Preclinical Characterization of Vadadustat (AKB-6548), an Oral Small Molecule Hypoxia Inducible Factor Prolyl-4-Hydroxylase Inhibitor, for the Potential Treatment of Renal Anemia

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PRESENTED AT ASN KIDNEY WEEK 2019

Disclosures

- The authors are employees of Akebia Therapeutics, which funded the studies

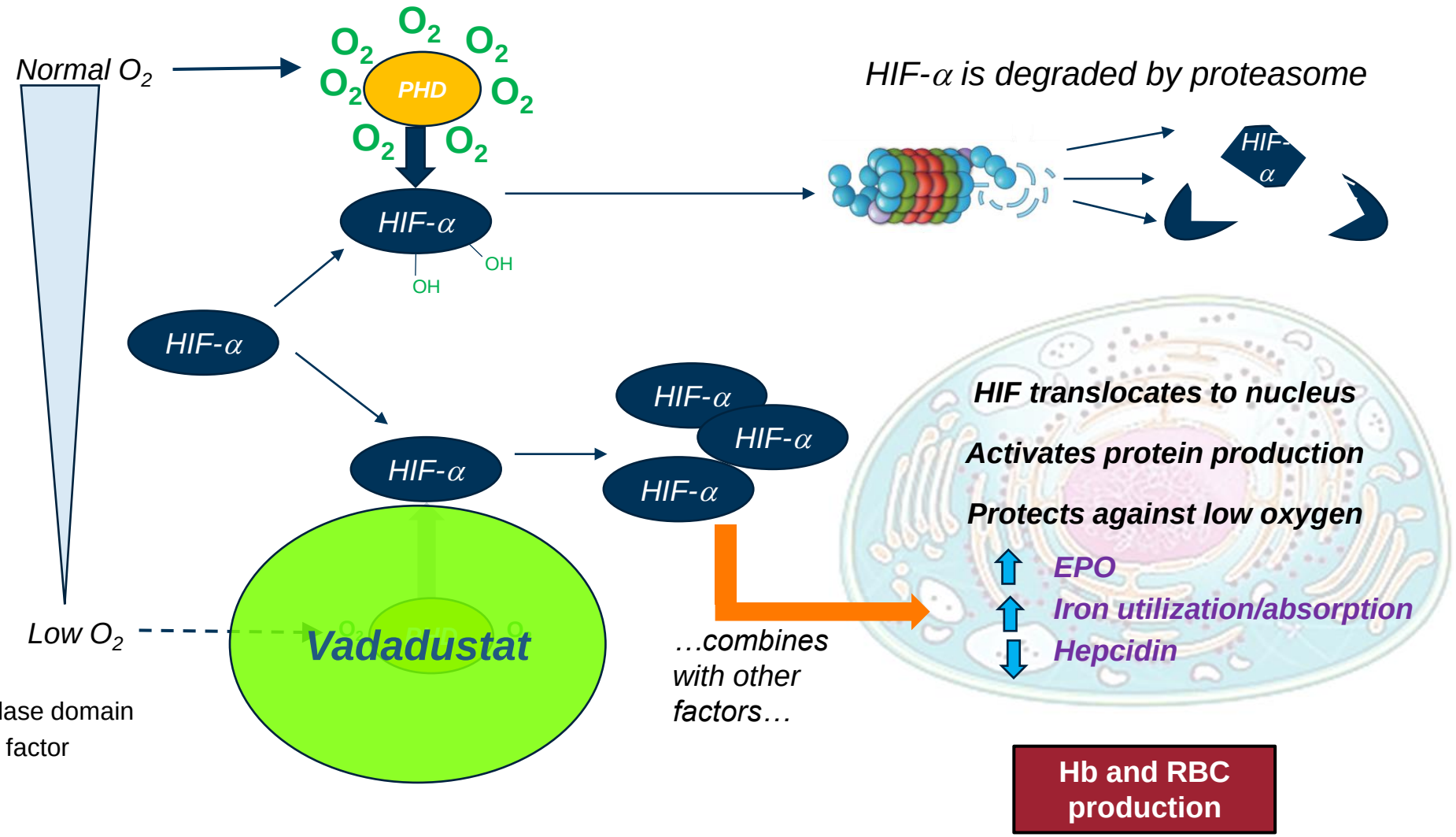
Disclaimers

- Vadadustat is an investigational drug. Vadadustat is not approved by the United States Food and Drug Administration or any regulatory authority.

