

RESEARCH MONOGRAPH . COMPOUND PROFILE

Roxadustat

A first-in-class oral hypoxia-inducible factor prolyl hydroxylase inhibitor developed by FibroGen for the treatment of anemia in chronic kidney disease, distinguished from conventional erythropoiesis-stimulating agents by its mechanism of HIF-alpha stabilization, endogenous erythropoietin induction, hepcidin suppression, and improved iron homeostasis.

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ABSTRACT

Roxadustat (FG-4592), the N-[(4-hydroxy-1-methyl-7-phenoxyisoquinolin-3-yl)carbonyl]glycine derivative and first-in-class hypoxia-inducible factor prolyl hydroxylase inhibitor (HIF-PHI) to receive regulatory approval worldwide, is an orally bioavailable small molecule developed by FibroGen, Inc. for the treatment of anemia associated with chronic kidney disease (CKD) in both dialysis-dependent and non-dialysis-dependent patient populations. The compound acts by competitively inhibiting the three isoforms of prolyl hydroxylase domain enzymes (PHD1, PHD2, PHD3) that, under normoxic conditions, hydroxylate proline residues on the oxygen-dependent degradation domain of hypoxia-inducible factor alpha subunits (HIF-1alpha and HIF-2alpha), thereby targeting them for von Hippel-Lindau (VHL) E3 ubiquitin ligase-mediated proteasomal degradation. By reversibly occupying the 2-oxoglutarate binding pocket of the PHD enzymes, roxadustat stabilizes HIF-alpha, permitting its nuclear translocation, heterodimerization with HIF-beta (ARNT), and transcriptional activation of hypoxia-responsive element (HRE)-containing target genes. The principal therapeutic consequence is a dose-dependent, transient elevation of endogenous erythropoietin (EPO) production that remains within or near the physiological range, in contrast to the supraphysiological EPO concentrations produced by injectable erythropoiesis-stimulating agents (ESAs). In addition to EPO induction, roxadustat suppresses hepatic hepcidin expression, upregulates duodenal ferroportin and divalent metal transporter 1 (DMT1), increases transferrin receptor expression, and thereby improves iron absorption, mobilization, and utilization, an effect of particular

clinical relevance in the iron-restricted erythropoiesis commonly observed in CKD patients with chronic inflammation. Roxadustat received its first global approval in China in December 2018 for dialysis-dependent CKD anemia, followed by approvals in Japan (2019, Astellas Pharma, marketed as Evrenzo), the European Union (August 2021, European Commission), South Korea, and Chile. In the United States, the Cardiovascular and Renal Drugs Advisory Committee of the Food and Drug Administration voted against approval in July 2021, citing unresolved cardiovascular safety signals (including increased rates of vascular access thrombosis and deep venous thrombosis in dialysis-dependent populations relative to epoetin alfa) and concerns regarding data integrity in the sponsor's cardiovascular safety analyses. A large Phase 3 clinical program encompassing the ALPS, ANDES, OLYMPUS, ROCKIES, SIERRAS, HIMALAYAS, and PYRENEES trials demonstrated non-inferiority or superiority to placebo and non-inferiority to epoetin alfa for hemoglobin correction and maintenance across CKD populations, with pooled analyses in incident dialysis patients suggesting a 30 percent reduction in major adverse cardiovascular events (MACE) relative to epoetin alfa. Pharmacokinetics are characterized by rapid oral absorption (Tmax approximately 1 to 2 hours), high plasma protein binding (approximately 99 percent), hepatic metabolism predominantly through CYP2C8 oxidation and UGT1A9 glucuronidation, and an elimination half-life of approximately 12 to 15 hours in CKD patients. Clinically significant drug-drug interactions include increased exposure with CYP2C8 inhibitors (gemfibrozil), reduced absorption with phosphate binders (sevelamer, calcium acetate), and inhibition of BCRP and OATP1B1 transporters leading to elevated statin exposures when co-administered. Beyond anemia, preclinical and early clinical evidence suggests potential

renoprotective, anti-inflammatory, and cardioprotective properties mediated through HIF-dependent pathways, including protection against ischemia-reperfusion injury, suppression of NF-kappaB-driven inflammatory cytokines, and modulation of lipid metabolism. This monograph reviews the chemistry, synthesis, and structural pharmacology of roxadustat; the molecular mechanism of HIF-PHD inhibition in biochemical and cellular detail; the comprehensive human pharmacokinetic record; preclinical pharmacology across renal, inflammatory, and metabolic models; the clinical evidence base from the global Phase 3 program; sourcing and quality verification considerations; reconstitution and handling; drug interaction and combination considerations; adverse-event and safety signals including the cardiovascular and thrombotic concerns that limited United States approval; and a comparative assessment of five HIF-PHI class members (daprodustat, vadadustat, molidustat, enarodustat, desidustat) against roxadustat on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

International Nonproprietary Name: Roxadustat.

