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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ABSTRACT
Pharmacological inhibition of prolyl-4-hydroxylase domain (PHD) enzymes stabilizes hypoxia-inducible factors (HIFs), transcription factors that activate target genes that, among others, increase erythropoietin (EPO) synthesis, resulting in the production of new red blood cells (RBCs). Herein, we summarize the preclinical characteristics of the small molecule HIF prolyl-4-hydroxylase inhibitor vadadustat (AKB-6548), which is in development for the treatment of anemia in patients with chronic kidney disease (CKD). Vadadustat inhibits the enzyme activity of all three human PHD isozymes, PHD1, PHD2, and PHD3, with similar low nanomolar inhibitory constant values. PHD enzyme inhibition by vadadustat is competitive with endogenous cofactor 2-oxoglutarate and is insensitive to free iron concentration. In the human hepatocarcinoma cell line (Hep 3B) and human umbilical vein endothelial cells, PHD inhibition by vadadustat leads to the time- and concentration-dependent stabilization of HIF-1α and HIF-2α. In Hep 3B cells, this in turn results in the synthesis and secretion of EPO; vascular endothelial growth factor is not measured at detectable levels. A single oral dose of vadadustat in rats potently increases circulating levels of EPO, and daily oral dosing for 14 days increases RBC indices in healthy rats and in the 5/6 nephrectomy model of CKD. In mice and dogs, once-daily repeat oral dosing increases hemoglobin and hematocrit. Vadadustat has a relatively short half-life in all nonclinical species evaluated and does not accumulate when administered as a single bolus dose (oral or intravenous) or upon repeat oral dosing. The pharmacological profile of vadadustat supports continued development for treatment of renal anemia.

SIGNIFICANCE STATEMENT
Vadadustat (AKB-6548) is an orally bioavailable small molecule prolyl-4-hydroxylase inhibitor in development for anemia of chronic kidney disease. It is an equipotent inhibitor of the three human prolyl-4-hydroxylase domain isoforms, which activates erythropoiesis through stabilization of hypoxia-inducible factor (HIF)-1α and HIF-2α, increasing production of erythropoietin, without detectable stimulation of vascular endothelial growth factor.

Introduction
Chronic kidney disease (CKD) is a major source of morbidity and mortality worldwide and costs the healthcare industry billions of dollars annually. In addition to the risk of heart disease, stroke, and early death, patients with CKD face higher risks of developing anemia, a result of failing kidney function (US Department of Health and Human Services, 2021). Kidneys are highly metabolically active organs requiring energy to remove waste from the blood, reabsorb nutrients, regulate the balance of electrolytes and fluid, maintain acid-base balance, and excrete nitrogenous waste products. Therefore, CKD leads to edema, maintain acid-base balance, and excrete nitrogenous waste products. Therefore, CKD leads to edema, metabolic acidosis, anemia, and electrolyte disturbances (Wesson and Lassiter, 2021). Chronic kidney disease is associated with increased risks of heart disease and increased mortality, and accounts for up to 16% of all deaths worldwide (Hansen et al., 2012). The annual incidence of CKD in the United States is 4.4 per 1000 individuals (National Kidney Foundation, 2020). In addition to the health risks to patients with CKD, the disease has a significant economic impact in the United States. In 2016, CKD patients and their caregivers spent $145 billion or 12% of the total health spending in the United States (National Kidney Foundation, 2020). In 2017, the cost of treating CKD in the United States was $24 billion (National Kidney Foundation, 2018).CKD is a major source of morbidity and mortality worldwide and costs the healthcare industry billions of dollars annually. In addition to the risk of heart disease, stroke, and early death, patients with CKD face higher risks of developing anemia, a result of failing kidney function (US Department of Health and Human Services, 2021). Kidneys are highly metabolically active organs requiring energy to remove waste from the blood, reabsorb nutrients, regulate the balance of electrolytes and fluid, maintain acid-base balance, and excrete nitrogenous waste products. Therefore, CKD leads to edema, maintain acid-base balance, and excrete nitrogenous waste products. Therefore, CKD leads to edema, metabolic acidosis, anemia, and electrolyte disturbances (Wesson and Lassiter, 2021). Chronic kidney disease is associated with increased risks of heart disease and increased mortality, and accounts for up to 16% of all deaths worldwide (Hansen et al., 2012). The annual incidence of CKD in the United States is 4.4 per 1000 individuals (National Kidney Foundation, 2020). In addition to the health risks to patients with CKD, the disease has a significant economic impact in the United States. In 2016, CKD patients and their caregivers spent $145 billion or 12% of the total health spending in the United States (National Kidney Foundation, 2020). In 2017, the cost of treating CKD in the United States was $24 billion (National Kidney Foundation, 2018).
base homeostasis, and regulate blood pressure (Bhargava and Schnellmann, 2017). The kidneys are keenly sensitive to changes in local oxygen supply and respond to reduced oxygen (hypoxia) by interstitial fibroblasts in the cortico-medullary region producing the glycoprotein hormone erythropoietin (EPO) (Lacombe et al., 1988; Pan et al., 2011; Souma et al., 2013; Sugahara et al., 2020). EPO activates signaling through its receptor on erythroid progenitor cells in the bone marrow to stimulate proliferation and differentiation into red blood cells (RBCs), increasing the oxygen-carrying capacity of the blood (Youssoufian et al., 1993; Broxmeyer, 2013).

As the kidneys are a major source of EPO, a decline in kidney function leads to decreased EPO production that may result in anemia. The first line of treatment of anemia associated with CKD is intravenous or oral iron, followed by recombinant human EPO or similar erythropoiesis-stimulating agents (ESAs), which are the standard of care. These agents benefit patients by improving the debilitating symptoms (e.g., fatigue, shortness of breath, weakness, dizziness, depression) and reducing the dependence on blood transfusions, which are associated with secondary complications such as iron overload, infections, and sensitization impeding transplantation (Winearls et al., 1986; Eschbach, 1989). However, patients receiving ESAs also face potential adverse effects such as worsening hypertension and seizures (Winearls et al., 1986; Eschbach, 1989; Babitt and Lin, 2012). In addition, ESAs do not reduce adverse outcomes associated with anemia, such as mortality, nonfatal cardiovascular events, hospitalizations, and progression of kidney disease (KDOQI, 2006; Babitt and Lin, 2012). Thus, there remains a clinical need for effective and safer alternatives to treat anemia associated with CKD.

