for Disease Control (CDC), Health Resources and Service's Administration (HRSA) and Patient Centered Outcomes Research Institute (PCORI). I.O was an employee of Forma between February and October 2022 and is currently an employee of Novo Nordisk since October 2022. S.L.S reports consultancies with Forma Therapeutics, Inc., Novo Nordisk, GBT/Pfizer, ORIC, Agios, and Beam Therapeutics, membership on the advisory committees of GBT/Pfizer and Novartis, and research funding from Forma Therapeutics, Inc., Novo Nordisk, GBT, Novartis, and Pfizer. R.H. reports consultancies with Bristol Myers Squib, GBT, Imara, NIH, Novartis, and research funding from Chiesi, Forma Therapeutics, Inc., and University of Pittsburgh. M.I. reports consultancies with GBT, receives research funding from Novartis, Pfizer, GBT, Agios, Alexion, Novo Nordisk, and Forma, serves on the GBT speaker's bureau, and is a member of the board/advisory committee of GBT. R.C.B. is a former principal investigator at Children's Healthcare of Atlanta and formally held consultancies with GBT, Imara, Novartis, and received funding from GBT, Novartis, Forma Therapeutics, and Imara; He has been an employee of Global Blood Therapeutics, Inc., a wholly-owned subsidiary of Pfizer as of October 2023, since July 2022. F.A.K. received research funding from Forma. T.A.K. reports consultancies, membership on advisory boards, and research funding from Forma Therapeutics, Inc., Novo Nordisk, Agios Pharmaceuticals, Inc., and the National Institutes of Health. M.J.T. reports consultancies with GlycoMimetics, Inc., served on a data safety monitoring board of Novartis, and received research funding from Forma Therapeutics, Inc., CSL Behring, Inc., Doris Duke Charitable Foundation, and the National Institutes of Health. K.C. has no conflicts of interest to disclose. -

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Agreement to Share Publication-Related Data and Data Sharing Statement: Data will be shared with researchers after approval of a research proposal requesting access to data. Data will be shared for use as approved by the IRB according to the IRB Charter (see novonordisk-trials.com). The access request proposal form and the access criteria can be found at novonordisk-trials.com. Individual participant data will be shared in data sets in a de-identified/anonymized format. The data will be made available on a specialized SAS data platform.

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Title: Multicenter, phase 1 study of etavopivat (FT-4202) treatment for up to 12 weeks in patients with sickle cell disease

Short title: Etavopivat in sickle cell disease

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Clinical Trial Data Sharing

Data will be shared with researchers after approval of a research proposal requesting access to data. Data will be shared for use as approved by the IRB according to the IRB Charter (see novonordisk-trials.com). The access request proposal form and access criteria can be found at novonordisk-trials.com. Only de-identified/anonymized individual participant data will be shared on a specialized SAS data platform.

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- Estepp, et al. Phase 1 Single (SAD) and Multiple Ascending Dose (MAD) Study of the Safety, Pharmacokinetics (PK)and Pharmacodynamics (PD) of FT-4202, a PKR Activator, in Healthy and Sickle Cell Disease Subjects, European Hematology Association, June 11-21, 2020, virtual (poster).
- Kelly P, et al. Forma Therapeutics & FT-4202 for the Treatment of Sickle Cell Disease, Sickle Cell Disease Association of America (SCDAA), 48th Annual National Convention, October 13, 2020, virtual (oral presentation).
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- Saraf, et al. Etavopivat Treatment for up to 12 Weeks in Patients with Sickle Cell Disease Was Well Tolerated and Improved Red Blood Cell Health, European

Hematology Association (EHA), June 9-12, 2022, Hybrid/Vienna Austria (poster presentation)

Key points:

- Consistent with erythrocyte pyruvate kinase activation, ATP increased and 2,3-DPG decreased with etavopivat treatment.
- Clinically, this translated to 73.3% of etavopivat-treated patients with SCD having a Hb increase >1 g/dL at any time during treatment.

Key Words: Etavopivat; pyruvate kinase; 2,3-diphosphoglycerate; sickle cell disease; hemoglobin

Explanation of Novelty:

Etavopivat is an investigational, once daily, oral erythrocyte pyruvate kinase (PKR) activator. In this multicenter phase 1 trial of patients with sickle cell disease (SCD), ATP increased, and 2,3 DPG decreased with etavopivat treatment for up to 12 weeks. This translated to 73.3% of etavopivat-treated patients with SCD achieving a hemoglobin increase >1 g/dL at any time during 12 weeks of treatment.

Abstract

Etavopivat is an investigational, once-daily, oral, selective erythrocyte pyruvate kinase (PKR) activator. A multicenter, randomized, placebo-controlled, double-blind, 3-part, phase 1 study (https://clinicaltrials.gov/study/NCT03815695) was conducted to characterize the safety and clinical activity of etavopivat. Thirty-six patients with sickle cell disease (SCD) were enrolled into 4 cohorts: one single-dose; two multiple ascending doses; one open-label [OL]. In the OL cohort, 15 patients (median age 33.0 [range, 17–55] years received 400-mg etavopivat once daily for 12 weeks; 14 completed treatment. Consistent with the mechanism of PKR activation, increases in ATP and decreases in 2,3-diphosphoglycerate were observed and sustained over 12 weeks' treatment. This translated clinically to an increase in hemoglobin (mean maximal increase 1.6 [range, 0.8–2.8] g/dL), with >1 g/dL increase in 11 (73%) patients during treatment. Additionally, oxygen tension at which hemoglobin is 50% saturated was reduced (*P*=.0007) with concomitant shift in point-of-sickling (*P*=.0034) to lower oxygen tension in oxygen-gradient ektacytometry. Hemolysis markers (absolute reticulocyte count, indirect bilirubin, lactate dehydrogenase) decreased from baseline, along with matrix metalloproteinase-9 and erythropoietin. In the OL cohort, adverse events (AEs) were mostly grade 1/2, consistent with underlying SCD; 5 patients had serious AEs. Vaso-occlusive pain episode was the most common treatment-emergent AE (n=7) in the OL cohort. In this first study of etavopivat in SCD, 400 mg once daily for 12 weeks was well-tolerated, resulting in rapid and sustained increases in hemoglobin, improved RBC physiology, and decreased hemolysis.

Introduction

Sickle cell disease (SCD) is an inherited hemolytic anemia affecting 300,000 newborns/year globally in 2010 and expected to affect more than 400,000 newborns/year by 2050.[1-4] Impaired red blood cell (RBC) physiology is the hallmark of SCD, which is caused by a single mutation in the β-globin gene, resulting in formation of hemoglobin S (HbS) rather than hemoglobin A (HbA).[1-3] Clinical consequences include vaso-occlusion and hemolytic anemia, causing vaso-occlusive pain episodes (VOEs), acute and progressive end-organ damage, and diminished quality of life. [1-3] In high-resource countries, survival has improved due to newborn screening, penicillin prophylaxis, and clinically validated treatment strategies. [1,5] However, even there, the average lifespan of a person with SCD remains 20–30 years shorter than peers.[1,6,7]

Current SCD treatments include supportive care, transfusions, disease-modifying therapies such as hydroxyurea (HU), and hematopoietic stem cell transplantation.[1,3,5,8-10] Potentially curative gene therapies have recently been FDA-approved but are costly.[11-13] Additionally, disease modifying therapies are hampered by barriers to access, toxicity profiles, and for the newer agents, uncertain long-term benefit. Of available therapies, hematopoietic stem cell transplantation is potentially curative, but poor donor availability, risk, and cost limit its use.[1,5,10]. There is an unmet need for therapeutic agents that can be initiated early, target underlying SCD pathophysiology, reduce hemolysis and VOEs, limit end-organ damage, and improve quality of life, while having a favorable risk–benefit ratio. [1,5,10,14]

Several glycolytic enzymes and the Rapoport-Luebering shunt are activated in RBCs under hypoxic conditions, leading to increased 2,3 diphosphoglycerate (2,3-DPG) production.[15,16] In sickle RBCs, increased 2,3-DPG reduces the oxygen (O_2) affinity of HbS, causing increased dissociation of O_2 at higher partial pressure of dissolved O_2 (pO_2) compared with normal RBCs.[1,15] The increase in deoxygenated HbS induces Hb polymerization and precipitates a cascade of pathologic events, including RBC sickling, hemolysis, endothelial dysfunction, and abnormal activation of inflammatory, coagulation, and oxidative pathways.[1] This causes oxidative stress, vaso-occlusion, and tissue ischemia-reperfusion injury.[1,3,9] Concurrently with increased intracellular 2,3-DPG in sickle RBCs, adenosine triphosphate (ATP) levels are reduced.[17] ATP is necessary for normal ion channel function and RBC membrane homeostasis;[15,18] therefore, RBCs with reduced ATP levels are less flexible than normal RBCs, contributing to premature hemolysis.[19]

Erythrocyte pyruvate kinase (PKR) catalyzes the last, rate-limiting glycolysis step (phosphoenolpyruvate to pyruvate), generating ATP from adenosine diphosphate. PKR deficiency causes moderate-to-severe hemolytic anemia.[20] Etavopivat is an investigational,

once-daily PK activator selective for the RBC isozyme (PKR). PKR activation increases Hb-O_2 affinity, decreases HbS polymerization, and improves RBC function and lifespan by decreasing intracellular 2,3-DPG and increasing intracellular ATP.[21,22] Proof of pharmacodynamic (PD) activity for etavopivat was demonstrated in nonhuman primates, healthy humans, and ex vivo-treated RBCs from patients with SCD.[21] Etavopivat decreased whole blood 2,3-DPG levels, increased ATP levels, and increased Hb-O_2 affinity (decreased P_{50}) in RBCs from healthy subjects after a single 700-mg dose. In ex vivo studies involving RBCs from patients with SCD, etavopivat increased Hb-O_2 affinity and reduced RBC sickling. Another allosteric activator of PKR has also recently demonstrated in Phase 1 and Phase 2 clinical studies that targeting this pathway may lead to clinical benefit in patients with SCD and was also relatively well tolerated and associated with improvements in Hb concentration and markers of hemolysis.[23,24]

We report here the first study of etavopivat in patients with SCD. The aim of this phase 1 study was to assess the safety and clinical efficacy of etavopivat in single-dose, multiple ascending doses (MAD), followed by open-label (OL) treatment in patients with SCD.

METHODS

Clinical Trial and Human Subjects

Study 4202-HVS-101 (NCT03815695) was a first-in-human, randomized, placebo-controlled, double-blind, single-dose and MAD, phase 1 trial in SCD. Results from healthy volunteers have been reported.[25]

The protocol and amendments were reviewed and approved by appropriate institutional review boards (IRBs)/independent ethics committees. Patients provided written informed consent before undergoing study-related procedures. The study was conducted in accordance with the principles of the Declaration of Helsinki, Good Clinical Practice, and relevant laws/regulations. Data were analyzed by the study statistician (EW) and multiple authors. The authors had access to the data.

Key inclusion criteria were age 12–65 years (inclusive) at screening, minimum weight 40 kg, and confirmed SCD (HbSS, HbSβ^0-thalassemia, HbSβ^+-thalassemia, or HbSC). Patients with reproductive potential agreed to use a medically accepted contraceptive during the study and for 90 days after the last dose of study medication.

Key exclusion criteria were >6 episodes of VOEs within the past year requiring a hospital, emergency department, or clinic visit; hospitalization for VOE or other SCD-related event within 14 days of consent or 28 days before study treatment; ≥1 episode of acute chest

syndrome requiring hospitalization, intubation, and mechanical ventilatory support within 6 months before screening; pulmonary hypertension; use of HU if started <90 days before study treatment, crizanlizumab if started within 14 days of study treatment, or voxelotor within 7 days of study treatment until the end of the study period. SCD patients with >6 VOE were excluded to minimize the risk of including those with chronic pain disorders. Patients were allowed crizanlizumab as scheduled infusions every ≥4 weeks. Stable doses of HU and L-glutamine were permitted.