Marketed names: Evrenzo (Astellas Pharma; Japan, European Union member states), Ai Rui Zhuo (AstraZeneca; People's Republic of China), Enalvo (selected markets).

Development codes: FG-4592 (FibroGen), ASP1517 (Astellas). The FibroGen code FG-4592 is the canonical identifier in the preclinical and early clinical pharmacology literature. ASP1517 appears in the Japanese and European regulatory filings.

Chemical Abstracts Service registry number: 808118-40-3 (free acid form).

PubChem Compound Identifier: CID 11256664.

International Union of Pure and Applied Chemistry name: 2-[(4-hydroxy-1-methyl-7-phenoxyisoquinoline-3-carbonyl)amino]acetic acid. The compound is an N-acylglycine derivative bearing a 4-hydroxyisoquinoline-3-carboxamide core with a phenoxy substituent at the 7-position and a methyl group at the 1-position of the isoquinoline ring.

1.2 Structural class and chemistry

Roxadustat belongs to the isoquinoline-derived glycinamide class of 2-oxoglutarate (2-OG) mimetics. The structural class places it within the broader family of alpha-ketoglutarate analog enzyme inhibitors that compete for the 2-OG binding site on Fe(II)/2-OG-dependent dioxygenases, specifically the prolyl hydroxylase domain (PHD) enzymes [1]. The isoquinoline scaffold provides the iron-chelating 4-hydroxycarbonyl pharmacophore that coordinates the active-site Fe(II), while the glycine carboxylate occupies the 2-OG carboxylate binding pocket. The 7-phenoxy group provides hydrophobic complementarity to the PHD substrate channel and contributes to isoform selectivity.

Molecular formula: C₁₉H₁₆N₂O₅.

Exact mass: 352.1059 g/mol (monoisotopic).

Molecular weight: 352.34 g/mol (free acid).

Physical properties: White to off-white crystalline powder. Sparingly soluble in water at neutral pH; solubility increases at alkaline pH owing to ionization of the glycine carboxylate (pKa approximately 3.5) and the 4-hydroxyisoquinoline hydroxyl (pKa approximately 8.5). Freely soluble in dimethyl sulfoxide and dimethylformamide. Melting point has been reported in the range of 220 to 225 degrees Celsius with decomposition.

Stereochemistry: Roxadustat contains no stereocenters and is achiral. No enantiomeric or diastereomeric considerations apply to sourcing or quality verification.

1.3 Synthesis

The published synthesis of roxadustat proceeds through construction of the 1-methyl-4-hydroxy-7-phenoxyisoquinoline-3-carboxylic acid core followed by amide coupling to glycine [2]. The isoquinoline ring system is typically assembled through a Pfitzinger-type or modified Doebner reaction starting from a substituted 2-aminobenzaldehyde or equivalent precursor, with introduction of the 7-phenoxy group through nucleophilic aromatic substitution or through use of a pre-functionalized phenoxy-bearing precursor. The 4-hydroxy group is intrinsic to the isoquinoline-4-ol tautomer and does not require separate introduction. The 1-methyl group is installed during ring cyclization or through subsequent N-alkylation depending on the synthetic route. The 3-carboxylic acid is activated (typically as an acid chloride, mixed anhydride, or using carbodiimide/hydroxybenzotriazole activation) and coupled with glycine ethyl ester, followed by saponification to yield the free acid. Final purification is by recrystallization from appropriate solvent systems. The compound is characterized by nuclear magnetic resonance spectroscopy, high-resolution mass spectrometry, and high-performance liquid chromatography. Industrial-scale synthesis has been optimized for yield and purity by FibroGen and its manufacturing partners; process chemistry details are disclosed in patent filings (US Patent 7,928,120 and related family members).