A recent trend in anemia management is to pharmacologically mimic mild states of physiologic hypoxia to stabilize hypoxia-inducible factors (HIFs) by preventing their degradation under normoxia (Rabinowitz, 2013). Stabilization of HIF drives gene expression and production of factors necessary for cellular adaptation to hypoxia and includes EPO, which increases erythropoiesis (Semenza and Wang, 1992). HIFs are dimeric transcription factors with three known z subunits (HIF-1x, HIF-2x, and HIF-3x) and two / subunits (HIF-1/ and HIF-2/) (Wang et al., 1995; Lisý and Peet, 2008; Graham and Presnell, 2017). The z subunits are constitutively synthesized and rapidly degraded under normal (physiologic) tissue oxygenation. Oxygen-dependent HIF degradation is initiated by three iron-containing 2-oxoglutarate (2-OG)-dependent oxygenases known as the prolyl-4-hydroxylase domain (PHD) enzymes PHD1, PHD2, and PHD3 (also known as EGLN2, EGLN1, and EGLN3, respectively) (Epstein et al., 2001; Haase, 2017; Scheldel and Ratcliffe, 2019). PHDs use molecular oxygen to hydroxylate specific proline residues on HIF-z subunits, which then serve as recognition tags for binding of the von Hippel Lindau (VHL) E3 ubiquitin ligase complex that subsequently leads to ubiquitylation and proteasomal degradation of the z subunits (Ivan et al., 2001; Jaakkola et al., 2001). Enzyme activity of PHDs is inhibited via substrate (molecular oxygen) depletion during hypoxia, allowing the HIF-z subunits to escape proline hydroxylation-mediated degradation, dimerize with the HIF-/ subunit, translocate to the nucleus, and initiate transcription of a large set of genes, such as EPO, that are controlled with a hypoxia response element in their promoter (Wenger et al., 2005).

PHD enzyme activity also can be inhibited by nonlabile structural analogs of 2-OG that bind to the active site and block both 2-OG and catalytic enzyme activity (Rabinowitz, 2013). Several small molecule PHD inhibitors (PHIs) are undergoing clinical evaluation for safety and efficacy in anemia associated with CKD to serve as orally bioavailable alternative treatment options to ESAs (Sanghani and Haase, 2019), thereby initiating erythropoiesis in a potentially more orchestrated manner than with ESAs alone. Vadadustat (Akebia Therapeutics, Inc., Cambridge, MA) is one such PHI under development for the treatment of anemia associated with CKD (Markham, 2020). Herein, we summarize the preclinical characterization of vadadustat determined by in vitro assays, which describe mechanism of action, and in vivo models, which describe exposure and pharmacodynamic effect.

Materials and Methods

Vadadustat

Vadadustat (molecular weight, 306.7 g/mol) was synthesized and micronized by Evonik Corporation (Lafayette, IN) (Sanghani and Haase, 2019). Chemical identity and purity were confirmed by infrared spectroscopy, X-ray powder diffraction, NMR, and high-performance liquid chromatography. For in vitro studies, vadadustat was dissolved in DMSO as a 10 mM stock solution and stored at −20°C, protected from light. For in vivo studies, vadadustat was prepared as described in the respective sections below.

Vadadustat-O-glucuronide (molecular weight, 483 g/mol) was synthesized by Synom (Groningen, The Netherlands). Identity was confirmed by mass spectrometry, and purity > 98.5% was measured by high performance liquid chromatography-ultraviolet (254 nm). Vadadustat-O-glucuronide was dissolved in DMSO as a 10 mM stock solution and stored at −20°C, protected from light.

X-ray Crystallography

Purified PHD2181-416 was generated as described by Hewitson et al. (2007) and used at 12 mg/mL (0.45 mM) in a buffer consisting of 10 mM Tris pH 7, 100 mM NaCl, and 0.5 mM tris(2-carboxyethyl) phosphine; 0.1 mM iodoacetate was flash-frozen for crystallization trials. For analysis, cocrystals with vadadustat were produced by sitting drop vapor diffusion against 100 mM Na acetate, pH 5.5, 21% PEG1500, 15% 2-methyl-2,4-pentanediol, and 0.3 M MgSO4. The dataset was collected at the Argonne Advanced Photon Source (Argonne National Laboratory, Lemont, IL) using beamline 24ID. The structure of PHD2181-416 and vadadustat was solved using the Phenix suite of programs. Specifically, the Phenix.ligand pipeline module was used to solve the structure by molecular replacement using the program Phaser with a protein-only version of Protein Data Bank ID 30UH as a search model and subjecting the resulting solution to autobuilding and restrained refinement. The electron density map from the pipeline solution was visually examined for evidence of ligand binding. The model of PHD2-ligand complexes was then refined using alternating rounds of manual rebuilding in Coot and restrained refinement with Phenix. Ligand model restraints were generated using the program GAMESS and Akebia-provided SMILES strings as starting points.

Enzymatic Assay for PHD1, PHD2, and PHD3

The time-resolved fluorescence resonance energy transfer (TR-FRET) assay was used to measure the enzymatic activity of vadadustat against recombinant human PHD1, PHD2, and PHD3.

All chemicals and materials unless otherwise noted were of standard laboratory grade and were purchased from Sigma-Aldrich (St. Louis, MO).
Reagents. TR-FRET reagents were as follows: monoclonal antibody anti-6His-Tb-cryptate Gold and streptavidin (SA)-D2 were purchased from CsBio International (Bedford, MA). N terminus biotinylated HIF-1α C35 synthetic peptide representing amino acids 547 to 581 and including the proline 564 PHD2 hydroxylation site was purchased from California Peptide Research (Salt Lake City, UT).