Additional exclusion criteria included use of moderate or strong inducers/inhibitors of cytochrome P450 3A4/5 within 2 weeks of study treatment; RBC transfusion within 30 days of study treatment; history of deep vein thrombosis (DVT) requiring systemic anticoagulation therapy for ≥6 weeks occurring within 6 months of study treatment; and Hb <7.0 g/dL or >10.5 g/dL during screening.

Study Design and Treatment

Single dose segment

The randomized, placebo-controlled, single-dose portion of the study (Figure 1) was conducted to confirm the safety and pharmacokinetic/pharmacodynamic (PK/PD) response to 700-mg etavopivat (previously shown to be safe and tolerable in healthy volunteers[25]). End of treatment (EOT) was on day 2, 24 hours after dosing (supplemental Appendix 1).

Seven patients received 1 oral dose of etavopivat 700 mg (N = 5) or placebo (N = 2).

MAD segment

The MAD study had 2 cohorts (MAD1 and MAD2) (Figure 1) with a randomized, placebo-controlled, double-blind design. Patients were randomized (3:1) to receive daily etavopivat 300 mg (MAD1) or 600 mg (MAD2) or placebo for 14 days. EOT was on day 14/15, 24 hours after completion of dosing (supplemental Appendix 1). Etavopivat/placebo dosing could extend by 48 hours to enable a 2-day stepwise dose reduction in patients demonstrating Hb increase >2.0 g/dL over baseline.

MAD segment included 20 patients who received etavopivat 300 mg (MAD1, N = 8), etavopivat 600 mg (MAD2, N = 8), or placebo (N = 4).

OL segment

During OL segment, 15 patients received ≤84 consecutive 400-mg daily oral doses of etavopivat (Figure 1). EOT was on day 84/85, 24 hours after the last dose (supplemental Appendix 1). Patients returned to the clinic on day 84 for the last etavopivat dose and on days 85, 88, 91, 98,

and 112 (EOS) for follow-up visits to monitor disease parameters post study drug discontinuation.

Protocol amendment 7.0 allowed etavopivat dosing to extend from 2 days to up to 2 weeks beyond day 84, allowing a stepwise dose decrease in patients with >2.0 g/dL Hb increase over baseline, or if clinically indicated.

Safety and tolerability

Adverse events (AE) were monitored from time of written consent to the last protocol-defined end-of-study (EOS) visit. Safety/tolerability monitoring has been described.[25] A treatment-emergent AE (TEAE) was any AE new in onset or aggravated in severity/frequency following the first dose of study medication, up to and including the EOS visit. AE severity was assessed by the investigator using Common Terminology Criteria for Adverse Events v5.0.[2623] The potential relationship of each AE to study drug (treatment) was categorized by the investigator as "yes" (possibly, probably or definitely related) or "no" (unrelated or unlikely to be related).

PK/PD

Venous blood was collected at prespecified timepoints for PK/PD, RBC functional assessments, and biomarkers. PK parameters for etavopivat were derived using Phoenix WinNonlin (version 6.4 or higher) software for noncompartmental analysis of plasma concentration data at actual sampling times. Plasma concentrations of etavopivat were determined using liquid chromatography-tandem mass spectrometry.[21,25]

PD assessments included RBC 2,3-DPG, ATP, pO_2 at which 50% of Hb is O_2-saturated (P_{50}), and exploratory laboratory assessments (RBC functional studies, and biomarkers of inflammation and coagulation). ATP and 2,3-DPG concentrations in whole blood were measured using liquid chromatography-tandem mass spectrometry.[21,25] The impact of 2,3-DPG reduction on Hb-O_2 affinity was assessed before/after dosing using P_{50} values.[21,25]

Clinical activity

Indirect bilirubin (iBIL), lactate dehydrogenase (LDH), reticulocyte counts, and Hb were measured at local laboratories. Hb response was defined as >1g/dL change from baseline at any time during treatment.

RBC function

Complete blood counts and hematologic parameters were analyzed by local laboratories. Additional hematology parameters, such as cellular Hb concentration mean (CHCM) and dense

RBCs (DRBCs), were centrally analyzed using an ADVIA® 2120i system (Siemens Healthineers, Hoffman Estates, IL).

Hb-O_2 equilibrium curves were collected using a HEMOX Analyzer (TCS Scientific Corp, New Hope, PA).[21,25] RBC deformability was measured using O_2 gradient ektacytometry (Oxygenscan) with the Laser Optical Rotational Red Cell Analyzer (Lorrca®; RR Mechatronics, Zwaag, The Netherlands).[21] RBC deformability was defined by the elongation index (EI) derived from the laser diffraction pattern in a suspension of RBCs subjected to a cycle of deoxygenation and reoxygenation. pO_2 in the RBC suspension was calculated every 20 seconds based on signal quenching using a luminophore O_2 sensor. Point of sickling (PoS) was calculated as the pO_2 (mmHg) at which the EI dropped below 5% of maximum EI during deoxygenation, thus indicating the O_2 pressure at which the polymerization of HbS begins to impact RBC deformability.[21]

Biomarkers

Biomarkers of inflammation (plasma tumor necrosis factor-α, matrix metalloproteinase-9, white blood cell count), hypercoagulability (prothrombin fragment 1.2 [F1.2]; D-dimer), and tissue hypoxia (erythropoietin) were assessed using commercially available kits.

Statistical analyses

Sample size was based on clinical considerations and was not powered for hypothesis testing. Data were analyzed by cohort.

The safety population comprised all patients who received ≥1 dose of study treatment. The PK population included all patients in the safety population with ≥1 evaluable PK profile and no important protocol deviations or other reasons for exclusion from analysis. The PD population included all patients in the safety population with ≥1 post-dose PD assessment.

Statistical analyses were performed using SAS software version 9.4. Wilcoxon tests or unadjusted mixed models for repeated measures statistical tests were used as appropriate. A *P* value < .05 was statistically significant.

Figures were plotted using GraphPad Prism version 9.

The protocol and amendments were reviewed and approved by appropriate institutional review boards (IRBs)/independent ethics committees: - Duke University Health System Institutional Review Board - University of California, San Francisco Human Research Protection Program - Advarra Institutional Review Board - University of Illinois at Chicago Office for the Protection of

Research Subjects - Children's Healthcare of Atlanta Institutional Review Board - Institutional Review Board Office, Augusta University

Results

Study Population

Thirty-six patients were enrolled and treated (supplemental Figure 1). Randomization began in November 2019 with the last patient completing in December 2021. All patients in the single-dose (N = 7) and MAD cohorts (N = 20) completed the study. Fourteen of 15 patients in the OL cohort (including 6 patients from the MAD cohorts who elected to roll over) completed the study; 1 withdrew due to an AE. All 15 patients in the OL cohort were included in the analyses.

Table 1 shows baseline patient demographics and clinical characteristics.

Exposure

Patients in the single-dose cohort received 1 dose of etavopivat at 700 mg. Patients in the MAD1 and MAD2 cohorts received etavopivat 300 mg and 600 mg once daily, respectively (median 14 days [range 14–16 days for 300 mg and 14–14 days for 600 mg]). All patients in the 2 MAD cohorts had ≥ 80% compliance. One patient each in the MAD placebo and 600-mg etavopivat-treated groups had a dose interruption (unspecified nonadherence and “other” [nausea], respectively).

Patients in the OL cohort had a median exposure of 85 (range 14–97) days. Fourteen patients had ≥80% compliance; 1 had <80% compliance. Median exposure was 33,000 (range 5,600–34,400) mg. Two patients experienced dose interruption due to an AE (nausea) and “other” (self-decreased dose due to headache). Another patient had drug withdrawn due to an AE (DVT).

Safety and Tolerability

In the single-dose cohort, 2 (100%) placebo-treated patients and 2 (40%) etavopivat-treated patients had 3 TEAEs each, with 1 etavopivat-treated patient experiencing treatment-related palpitations. All TEAEs were grade 1 (Supplemental Tables 1-3).

In the MAD cohorts, 1 (25%) placebo-treated patient experienced 7 TEAEs, 7 (87.5%) patients in MAD1 had 14 TEAEs, and 6 (75%) patients in MAD2 had 16 TEAEs. Three patients experienced 1 treatment-related TEAE (MAD1, headache and nausea; MAD2, increased total bilirubin). Among etavopivat-treated patients in the MAD cohorts, 10 had grade 1, 9 had grade 2, and 1 had grade 3 TEAEs. One patient in MAD2 experienced a serious unrelated TEAE

(VOE). The most frequently reported all-causality TEAEs were VOEs (N = 6 patients), headache (N = 4), and nausea (N = 2) (supplemental Tables 1-3).

During the 12-week etavopivat 400mg daily OL treatment, 15 (100%) patients experienced 63 TEAEs. The most frequently reported all-causality TEAEs were VOEs (N = 7 patients), headache (N = 4), nausea (N = 3), upper respiratory tract infection (N = 3), and dizziness, migraine, increased gamma-glutamyl transferase, musculoskeletal chest pain, and noncardiac chest pain (N = 2 each) (Supplemental Tables 1-3). TEAEs assessed as possibly or probably treatment-related by the investigator were reported in eight patients; the most common were VOEs (N = 3 patients) occurring on Day 89 (last dose Day 85 [400 mg], Day 89 (stepwise reduction, last dose Day 87 [100 mg), and Day 96 (stepwise reduction, last Day 87 [100 mg]).

In the OL cohort, 13, 8, and 6 patients had grade 1, 2, and ≥3 TEAEs, respectively. Five patients had serious TEAEs—VOE and COVID-19 infection, acute chest syndrome and VOE, DVT, noncardiac chest pain, and syncope (Table 2). On day 15, 1 patient discontinued treatment due to grade 3 DVT (possibly related), which resolved with mild residual swelling on day 80. No deaths were recorded.

Following etavopivat treatment, there were no clinically meaningful adverse shifts in vital signs or, physical examination findings, chemistry, liver function, or hematology laboratory parameters, and no clinically meaningful laboratory abnormalities reported as serious AEs or resulting in study discontinuation. Supplemental Appendix 2 has additional details.

The frequency of pain-related TEAEs decreased over time (supplemental Table 4).

No patient received a transfusion during the study.

PK

Etavopivat was rapidly absorbed with time to maximum observed plasma concentration ranging 1–4 hours post-dose (Table 2; Figure 2). Across cohorts, total exposure (area under the plasma concentration-time curve from 0 to 24 hours) and maximum observed plasma concentration increased with increasing etavopivat dose. The estimated elimination half-life of etavopivat was 16.9 hours in the 700-mg single-dose cohort and 4–4.9 hours in the 300-, 600-, and 400-mg cohorts (Table 2). Apparent etavopivat clearance was similar across cohorts.

PD

Following etavopivat administration, mean whole blood 2,3-DPG levels (µg/mL per g/mL of Hb) declined rapidly from day 1 to day 2 and remained stable throughout the 14-day MAD and

84-day OL periods (Figure 3A-B). At EOT, mean 2,3-DPG levels were significantly lower than baseline in the MAD1 and OL cohorts (Figure 3A-B; Table 3).

Consistent with the mechanism of PKR activation, ATP levels in whole blood rose concomitantly with 2,3-DPG reductions and remained stable throughout treatment. At EOT, mean normalized ATP levels were significantly higher than baseline in the MAD and OL cohorts **(**Figure 3C-D; Table 3).

Following etavopivat discontinuation, 2,3-DPG levels rose to baseline or above over the next 1–4 weeks (Figure 3A-B) while ATP levels decreased toward baseline (Figure 3C-D). Decreased 2,3-DPG was associated with lower P_{50} (Figure 3E).