2. Abstract

Roxadustat (FG-4592) is a first-in-class, orally bioavailable, reversible inhibitor of the three prolyl hydroxylase domain enzyme isoforms (PHD1, PHD2, PHD3) that regulate the stability and transcriptional activity of hypoxia-inducible factor alpha subunits (HIF-1alpha and HIF-2alpha) [1, 3]. The compound functions as a structural mimetic of 2-oxoglutarate, the obligate co-substrate of PHD enzymes, and competitively occupies the 2-OG binding pocket in the enzyme active site while coordinating the catalytic Fe(II) center through its 4-hydroxycarbonyl chelating pharmacophore. The net pharmacological effect is stabilization of HIF-alpha, which under normoxic conditions is continuously hydroxylated by PHD enzymes, ubiquitinated by the von Hippel-Lindau (VHL) E3 ubiquitin ligase complex, and degraded by the 26S proteasome [4].

The principal clinical application is the treatment of anemia associated with chronic kidney disease (CKD). In CKD, erythropoietin (EPO) production by peritubular interstitial fibroblasts of the renal cortex is impaired owing to tubulointerstitial fibrosis, chronic inflammation, and loss of the hypoxia-sensing apparatus. Conventional treatment with injectable erythropoiesis-stimulating agents (ESAs) such as epoetin alfa and darbepoetin alfa produces supraphysiological EPO concentrations and has been associated with cardiovascular risk at higher hemoglobin targets [5]. Roxadustat offers a mechanistically distinct approach: by stabilizing HIF-2alpha (the principal isoform driving renal EPO gene transcription), it induces endogenous EPO production within or near the physiological range, producing hemoglobin correction without the peak-and-trough EPO pharmacokinetics characteristic of injectable ESAs [6].

A second therapeutically relevant consequence of HIF stabilization is the modulation of iron homeostasis. Roxadustat suppresses hepatic hepcidin production (mediated through HIF-dependent and erythroferrone-dependent pathways), up-regulates intestinal iron absorption through induction of ferroportin and divalent metal transporter 1 (DMT1), increases transferrin and transferrin receptor expression, and thereby addresses the functional iron deficiency and iron-restricted erythropoiesis that characterize CKD anemia in the setting of chronic inflammation [7, 8]. This iron-mobilizing effect is particularly relevant in patients with elevated in-

inflammatory markers (C-reactive protein, interleukin-6) and elevated hepcidin, in whom conventional ESA therapy often requires concomitant intravenous iron supplementation to achieve adequate erythropoietic response.

Roxadustat received its first global regulatory approval in China in December 2018 for dialysis-dependent CKD anemia, followed by approvals in Japan (2019), the European Union (2021), South Korea, and Chile [9]. The compound has not been approved by the United States Food and Drug Administration; in July 2021, the FDA Cardiovascular and Renal Drugs Advisory Committee voted 13-1 (non-dialysis-dependent CKD) and 12-2 (dialysis-dependent CKD) against approval, citing cardiovascular and thrombotic safety signals and concerns regarding data integrity in the sponsor's pooled cardiovascular analyses [10].

The global Phase 3 clinical program encompassed seven principal trials (ALPS, ANDES, OLYMPUS for non-dialysis-dependent CKD; ROCKIES, SIERRAS, PYRENEES for dialysis-dependent CKD; HIMALAYAS for incident dialysis) involving more than 9,600 patients across multiple geographies. Efficacy endpoints (hemoglobin correction and maintenance) were met across all trials, demonstrating non-inferiority or superiority to placebo and non-inferiority to epoetin alfa [11, 12, 13, 14]. Pooled cardiovascular safety analyses produced heterogeneous results depending on population and analytic approach, with the incident dialysis subgroup showing a 30 percent reduction in MACE relative to epoetin alfa and the non-dialysis-dependent population showing a numerical (but not statistically significant) increase in MACE relative to placebo [15].

Beyond CKD anemia, preclinical evidence supports potential renoprotective effects (protection against cisplatin-induced and ischemia-reperfusion acute kidney injury), anti-inflammatory activity (suppression of NF-kappaB-mediated cytokine release), cardioprotective effects (reduced infarct size in myocardial ischemia-reperfusion models), and modulation of lipid and cholesterol metabolism [16, 17, 18]. These pleiotropic HIF-dependent effects are under active investigation but have not yet produced registration-quality clinical evidence for indications beyond CKD anemia.

3. Discovery and Development History

3.1 Origin

FibroGen, Inc. (San Francisco, California) initiated a program targeting the HIF-prolyl hydroxylase pathway in the late 1990s following the elucidation of the oxygen-sensing mechanism by which PHD enzymes regulate HIF- α stability. The founding science was informed by the discoveries of Semenza, Ratcliffe, and Kaelin (awarded the 2019 Nobel Prize in Physiology or Medicine for their characterization of cellular oxygen sensing), which established that PHD-mediated hydroxylation of HIF- α proline residues (Pro402 and Pro564 in human HIF-1 α) is the molecular switch governing the hypoxic transcriptional response [4]. FibroGen recognized that pharmacological inhibition of PHD enzymes could stabilize HIF- α under normoxic conditions and thereby activate the endogenous erythropoietin gene, offering an oral, non-injectable alternative to recombinant ESAs for CKD anemia.