Objective

- To summarize the preclinical pharmacological characterization of vadadustat

HIF and the prolyl-4-hydroxylase domain enzymes



Abbreviations:

O_2 = oxygen

PHD = prolyl-4-hydroxylase domain

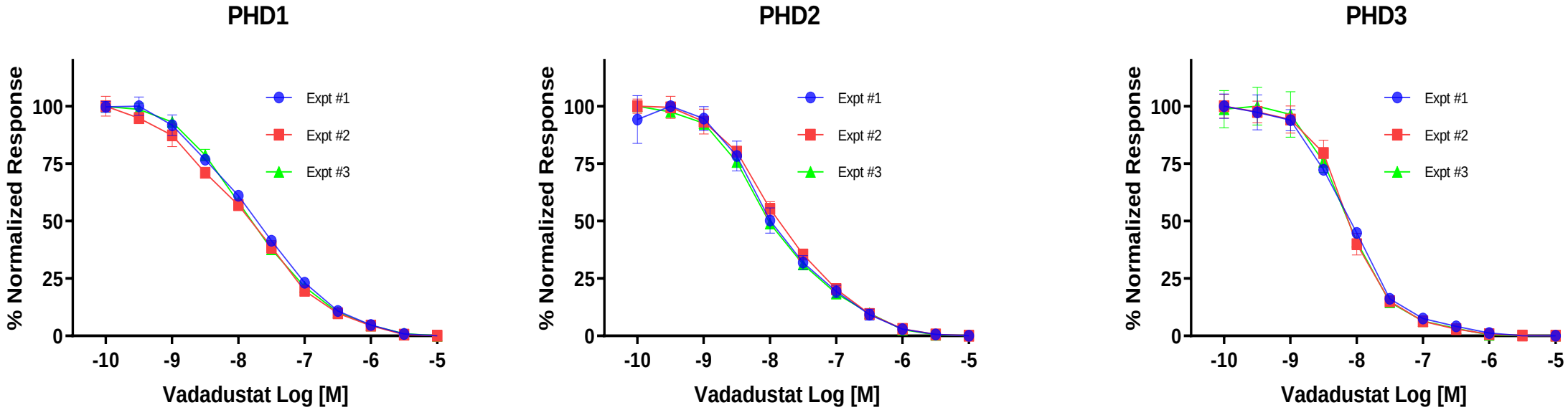
HIF = hypoxia inducible factor

EPO = erythropoietin

Hb = hemoglobin

RBC = red blood cell

Vadadustat inhibits recombinant human PHD1, PHD2 and PHD3 at equivalent nanomolar concentrations*



*Measured by Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) Assay. Data represent Mean ± SD.

	Mean (95% Confidence Interval)	
	IC_{50} value (nM)	pIC_{50} value
PHD1	15.36 (11.96, 19.73)	7.81 (7.71, 7.92)
PHD2	11.83 (8.20, 17.07)	7.93 (7.77, 8.09)
PHD3	7.63 (7.21, 8.07)	8.12 (8.09, 8.14)

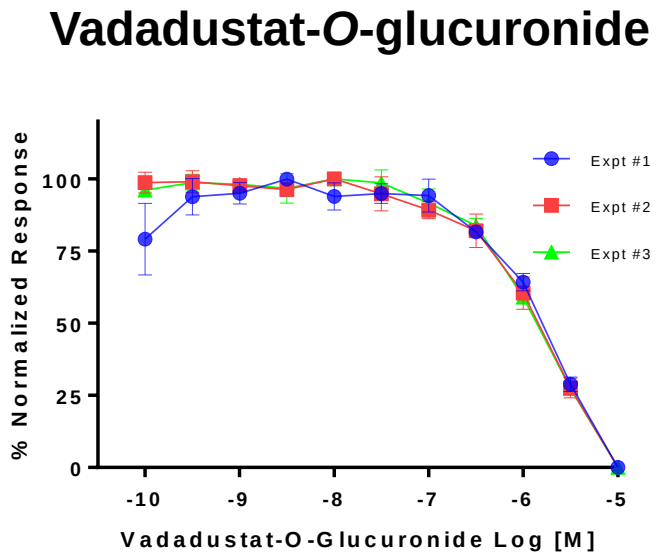
Abbreviations:

PHD = prolyl-4-hydroxylase domain

IC_{50} = half maximal inhibitory concentration

pIC_{50} = negative log of the IC_{50} value in molar

Vadadustat-O-glucuronide inhibits recombinant human PHD2 at micromolar concentration*



*Measured by TR-FRET Assay. Data represent Mean ± SD.

	IC_{50} value (µM)	pIC_{50} value
Mean (95% Confidence Internal)	2.31 (1.74, 3.08)	5.64 (5.51, 5.77)

Inhibition is approximately 200-fold less potent than the parent compound at the IC_{50}