In the OL cohort, reductions from baseline in P_{50} occurred by day 14 and persisted through day 84; mean changes from baseline were 3.5, 2.9, 4.5, and 3.3 mmHg on days 14 (earliest timepoint), 28, 56, and 84, respectively (Table 3). At EOT, the decrease from baseline in P_{50} was statistically significant in the OL and MAD cohorts (Figure 3F; Table 3; supplemental Figure 3).

Clinical Activity

Hb

An increase in mean Hb concentration occurred on day 2 of treatment in the MAD and OL cohorts (Figure 4A-B). From baseline to EOT, there were statistically significant increases in mean Hb levels of 1.2 (range, −0.1 to 2.3) g/dL, 1.1 (range, −0.1 to 3.5) g/dL, and 1.1 (range, −0.2 to 2.7) g/dL in the MAD1, MAD2, and OL cohorts, respectively (Figure 4A-B; Table 3).

Overall, 87.5%, 50.0%, and 73.3% of patients in the MAD1, MAD2, and OL cohorts, respectively, were Hb responders (>1 g/dL at any time during treatment). In the OL cohort, the mean maximal Hb increase for each patient was 1.6 (range, 0.8–2.8) g/dL (Table 4) regardless of responder status during treatment; among Hb responders, the mean maximal Hb increase during treatment was 1.9 (range, 1.2–2.8) g/dL.

By-patient analyses showed that Hb levels increased in most patients during treatment (Figure 4C-F).

Hemolysis markers

In the MAD and OL cohorts, hemolysis markers (reticulocytes, iBIL, LDH) decreased over the first 1–2 weeks of treatment and remained stable in the OL cohort for the treatment duration (Figure 5). At EOT, mean decreases from baseline in hemolysis markers were statistically significant in the MAD and OL cohorts, except iBIL in MAD1 and LDH in MAD2 (Figure 5; Table 3). Individual patient data at baseline and EOT are shown in supplemental Figure 2.

Impact on RBC Function

P_{50} reduction was associated with a shift in mean PoS to lower pO_2 values (Table 3). At EOT, decreases in PoS from baseline were statistically significant in the MAD and OL cohorts (Figure 6; Table 3; supplemental Figure 3). Although the mean change from baseline to EOT was not statistically significant for RBC deformability (EI_{min} and EI_{max}), hydration (dense RBCs [DRBCs], and CHCM (MAD2) (Table 3), by-patient plots suggest these parameters may have been favorably impacted (decreased DRBCs and increased EI_{min} and EI_{max}) in many of the etavopivat-treated patients (Figure 6; supplemental Figure 3).

Systemic Markers of SCD Pathophysiology

At EOT in the OL cohort, there were statistically significant reductions from baseline in matrix metalloproteinase-9 and erythropoietin mean levels (Figure 7; Table 3). Mean changes from baseline in tumor necrosis factor-α, leukocytes, prothrombin 1.2, and d-dimer were not statistically significant (Figure 7; Table 3). By-patient plots for the MAD cohorts are shown in supplemental Figure 4.

DISCUSSION

Etavopivat is a novel, selective erythrocyte PKR activator with multimodal PD effects — it decreases 2,3-DPG and increases ATP in whole blood. In this phase 1 study, etavopivat, 300 or 600 mg daily for 2 weeks and 400 mg daily for up to 12 weeks, was well tolerated in SCD patients. Decreased intracellular 2,3-DPG and increased intracellular ATP at all doses support the proof-of-mechanism of etavopivat, which resulted in rapid and sustained improvement in Hb levels and reduction of hemolysis as demonstrated by improvements in hemolytic biomarkers. Notably, 11 of 15 (73%) patients in the etavopivat 400mg daily OL cohort achieved >1.0 g/dL Hb increase from baseline during treatment; improved Hb levels were generally accompanied by decreases in hemolytic markers (reticulocytes, iBIL, LDH).

Hb-O_2 affinity was significantly increased by etavopivat, with significant reduction in P_{50} by hemoximetry. Decreased PoS on O_2 gradient ektacytometry (Oxygenscan), a functional biomarker of sickle RBC pathophysiology,[27-29] is associated with lower risk of acute complications in SCD (e.g., cerebral infarction, acute chest syndrome, VOEs).[29-31] Etavopivat improved PoS to lower O_2 pressures from study baseline to EOT in the MAD and OL cohorts. The study was not powered to determine if there were fewer VOEs over time. .

In this study, 87.5%, 50%, and 73.3% of etavopivat-treated patients in the MAD1, MAD2, and OL cohorts, respectively, were Hb responders (>1 g/dL at any time during treatment).

These response rates are consistent with the data reported for another allosteric activator of both wild type and mutant forms of PKR.[33] Although, left shifting of the oxygen-dissociation curve may reduce tissue oxygen delivery and raise erythropoietin levels, leading to increase in Hb concentration with PKR activators, most patients in our study were found to have a decline in serum erythropoietin levels (Figure 7F). Reasons for lack of Hb response in some patients are not yet completely understood. In exploratory analyses of Hb responders versus nonresponders in our study, we did not observe differences in the change in ATP (+102 μg/mL versus +72.6 μg/mL, respectively; P = 0.7) or in 2,3-DPG (-113 μg/mL versus -159 μg/mL, respectively; P = 0.9). Nonresponders in the MAD and OL cohorts had lower baseline reticulocytes and higher baseline erythropoietin (supplemental Table 5). Overall, there was a reduction from baseline in erythropoietin and reticulocyte levels in the OL cohort, but the change from baseline was not significant among Hb nonresponders. Patients with higher baseline hemolysis as indicated by the higher baseline reticulocyte count may be more likely to respond to etavopivat because treatment will decrease hemolysis. Further work is needed to determine whether baseline reticulocyte number is a predictor of etavopivat response. Response to etavopivat may also vary by SCD genotype. We included patients with non-Hb SS SCD because they experience varying degrees of hemolytic anemia and SCD-related complications, and effective therapies are needed in this group of patients. However, with only few non-Hb SS SCD patients enrolled, we are unable to assess the genotype-related effect of etavopivat, and this will be evaluated in the ongoing Phase 2/3 study.

Etavopivat was well-tolerated with a safety profile consistent with the data reported with another PKR activator used in SCD. VOEs were the most common TEAE, occurring in 3 (37.5%), 3 (37.5%) and 7 (46.7%) patients in the MAD1, MAD2, and OL groups, respectively. Three patients in the OL cohort were assessed to have treatment-related VOEs. One patient (12.5%) in the MAD2 group and 2 patients (13.3%) during the 12-week OL period had serious VOEs, all assessed by the investigator as unrelated to treatment. These numbers are comparable to the phase 1 data reported with another PKR activator in SCD, where 4 of 17 (23.5%) patients had serious VOEs.[23] Of the 15 patients receiving etavopivat in the OL cohort, 1 had drug withdrawn due to a serious, possibly treatment-related, grade 3 DVT. Despite the increased risk of thrombosis in adults with SCD,[34] an association with etavopivat treatment could not be excluded by the investigator due to the temporal relationship with study drug initiation. The number of patients with TEAEs related to SCD pain decreased during the 12-week OL treatment and returned to week 1–4 levels after etavopivat was discontinued. Given

the small number of patients, an AE withdrawal event cannot be confirmed or refuted; phase 3 data are needed to further inform any causal relationship.

In conclusion, daily etavopivat, up to 600 mg for 2 weeks and 400 mg for up to 12 weeks, was well-tolerated in patients with SCD. Consistent with the mechanism of PKR activation, increases in whole blood ATP and decreases in 2,3-DPG levels were sustained over 12 weeks. Improvements in Hb oxygenation, RBC physiology, and biomarkers of SCD pathophysiology, translated clinically to 73% of patients in the OL cohort achieving a Hb response (increase from baseline >1 g/dL) during etavopivat treatment. This new, once-a-day PKR activator demonstrated persistent improvement in Hb markers and RBC physiology over a sustained time period (12 weeks) in patients with SCD, in this multicenter placebo-controlled blinded study for 2 weeks as well as in a multicenter OL study for 12 weeks. We recognize the limitation of a small sample size and relatively short treatment period; in addition, in the OL cohort, patients with Hb <7 or >10.5 g/dL were excluded during screening, and males and adolescent patients were under-represented in this study. The safety and efficacy of etavopivat in individuals with SCD aged 12–65 years is being further evaluated in HIBISCUS, a registrational, randomized, placebo-controlled, double-blind, multicenter, phase 2/3 trial (NCT04624659).[32] These longer term data (52 weeks double-blind treatment followed by a 52-week OL extension) will further inform the benefit–risk profile of etavopivat and the potential of this PKR activator to modify the course of SCD.

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Authorship

Contribution: S.L.S., T.A.K., P.F.K., and M.J.T.: Conceptualization, methodology, validation, data analysis, investigation, resources, data curation, writing, preparation, creation and/or presentation of the published work, and supervision of research. R.H., M.I., M.R., K.C., and R.C.B.: Investigation and writing. E.W., P.S., and S.F: Data analysis, investigation, and writing. I.O.: Investigation, resources, and writing. F.A.K.: Methodology, validation, data analysis, investigation, resources, and writing. J.G.: Conceptualization, methodology, validation, data analysis, investigation, resources, data curation, and writing. All authors approved the final version of the manuscript for submission.

Conflict-of-interest disclosure:

E.W., S.F., J.G., I.O., P.S., and M.R. were employees of and held stock in Forma Therapeutics, Inc., at the time of this study. E.W., S.F., I.O., P.S., and M.R. are currently employees of Novo Nordisk. P.F.K is a consultant to Forma Therapeutics, Inc., and was an employee when the study was performed, and holds stock in Forma Therapeutic, Inc.

I.O. is a former principal investigator at Levine Cancer Institute/Atrium health and formerly held consultancies with Forma therapeutics, Novo Nordisk, Agios, GBT, Novartis, Cheisi, Acceleron and Emmaus, and served on speaker's bureau for Novartis, GBT; she received research funding from the Centers for Disease Control (CDC), Health Resources and Service's Administration (HRSA) and Patient Centered Outcomes Research Institute (PCORI). I.O was an employee of Forma between February and October 2022 and is currently an employee of Novo Nordisk since October 2022. S.L.S reports consultancies with Forma Therapeutics, Inc., Novo Nordisk, GBT/Pfizer, ORIC, Agios, and Beam Therapeutics,

membership on the advisory committees of GBT/Pfizer and Novartis, and research funding from Forma Therapeutics, Inc., Novo Nordisk, GBT, Novartis, and Pfizer. R.H. reports consultancies with Bristol Myers Squib, GBT, Imara, NIH, Novartis, and research funding from Chiesi, Forma Therapeutics, Inc., and University of Pittsburgh. M.I. reports consultancies with GBT, receives research funding from Novartis, Pfizer, GBT, Agios, Alexion, Novo Nordisk, and Forma, serves on the GBT speaker's bureau, and is a member of the board/advisory committee of GBT. R.C.B. is a former principal investigator at Children's Healthcare of Atlanta and formally held consultancies with GBT, Imara, Novartis, and received funding from GBT, Novartis, Forma Therapeutics, and Imara; He has been an employee of Global Blood Therapeutics, Inc., a wholly-owned subsidiary of Pfizer as of October 2023, since July 2022. F.A.K. received research funding from Forma. T.A.K. reports consultancies, membership on advisory boards, and research funding from Forma Therapeutics, Inc., Novo Nordisk, Agios Pharmaceuticals, Inc., and research funding from the National Institutes of Health. M.J.T. reports consultancies with GlycoMimetics, Inc., served on a data safety monitoring board of Novartis, and received research funding from Forma Therapeutics, Inc., CSL Behring, Inc., Doris Duke Charitable Foundation, and the National Institutes of Health.