The initial FibroGen PHD inhibitor program produced FG-2216, a first-generation compound that entered Phase 2 clinical trials but was associated with a fatal case of hepatic necrosis in 2007, leading to a clinical hold by the FDA [19]. FibroGen subsequently optimized the chemical series, producing FG-4592 (roxadustat) as a structurally refined compound with improved selectivity, metabolic stability, and safety profile. Roxadustat entered Phase 1 clinical development in approximately 2007 to 2009.

3.2 Partnership and licensing

FibroGen established two major partnership agreements for the global development and commercialization of roxadustat. In 2006, an exclusive licensing agreement was executed with Astellas Pharma for development and commercialization in Japan, Europe, the Commonwealth of Independent States, the Middle East, and South Africa, with Astellas providing a \$300 million upfront payment and up to \$465 million in milestone payments [20]. In 2013, a strategic collaboration was executed with AstraZeneca for co-development and commercialization in China, the United States, and all territories not covered by the Astellas agreement, with

AstraZeneca providing a \$350 million upfront payment and up to \$465 million in milestone payments [20]. FibroGen retained co-promotion rights in the United States and China.

3.3 Clinical development program

Phase 2 clinical trials were conducted in China (beginning 2012), Japan, the United States, and Europe. The Chinese Phase 2 program was notable for its rapid enrollment and for producing the first positive dose-ranging efficacy data in both dialysis-dependent and non-dialysis-dependent CKD populations [21]. The Phase 3 program was initiated in 2015 to 2016 across seven principal trials with a combined enrollment of more than 9,600 patients.

3.4 Regulatory history

Roxadustat received its first global approval from the National Medical Products Administration (NMPA) of China on December 17, 2018, for the treatment of anemia in adult CKD patients on dialysis [9]. This was the first approval of an HIF-PHI worldwide and represented a landmark in the translation of the HIF oxygen-sensing biology to clinical therapeutics.

Japan's Ministry of Health, Labour and Welfare (MHLW) approved roxadustat (marketed as Evrenzo by Astellas) in September 2019 for dialysis-dependent CKD anemia, with subsequent extension to non-dialysis-dependent CKD.

The European Commission granted marketing authorization for Evrenzo in August 2021 for the treatment of symptomatic anemia associated with CKD in adult patients.

In the United States, AstraZeneca and FibroGen submitted a New Drug Application (NDA) to the FDA. In July 2021, the FDA Cardiovascular and Renal Drugs Advisory Committee held a two-day hearing and voted against approval for both dialysis-dependent (12-2) and non-dialysis-dependent (13-1) indications [10]. The committee cited increased rates of thrombotic events (particularly vascular access thrombosis and deep venous thrombosis), a numerical imbalance in MACE in the non-dialysis-dependent placebo-controlled population, and concerns that FibroGen had altered parameters in its cardiovascular safety analyses in a manner

that understated risk. The FDA subsequently issued a Complete Response Letter in August 2021. As of the most recent monograph revision, roxadustat has not received FDA approval for any indication.

South Korea and Chile have also approved roxadustat for CKD anemia.

3.5 Significance

Roxadustat was the first compound to demonstrate that pharmacological activation of the endogenous oxygen-sensing pathway could produce clinically meaningful erythropoiesis in humans, validating the HIF-PHI mechanism and opening the field for the subsequent development of daprodustat (GlaxoSmithKline), vadadustat (Akebia Therapeutics), molidustat (Bayer), enarodustat (Japan Tobacco), and desidustat (Cadila Healthcare/Zydus). The regulatory path for roxadustat, including the divergent approval decisions between Asian/European and United States regulators, established the precedents for cardiovascular safety evaluation of the HIF-PHI class.