Recombinant proteins included the following: For the VBC complex, His-tagged recombinant VHL protein, EloB, EloC complex (His-VBC) was supplied by Axxam (Milan, Italy). Recombinant human VHL (National Center for Biotechnology Information (NCBI) accession number NP_065421.2) contained a His-tag at the C terminus of amino acids 55–213 and is referred to as VHL-His. VHL-His was coexpressed in Escherichia coli with full-length human EloB (NCBI accession number Q15370.1) and full-length human EloC (NCBI accession number Q15369.1) and purified by affinity chromatography on a nickel-nitritotriacetic acid column as the His-VBC complex. Purity (~80%) was assessed by SDS-PAGE. Recombinant human PHD1 protein was purchased from Active Motif (Carlsbad, CA). PHD1 was expressed in a baculovirus expression system as the full-length protein (NCBI accession number NP_542770.2) with an N-terminal FLAG tag (molecular mass, 44.9 kDa). Purity (>90%) was assessed by SDS-PAGE. The full-length human PHD2 enzyme was produced with a baculovirus-infected insect cell expression system manufactured by Beryllium (Bedford, MA). The PHD2 construct contained amino acids 1–426 of PHD2 [UniProt Knowledgebase (UniProtKB)/Swiss-Prot accession number Q69729.1] and a His-tag and a tobacco etch virus protease cleavage site at the N terminus. The construct was expressed in Sf9 insect cells, purified by nickel-nitritotriacetic acid column, and digested with tobacco etch virus protease to remove the His tag. The purity of fully cleaved protein was assessed by SDS-PAGE and was found to be >94% pure. Recombinant human PHD3 protein (molecular mass, 31.1 kDa) was purchased from Active Motif (Carlsbad, CA). It was expressed in E. coli as the full-length protein (NCBI accession number NP_071356.1) with an N-terminal 6-His tag. Purity was assessed by SDS-PAGE and was found to be >75%.

TR-FRET Assay Procedure. Vadadustat was preincubated with PHD enzyme in a 10 μL reaction volume in white 384-well OptiPlate microplates (PerkinElmer, Waltham, MA). This entailed serial dilution of 5 μL vadadustat with dilution buffer (50 mM HEPES pH 7.5, 50 mM NaCl, 0.01% Tween 20, and 0.01% purified bovine serum albumin) and mixed with 5 μL PHD enzyme mix prepared as a 4X concentrate in the dilution buffer containing PHD enzyme (50 mM PHD1, 20 mM PHD2, and 140 mM PHD3), 40 mM ferrous ammonium sulfate (FAS), and 4 mM Na ascorbate. The plates were incubated for 30 minutes at room temperature (RT) without rotation.

Five microliters of the VBC/anti-6His-Tb-cryptate Gold mix, prepared as a 4X concentrate in dilution buffer containing 20 mM His-VBC and 1.32 mM monoclonal antibody anti-6His-Tb-cryptate Gold, was then added. This step was followed immediately by the addition of 5 μL of the HIF-1α C35 substrate mix prepared as a 4X concentrate in the dilution buffer containing 120 nM biotin-labeled HIF-1α C35, 132 nM SA-D2, and 4 μM 2-OG to reach a final reaction volume of 20 μL.

The final assay reaction contained 50 mM HEPES pH 7.5, 50 mM NaCl, 1 μM 2-OG, 10 μM FAS, 1 mM Na ascorbate, 0.01% Tween 20, 0.01% purified bovine serum albumin, 30 mM biotin-labeled HIF-1α C35, 5 mM His-VBC, 0.33 mM monoclonal antibody anti-6His-Tb-cryptate Gold, 35 nM SA-D2, and PHD enzyme (15 nM PHD1, 5 nM PHD2, or 35 nM PHD3) with the diluted compound.

For the measurement of the IC50 of vadadustat, reactions were incubated for 10 minutes at RT and then read on an EnVision plate reader (PerkinElmer) at an excitation wavelength of 340 nm and an emission wavelength of 515 nm. The dequenched fluorescence measured in the presence of 2-OG was used to determine the IC50 values, calculated by EnVision Manager software (PerkinElmer). The IC50 values (mean, standard deviation, standard error of the mean, geometric mean, and 95% confidence interval (CI)) were determined using a four-parameter curve fit using GraphPad Prism 7.0 (GraphPad, La Jolla, CA) and represent the compound concentration plotted against the calculated ratio of 655 nm and 615 nm. TR-FRET assays were performed in triplicate at each concentration of compound, and the assays were repeated independently three times.

Estimation of Inhibition Constants for Vadadustat. The IC50 values were converted to inhibition constants (Ki) using the Cheng-Prusoff equation as described previously for another similar PHD inhibitor (Barrett et al., 2011):
humidified, 95% air/5% CO₂ atmosphere. When a confluent monolayer was achieved, the cell culture media was replaced with media containing varying concentrations of vadadustat, and the plate was returned to the incubator. After 6 or 24 hours of incubation, the media was aspirated, and cells were rinsed with PBS and lysed with a nonen-darting Tris lysis solution from MSD. The lysate was clarified by centrifugation, and the protein content was measured using the BCA protein assay (ThermoFisher Scientific, Waltham, MA). The cell lysate from the cells of the cell culture plate was applied to corresponding wells of a 96-well MSD plate precoated either with a HIF-1α or HIF-2α capture antibody (R&D Systems, Minneapolis, MN). After incubation at room temperature for 1 hour, the wells were washed three times with a MSD Tris Wash Buffer and then incubated for another hour with a detection antibody, which, in the case of HIF-1α, was pre-conjugated with a SulfoTag. For HIF-2α, a biotinylated detection antibody (R&D Systems) was added. For HIF-2α, the detection antibody step was followed by an additional wash and further incubation with a SulfoTag-conjugated streptavidin. The plates were washed, an enhanced chemiluminescence read buffer was added to the wells, and chemiluminescence was measured on a MesoQuickPlex SQ120 plate reader. The readings were converted to pg/mL of HIF-1α or HIF-2α by interpolating to a standard curve for the respective analyte. Finally, the analyte concentrations were converted to pg/mg of lysate by normalizing to the amount of total protein in each lysate and plotted against the vadadustat concentration to generate a concentration response curve.

Quantification of EPO and Vascular Endothelial Growth Factor A Release

The effect of vadadustat effect on EPO release was quantified from culture supernatants of Hep 3B cells using the Human Erythropoietin Quantikine ELISA kit (R&D Systems). Cells were grown in Eagle’s Minimal Essential Media, supplemented with 2 mM GlutaMax, 1 mM Na-pyruvate, and 10% FBS (growth medium). For this assay, Hep 3B cells were seeded at a density of 40,000 cells per well in growth medium in a 96-well plate format. After incubation at 37°C, 5% CO₂ for 6 hours, the medium was replaced by growth medium without FBS and cultured overnight. The following morning, different concentrations of vadadustat were added to the wells in a 100 μL per well final volume and the cells were incubated for an additional 24 hours. At the end of the treatment, supernatants were collected from the wells, and EPO release was immediately measured with the human EPO sandwich ELISA kit following the manufacturer’s protocol. Briefly, equal amount of culture supernatants from each well or diluted standards (recombinant human EPO) were added to the appropriate antibody precoated wells and incubated for 2 hours at RT, protected from light. Finally, light activity was measured by EnVision plate reader, and VEGF concentration was calculated using the standard curve.