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TABLES

Table 1. Baseline demographic and clinical characteristics

	Single dose		MAD			OL
	Placebo	Etavopivat single dose	Pooled Placebo	Etavopivat once daily for 2 weeks		Etavopivat once daily for 12 weeks
Characteristic, median (range) except where indicated	**(N = 2)**	**700 mg (N = 5)**	**(N = 4)**	**MAD1, 300 mg (N = 8)**	**MAD2, 600 mg (N = 8)**	**400 mg (N = 15)**
Age, years	45 (42–48)	32 (15–42)	26.5 (17–36)	24.0 (19–43)	29.5 (22–64)	33.0 (17–55)
Male sex, n (%)	1 (50.0)	1 (20.0)	3 (75.0)	2 (25.0)	1 (12.5)	5 (33.3)
Genotype, n (%)						
HbSS	2 (100)	5 (100)	4 (100)	7 (87.5)	6 (75.0)	13 (86.7)
HbSC	0	0	0	0	1 (12.5)	2 (13.3)
HbSβ^+-thalassemia	0	0	0	1 (12.5)	1 (12.5)	0
Current hydroxyurea therapy, n (%)	2 (100)	5 (100)	3 (75.0)	6 (75.0)	7 (87.5)	13 (86.7)
Hb, g/dL	7.2 (6.7–7.7)	9.7 (7.7–10.4)	7.6 (7.1–8.0)	9.1 (6.9–10.1)	8.9 (7.3–10.2)	8.7 (7.2–10.1)
% HbS	79.7 (70.2–89.1)	78.8 (70–86.5)	84.6 (76.6–92.7)	83.3 (67.0–92.9)	80.1 (78.2–87.8)	80.3 (46.2–92.7)
% HbF	17.4 (7.3–27.5)	11.4 (5.5–20.5)	10.0 (4.4–16.6)	9.8 (3.5–20.1)	15.3 (5.2–19.2)	11.5 (1.2–23)
Advia MCV, fL*	113.3 (101.6–125.0)	108.7 (96.5–122.8)	107.4 (100.1–131.5)	112.9 (75.0–117.6)	114.7 (68.5–129.6)	108.1 (77.1–122.7)

ARC, 10^9/L[†]	178.1 (72.9–283.4)	205.5 (136.0–366.4)	238.4 (227.0–360.6)	274.1 (125.6–329.6)	226.8 (29.4–366.0)	219.3 (80.5–511.0)
Indirect bilirubin, mg/dL[‡]	3.8 (2.1–5.4)	2.3 (1.6–5.1)	2.8 (2.0–5.0)	1.7 (0.5–10.5)	1.3 (0.7–4.5)	1.3 (0.8–5.2)
LDH, U/L	374.5 (348–401)	405.0 (308–543)	352.0 (180–683)	381.5 (207–699)	368.5 (251–683)	367.0 (186–683)
% F cells[§]	62.6 (33.3–91.8)	50.6 (34.4–75.5)	26.2 (22.3–30.1)	36.1 (16.4–67.2)	54.4 (13.3–64.8)	54.4 (6.1–76.9)

ARC, absolute reticulocyte count; Hb, hemoglobin; HbF, fetal hemoglobin; HbS, sickle hemoglobin; LDH, lactate dehydrogenase; MAD, multiple ascending dose; MCV, mean corpuscular volume; OL, openlabel.

*N = 3 for MAD pooled placebo; N = 7 for MAD1 (300 mg); N = 14 for the 12-week cohort.

[†]N = 3 for MAD pooled placebo.

[‡]N = 4 for single dose 700 mg; N = 3 for MAD pooled placebo.

[§]N = 2 for MAD pooled placebo; N = 7 for MAD1 (300 mg); N = 14 for the 12-week cohort.

Table 2. Pharmacokinetic parameters (pharmacokinetic population)

Exposure in patients who received etavopivat	T_{max}, h	C_{max}, ng/mL	AUC_{0-24}, ng·h/mL	$t_{½}$, h*	CL/F, L/h
Single dose					
700 mg (N = 5)	2.0 (1.0–4.0)	2894 (1450); 50.1	7552 (3294); 43.6	16.9 (7.1); 41.8†	102.0 (50.8); 49.8†
Once-daily multiple doses					
300 mg for 2 weeks					
Day 1 (N = 8)	1.0 (0.9–2.1)	884 (339); 38.3	2508 (995); 39.7‡	4.9 (0.9); 18.4‡	136.6 (61.5); 45.0‡
Day 14 (N = 7)	–	760 (412); 54.2	2747 (1047); 38.1	–	123.5 (46.9); 38.0§
600 mg for 2 weeks					
Day 1 (N = 8)	1.8 (1.0–4.1)	1724 (1246); 72.3	6177(2944); 47.7	4.0 (0.6); 14.4‖	107.2 (45.0); 42.0‖
Day 14 (N = 8)	–	3465 (2136); 61.7	7728 (4218); 54.6	–	98.8 (50.5); 51.1§
400 mg for 12 weeks					
Day 1 (N = 15)	1.8 (1.0–3.9)	1139 (510); 44.8	3474 (1283); 36.9¶	4.7 (1.2); 25.6#	121.8 (31.6); 26.0#
Day 84 (N = 13)	–	1288 (684); 53.1	3105 (901); 29.0**	–	138.2 (37.7); 27.3§,**

Note: A dash indicates not done.

* Data are presented as arithmetic mean (standard deviation) and %CV for C_{max}, AUC_{0-24}, $t_{½}$, and CL/F. Data are presented as median (range) for T_{max}.

AUC_{0-24}, area under the concentration–time curve from time 0 to 24; CL/F, apparent clearance; C_{max}, maximum concentration; $t_{½}$, terminal elimination half-life; T_{max}, time to maximum concentration; %CV, percent coefficient of variation.

*The difference between the 700-mg dose and the 300-mg, 400-mg, and 600-mg doses in estimated $t_{½}$ is likely due the reduced sampling schedule during the elimination phase of the pharmacokinetic profile in the MAD and OL cohorts.

†N = 4.

‡N = 7.

§Steady state.

‖N = 6.

¶N = 11.

#N = 10.

**N = 9.

Table 3. Change from baseline and percentage change from baseline at end of treatment[*, †, ‡, §, ‖]

	OL 400 mg, 12-week cohort (N = 15)		MAD pooled placebo (N = 4)		MAD1, 300 mg (N = 8)		MAD2, 600 mg (N = 8)	
	Mean (SD)	Median (min, max)	Mean (SD)	Median (min, max)	Mean (SD)	Median (min, max)	Mean (SD)	Median (min, max)
2,3 DPG, µg/mL per g/mL Hb								
Baseline	5956.5 (2050.8)	6181.8 (541, 8350)	4349.7 (2904.8)	4689.5 (541, 7479)	6109.7 (621.8) N = 7	6053.8 (5368, 7071) N = 7	6821.8 (1764.2)	6103.9 (5067, 10693)
EOT	4099.2 (833.8) N = 14	4090.2 (2732.1, 5555.6) N = 14	5890.9 (800.4)	5642.6 (5253, 7025)	4263.6 (507.3) N = 7	4193.5 (3595, 4915) N = 7	4643.8 (2374.7)	3968.8 (2852, 10338)
CFB EOT	−1732.9* (2213.6) N = 14	−1988.4* (−4511.8,3595.3) N = 14	1541.2 (2430.4)	873.1 (−454, 4872)	−1846.1* (494.2) N = 7	−1860.2* (−2665,-1156) N = 7	−2177.9 (2919.3)	−2115.2 (−7457, 3310)
ATP, µg/mL per g/mL Hb								
Baseline	2037.7 (947.3) N = 13	2250.0 (321, 3111) N = 13	1284.5 (1128.5)	1152.2 (313, 2521)	2355.5 (418.5) N = 7	2149.4 (1989, 3170) N = 7	2117.9 (601.6)	2217.5 (989, 3033)
EOT	3802.0 (1276.1) N = 11	4117.0 (1484.9, 5276.8) N = 11	1854.7 (1096.4)	2209.5 (333, 2667)	3202.9 (516.2) N = 7	3265.0 (2577, 4150) N = 7	3218.1 (739.2)	3391.6 (2024, 4255)
CFB EOT	1939.4* (1483.7) N = 11	2067.5* (−802.4,4112.1) N = 11	570.2 (1196.2)	57.7 (−189, 2354)	847.3* (656.4) N = 7	680.1* (127, 2101) N = 7	1100.2* (528.0)	1282.3* (251, 1596)
Hemoglobin, g/dL								
Baseline	8.7 (1.0)	8.7 (7.2, 10.1)	7.6 (0.4)	7.6 (7.1, 8.0)	9.0 (1.1)	9.1 (6.9, 10.1)	8.7 (0.9)	8.9 (7.3, 10.2)
EOT	9.8 (1.1)	9.5 (8.4, 12.0)	7.7 (.2)	7.7 (7.5, 7.9)	10.1 (1.6)	10.8 (6.8, 11.7)	9.8 (1.5)	9.8 (7.4, 12.3)

	OL 400 mg, 12-week cohort (N = 15)		MAD pooled placebo (N = 4)		MAD1, 300 mg (N = 8)		MAD2, 600 mg (N = 8)	
	Mean (SD)	Median (min, max)	Mean (SD)	Median (min, max)	Mean (SD)	Median (min, max)	Mean (SD)	Median (min, max)
CFB EOT	1.1* (0.8) N = 14	1.2* (−0.2, 2.7) N = 14	0.1 (0.5)	0.0 (−0.4, 0.8)	1.2* (0.9)	1.2* (−0.1, 2.3)	1.1* (1.1)	1.0* (−0.1, 3.5)
Absolute reticulocytes, 10^9/L								
Baseline	229.4 (116.9)	219.3 (80.5, 511.0)	275.3 (74.1) N = 3	238.4 (227.0, 360.6) N = 3	252.3 (70.4)	274.1 (125.6, 329.6)	227.3 (105.8)	226.8 (29.4, 366.0)
EOT	163.3 (85.8) N = 14	133.1 (48.7, 351.6) N = 14	241.9 (115.7)	261.8 (85.2, 358.9)	144.0 (121.1)	99.2 (60.0, 433.9)	130.1 (64.0)	135.2 (24.8, 219.1)
CFB EOT	−66.8* (107.0) N = 14	−44.6* (−305.0, 118.1) N = 14	18.8 (98.0) N = 3	11.0 (−75.0, 120.5) N = 3	−108.3* (114.7)	−142.0* (−233.8, 133.4)	−97.2* (66.3)	−87.4* (−217.2, −4.6)
Indirect bilirubin, mg/dL								
Baseline	1.9 (1.4)	1.3 (0.8, 5.2)	3.3 (1.6) N = 3	2.8 (2.0, 5.0) N = 3	3.1 (3.4)	1.7 (0.5, 10.5)	1.9 (1.4)	1.3 (0.7, 4.5)
EOT	1.2 (0.7) N = 13	0.9 (0.5, 2.9) N = 13)	2.6 (1.5)	2.6 (0.8, 4.4)	1.8 (1.5)	0.9 (0.3, 3.8)	1.1 (0.8)	0.7 (0.5, 2.6)
CFB EOT	−0.5* (0.8) N = 13	−0.5* (−2.3, 0.5) N = 13	−0.03 (0.5) N = 3	0.21 (−0.6, 0.3) N = 3	−1.3 (2.5)	−0.5 (−7.0, 0.9)	−0.8* (0.7)	−0.6* (−1.9, −0.1)
Lactate dehydrogenase, U/L								
Baseline	375.2 (142.9)	367.0 (186, 683)	391.8 (210.5)	352.0 (180, 683)	430.4 (159.0)	381.5 (207, 699)	391.4 (129.7)	368.5 (251, 683)
EOT	319.1 (87.7) N = 14	323.0 (193, 470) N = 14	449.8 (209.5)	486.5 (192, 634)	311.4 (155.3)	280.0 (159, 641)	315.5 (94.0)	308.5 (199, 492)
CFB EOT	−38.4* (77.7)	−23.0*	58.0 (151.4)	17.0	−119.0* (113.3)	−97.5*	−75.9 (173.2)	−54.5