4. Molecular Pharmacology

4.1 The HIF oxygen-sensing pathway

The hypoxia-inducible factor (HIF) pathway is the master transcriptional regulator of the cellular response to oxygen availability. Under normoxic conditions, HIF- α subunits (HIF-1 α , HIF-2 α , HIF-3 α) are continuously synthesized and rapidly degraded through a post-translational hydroxylation-ubiquitination-proteasome sequence. Three prolyl hydroxylase domain enzymes (PHD1/EGLN2, PHD2/EGLN1, PHD3/EGLN3), which are members of the Fe(II)/2-oxoglutarate-dependent dioxygenase superfamily, hydroxylate conserved proline residues in the oxygen-dependent degradation domain (ODDD) of HIF- α (Pro402 and Pro564 in human HIF-1 α ; Pro405 and Pro531 in human HIF-2 α) [4]. The hydroxylated proline residues are recognized by the von Hippel-Lindau (VHL) tumor suppressor protein, which serves as the substrate recognition component of an E3 ubiquitin ligase complex (VHL-elongin B-elongin C-Cullin2-Rbx1). Polyubiquitinated HIF- α is then degraded by the 26S proteasome, maintaining very low steady-state HIF- α protein levels under normoxic conditions.

Under hypoxic conditions, molecular oxygen (O₂), which is a required co-substrate for PHD catalytic activity, becomes limiting. PHD activity declines, HIF- α hydroxylation ceases, and the unhydroxylated HIF- α escapes VHL recognition and proteasomal degradation. Stabilized HIF- α translocates to the nucleus, heterodimerizes with HIF-1 β (also known as ARNT, the aryl hydrocarbon receptor nuclear translocator), and binds to hypoxia-responsive elements (HREs; consensus sequence 5'-RCGTG-3') in the promoter and enhancer regions of target genes. A second hydroxylation event, mediated by factor inhibiting HIF (FIH), hydroxylates an asparagine residue (Asn803 in human HIF-1 α) in the C-terminal transactivation domain, blocking recruitment of the p300/CBP coactivator; under hypoxia, FIH activity also declines, permitting full transcriptional activation [4].

4.2 PHD inhibition by roxadustat

Roxadustat inhibits all three PHD isoforms by competitively occupying the 2-oxoglutarate binding pocket in the enzyme active site [1, 3]. The isoquinoline 4-hydroxycarbonyl moiety chelates the active-site Fe(II) in a bidentate fashion, mimicking the interaction of the alpha-keto acid and carboxylate groups of 2-OG with the metal center. The glycine carboxylate extends into the 2-OG carboxylate binding subsite, providing additional electrostatic complementarity. X-ray crystallographic studies of PHD2 in complex with related isoquinoline inhibitors have confirmed this binding mode [22].

The inhibition is reversible and competitive with respect to 2-OG, producing transient HIF- α stabilization that is governed by the plasma pharmacokinetics of the drug. After oral dosing and systemic clearance, PHD activity recovers, HIF- α is again hydroxylated and degraded, and the transcriptional response subsides. This pharmacokinetic-pharmacodynamic relationship produces a pulsatile pattern of EPO induction: serum EPO concentrations rise within 4 to 8 hours of dosing, peak at approximately 8 to 12 hours, and return toward baseline within 24 to 48 hours [6, 23]. The intermittent dosing schedule (typically three times weekly) therefore produces intermittent EPO elevations that approximate the physiological pattern of EPO secretion more closely than the sustained supraphysiological concentrations produced by injectable ESAs.

Selectivity among the PHD isoforms has been characterized in biochemical assays. Roxadustat inhibits PHD2 (the principal isoform regulating erythropoietic HIF-2 α in renal interstitial cells) with an IC₅₀ in the low micromolar range. PHD1 and PHD3 are inhibited at similar concentrations. The compound does not substantially inhibit FIH at therapeutic concentrations, an observation that has been cited as favorable for the safety profile since FIH regulates a broader spectrum of HIF target genes including those involved in metabolism and apoptosis [3].

4.3 Downstream transcriptional targets

The stabilization of HIF-2 α by roxadustat activates transcription of multiple genes relevant to erythropoiesis, iron homeostasis, and related physiological processes:

Erythropoietin (EPO): The EPO gene is principally regulated by HIF-2alpha in renal interstitial fibroblasts and hepatocytes. Roxadustat-induced HIF-2alpha stabilization produces dose-dependent increases in serum EPO, typically peaking at 100 to 500 mIU/mL (within or near the physiological range) compared to the 10,000 to 100,000 mIU/mL peak concentrations produced by injectable ESA dosing [6].

EPO receptor (EPOR): HIF activation upregulates EPOR expression on erythroid progenitor cells, potentially enhancing erythropoietic responsiveness to endogenous EPO.

Iron transport and metabolism genes: HIF-2alpha induces expression of divalent metal transporter 1 (DMT1, SLC11A2) and duodenal cytochrome b reductase (DCYTB, CYBRD1) on the apical surface of duodenal enterocytes, increasing intestinal non-heme iron absorption. HIF also induces ferroportin (SLC40A1) expression on the basolateral surface of enterocytes and on macrophages, promoting iron export into plasma [7].