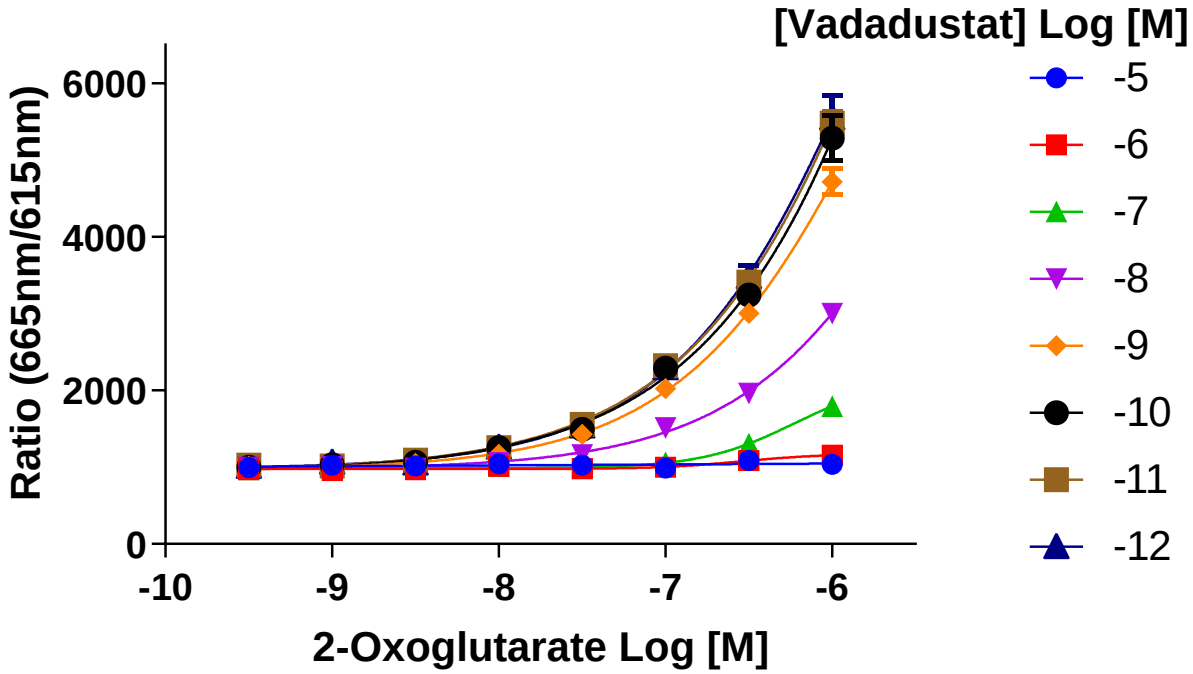
Abbreviations:

PHD2 = prolyl-4-hydroxylase domain 2

IC_{50} = half maximal inhibitory concentration

pIC_{50} = negative log of the IC_{50} value in molar

Vadadustat is a competitive inhibitor of 2-oxoglutarate for recombinant human PHD2*

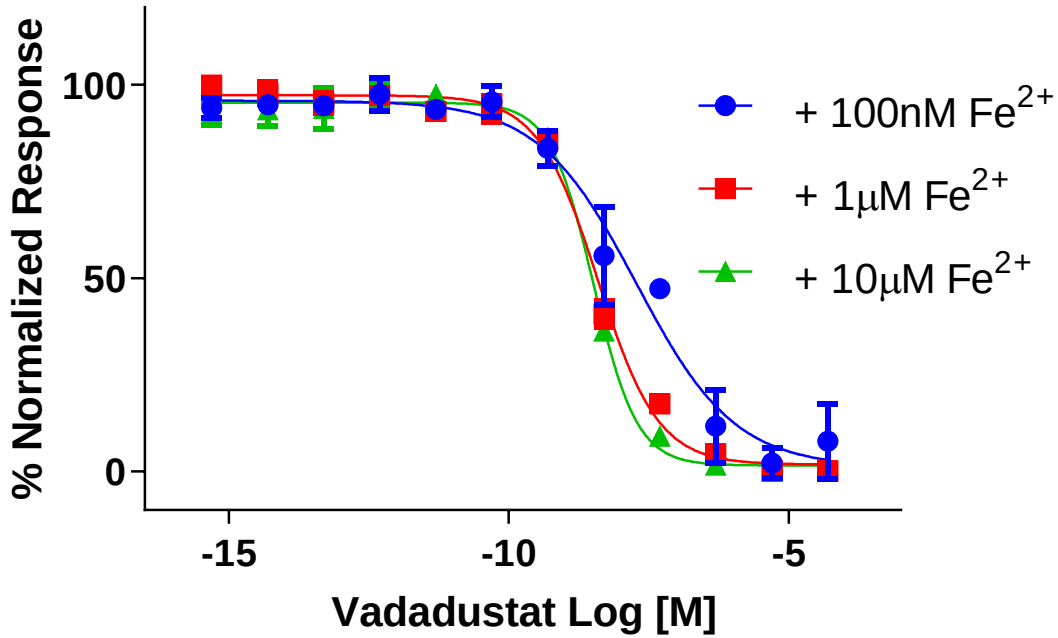


*Measured by TR-FRET Assay. Data represent Mean ± SD.

Abbreviations:

PHD2 = prolyl-4-hydroxylase domain 2

Vadadustat inhibition of recombinant human PHD2 is not sensitive to iron concentration in vitro*



*Measured by TR-FRET Assay. Data represent Mean ± SD.