	OL 400 mg, 12-week cohort (N = 15)		MAD pooled placebo (N = 4)		MAD1, 300 mg (N = 8)		MAD2, 600 mg (N = 8)	
	Mean (SD)	**Median (min, max)**	**Mean (SD)**	**Median (min, max)**	**Mean (SD)**	**Median (min, max)**	**Mean (SD)**	**Median (min, max)**
	N = 14	(−213, 57) N = 14		(−77, 275)		(−283, 61)		(−461, 90)
P_{50}, mmHg								
Baseline	29.9 (2.4) N =14	30.3 (25.8, 34.4) N = 14	29.2 (5.9) N = 3	30.5 (22.8, 34.4) N = 3	30.4 (1.3) N = 6	30.8 (28.3, 31.6) N = 6	30.1 (2.2)	30.4 (26.2, 33.2)
EOT	26.6 (2.5) N = 14	25.8 (23.8, 32.1) N = 14	31.4 (2.6)	31.3 (28.5, 34.6)	26.4 (1.6) N = 7	26.6 (23.8, 28.4) N = 7	26.3 (1.9)	26.5 (23.2, 28.9)
CFB EOT	−3.3* (2.0) N = 13	−3.8* (−5.9, 1.9) N = 13	2.0 (3.2) N = 3	0.2 (0.2, 5.7) N = 3	−4.3* (1.9) N = 6	−4.3* (−7.2, −1.6) N = 6	−3.9* (1.6)	−3.5* (−6.7, −2.3)
PoS, mmHg								
Baseline	43.2 (7.1) N = 14	43.1 (22.0, 50.0) N = 14	45.8 (4.9) N = 3	48.1 (40.1, 49.2) N = 3	36.3 (9.2) N = 6	39.1 (19.0, 45.0) N = 6	38.5 (8.6)	39.7 (26.2, 49.2)
EOT	35.1 (12.3) N = 14	36.5 (9.5, 51.0) N = 14	63.2 (34.2)	52.8 (34.6, 112.7)	31.0 (9.7)	28.5 (18.1, 47.3)	31.3 (3.8)	31.1 (25.6, 36.2)
CFB EOT	−8.6* (8.2) N = 13	−8.6* (−22.8, 3.0) N = 13	19.8 (38.1) N = 3	1.3 (−5.6, 63.5) N = 3	−8.0* (5.7) N = 6	−9.0* (−15.1, −0.9) N = 6	−7.3* (7.0)	−7.2* (−15.4, 4.6)

	OL 400 mg, 12-week cohort (N = 15)		MAD pooled placebo (N = 4)		MAD1, 300 mg (N = 8)		MAD2, 600 mg (N = 8)	
	Mean (SD)	**Median (min, max)**	**Mean (SD)**	**Median (min, max)**	**Mean (SD)**	**Median (min, max)**	**Mean (SD)**	**Median (min, max)**
EI_{min}								
Baseline	0.12 (0.07) N = 14	0.11 (0.0, 0.3) N = 14	0.13 (0.15) N = 3	0.18 (0, 0.3) N = 3	0.11 (0.10) N = 6	0.07 (0, 0.3) N = 6	0.19 (0.11)	0.15 (0.1, 0.4)
EOT	0.16 (0.14) N = 14	0.12 (0.0, 0,5) N = 14	0.06 (0.11)	0.03 (0.0, 0.2)	0.18 (0.15)	0.12 (0.1, 0.5)	0.23 (0.10)	0.21 (0.1, 0.5)
CFB EOT	0.05 (0.10) N = 13	0.04 (−0.1, 0.2) N = 13	−0.06 (0.19) N = 3	0.04 (−0.3, 0.1) N = 3	0.10 (0.11) N = 6	0.05 (0, 0.2) N = 6	0.04 (0.05)	0.06 (0, 0.1)
EI_{max}								
Baseline	0.45 (0.10) N = 14	0.46 (0.2, 0.5) N = 14	0.38 (0.19) N = 3	0.49 (0.2, 0.5) N = 3	0.43 (0.14) N = 6	0.43 (0.3, 0.6) N = 6	0.48 (0.06)	0.49 (0.4, 0.6)
EOT	0.47 (0.08) N =14	0.49 (0.2, 0.5) N = 14	0.40 (0.14)	0.44 (0.2, 0.5)	0.49 (0.08)	0.51 (0.3, 0.6)	0.51 (0.04)	0.51 (0.4, 0.6)
CFB EOT	0.02 (0.05) N = 13	0.01 (−0.1, 0.1) N = 13	0.00 (0.05) N = 3	0.02 (−0.1, 0) N = 3	0.05 (0.08) N = 6	0.02 (0, 0.2) N = 6	0.03 (0.049)	0.02 (0, 0.1)
Hyper (dense) RBCs, %								
Baseline	3.2 (2.5) N = 14	2.8 (1.0, 11.1) N = 14	5.0 (5.4) N = 3	2.7 (1.1, 11.1) N = 3	4.0 (3.1) N = 7	3.3 (0.9, 8.9) N = 7	2.5 (1.1)	2.6 (1.0, 4.1)
EOT	2.8 (2.1) N = 14	2.0 (1.2, 8.9) N = 14	4.8 (3.3)	3.9 (1.9, 9.6)	2.6 (1.8)	2.1 (1.1, 6.9)	1.9 (0.9)	1.9 (0.7, 3.3)
CFB EOT	−0.3 (1.6) N = 13	−0.4 (−2.5, 2.7) N = 13	0.2 (1.5) N = 3	0.8 (−1.5, 1.3) N = 3	−1.3 (1.7) N = 7	−1.8 (−4.2, 0.7) N = 7	−0.6 (1.1)	−0.8 (−2.1, 1.4)
CHCM, g/dL								
Baseline	33.0 (1.3) N = 14	32.8 (30.6, 35.3)	32.6 (2.3) N = 3	32.3 (30.5, 35.1)	33.0 (0.9) N = 7	33.0 (31.7, 34.3)	32.3 (1.3)	32.6 (30.3, 34.3)

	OL 400 mg, 12-week cohort (N = 15)		MAD pooled placebo (N = 4)		MAD1, 300 mg (N = 8)		MAD2, 600 mg (N = 8)	
	Mean (SD)	Median (min, max)	Mean (SD)	Median (min, max)	Mean (SD)	Median (min, max)	Mean (SD)	Median (min, max)
		N = 14		N = 3		N = 7		
EOT	32.5 (1.5) N = 14	32.2 (30.8, 36.1) N = 14	33.5 (1.0)	33.4 (32.5, 34.8)	32.4 (0.9)	32.2 (31.3, 33.8)	31.7 (1.3)	31.6 (29.2, 33.6)
CFB EOT	−0.6 (1.1) N = 13	−0.9 (−1.8, 2.3) N = 13	1.0 (1.2) N = 3	1.3 (−0.3, 2.0) N = 3	−0.7* (0.6) N = 7	−0.7* (−1.4, 0.3) N = 7	−0.6 (0.8)	−0.9 (−1.3, 0.6)
TNF-α, pg/mL								
Baseline	1.2 (0.5) N = 14	1.1 (0.6, 2.2) N = 14	1.7 (0.7) N = 2	1.7 (1.3, 2.2) N = 2	1.2 (0.5)	1.1 (0.6, 2.0)	1.4 (0.5) N = 7	1.2 (0.8, 2.0) N = 7
EOT	0.8 (0.4) N = 13	0.9 (0.2, 1.6) N = 13	1.3 (0.1)	1.2 (1.1, 1.5)	1.4 (0.6) N = 7	1.4 (0.7, 2.3) N = 7	0.7 (0.4)	0.8 (0.2, 1.3)
CFB EOT	−0.3 (0.7) N = 12	−0.1 (−1.7, 0.4) N = 12	−0.5 (0.6) N = 2	−0.5 (−0.9, −0.1) N = 2	0.1 (0.6) N = 7	0.0 (−0.6, 1.0) N = 7	−0.5* (0.6) N = 7	−0.3* (−1.7, 0) N = 7
MMP-9, ng/mL								
Baseline	440.1 (282.4) N = 13	434.7 (90.3, 929.4) N = 13	573.1 N = 1	573.1 (573.1, 573.1) N = 1	ND	ND	451.2 (313.5) N = 7	434.7 (97.2, 929.4) N = 7
EOT	296.0 (354.0) N = 13	175.6 (0.0, 1280.9) N = 13	280.9 (96.6) N = 2	280.9 (212.6, 349.1) N = 2	ND	ND	282.2 (176.2)	242.4 (69.3, 602.3)
CFB EOT	−149.8* (259.3) N = 11	−175.6* (−627.8, 351.5) N = 11	−224.0 N = 1	−224.0 (−224.0, −224.0) N = 1	ND	ND	−193.3* (215.4) N = 7	−96.3* (−615.5, −18.5) N = 7
Leukocytes, 10^9/L								

	OL 400 mg, 12-week cohort (N = 15)		MAD pooled placebo (N = 4)		MAD1, 300 mg (N = 8)		MAD2, 600 mg (N = 8)	
	Mean (SD)	Median (min, max)	Mean (SD)	Median (min, max)	Mean (SD)	Median (min, max)	Mean (SD)	Median (min, max)
Baseline	9.6 (4.9)	7.9 (5.0, 24.5)	11.2 (4.6)	10.4 (6.9, 17.3)	8.8 (4.4)	7.5 (4.4, 15.8)	8.6 (2.9)	7.9 (6.0, 14.7)
EOT	8.2 (3.3) N = 14	8.9 (2.7, 13.1) N = 14	11.1 (6.1)	9.0 (6.3, 20.1)	7.1 (3.4)	5.9 (3.9, 14.4)	6.2 (2.1)	5.5 (4.1, 9.9)
CFB EOT	−1.4 (3.7) N = 14	−1.1 (−12.1, 3.0) N = 14	−0.1 (2.3)	−0.3 (−2.8, 2.8)	−1.7* (2.1)	−1.5* (−6.4, 0.2)	−2.4 (2.7)	−1.7 (−6.9, 1.2)
Prothrombin fragment 1.2, pmol/L								
Baseline	672.4 (1235.1) N = 14	350.5 (150, 4900) N = 14	377.0 (234.7) N = 3	366.0 (148, 617) N = 3	2305.7 (4750.1) N = 6	380.5 (252, 12 000) N = 6	1341.2 (1768.7) N = 6	665.0 (371, 4900) N = 6
EOT	297.0 (151.0) N = 13	260.0 (106, 659) N = 13	352.3 (23.7)	352.5 (323, 381)	408.5 (189.3) N = 6	394.0 (177, 663) N = 6	1923.6 (4078.4)	543.0 (154, 12000)
CFB EOT	−91.1 (249.2) N = 12	1.0 (−720, 154) N = 12	−25.0 (205.7) N = 3	−14.0 (−236, 175) N = 3	25.0 (171.8) N = 5	35.0 (−199, 278) N = 5	−912.3 (1843.3) N = 6	−323 (−4611, 353) N = 6
D-dimer, µg/mL FEU								
Baseline	2.4 (1.4) N = 13	2.1 (0.6, 5.9) N = 13	2.5 (1.2) N = 3	2.1 (1.6, 3.8) N = 3	2.5 (2.3) N = 5	1.9 (0.2, 6.3) N = 5	3.4 (1.5) N = 6	3.3 (1.6, 5.9) N = 6
EOT	1.9 (1.0) N = 13	1.8 (0.2, 3.7) N=13	2.2 (0.6)	2.2 (1.6, 2.9)	3.0 (1.7) N = 6	3.4 (1.1, 5.1) N = 6	2.7 (1.5)	2.6 (0.9, 5.0)
CFB EOT	−0.6 (1.5) N = 12	−0.4 (−3.2, 1.6) N = 12	−0.3 (1.7) N = 3	−0.2 (−2.1, 1.4) N = 3	0.3 (1.2) N = 5	0.7 (−1.2, 1.8) N = 5	−0.6 (1.5) N = 6	−0.6 (−3.0, 1.7) N = 6
Erythropoietin, mIU/mL								
Baseline	104.7 (63.6) N = 13	94.4 (17.0, 244.9)	157.1 N = 1	157.1 (157.1, 157.1)	92.1 (50.1) N = 2	92.1 (56.6, 127.5)	152.0 (130.4) N = 7	106.7 (65.9, 441.6)