Hepcidin suppression: Roxadustat reduces serum hepcidin levels through both direct HIF-dependent suppression of hepcidin gene (HAMP) transcription in hepatocytes and indirect suppression through erythroferrone (ERFE), a hormone released by erythroid precursors in response to EPO stimulation that acts on hepatocytes to suppress hepcidin [8]. The reduction in hepcidin relieves ferroportin internalization and degradation, permitting iron release from enterocytes, macrophages, and hepatic stores into plasma.

Transferrin and transferrin receptor: HIF-dependent induction of transferrin receptor 1 (TFRC) on erythroid precursors enhances iron uptake from circulating transferrin, while increased transferrin synthesis supports iron transport capacity.

Vascular endothelial growth factor (VEGF): A HIF-1alpha target gene. VEGF induction is a theoretical safety consideration for long-term PHD inhibition owing to its role in angiogenesis and vascular permeability; however, clinical studies have not demonstrated clinically significant VEGF elevations at therapeutic roxadustat doses [24].

4.4 Composite pharmacology

The composite pharmacological effect of roxadustat at therapeutic doses is therefore a coordinated erythropoietic response comprising: (a) increased endogenous EPO production; (b) enhanced erythroid progenitor responsiveness to EPO; (c) im-

proved iron availability through hepcidin suppression and upregulation of iron transport; and (d) potential anti-inflammatory modulation of the anemia of chronic disease. This multi-target mechanism distinguishes roxadustat and the HIF-PHI class from conventional ESAs, which address only the EPO deficiency component of CKD anemia without modifying the iron-restricted, inflammation-driven erythropoietic impairment.

5. Pharmacokinetics

5.1 Absorption

Roxadustat is rapidly absorbed after oral administration, with time to peak plasma concentration (T_{max}) of approximately 1 to 2 hours in healthy volunteers and CKD patients [23, 25]. Oral bioavailability has not been directly determined by intravenous crossover study owing to the limited aqueous solubility of the compound; however, urinary recovery of total radioactivity (45.8 percent of the administered dose as roxadustat and metabolites) in a mass balance study in healthy volunteers indicates at least moderately high absorption [25]. Food modestly delays T_{max} without significantly affecting overall exposure (AUC); the compound is administered without regard to meals in some prescribing labels and with fasting instructions in others.

Phosphate binders, which are commonly co-prescribed in CKD patients, substantially reduce roxadustat absorption when co-administered. Sevelamer carbonate reduced roxadustat AUC to 59 percent and calcium acetate reduced AUC to 69 percent of the fasted reference value [26]. Temporal separation of at least 1 hour between roxadustat and phosphate binder administration mitigates this interaction.

5.2 Distribution

The apparent volume of distribution after oral administration (V_d/F) is 22 to 57 liters in healthy volunteers, suggesting moderate tissue distribution [25]. Plasma protein binding is approximately 99 percent, predominantly to albumin. The high protein binding limits renal filtration of the parent compound and limits removal by hemodialysis; the fraction eliminated by hemodialysis is approximately 2.3 percent [25].

5.3 Metabolism

Roxadustat is extensively metabolized in the liver through two principal pathways: phase I oxidation by cytochrome P450 2C8 (CYP2C8) and phase II glucuronidation by UDP-glucuronosyltransferase 1A9 (UGT1A9) [25, 26]. In a mass balance study, the

O-glucuronide conjugate of roxadustat was the major urinary metabolite (20.3 percent of dose), followed by the O-glucoside (7.2 percent) and the sulfate conjugate of hydroxy-roxadustat (2.0 percent). Unchanged roxadustat accounted for only 1.3 percent of the urinary dose, confirming that elimination is predominantly metabolic.

Co-administration with gemfibrozil (a strong CYP2C8 and OATP1B1 inhibitor) or probenecid (a UGT and OAT1/OAT3 inhibitor) increased roxadustat AUC by approximately 2.3-fold and C_{max} by approximately 1.4-fold, confirming the clinical significance of both metabolic pathways [26]. Dose adjustment or enhanced hemoglobin monitoring is recommended when roxadustat is co-administered with strong CYP2C8 or UGT1A9 inhibitors.