In Vivo Studies

All in vivo studies were carried out at Charles River Laboratories, accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, in accordance with the Guide for the Care and Use of Laboratory Animals from the USA National Research Council and the guidelines from the Canadian Council on Animal Care for those studies conducted in Canada.

Pharmacokinetic Parameters of Vadadustat

To characterize the pharmacokinetic (PK) parameters of vadadustat, single or repeat doses of vadadustat were administered to Sprague-Dawley Crl:CD (SD) rats, beagle dogs, and CbyB6F1 hybrid mice as described below. Blood samples (approximately 0.5 mL each) were collected via percutaneous cardiotocentesis or from the vena cava (from animals anesthetized with isoflurane) into tubes containing K₃EDTA and 1% (v/v of blood collected) of 1 M citric acid solution, mixed by inversion, and stored on wet ice until centrifuged at 1800×g within 30 minutes of collection to separate the plasma. Plasma was placed into chilled polypropylene tubes containing approximately 2% (v/v of plasma in tube) glacial acetic acid and stored frozen (approximately 70°C) until analyzed for the concentration of vadadustat using a validated ultra-high performance liquid chromatography-tandem mass spectrometry bioanalytical procedure.

Single-Dose PK of Vadadustat. To evaluate the PK properties of a single dose of vadadustat, male and female SD rats (n = 6 per sex per dosing group) and beagle dogs (n = 3 per sex per dosing group) were administered a single dose of intravenous vadadustat at 5.57 mg/kg (rats) or 5 mg/kg (dogs) prepared in pH-adjusted sterile water or by oral gavage at 20 mg/kg (rat) or 30 mg/kg (dog) as a suspension in 0.25% (w/v) hydroxypropyl methyl cellulose (HPMC; 4000 cP) and stored frozen (approximately 70°C) until analyzed for the concentration of vadadustat using a validated ultra-high performance liquid chromatography-tandem mass spectrometry bioanalytical procedure.

Repeat-Dose PK of Vadadustat. SD rats (n = 5 per sex per dosing group) and beagle dogs (n = 5 per sex per dosing group), and CbyB6F1 hybrid mice (n = 18 per sex per dosing group; n = 3 per sex per timepoint) were administered 100 mg/kg (mouse) or 120 mg/kg (rats and dogs) of vadadustat once daily by oral gavage formulated in 150 mg/kg vadadustat formulated in 0.25% (w/v) HPMC; 4000 cP and stored frozen (approximately 70°C) until analyzed for the concentration of vadadustat using a validated ultra-high performance liquid chromatography-tandem mass spectrometry bioanalytical procedure.

In Vivo Hematologic Effects of Vadadustat

A detailed analysis of the pharmacodynamic effects of vadadustat was conducted in male SD rats after single oral gavage doses of 50 or 150 mg/kg vadadustat formulated in 0.25% (w/v) HPMC; 4000 cP and stored frozen (approximately 70°C) until analyzed for the concentration of vadadustat using a validated ultra-high performance liquid chromatography-tandem mass spectrometry bioanalytical procedure.
Serum samples underwent EPO analysis using the LEGEND MAX Rat Erythropoietin ELISA Kit (BioLegend, San Diego, CA). Rat serum samples were analyzed in duplicate using qualified analytical procedures.

Hematologic parameters were measured in male SD rats after daily oral gavage dosing of vehicle or vadadustat at 30 or 90 mg/kg for 14 days, formulated as described above (n = 10–12 rats per group). On day 14, 0.5 mL of whole blood was collected into EDTA-containing tubes and analyzed on an Advia 120 Automated Hematology Analyzer (Siemens, Malvern, PA).

To assess the exposure response relationship between hematocrit (HCT), hemoglobin (Hgb), and area under the curve (AUC), data were combined from multiple studies conducted in male and female SD rats (1–48 months in duration), beagle dogs (1–9 months in duration), and C57Bl6F1 hybrid mice (2–6 months in duration). Animal numbers were as follows: for rats, 181 males and 184 females; for dogs, 89 males and 89 females; and for mice, 174 males and 170 females. In brief, rats, dogs, and mice were administered a range of doses (5–300 mg/kg/d) of vadadustat once daily by oral gavage formulated in 0.25% (w/v) HPMC; 4000 cP/0.1% (w/v) Tween 80 in ultrapure water.

**5/6 Nephrectomy Model of CKD.** Male SD rats (250 ± 20 g) underwent 5/6 nephrectomy by ligation and removal of the upper and lower third of the left kidney, followed by removal of the contralateral right kidney 1 week later. Six weeks after surgery completion, treatment with vehicle (n = 20) or vadadustat (n = 25 per dose group) was started by daily oral dosing (30 mg/kg or 90 mg/kg) for 14 days. Vadadustat was prepared in 0.25% (w/v) HPMC/0.1% (w/v) Tween 80 in ultrapure water. Blood samples were collected from the retro-orbital sinus in K_{2}EDTA on day 0 and day 14 for hematology; RBC indices were measured on an Advia 120 Automated Hematology Analyzer. Serum was collected into serum separator tubes and measured for creatinine and urea nitrogen on an Advia 1800 Chemistry Analyzer (Siemens, Malvern, PA). Kidneys were excised and fixed in 10% neutral buffered formalin, followed by paraffin embedding, sectioning, and staining with hematoxylin-eosin.

**Statistical Analyses**

The data were analyzed and/or graphed using GraphPad Prism. Differences in EPO and VEGF secretion were evaluated by Tukey multiple comparison test. Differences in RBC indices were evaluated by Kruskal-Wallis and Dunn test or ANOVA and Dunnnett’s multiple comparison test.

**Results**

**In Vitro Pharmacological Characterization of Vadadustat**

Vadadustat (2-[[5-(3-chlorophenyl)-3-hydroxypridine-2-carbonyl]amino]acetic acid) (Fig. 1A) was designed as a nonlabile active site 2-OG mimetic inhibitor of the HIF-PHD enzymes. Electron density measurements of the X-ray structure of a co-crystal of vadadustat and an iron-containing catalytic site construct of the PHD2 isoform (Fig. 1B) show the presence of two Cl-phenyl ring rotamers in roughly equal populations. As expected, vadadustat bound to the catalytic site of PHD2 at a resolution of 1.8Å and made bidentate chelation interactions with the active site Fe atom as well as a salt bridge interaction with Arg383 deep in the binding pocket, H-bond interaction of the vadadustat phenolic OH with Tyr303, and non-specific lipophilic interactions of the chlorophenyl ring with residues near the HIF binding site. Resolution achieved was 1.8Å.