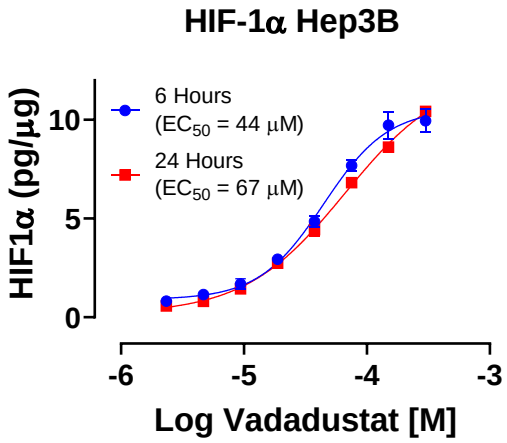
	Vadadustat + 100 nM Fe^{2+}	Vadadustat + 1 µM Fe^{2+}	Vadadustat + 10 µM Fe^{2+}
IC_{50} (nM)	19.25 ± 5.74	3.91 ± 0.38	3.26 ± 0.24

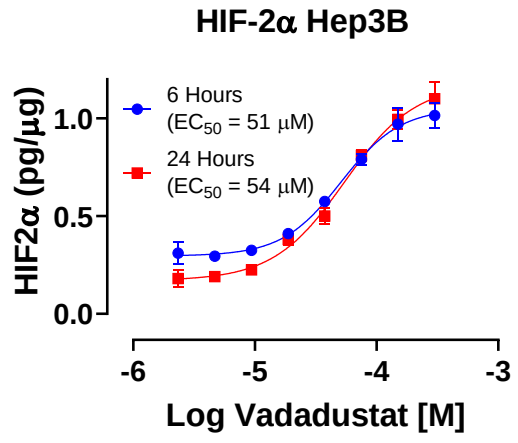
Abbreviations:

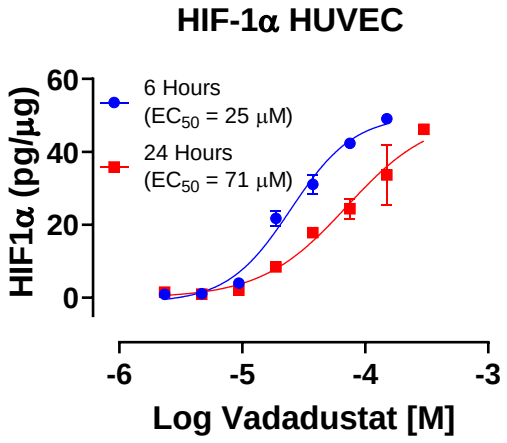
PHD2 = prolyl-4-hydroxylase domain 2

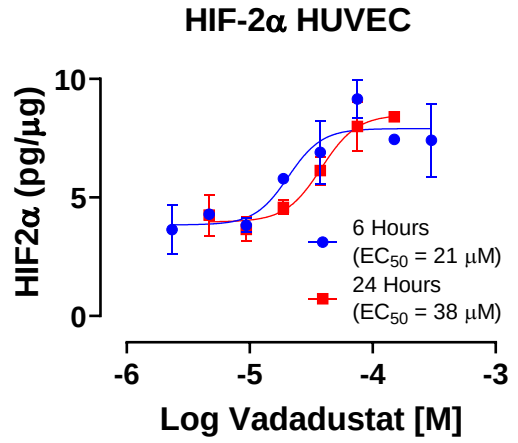
IC_{50} = half maximal inhibitory concentration

Vadadustat was shown to stabilize both HIF-1α and HIF-2α in Hep3B and HUVEC cell lines in a dose and time dependent manner*









Abbreviations:

HIF1α = hypoxia inducible factor-1 alpha

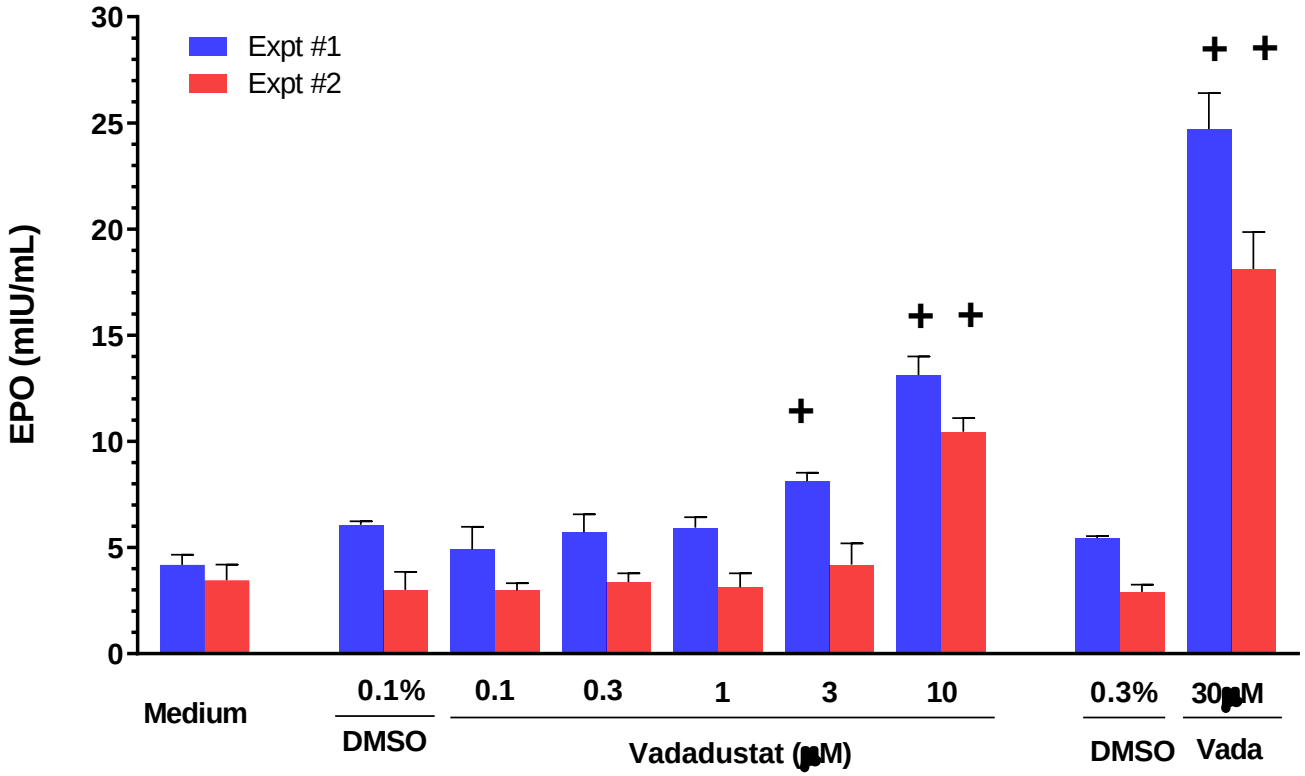
HIF2α = hypoxia inducible factor-2 alpha