	OL 400 mg, 12-week cohort (N = 15)		MAD pooled placebo (N = 4)		MAD1, 300 mg (N = 8)		MAD2, 600 mg (N = 8)	
	Mean (SD)	Median (min, max)	Mean (SD)	Median (min, max)	Mean (SD)	Median (min, max)	Mean (SD)	Median (min, max)
		N = 13		N = 1		N = 2		N = 7
EOT	88.0 (65.9) N = 13	56.7 (17.5, 234.1) N = 13	159.0 (94.6) N = 2	159.0 (92.1, 225.9) N = 2	82.8 (35.6) N = 2	82.8 (57.6, 108.0) N = 2	149.3 (142.5)	128.2 (17.8, 473.8)
CFB EOT	−18.6* (44.8) N = 11	−29.4* (−76.0, 73.9) N = 11	−65.0 N = 1	−65.0 (−65.0, −65.0) N = 1	−69.9 N = 1	−69.9 (−69.9, −69.0) N = 1	6.3 (70.5) N = 7	32.2 (−109.6, 81.5) N = 7

ATP, adenosine triphosphate; CFB, change from baseline; CHCM, cellular hemoglobin concentration mean; El_{max}, maximum elongation index; El_{min}, minimum elongation index; EOT, end of treatment; Hb, hemoglobin; LDH, lactate dehydrogenase; LS, least squares; MAD, multiple ascending dose; ND, not done; max, maximum; min, minimum; MMP-9, matrix metalloproteinase-9; OL, openlabel; PoS, point of sickling; P_{50}, oxygen tension at which hemoglobin is 50% saturated; RBC, red blood cell; TNF-α, tumor necrosis factor-alpha; 2,3-DPG, 2,3 diphosphoglycerate.

Note: The N-values represent patients with non-missing values.

*P < .05 for baseline versus EOT comparison. For the MAD cohorts, P values were obtained from a Wilcoxon signed rank test. For the OL cohort, P values for hemoglobin, LDH, reticulocytes, indirect bilirubin, were derived from LS means using a mixed model for repeated measurement, with hematology/hemolysis assessment as dependent variable and scheduled visit during treatment period as a fixed effect. An unstructured covariance was used for hemoglobin, LDH, and reticulocytes. A compound symmetry covariance was used for indirect bilirubin, normalized ATP, and normalized 2,3-DPG. In the OL cohort, P values for normalized 2,3-DPG, normalized ATP, P_{50}, PoS, El_{min}, El_{max}, hyper RBCs, CHCM, TNF-α, MMP-9, leukocytes, prothrombin fragment 1.2, D-dimer, and erythropoietin were derived from a Wilcoxon signed rank test.

†For the MAD cohorts, baseline was defined as the last measurement obtained prior to the first dose of study drug. For the 12-week cohort, baseline was defined as average of prior-treatment measurements (screening and predose on day 1) for patients who were newly enrolled in the 12-week cohort; for patients who were enrolled in the MAD2 (600 mg) cohort and later rolled over into the 12-

week cohort, baseline was defined as the average of prior treatment measurements (screening and predose on day 1) in the MAD2 (600 mg) period.

‡Sample sizes that deviate from those in the column header are indicated in the appropriate cells.

§EOT was day 14/15 (24 hours after last dosing) in the MAD cohorts and day 84/85 (24 hours after last dosing) in the OL cohort.

‖One MAD1 (300 mg) patient was excluded from 2,3 DPG, ATP, and P_{50} analyses because the patient only took 1 dose of study drug on day 1.

Table 4. Hemoglobin responders at any time during treatment (all cohorts)

	Placebo or etavopivat once daily			
	MAD Placebo	**MAD1**	**MAD2**	**OL**
	0 mg	**300 mg**	**600 mg**	**400 mg**
Weeks	2	2	2	12
N	4	8	8	15
Maximal Hb increase, mean (range), g/dL	0.4 (0.0–0.6)	1.4 (0.4–2.4)	1.4 (0.2–3.5)	1.6 (0.8–2.8)
Hb increase >1 g/dL response on treatment, n (%)	0	7 (87.5)	4 (50.0)	11 (73.3)
Maximal Hb increase in patients with >1 g/dL response, mean (range), g/dL	NA	1.5 (1.1–2.4)	2.2 (1.5–3.5)	1.9 (1.2–2.8)

Hb, hemoglobin; MAD, multiple ascending dose; NA, not assessed; OL, openlabel.

Note: Only measurements up to end of treatment were included.

FIGURE LEGENDS

Figure 1. Study design. Patients in the MAD2 cohort could directly rollover into the OL cohort at the time of their end-of-study visit if they tolerated the 2-week treatment period and continued to meet eligibility criteria. Patients from other cohorts and the study sites could also enroll in the OL cohort. In the OL cohort, protocol amendment 7.0 allowed etavopivat dosing to extend from 2 days to up to 2 weeks beyond day 84, allowing a stepwise dose decrease in patients with a >2.0 g/dL increase in hemoglobin over baseline or if clinically indicated. MAD, multiple ascending dose; OL, openlabel.

Figure 2. Etavopivat Concentration versus time following daily dosing in patients with sickle cell disease (MAD and OL cohorts). Mean (standard deviation) etavopivat concentrations following daily dosing on day 14 (MAD) or day 84 (OL) at the indicated time point (hours). MAD, multiple ascending dose; OL, openlabel; SD, standard deviation.

Figure 3. Pharmacodynamics in patients with sickle cell disease. Mean RBC 2,3-DPG and ATP concentrations in the MAD (A, C), and OL (B, D) cohorts. Values were normalized by dividing the hemoglobin value at each time point to adjust for a dilution effect from increased hemoglobin (A, B, C, D). The P_{50} value as a function of intracellular 2,3-DPG concentration in the MAD (excluding placebo patients) and OL cohorts 24 hours after the last dose (E). Scatter plot at baseline and EOT for P_{50} in the OL cohort (Median BL and EOT values shown in red and blue diamonds, respectively) (F); each data point corresponds to data from 1 patient. Paired baseline and end-of-treatment data points from each patient are connected by a line. In the MAD cohorts (A, C), *P* values were based on Wilcoxon signed rank tests to test the changes at EOT from baseline. In the OL cohort (B, D), PD values with statistical significance compared to baseline were identified with an asterisk (*$P < .05$) at their scheduled visits, based on MMRM, which included PD values as dependent variable, and a fixed effect of scheduled visit during the treatment period with compound symmetry covariance matrix to model the within-patient variance-covariance errors; the EOT *P* values were derived from Wilcoxon signed rank tests. Statistical tests were not performed for the visits after EOT. *P* values in the scatter plot are from a Wilcoxon matched-pairs signed rank test (F). One MAD1 (300 mg) patient was excluded from 2,3 DPG, ATP, and P_{50} analyses because the patient only took 1 dose of study drug on day 1. ATP, adenosine triphosphate; BL, baseline; CFB, change from baseline; EOT, end of treatment; Hb, hemoglobin; MAD, multiple ascending dose; MMRM, mixed model for repeated

measurement; OL, openlabel; P_{50}, the partial pressure of dissolved O_2 at which hemoglobin is 50% saturated with oxygen; PD, pharmacodynamic; 2,3-DPG, 2,3-diphosphoglycerate.

Figure 4. Change in hemoglobin response in patients with sickle cell patients (MAD and OL cohorts). Mean (± SE) hemoglobin concentration over time in the MAD (A) and OL (B) cohorts. Values for mean change from baseline at EOT are shown on the graphs (A, B). In the MAD cohorts, EOT was equal to the day 15 value if available, otherwise EOT was equal to day 14 (A). In the OL cohort, EOT was equal to the day 85 value if available, otherwise EOT was equal to day 84 (B). Scatter plots at baseline and EOT for MAD pooled placebo (C), MAD1 (D), MAD2 (E), and OL (F); each data point corresponds to data from 1 patient. Median BL and EOT values shown in red and blue diamonds, respectively (C-F). Paired baseline and end-of-treatment data points from each patient are connected by a line. In the MAD cohorts (A), *P* values were based on Wilcoxon signed rank tests to test the changes at EOT from baseline. In the OL cohort (B), hemoglobin values with statistical significance as compared to baseline were identified using asterisks (*$P \leq .0001$, **$P < .01$) at their scheduled visits, based on MMRM, which included hemoglobin values as a dependent variable, and a fixed effect of scheduled visits during the treatment period, with unstructured covariance matrix to model the within-patient variance-covariance errors. Statistical tests were not performed for the visits after EOT. *P* values in the scatter plots are from a Wilcoxon matched-pairs signed rank test (C, D, E, F). BL, baseline; CFB, change from baseline; EOT, end of treatment; MAD, multiple ascending dose; MMRM, mixed model for repeated measurement; OL, openlabel; SE, standard error.

Figure 5. Hemolysis markers in patients with sickle cell disease (MAD and OL cohorts). Mean (± SE) absolute reticulocytes, indirect bilirubin, and LDH over time in the MAD cohorts (A, B, C, respectively) and OL cohorts (D, E, F, respectively). In the MAD cohorts (A,B,C), *P* values were based on Wilcoxon signed rank tests to test the changes at EOT from baseline. In the OL cohort (D,E,F), hemolysis marker values with statistical significance as compared to baseline were identified using an asterisk (*$P \leq .05$) at their scheduled visits, based on MMRM, which included hemolysis marker values as a dependent variable, and a fixed effect of scheduled visits during the treatment period. An unstructured covariance was used for LDH and reticulocytes, and a compound symmetry covariance was used for indirect bilirubin. Statistical tests were not performed for the visits after EOT. BL, baseline; CFB, change from baseline; EOT, end of treatment; LDH, lactate dehydrogenase; MAD, multiple ascending dose; MMRM, mixed model for repeated measurement OL, openlabel.

Figure 6. Markers of RBC physiology (OL cohort). Scatter plots for PoS (A), El_{min} (B), El_{max} (C), and dense (hyper) RBCs (D) at baseline and EOT. Each data point corresponds to data from 1 patient. Paired baseline and EOT data points from each patient are connected by a line. Median BL and EOT values shown in red and blue diamonds, respectively. *P* values are from a Wilcoxon matched-pairs signed rank test. % hyper RBC is defined as the percent of RBCs with >41 g/dL of hemoglobin. BL, baseline; DRBC, dense red blood cell; El_{max}, maximum elongation index; $El_{min,}$ minimum elongation index; EOT, end of treatment; OL, openlabel; PoS, point of sickling; RBC, red blood cell.

Figure 7. Systemic markers of sickle cell disease pathophysiology in patients with sickle cell disease (OL cohort). Each data point corresponds to data from 1 patient. Paired baseline and EOT data points from each patient are connected by a line. Median BL and EOT values shown in red and blue diamonds, respectively. *P* values are from a Wilcoxon matched-pairs signed rank test. TNF-α (A), MMP-9 (B), leukocytes (C), prothrombin 1.2 (D), D-dimer (E), and erythropoietin (F). MMP-9, matrix metalloproteinase-9; OL, openlabel; EPO, erythropoietin; EOT, end of treatment; TNF-α, tumor necrosis factor-alpha.