Vadadustat inhibited full-length recombinant human PHD1, PHD2, and PHD3 enzymes. Based on the TR-FRET assay, which measures the interaction between the VBC complex and hydroxylated HIF-1α (produced by recombinant PHDs) in the presence of increasing concentrations of vadadustat, the pKi values (geometric mean and 95% CI) for PHD1, PHD2, and PHD3 were 9.72 (9.61, 9.82), 9.58 (9.42, 9.74), and 9.25 (9.23, 9.27), respectively (Fig. 2, A–C; Table 1). Enzymatic activity of vadadustat-O-glucuronide, a major metabolite of vadadustat, was also measured by the TR-FRET assay with PHD2 enzyme. The Ki (nM) and pKi values (geometric mean and 95% CI) were 51.1 (38.5, 68.2) and 7.29 (7.17, 7.41), respectively (Fig. 2D), suggesting that vadadustat-O-glucuronide metabolite was approximately 200-fold less potent a PHD inhibitor than vadadustat itself. Additionally, vadadustat is a competitive inhibitor of PHD2. Concentration-effect curves constructed across a range of 2-OG concentration versus a range of vadadustat concentrations using the TR-FRET assay with PHD2 enzyme indicated competitive inhibition with 2-OG (Fig. 2E). Inhibition of PHD2 enzyme by vadadustat in the presence of increasing iron concentrations was also measured to evaluate the effect of added iron on the IC_{50} of vadadustat (Fig. 2F). The IC_{50} value of vadadustat was found to be relatively insensitive to increasing iron concentrations, suggesting that vadadustat is not a
potent chelator of free iron under the assay conditions. The IC50 values (nM) of vadadustat in the presence of 100 nM Fe2+
and 10 µM Fe2+ were 211.10 ± 40.81, 8.46 ± 0.77, and 5.12 ± 0.82, respectively. Additionally, to assess the affinity of vadadustat for free iron, a protein-free assay based on calcein fluorescence was carried out (Barrett et al., 2011). As expected, DFO showed strong iron binding, whereas DMOG demonstrated minimal iron binding. Vadadustat exhibited weak iron chelation compared with DFO (Fig. 2G). Thus, based on in vitro pharmacological characterization, vadadustat was found to be a potent inhibitor of isolated and purified human PHD1, PHD2, and PHD3 enzymes that is competitive with 2-OG and not strongly affected by local iron levels.

### Table 1

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<tr>
<th>Geometric Mean (95% CI)</th>
<th>pKi value</th>
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<tr>
<td><strong>Ki value (nM)</strong></td>
<td><strong>pKi value</strong></td>
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<tr>
<td>PHD1 0.19 (0.15, 0.25)</td>
<td>9.72 (9.61, 9.82)</td>
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<tr>
<td>PHD2 0.26 (0.18, 0.38)</td>
<td>9.58 (9.42, 9.74)</td>
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<tr>
<td>PHD3 0.56 (0.53, 0.59)</td>
<td>9.25 (9.23, 9.27)</td>
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Vadadustat Stabilizes HIF-1α and HIF-2α in Multiple Cell Lines

Next, the ability of vadadustat to stabilize HIF-1α and HIF-2α was evaluated. Consistent with its ability to inhibit the three PHD enzyme isoforms, vadadustat stabilized HIF-1α and HIF-2α in Hep 3B cells and the HUVEC line in a dose-dependent manner (Fig. 3). Stabilization of both HIFs was sustained for the duration of vadadustat exposure (6 or 24 hours), measured with MSD technology. In Hep 3B cells, vadadustat...
treatment increased HIF-1α with a half-maximal EC50 of 44 μM and 67 μM over 6 and 24 hours, respectively, and increased HIF-2α with an EC50 of 51 μM and 54 μM over 6 and 24 hours, respectively. In the HUVEC line, vadadustat treatment increased HIF-1α with an EC50 of 25 μM and 71 μM over 6 and 24 hours, respectively, and increased HIF-2α with an EC50 of 21 μM and 38 μM over 6 and 24 hours, respectively.

**Vadadustat Stimulates EPO Secretion but Not VEGF**

Stabilization of HIF-1α and HIF-2α by PHD inhibition results in the mRNA transcription of HIF target genes, such as EPO and VEGF. The effect of vadadustat on EPO and VEGF release by Hep 3B cells was assayed in vitro at drug concentrations ranging from 0.1 to 100 μM (Fig. 4). At concentrations above 3 μM, vadadustat significantly increased EPO secretion by Hep 3B cells, reaching greater levels of EPO release at 30 μM (Fig. 4A). The calculated EC50 was 9.97 μM, and at this concentration, the maximal EPO release was 12.04 mIU/mL (Fig. 4A).

The effect of vadadustat on VEGF secretion was evaluated similarly. Vadadustat did not increase VEGF secretion by Hep 3B cells at the investigated doses of 3 μM, 10 μM, and 30 μM compared with the 1% hypoxia control (Fig. 4B).

**In Vivo Characterization of Vadadustat**

Based on the in vitro data demonstrating PHD inhibition, HIF stabilization, and EPO secretion with vadadustat treatment, the in vivo hematologic effects of vadadustat and PK were evaluated.

**Single-Dose Vadadustat PK.** PK parameters in SD rats (Fig. 5A; Table 2) and beagle dogs (Fig. 5B; Table 3) after the administration of an intravenous or oral dose of vadadustat were derived from concentration-time curves. In SD rats, plasma clearance (CL) after intravenous administration was low, with values of 145 mL/h per kg and 226 mL/h per kg for males and females, respectively. The apparent volume of distribution at steady state (Vss) after intravenous administration was low, with values of 182 mL/kg and 258 mL/kg for males and females, respectively. The time to maximum concentration (Tmax) after a single oral dose ranged from 0.5 to 2 hours. The half-life (T1/2) was relatively short, ranging from 1 to 2 hours. Vadadustat exhibited high oral bioavailability, with a calculated sex-combined estimated oral bioavailability (%F) > 90% normalized for a 20 mg/kg oral dose. There were no notable sex differences in systemic exposure. In beagle dogs, a linear elimination phase was not observed upon intravenous administration in both sexes or upon oral administration in males. A lack of an observed linear elimination phase precludes the acceptable calculation of PK parameters such
as CL, Vss, T1/2, and AUC0–inf. Following a single oral dose of 30 mg/kg, mean Cmax values were 50,900 ng/mL and 62,800 ng/mL, and AUC0–t values, defined as area under the curve to last measurable concentration, were 156,000 ng/C15h/mL and 109,000 ng/C15h/mL for males and females, respectively. There was no notable sex difference in plasma Cmax or AUC0–t values in this study. High oral bioavailability in the dogs was noted based on comparisons of dose-normalized AUC0–t following oral administration to AUC0–t following intravenous administration.