Hep3B = human hepatocarcinoma cell line

HUVEC = human umbilical vein endothelial cell

*Measured by Mesoscale Discovery (MSD) Electrochemiluminescence Assay. HIF1α and HIF2α were normalized to total cellular protein (pg/μg). Data represent Mean ± SD.

Erythropoietin (EPO) secretion is increased in vitro after exposure of Hep3B cells to vadadustat*



	EPO EC_{50} (μM)	EPO release (mIU/mL)/EC_{50}
Vadadustat	9.97	12.04

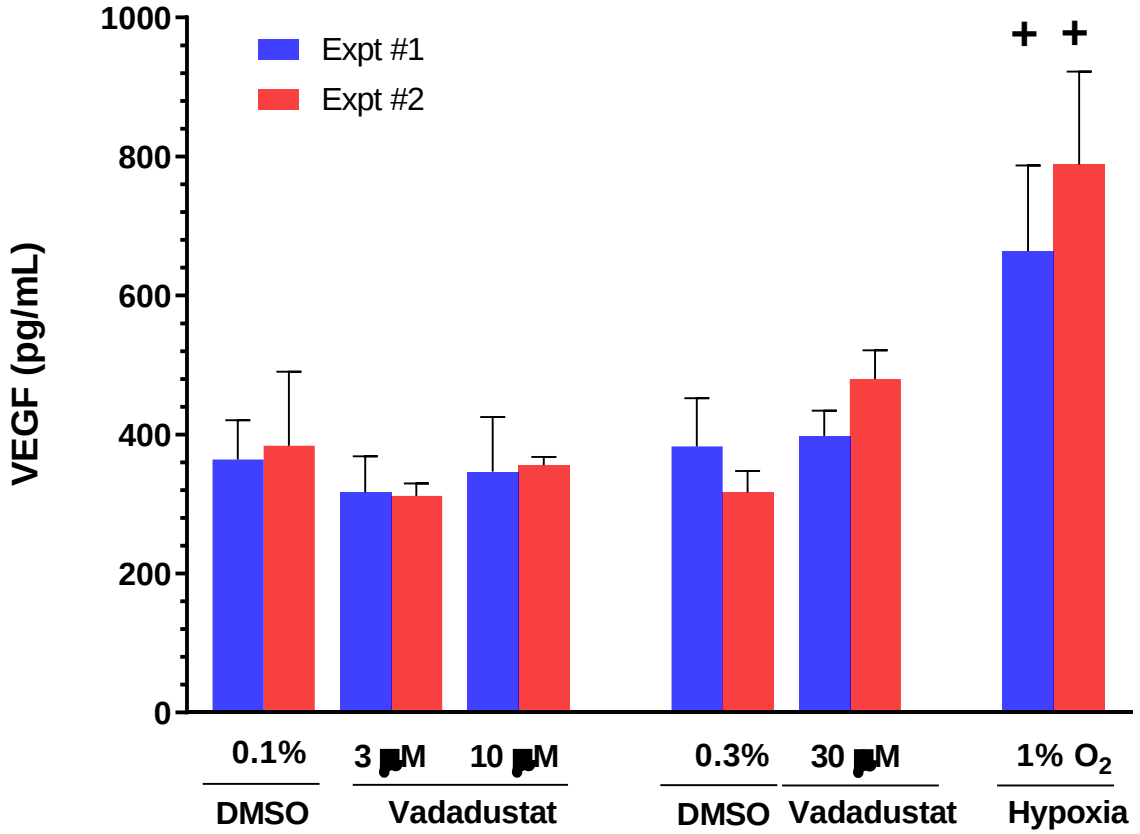
*Measured by an Enzyme Linked ImmunoSorbent Assay (ELISA) after 24 hrs incubation. Data represent Mean ± SD. + P < 0.05 vs respective DMSO Control, Tukey's Multiple Comparisons Test