5.4 Elimination

The mean elimination half-life is approximately 11.8 hours in healthy Caucasian volunteers, 12 hours in healthy Japanese volunteers, and approximately 15 hours in CKD patients [25]. The apparent oral clearance (CL/F) ranges from 1.2 to 2.65 L/h in healthy volunteers. Renal clearance is negligible (0.026 to 0.030 L/h), consistent with the high plasma protein binding and predominantly hepatic metabolic elimination.

Elimination occurs through both urinary (approximately 46 percent of radioactive dose as metabolites) and fecal (approximately 47 percent) routes. The fecal recovery includes both unabsorbed drug and biliary excretion of metabolites.

5.5 Effect of renal and hepatic impairment

Renal impairment does not substantially alter roxadustat pharmacokinetics, consistent with the predominantly hepatic metabolic elimination. No dose adjustment is required for renal impairment alone, and the compound is not significantly removed by hemodialysis [25]. Moderate hepatic impairment (Child-Pugh B) produced modest increases in roxadustat exposure (approximately 30 to 40 percent increase in AUC) in a dedicated hepatic impairment study [27]. The compound has not been studied in severe hepatic impairment and is generally not recommended in this population.

5.6 Dosing regimen

The approved dosing regimen is three times weekly (not on consecutive days), with starting doses of 70 mg (body weight less than 70 kg in some markets) or 100 mg, titrated based on hemoglobin response at 4-week intervals. The three-times-weekly schedule produces the desired intermittent, pulsatile EPO pharmacodynamic response. Doses range from 20 mg to 400 mg three times weekly in the approved labels, with most patients stabilizing at 50 to 200 mg three times weekly.

6. Preclinical Pharmacology

6.1 Erythropoietic activity

In preclinical models, roxadustat produced dose-dependent increases in serum EPO, reticulocyte counts, and hemoglobin in mice, rats, and cynomolgus monkeys [3, 28]. The erythropoietic response was observed after both oral and parenteral administration and was sustained with chronic intermittent dosing. In a 5/6 nephrectomy rat model of CKD anemia, roxadustat corrected hemoglobin to the normal range with intermittent oral dosing, demonstrating efficacy in the setting of reduced renal mass [28].

6.2 Iron metabolism

Preclinical studies confirmed that roxadustat reduces hepatic hepcidin mRNA and serum hepcidin protein levels in a dose-dependent manner. In models of inflammation-induced anemia (turpentine abscess model, lipopolysaccharide challenge), roxadustat suppressed the inflammation-driven hepcidin elevation and restored iron availability for erythropoiesis [7]. The effect was independent of the EPO-mediated erythropoietic stimulus, as demonstrated by experiments in which EPO-receptor signaling was blocked but hepcidin suppression persisted.

6.3 Renoprotection

Roxadustat demonstrated renoprotective effects in multiple preclinical models of acute kidney injury (AKI). In a cisplatin-induced nephrotoxicity model in mice, pre-treatment with roxadustat reduced serum creatinine elevation, attenuated tubular necrosis, and improved survival [16]. The mechanism was attributed to HIF-dependent induction of protective genes including heme oxygenase-1 (HO-1), VEGF, and glucose transporters. In ischemia-reperfusion injury models, roxadustat preconditioning reduced infarct size and improved functional recovery [16, 17]. These findings are consistent with the concept of pharmacological ischemic preconditioning through HIF stabilization.

6.4 Anti-inflammatory effects

HIF pathway activation by roxadustat has been associated with modulation of inflammatory signaling in preclinical models. In lipopolysaccharide-challenged macrophages, roxadustat reduced NF-kappaB-dependent production of tumor necrosis factor alpha (TNF-alpha), interleukin-1 beta (IL-1beta), and interleukin-6 (IL-6) [18]. In CKD patients with ESA resistance, roxadustat treatment was associated with reduced serum inflammatory markers and altered gut microbiome composition with increased abundance of short-chain fatty acid-producing bacteria [29]. The anti-inflammatory mechanism may contribute to the clinical efficacy of roxadustat in CKD patients with elevated baseline inflammatory markers, who characteristically respond poorly to conventional ESA therapy.

6.5 Lipid metabolism

Clinical observations have consistently demonstrated that roxadustat reduces total cholesterol and LDL cholesterol levels in CKD patients [24]. The mechanism is attributed to HIF-dependent induction of hepatic lipid metabolism genes, possibly including modulation of HMG-CoA reductase expression and cholesterol efflux pathways. The clinical significance of this lipid-lowering effect and its implications for long-term cardiovascular outcomes have not been fully characterized.

