SUPPLEMENTAL DATA

Etavopivat, a Pyruvate Kinase Activator in Red Blood Cells, for the Treatment of Sickle Cell Disease

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Text S1. 2,3-DPG PK/PD modeling

NHP PK and 2,3-DPG data were modeled using a simultaneous PK/PD approach utilizing Phoenix® NLME software (Certara, Princeton, NJ). The PK data were fit using a two-compartment model with first-order absorption. The 2,3-DPG data were fit using a basic indirect-response model (Dayneka et al., 1993; Sharma and Jusko, 1998) with a stimulation of loss function for 2,3-DPG shown in equation 1, where $2,3DPG_0$ is baseline 2,3-DPG levels, K_{out} is a first-order loss rate constant, S_{max} is the maximum ability of etavopivat to affect K_{out}, and SC_{50} is the etavopivat concentration resulting in 50% of the maximum stimulation achieved at the effect site.

$$\frac{d2,3DPG}{dt} = 2,3DPG_0 \cdot K_{out} - K_{out} \cdot \left(1 + \frac{S_{max} \cdot C}{C + SC_{50}}\right) \cdot 2,3DPG$$

The maximum 2,3-DPG suppression ($2,3DPG_{max}$) was estimated from equation 2.

$$2,3DPG_{max} = \left(\frac{2,3DPG_0}{1 + S_{max}}\right)$$

Half of the maximal 2,3-DPG response ($2,3DPG_{50}$) was estimated from equation 3

$$2,3DPG_{50} = 2,3DPG_0 - \frac{2,3DPG_0 - 2,3DPG_{max}}{2}$$

The plasma concentration resulting in half of the maximum decrease in 2,3-DPG (EC_{50}) was calculated using equation 4.

$$EC_{50} = \frac{SC_{50} \cdot (2,3DPG_0 - 2,3DPG_{50})}{2,3DPG_{50} \cdot (1 + S_{max}) - 2,3DPG_0}$$

Table S1. Etavopivat primary PK/PD parameters in non-human primates

Parameter	Value	S.D.
$2,3DPG_0$ (µg/mL)	841	9.79
K_{out} (h^{-1})	0.100	0.019
S_{max}	0.870	0.159
SC_{50} (ng/mL)	33.5	12.9

$2,3DPG_0$, baseline 2,3-DPG levels; K_{out}, first-order loss rate constant; PD, pharmacodynamic; PK, pharmacokinetic; S_{max}, maximum ability of etavopivat to affect K_{out}; SC_{50}, etavopivat concentration resulting in 50% of the maximum stimulation achieved at the effect site; S.D, standard deviation.

Table S2. Etavopivat exposure in non-human primates after single and repeated escalating doses

	Etavopivat mean (S.D.) PK parameters (oral dosing)			
	Day 1		Day 5	
Dose (mg/kg)	C_{max} (ng/ml)	$AUC_{0–24}$ (h·ng/ml)	C_{max} (ng/ml)	$AUC_{0–24}$ (h·ng/ml)
3	94 (44)	608 (292)	83 (45)	295 (52)
8	352 (95)	1116 (163)	725 (198)	1274 (86)
22	424 (320)	1728 (658)	689 (200)	2568 (803)
50	1823 (489)	8877 (1749)	ND	ND

n = 4 animals per dose

$AUC_{0–24}$, area under the concentration time curve from time 0–24 hours after dosing; C_{max}, maximum plasma concentration; ND, not determined; PK, pharmacokinetic; S.D, standard deviation.

Table S3. Change in P_{50} following etavopivat treatment in healthy subjects and ex vivo RBC from donors with SCD

	P_{50} Pre-dose (mmHg)	**P_{50} 24 hours post-dose (mmHg)**	**Change in P_{50} (mmHg)**
Healthy subjects: single 700-mg dose (*n* = 6)	26.5 (1.53)	21.6 (2.05)	4.85 (0.68)
	P_{50} - vehicle (DMSO) Treatment (mmHg)	**P_{50} - etavopivat Treatment (mmHg)**	**Change in P_{50} (mmHg)**
Ex vivo SCD RBC (HbSC disease) (*n* = 6)	26.3 (1.24)	24.8 (1.58)	1.47 (0.63)
Ex vivo SCD RBC (HbSS disease) (*n* = 13)	26.1 (1.99)	24.8 (1.82)	1.27 (0.76)

Data are mean (S.D.)

DMSO, dimethyl sulfoxide; P_{50}, the partial pressure of dissolved oxygen at which Hb is 50% saturated with oxygen; RBC, red blood cell; SCD, sickle cell disease; S.D., standard deviation.