Abbreviations:

DMSO = dimethylsulfoxide vehicle

EC_{50} = half maximal effective concentration

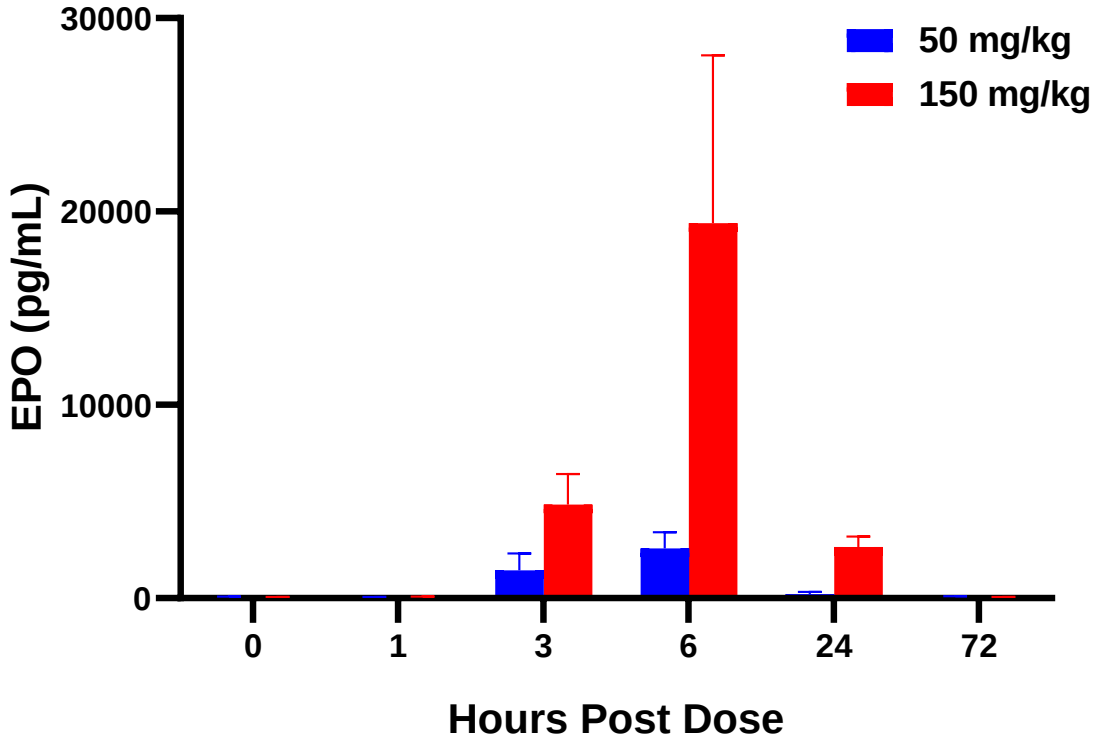
Production of vascular endothelial growth factor (VEGF) was not observed to increase in vitro after exposure of Hep3B cells to vadadustat*



*Measured by Enzyme Linked ImmunoSorbent Assay (ELISA) after 24 hrs incubation. Data represent Mean ± SD. + P < 0.05 vs 0.1% DMSO, Tukey's Multiple Comparisons Test.

Abbreviations:
DMSO = dimethylsulfoxide vehicle

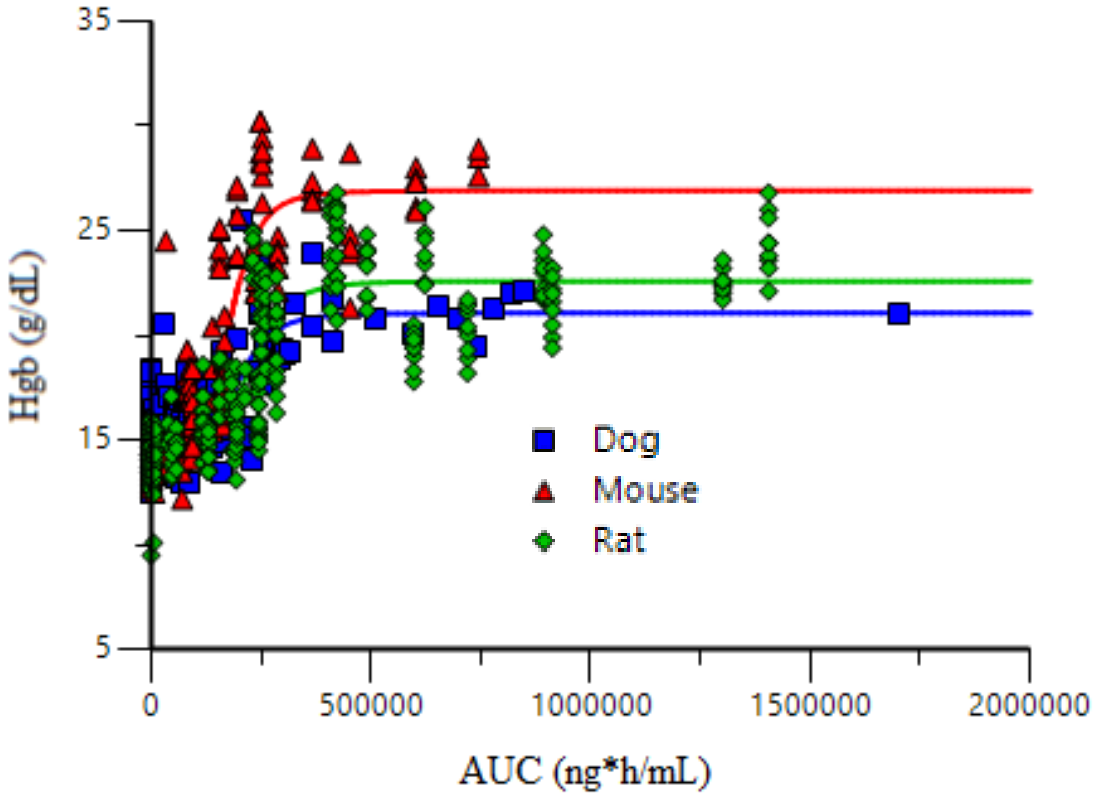
Single-dose administration of vadadustat in rats was shown to increase the circulating levels of EPO in a time and dose dependent manner*