**Repeat-Dose Vadadustat PK.** PK parameters of vadadustat were measured in mice, rats, and dogs after a single oral dose or after daily dosing for 28 or 56 days as shown in Table 4. Exposure to vadadustat, in terms of AUC0–t, was similar on study day 1 and at end of study (day 28 and day 56). The accumulation ratio for all three species was approximately 1 or less than a value of 1, suggesting no accumulation. The T1/2 for vadadustat in mice and rats was approximately 2 hours and in dogs was approximately 3 hours. The dose-normalized group mean AUClast was greatest for rats, followed by dogs and mice.

**Effect on Serum EPO in Normal Rats after a Single Dose.** A dose-dependent increase in serum EPO concentration was measured in normal SD rats after a single intravenous bolus dose (5.57 mg/kg) and a single oral dose (20 mg/kg). Data represent the mean plus or minus standard deviation of n = 3 per timepoint. (B) Mean plasma concentration for vadadustat in male and female beagle dogs after a single intravenous bolus dose of 5 mg/kg and a single oral dose of 30 mg/kg. Data represent the mean plus or minus standard deviation of 3 animals per timepoint. (C) EPO release in male Sprague-Dawley rats after a single oral administration of vadadustat at 50 mg/kg or 150 mg/kg. Data represent the mean plus or minus standard deviation of n = 3. Conc., concentration; IV, intravenous.

**Fig. 5.** In vivo pharmacokinetics of vadadustat and effect on serum EPO. (A) Composite plasma vadadustat concentration-time profiles in male and female Sprague-Dawley rats after a single intravenous bolus dose (5.57 mg/kg) and a single oral dose (20 mg/kg). Data represent the mean plus or minus standard deviation of n = 3 per timepoint. (B) Mean plasma concentration for vadadustat in male and female beagle dogs after a single intravenous bolus dose of 5 mg/kg and a single oral dose of 30 mg/kg. Data represent the mean plus or minus standard deviation of 3 animals per timepoint. (C) EPO release in male Sprague-Dawley rats after a single oral administration of vadadustat at 50 mg/kg or 150 mg/kg. Data represent the mean plus or minus standard deviation of n = 3. Conc., concentration; IV, intravenous.

**Table 4.** Exposure to vadadustat, in terms of AUC0–t, was similar on study day 1 and at end of study (day 28 and day 56). The accumulation ratio for all three species was approximately 1 or less than a value of 1, suggesting no accumulation. The T1/2 for vadadustat in mice and rats was approximately 2 hours and in dogs was approximately 3 hours. The dose-normalized group mean AUClast was greatest for rats, followed by dogs and mice.
postdose but returned to baseline levels at 72 hours postdose. The highest mean EPO concentrations noted at the 50 and 150 mg/kg dosage levels were 2,568.93 and 19,395.94 pg/mL, respectively; a greater-than-dose-proportionate increase in EPO level was noted at the 6-hour time point (7.6-fold compared with a threefold dose increase).

**Effect on RBC Indices in Normal Rats after 14 Days of Daily Oral Dosing.** RBC indices in normal rats increased in a dose-dependent manner after daily oral administration of vadadustat for 14 days at 30 mg/kg or 90 mg/kg (Fig. 6A). Hgb, HCT, reticulocyte count, mean corpuscular Hgb, and RBC distribution width were elevated compared with predose levels (day 0) after daily oral dosing for 14 days with 90 mg/kg of vadadustat.

**Exposure-Dependent Increase in Hematocrit and Hemoglobin across Multiple Species.** In mice, rats, and dogs, there was an exposure-response relationship between vadadustat plasma AUC0-12h and both HCT and Hgb levels (Fig. 6B). The duration of treatment of normal animals was as follows: mice up to 6 months, rats up to 2 years, and dogs up to 9 months. The maximal HCT increase from baseline for mice, rats, and dogs was 91.9%, 67.9%, and 66.5%, respectively, without any effect from animal sex. The maximum effect relative to baseline corresponded to net increases for mice, rats, and dogs of 94.7, 59.0, and 44.3%, respectively. Consistent with relative species differences in baseline HCT and Hgb were considered to be within the standard deviation and biologic variability and were considered comparable.

**Effect on RBC Indices in the Rat 5/6 Nephrectomy Model of CKD after 14 Days of Daily Oral Dosing.** The effect of vadadustat on hematologic parameters in the 5/6 nephrectomy model of CKD was investigated. Six weeks after the completion of surgical procedures to induce CKD, the rats were dosed daily for 14 days with 30 mg/kg or 90 mg/kg of vadadustat by oral gavage. RBC indices were measured before (day 0) and after (day 14) treatment (Fig. 7). The vehicle-treated group did not show any significant changes in RBC parameters between day 0 and day 14. In contrast, vadadustat dose-dependently increased Hgb, HCT, reticulocyte counts, mean corpuscular volume, mean corpuscular Hgb, and RBC distribution width between day 0 and day 14, with the greatest increases that reached statistical significance measured at 90 mg/kg vadadustat after 14 days of oral dosing in this model of kidney impairment. Changes in HCT were also statistically significant and were higher at 30 mg/kg.

There were no statistically significant changes in body weight (mean plus or minus standard deviation) between the vehicle (439 ± 29.6 g) and vadadustat-treated groups after 14 days of dosing. The highest mean EPO concentrations noted at the 50 and 150 mg/kg dosage levels were 2,568.93 and 19,395.94 pg/mL, respectively; a greater-than-dose-proportionate increase in EPO level was noted at the 6-hour time point (7.6-fold compared with a threefold dose increase).