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Figure 1

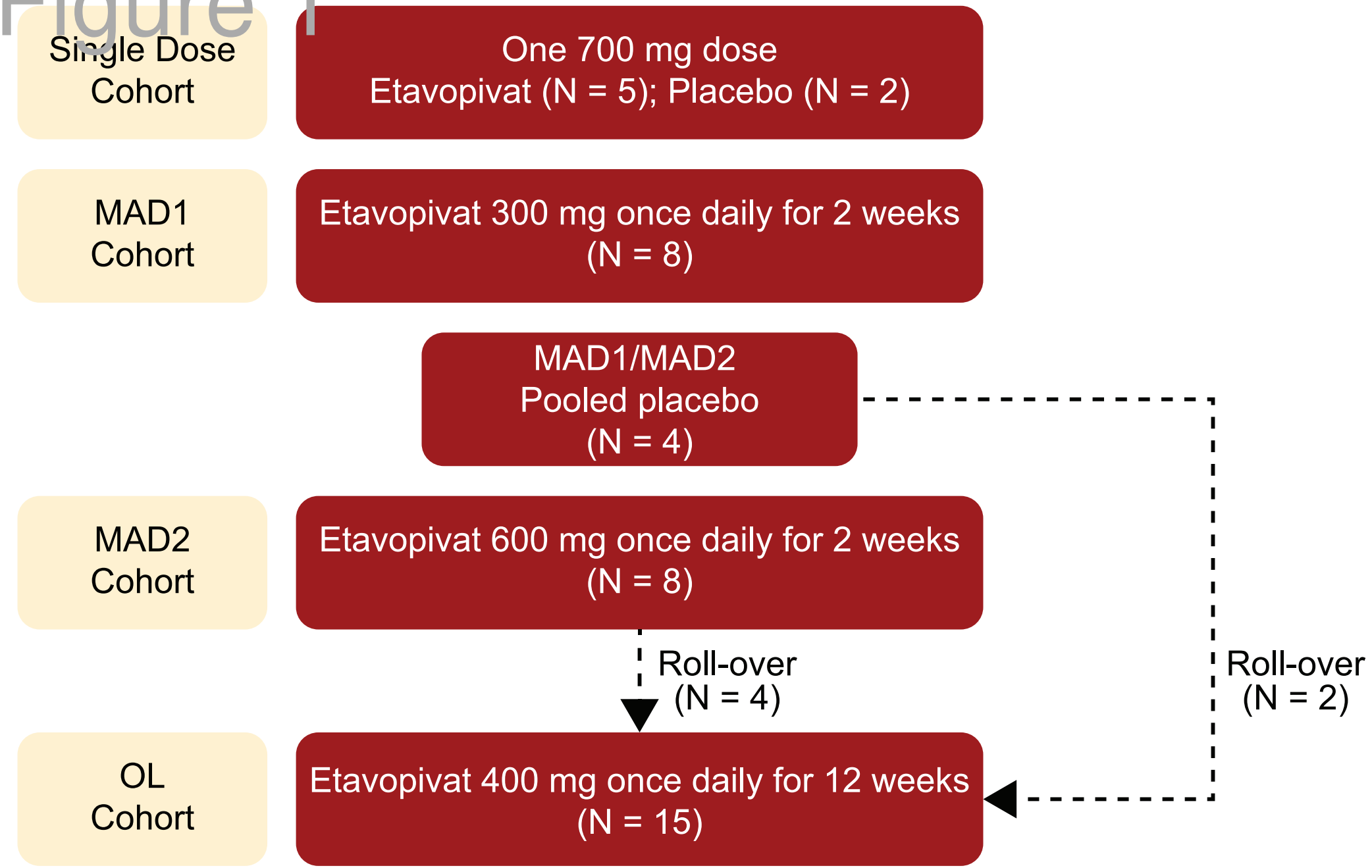
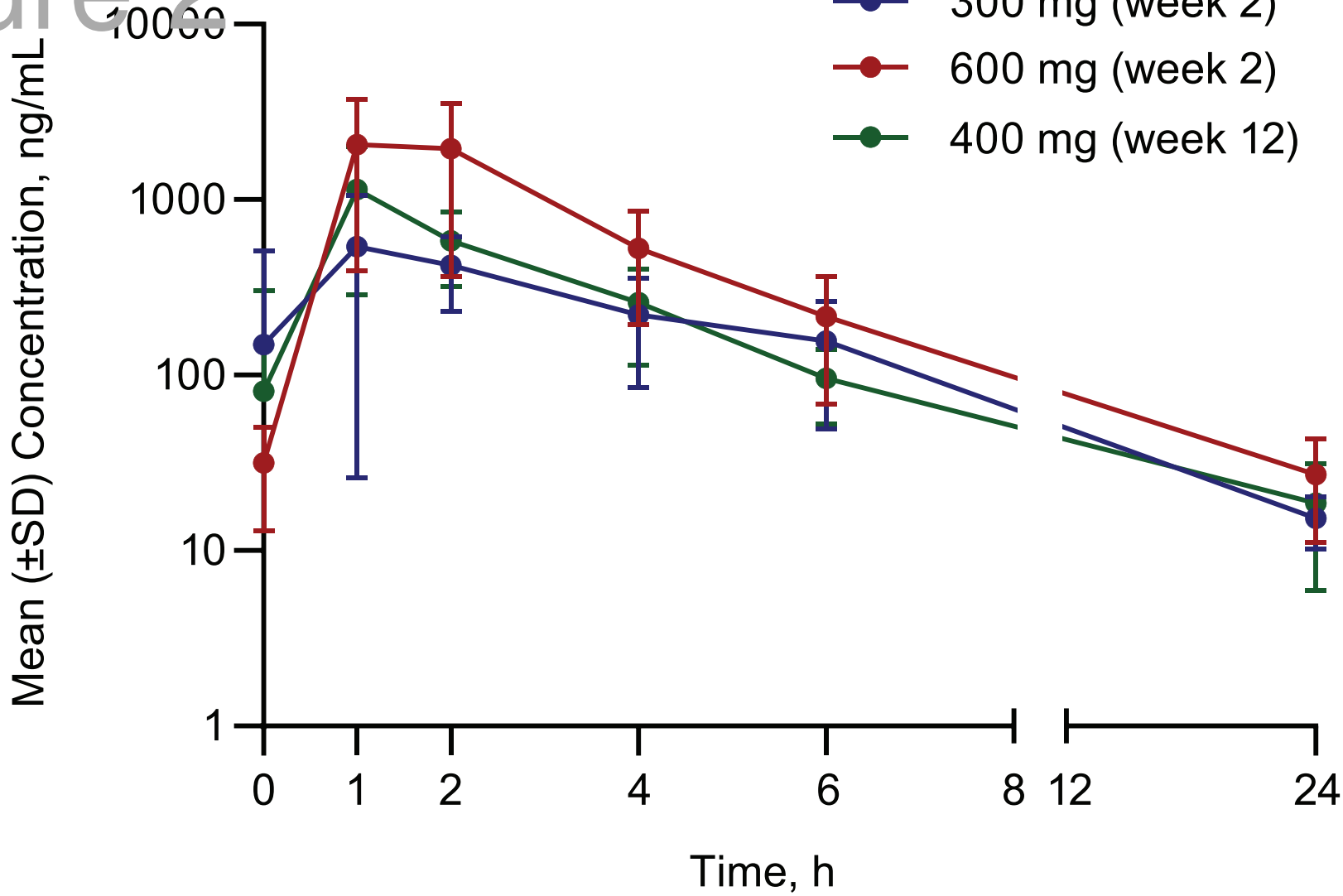


Figure 2



300 mg N =	7	7	7	7	7		6
600 mg N =	7	8	8	8	8		8
400 mg N =	12	13	13	13	13		14

Figure 3

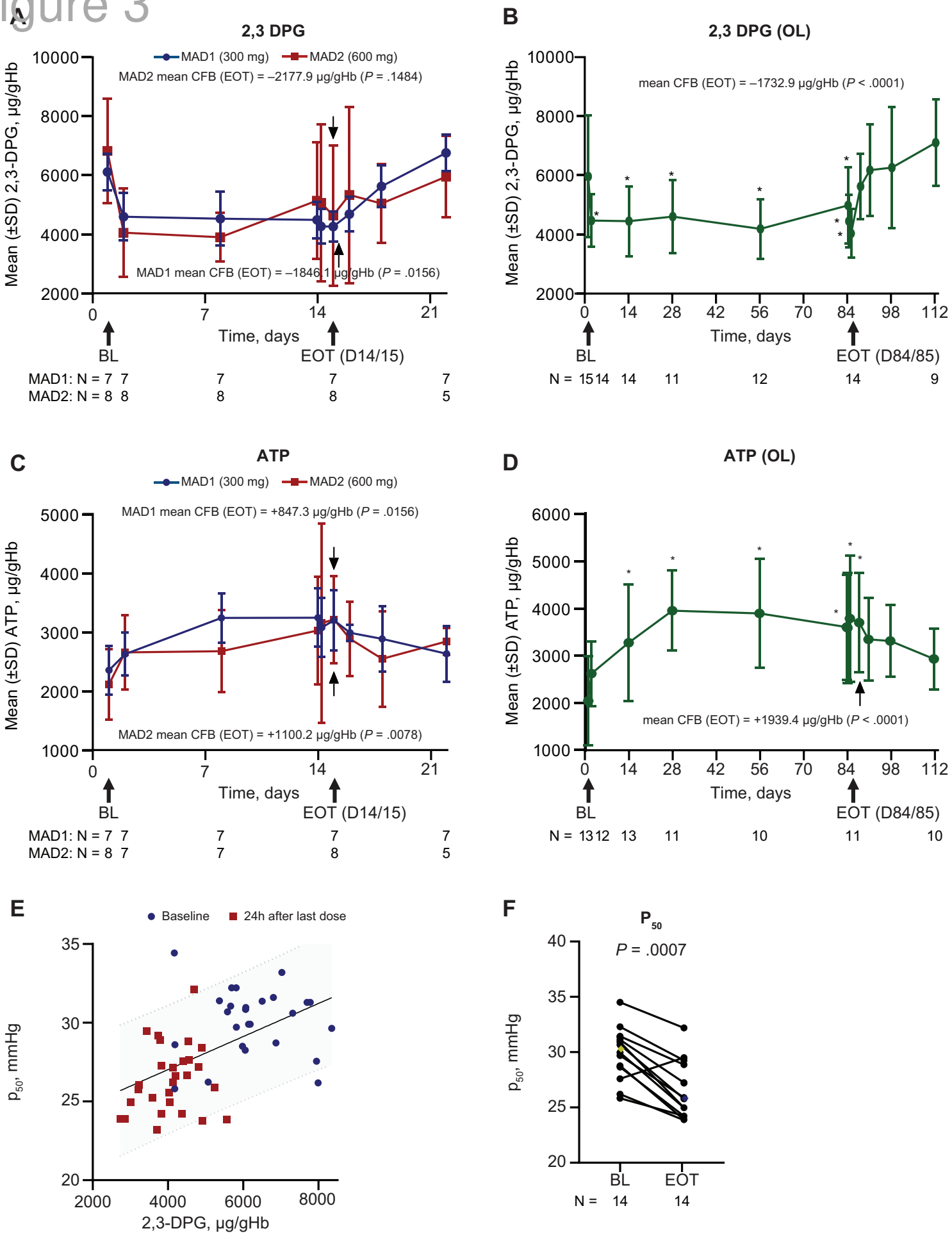
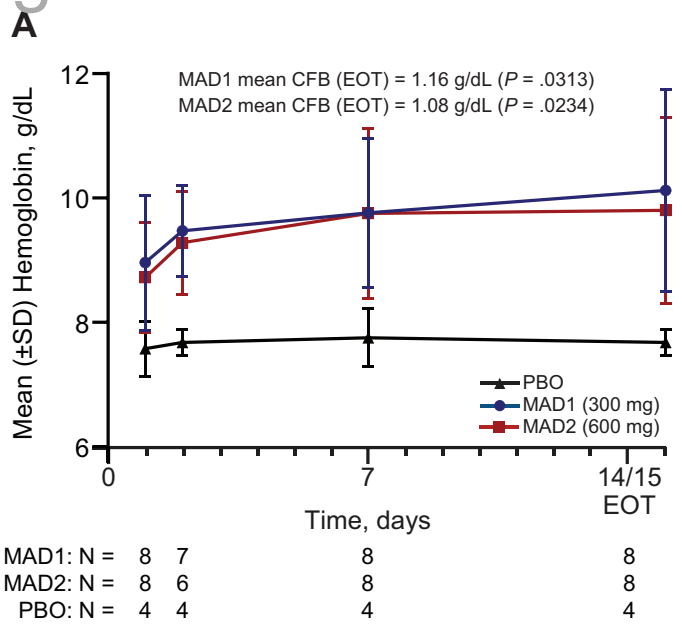
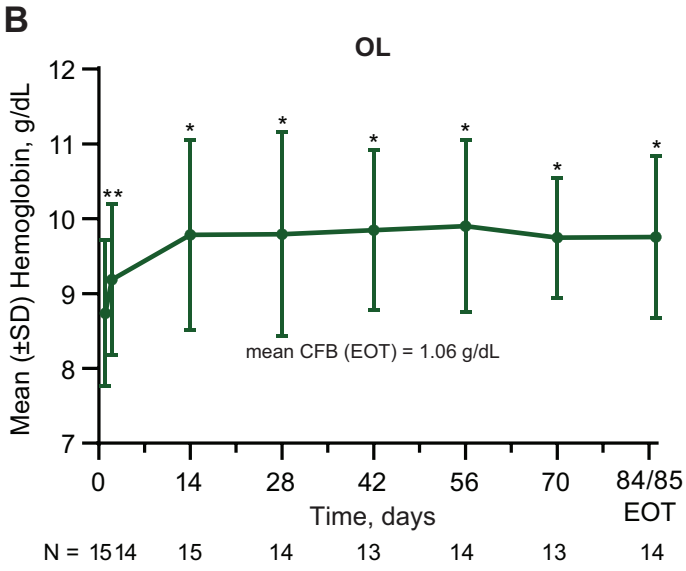
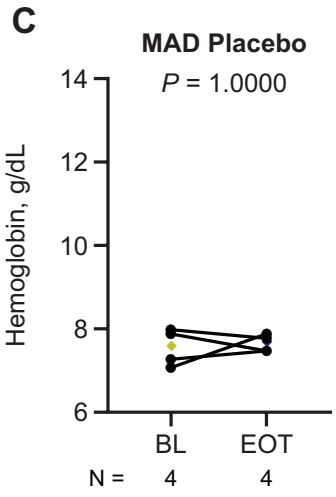
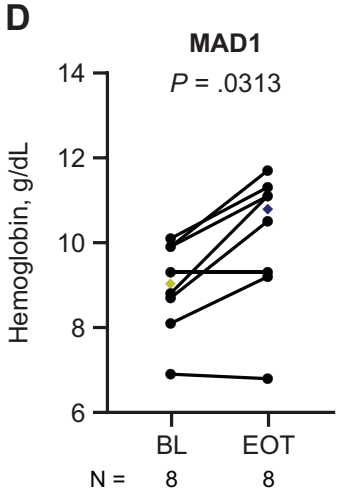