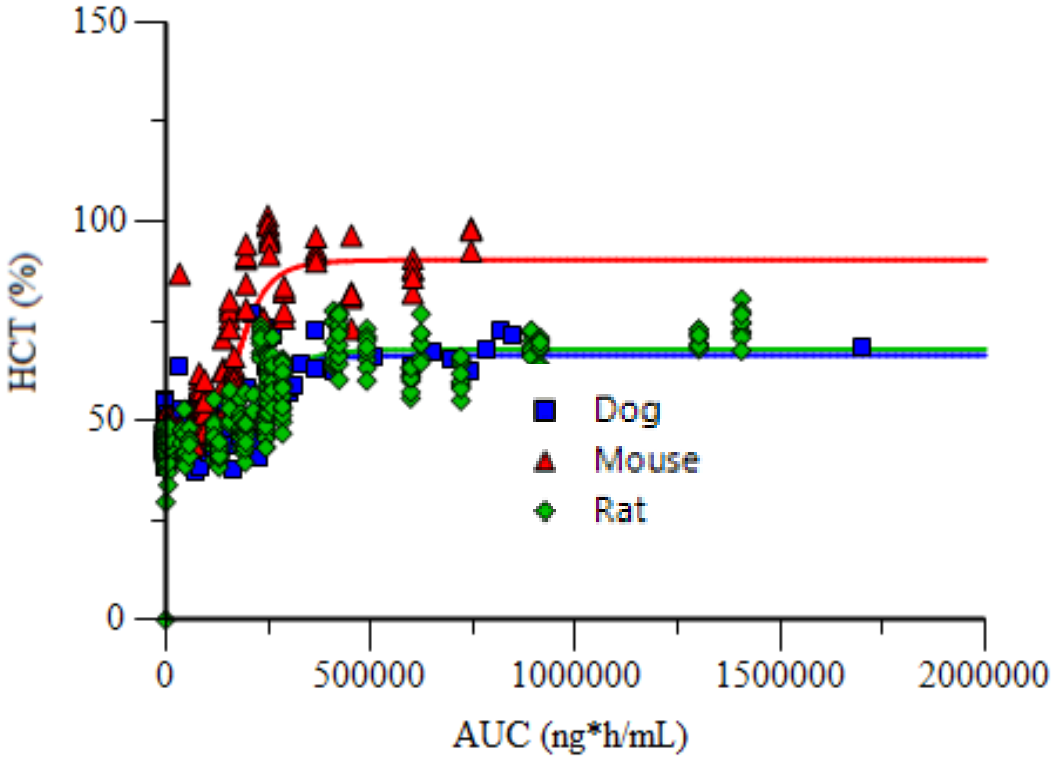
*Measured by Enzyme Linked ImmunoSorbent Assay (ELISA). Data represent Mean ± SD.

Multi-dose exposure to vadadustat in mouse, rat and dog demonstrated increases in hemoglobin and hematocrit

Hemoglobin



Hematocrit



Duration of treatment of normal animals:

- **Mouse = up to 6 months**
- **Rat = up to 2 years**
- **Dog = up to 9 months**

In mouse, rat and dog, vadadustat had a relatively short half-life and did not accumulate after repeat dosing

Species	Dose Level (mg/kg)	Day	Gender Combined $T_{1/2}$ (h)	Gender Combined AUC_{last} (µg*h/mL)	Accumulation Ratio
Mouse	100	1	2.40	234	NA
		56	1.90	197	0.84
Rat	120	1	2.09	993	NA
		28	2.05	902	0.90
Dog	120	1	2.86	740	NA
		28	3.59	776	1.05