**TABLE 2**

<table>
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<tr>
<th>Route</th>
<th>Dose</th>
<th>T1/2 (h)</th>
<th>Cmax (ng/mL)</th>
<th>Tmax (h)</th>
<th>AUC0-12h (ng*h/mL)</th>
<th>AUCinf (ng*h/mL)</th>
<th>DN AUCinf (ng*h/mL/mg/kg)</th>
<th>CL (mL/h/kg)</th>
<th>Vm (mL/kg)</th>
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<td>3,267</td>
<td>363</td>
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<td>2.7</td>
<td>2.7</td>
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<tr>
<td>Oral</td>
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<td>40,300</td>
<td>0.05</td>
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<td>3,840</td>
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<td>363</td>
<td>2.7</td>
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</table>

%F was calculated using the mean dose-normalized AUC0-12h oral/mean dose-normalized AUC0-12h intravenous × 100%.

**TABLE 3**

<table>
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<tr>
<th>Route</th>
<th>Dose</th>
<th>T1/2 (h)</th>
<th>Cmax (ng/mL)</th>
<th>Tmax (h)</th>
<th>AUC0-12h (ng*h/mL)</th>
<th>AUCinf (ng*h/mL)</th>
<th>DN AUCinf (ng*h/mL/mg/kg)</th>
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days of daily dosing at 30 mg/kg (435 ± 43.6 g) or 90 mg/kg (430 ± 34.9 g). Moreover, renal function did not change as measured by serum creatinine (0.82 ± 0.15 mg/dL, 0.97 ± 0.54 mg/dL, and 1.0 ± 0.22 mg/dL for vehicle, 30 mg/kg vadadustat, and 90 mg/kg vadadustat, respectively) and blood urea nitrogen (32.9 ± 7.6 mg/dL, 35.5 ± 12.1 mg/dL, and 33.7 ± 4.9 mg/dL for vehicle, 30 mg/kg vadadustat, and 90 mg/kg vadadustat, respectively). Histology did not identify differences in the severity of kidney damage between groups.

Discussion

Several HIF-PHI s have received marketing approval in Japan, China, and the European Union for the treatment of anemia in patients with dialysis-dependent (DD) and non–dialysis dependent (NDD) CKD (Hirota, 2021). Vadadustat, an oral HIF-PHI, became commercially available in Japan for the treatment of anemia in these same patient populations (Markham, 2020). Results of global phase 3 studies with HIF-PHIs were recently published for vadadustat, daprodustat, and roxadustat (Chertow et al., 2021; Eckardt et al., 2021; Fishbane et al., 2021; Provenzano et al., 2021; Singh et al., 2021a; Singh et al., 2021b). In this report, we present the first preclinical pharmacological characterization of vadadustat, detailing in vitro molecular, biochemical, and cellular characterization and in vivo evaluation in healthy and diseased animals.

We used the PHD2 enzyme to structurally and biochemically analyze vadadustat. Among the PHD enzymes, PHD2 is the main oxygen sensor (Berra et al., 2003); it is the most important PHD enzyme in setting steady-state levels of HIF-α and, hence, activity of the HIF system in oxygenated cells. PHD2 also is the most abundant PHD; it is expressed at moderate to high levels in the cytoplasm of almost all cells and tissues (Lieb et al., 2002; Metzen et al., 2003; Uhlen et al., 2015). Moreover, deletion of PHD2 is embryonic lethal because of placental and cardiac effects (Minamishima et al., 2008; Singh et al., 2013). In contrast, PHD3 is the most relevant PHD in hypoxia (Appelhoff et al., 2004). PHD1 and PHD3 have a more tissue-restricted expression (Lieb et al., 2002; Uhlen et al., 2015) and localize to the nucleus (PHD1) or to both the nucleus and cytoplasm (PHD3) (Metzen et al., 2003). PHD1 and PHD3 knockout mice are viable; deletion of PHD1 reduces exercise tolerance, whereas deletion of PHD3 affects adrenal and sympathetic nervous system development (Meneses and Wielockx, 2016). For these reasons, we used PHD2 to characterize the molecular and biochemical properties of vadadustat.

X-ray cocrystal structure determination of vadadustat bound to the catalytic domain of PHD2 revealed its target binding as an active site inhibitor, binding in the 2-OG pocket of the PHD2 enzyme in a binding pose similar to the endogenous ligand 2-OG (Rabinowitz, 2013). Concentration-responsive curves confirmed vadadustat effectively inhibited PHD enzyme in a 2-OG competitive manner. The activity of vadadustat was insensitive to added iron and did not show strong chelation of free iron, suggesting its activity is not as a general iron chelator and should not be affected by extra-enzymatic sources of iron.

Importantly, vadadustat is a pan-PHD inhibitor demonstrating equipotent inhibition of the three human PHD1, PHD2, and PHD3 enzymes. The Ki values generated by TR-FRET, which measures inhibition of HIF proline hydroxylation with increasing concentrations of vadadustat, were in the low nanomolar range. Because vadadustat displays competitive inhibition kinetics for 2-OG, and the concentration of 2-OG in the enzyme reaction will influence the IC₅₀ value, we calculated Ki values, which adjusts for the concentration of 2-OG required for inhibition of each of the PHD enzymes. Thus, based on the Ki values, our data indicate that vadadustat is a nonselective inhibitor of PHD enzymes. This contrasts with earlier reports suggesting a preference of vadadustat for PHD3 based on IC₅₀ values (Yeh et al., 2017; Sanghani and Haase, 2019). Other PHIs evaluated in clinical studies, FG-4592/roxadustat, GSK1278863/daprodustat, BAY85-3934/molidustat, and JTZ-51/enarodustat, also are pan inhibitors of the human PHD enzymes (Flamme et al., 2014; Ariazi et al., 2017; del Balzo et al., 2020). Biophysical, biochemical, and cellular comparisons of FG-4592, GSK1278863, BAY85-3934, and AKB-6548 have been reported (Yeh et al., 2017); all have similar effects upregulating HIF target genes but differ in the mode of binding to the active site of PHD2, the kinetics of their effects, and the extent of inhibition of the N- and C-terminal oxygen-dependent degradation domains (Yeh et al., 2017).

Inhibition of proline-hydroxylation activity of PHD enzymes results in stabilization of HIF-α (Semenza and Wang, 1992). Using two different cell lines, Hep 3B and HUVEC, we demonstrated that PHD inhibition by vadadustat stabilizes both HIF-1α and HIF-2α in a concentration- and time-dependent manner. Early internal studies suggested a preferential stabilization of HIF-2α by vadadustat (Shalwitz et al., 2011). However, the detailed quantitative analyses generated by MSD technology in two different cell lines in this report suggests vadadustat stabilizes both HIF-1α and HIF-2α, and stabilization of these two isoforms depends on the cell type examined (Yeh et al., 2017).