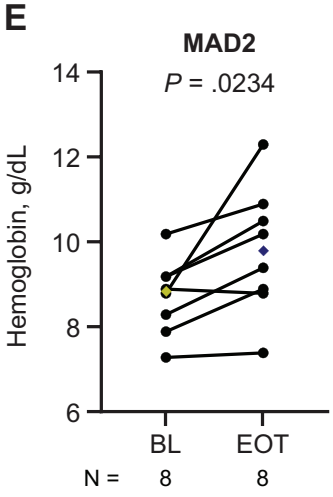
Figure 4











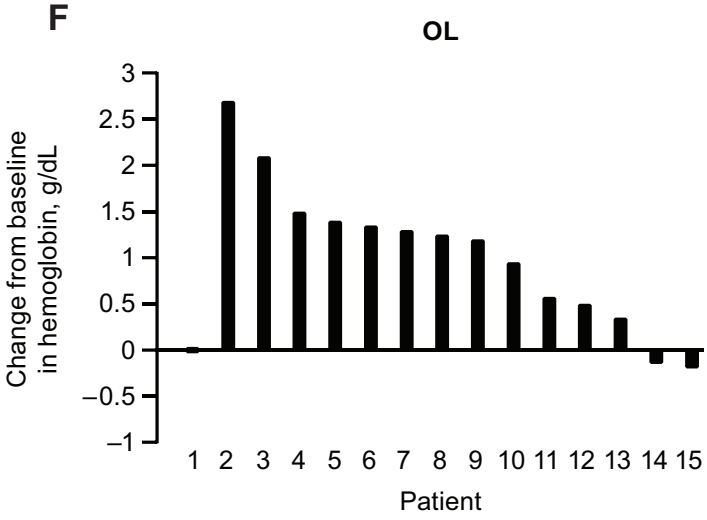


Figure 5

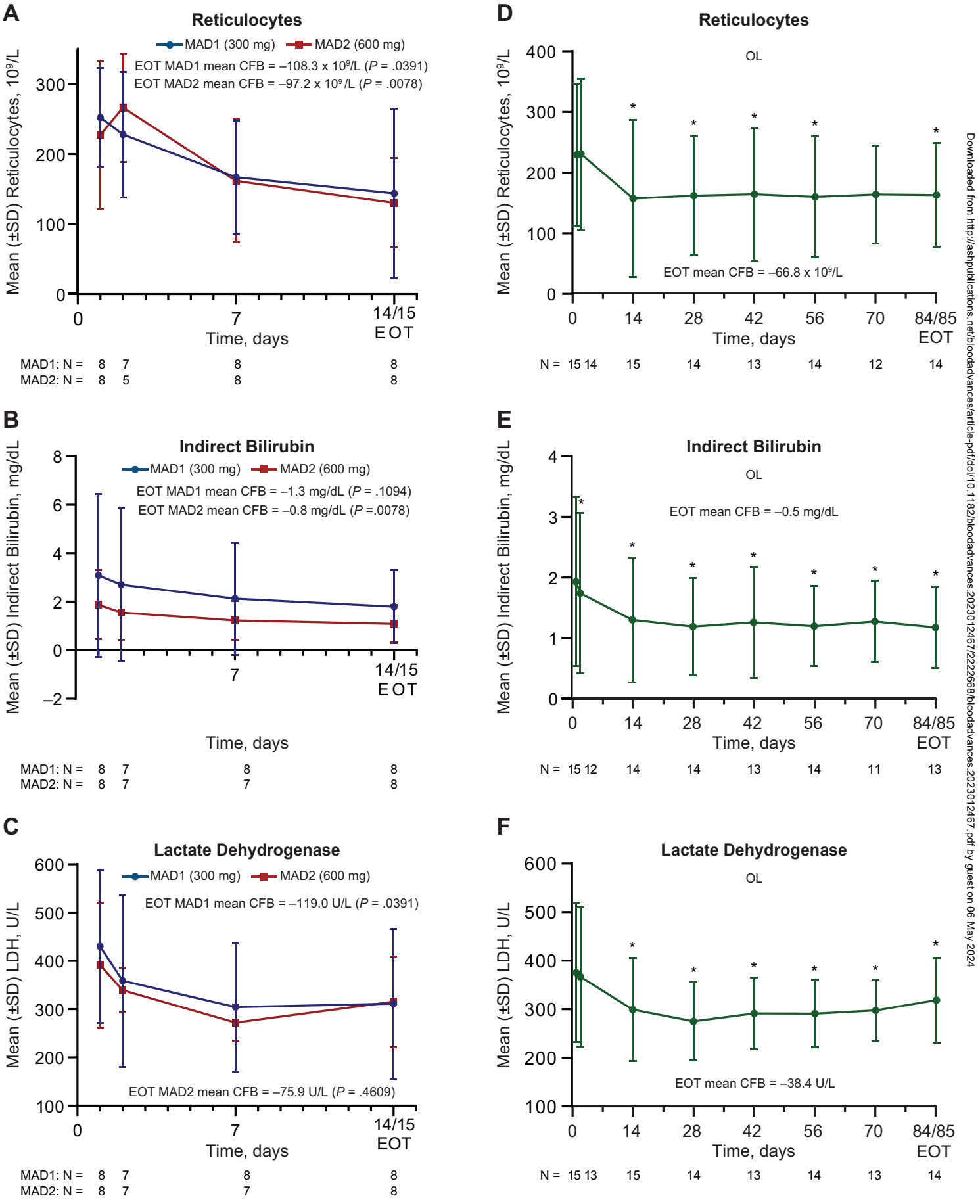


Figure 6

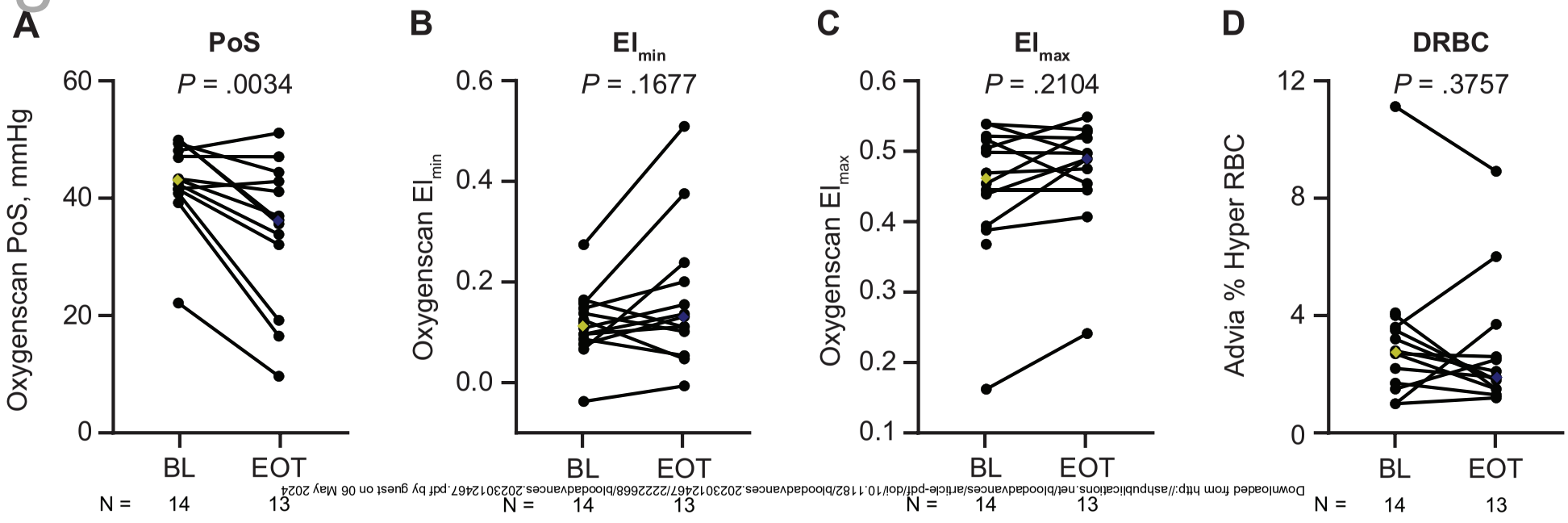


Figure 7