NA = Not Applicable

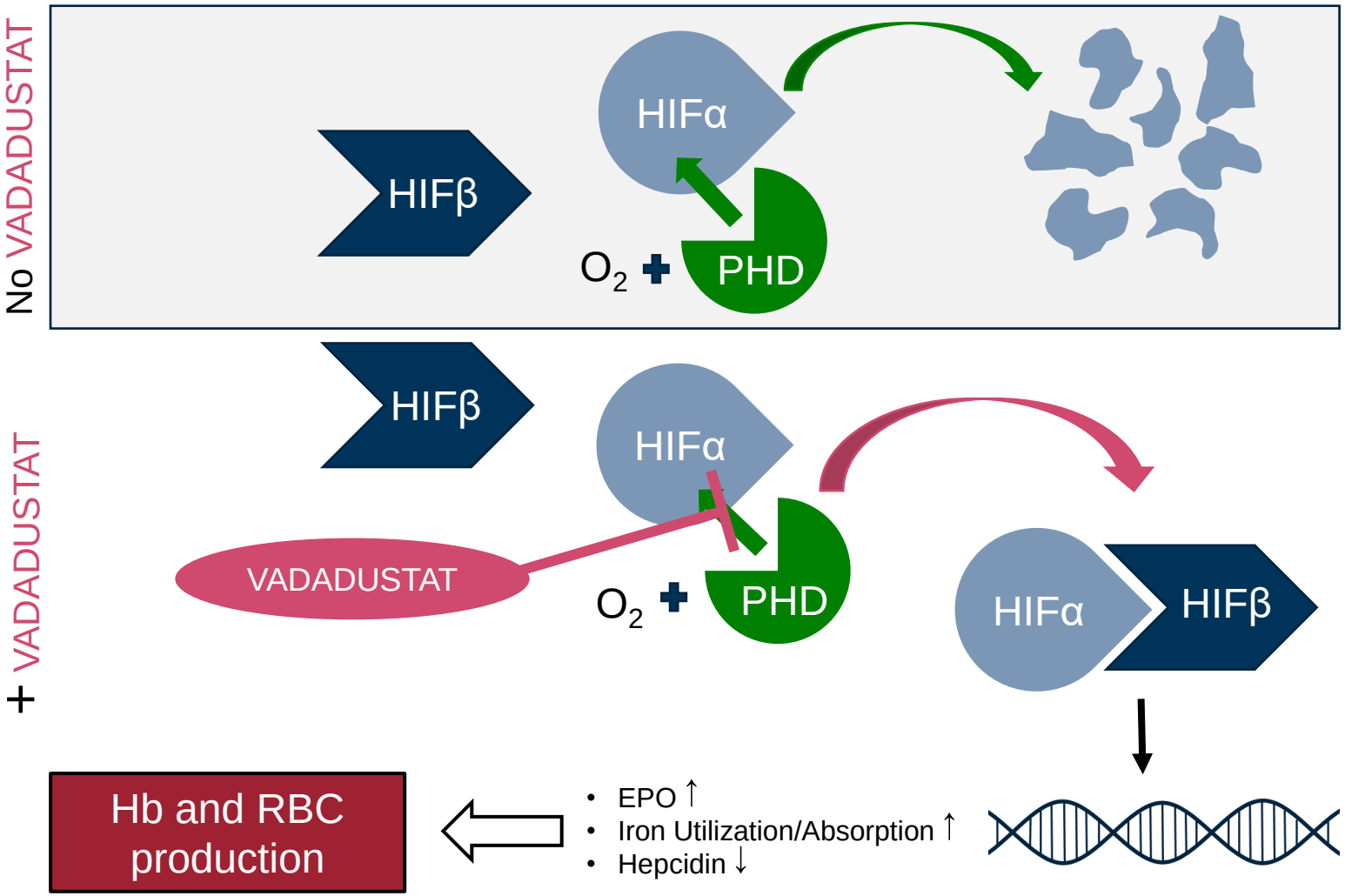
Conclusions

- In the preclinical setting, vadadustat
  - inhibited recombinant human PHD1, PHD2 and PHD3 isoenzymes at equivalent nanomolar concentrations
  - stabilized both HIF-1α and HIF-2α in vitro
  - stimulated EPO production in vitro and in vivo
  - increased hemoglobin and hematocrit in multiple species
  - did not stimulate VEGF production in vitro
- The pharmacology of vadadustat support development for anemia of CKD and ESRD

END

Possible backup slides

Background and Mechanism of Action



- Vadadustat is an orally bioavailable hypoxia-inducible factor prolyl hydroxylase inhibitor (HIF-PHI) in development for the potential treatment of anemia due to chronic kidney disease
- The HIF-PH enzymes are also referred to as EGLN proteins or prolyl 4-hydroxylase domains (PHDs)
- Pharmacological inhibition of PHD enzymes lead to the stabilization of hypoxia-inducible factor (HIF), a transcription factor that activates target genes to improve the O_2 carrying capacity of the blood

EPO, erythropoietin; Hb, hemoglobin; HIF, hypoxia-inducible factor, HIF-PHD, HIF prolyl-hydroxylase domain; RBC, red blood cell.