Consistent with PHD inhibition and stabilization of HIF-α is the subsequent increase in transcription of HIF-responsive genes, such as EPO, necessary for erythropoiesis (Semenza and Wang, 1992). Stabilization of HIF-α in Hep 3B cells by vadadustat resulted in concentration-dependent increases in secretion of EPO. In contrast, an increase in VEGF secretion was not detected. These data are consistent with the observed improvement of hematologic parameters in CKD patients, where Hgb and HCT were corrected in phase 2 and phase 3 trials of DD- and NDD-CKD patients, yet plasma levels of VEGF were not increased (Pergola et al., 2016; Martin et al., 2017; Nangaku et al., 2021a; Nangaku et al., 2021b). Why hypoxia and some PHIs increase VEGF (i.e., molidustat/
BAY85-3934) but vadadustat and others (i.e., roxadustat/FG-4592, daprodustat/GSK1278863, and enarodustat/JTZ-951) do not (Brigandi et al., 2016; Holdstock et al., 2016; Akizawa et al., 2019; Akizawa et al., 2021) is an area for future studies.

The observation that HIF-PHIs are erythropoietic at doses that do not stimulate VEGF also warrants investigation. Possible explanations for the dissociation in expression of EPO versus VEGF include the dose and pharmacokinetics of the

**Fig. 6.** In vivo hematologic effects of vadadustat. (A) Red blood cell indices in male Sprague-Dawley rats after daily oral administration of vadadustat for 14 days at 30 mg/kg or 90 mg/kg (n = 10–12 rats/group). Hemoglobin (g/dL), hematocrit (%), RETIC (number \( \times 10^9/L \)), MCV (fL), mean corpuscular hemoglobin (pg), and RDW (%) are shown. Data represent mean plus or minus standard deviation. Statistical analysis by Kruskal-Wallis and Dunn for hematocrit, RETIC, MCV, and MCH (**\( P \leq 0.001 \)) and ANOVA and Dunnett (*\( P \leq 0.05 \), **\( P \leq 0.01 \)) for hemoglobin and RDW versus vehicle. (B) Multidose exposure to vadadustat in male and female mice, rats, and dogs, measured as AUC (ng*h/mL), demonstrates increases in hemoglobin and hematocrit. For mice, rats, and dogs, there were 174, 181, and 89 males, respectively, and 170, 184, and 89 females, respectively. MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; RDW, red blood cell distribution width; RETIC, reticulocyte counts.

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HIF-PHI; the regulation of EPO expression, known to be highly sensitive to hypoxia compared with other HIF targets, such as VEGF (Sandner et al., 1996; Yeh et al., 2017), including epigenetic control of the promoter region (Steinmann et al., 2011; Chang et al., 2016); and the greater HIF stabilization that may be required to activate VEGF compared with EPO (Haase 2017).

Interestingly, the Ki values for PHD activity in the TR-FRET assay were in the nanomolar range, compared with the EC50 values in the cell-based assays, which were in the micromolar range. This difference could be explained by the small molecule properties of vadadustat, including protein binding and plasma membrane permeability. The concentration of intracellular citric acid (Kreb’s) cycle intermediates, specifically fumarate and succinate, could also be a factor. These intermediates inhibit all three PHD enzymes (Koivunen et al., 2007). Thus, higher concentrations of vadadustat in the cell-based assays could be required to overcome endogenous intracellular competition by citric acid cycle intermediates, potentially explaining the micromolar EC50 values in cell-based assays versus nanomolar concentrations with TR-FRET.

Based on biochemical and cellular data highlighting vadadustat as a nonselective PHD inhibitor that leads to the production of EPO, in vivo studies were conducted. A single oral dose of vadadustat in rats elevated plasma EPO concentrations within 6 hours after dosing, decreasing to baseline 72 hours postdose. When administered once daily for 14 days to healthy rats, vadadustat dose-dependently increased Hgb, HCT, reticulocytes, and other RBC parameters. Similar effects were measured in the 5/6 nephrectomy model of CKD, indicating vadadustat’s effect on erythropoiesis was not dependent on kidney status.

Pharmacokinetic parameters of vadadustat were evaluated in multiple nonclinical species. Upon oral administration as a suspension, vadadustat showed high oral bioavailability in rats and dogs with rapid absorption. Single-dose pharmacokinetic properties were characterized by low plasma CL in rats and low to moderate plasma CL in dogs. The Vss was lower than the volume of total body water in rats, whereas in dogs, the Vss was similar or slightly larger than the volume of total body water. The T1/2 values of vadadustat following oral administration were relatively short, approximately 2 hours in mice and rats and 3 hours in dogs; pharmacokinetic properties were generally similar in male and female animals, and there was no marked difference in vadadustat AUC0-last between the fed and fasted state in male beagle dogs.
Moreover, vadadustat did not show appreciable accumulation in the three species evaluated. There was a consistent positive correlation between vadadustat plasma AUC_0-24 and both HCT and Hgb levels across species. A 90 mg/kg dose in normal and 5/6 nephrectomized rats was associated with in vivo hematologic effects. Plasma drug concentrations associated with a 90 mg/kg dose in normal animals was approximately 2 times and 3 times the Cmax and AUC exposures, respectively, which associates with a 300 mg dose in humans where hematologic effects are measured (unpublished data).

Vadadustat’s mechanism of action supported progression into clinical trials for the treatment of anemia due to CKD. In phase 2 and 3 studies of DD- or NDD-CKD patients with anemia, vadadustat increased and maintained mean Hgb concentration (Pergola et al., 2016; Martin et al., 2017; Haase et al., 2019; Chertow et al., 2021; Eckardt et al., 2021). In phase 3 studies, vadadustat was noninferior to darbepeotin alfa with respect to cardiovascular safety in the DD-CKD population (INNOVATE) (Eckardt et al., 2021), however vadadustat did not meet the prespecified noninferiority criterion for cardiovascular safety in the NDD-CKD population (PROTECT) (Chertow et al., 2021).

Authorship Contributions

**Participated in research design:** Zuk, Si, Loh, Bommegowa, Danthi, Molnar, Rabiniowizt.

**Conducted experiments:** Si, Loh, Danthi, Molnar, Caszmidz.

**Performed data analysis:** Zuk, Si, Loh, Bommegowa, Hoivik, Danthi, Molnar, Caszmidz, Rabiniowiz.

**Wrote or contributed to the writing of the manuscript:** Zuk, Si, Hoivik, Danthi, Molnar, Rabiniowiz.

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References