6.6 Cardioprotection

In preclinical myocardial ischemia-reperfusion models, roxadustat preconditioning reduced infarct size, preserved left ventricular function, and attenuated post-ischemic inflammatory infiltration [17]. The cardioprotective mechanism is attributed to HIF-1alpha-dependent induction of glycolytic enzymes, antioxidant defenses, and pro-survival signaling. The translational relevance of these preclinical cardioprotective findings to human cardiovascular outcomes in CKD patients remains under investigation.

7. Clinical Evidence Base

7.1 Phase 2 trials

Phase 2 dose-ranging studies were conducted in China, Japan, the United States, and Europe between 2012 and 2015. The Chinese Phase 2 trial in non-dialysis-dependent CKD patients (Chen et al. 2019, published in the New England Journal of Medicine) was a pivotal early efficacy demonstration, showing dose-dependent hemoglobin correction at roxadustat doses of 1.0 to 2.0 mg/kg three times weekly over 8 weeks, with response rates exceeding 80 percent at the higher dose levels [21]. The study also confirmed the hepcidin-lowering and iron-mobilizing effects.

Phase 2 studies in hemodialysis patients demonstrated that roxadustat maintained hemoglobin at target levels (10 to 12 g/dL) in patients previously stabilized on injectable ESAs, supporting the transition from injectable to oral therapy [30].

7.2 Phase 3 program: non-dialysis-dependent CKD

Three principal Phase 3 trials evaluated roxadustat versus placebo in non-dialysis-dependent CKD patients:

OLYMPUS (global; N = 2,781): Roxadustat demonstrated superiority to placebo for the primary endpoint of mean change in hemoglobin from baseline averaged over weeks 28 to 52. The mean hemoglobin increase was approximately 1.75 g/dL with roxadustat versus 0.40 g/dL with placebo [11].

ALPS (United States; N = 594): Roxadustat was superior to placebo for hemoglobin response, with mean hemoglobin change from baseline of 1.85 g/dL versus 0.13 g/dL with placebo over the same evaluation window [12].

ANDES (global; N = 922): Consistent with OLYMPUS and ALPS, roxadustat demonstrated statistically significant hemoglobin correction and maintenance relative to placebo [12].

Across the non-dialysis-dependent trials, roxadustat reduced the need for rescue therapy (ESA or red blood cell transfusion) compared to placebo and demonstrated consistent hepcidin reduction and improvement in iron parameters.

7.3 Phase 3 program: dialysis-dependent CKD

Four principal Phase 3 trials evaluated roxadustat versus epoetin alfa in dialysis-dependent CKD patients:

ROCKIES (global; N = 2,133): Roxadustat demonstrated non-inferiority to epoetin alfa for hemoglobin correction and maintenance. Mean hemoglobin change from baseline was comparable between treatment arms over the evaluation period [13].

SIERRAS (United States; N = 1,043): Non-inferiority of roxadustat to epoetin alfa was demonstrated for hemoglobin maintenance in patients previously stabilized on ESAs [14].

PYRENEES (Europe; N = 836): Consistent efficacy results in a European dialysis population [14].

HIMALAYAS (global; N = 1,043): Evaluated roxadustat versus epoetin alfa in incident dialysis patients (patients initiating dialysis). Non-inferiority was demonstrated. Notably, pooled analyses of the HIMALAYAS population showed a 30 percent reduction in MACE (hazard ratio 0.70; 95% CI 0.51 to 0.97) and a 34 percent reduction in MACE-plus (MACE plus unstable angina and heart failure hospitalization) relative to epoetin alfa [15]. This subgroup finding was the most favorable cardiovascular signal in the roxadustat program.

7.4 Cardiovascular safety

Cardiovascular safety was the most contentious aspect of the roxadustat clinical program. The analysis approach was pre-specified as a pooled evaluation across trial populations:

Dialysis-dependent population (vs. epoetin alfa): Pooled analysis of ROCKIES, SIERRAS, PYRENEES, and HIMALAYAS demonstrated no increased risk of MACE (hazard ratio 0.96; 95% CI 0.84 to 1.10), MACE-plus (HR 0.98; 95% CI 0.87 to 1.10), or all-cause mortality compared to epoetin alfa [15]. The incident dialysis subgroup showed the favorable 30 percent MACE reduction noted above.

Non-dialysis-dependent population (vs. placebo): Pooled analysis of OLYMPUS, ALPS, and ANDES showed a hazard ratio for MACE of 1.10 (95% CI 0.96 to 1.27), which was not statistically significant but represented a numerical increase that

the FDA advisory committee found concerning in the context of a placebo-controlled comparison [10]. The hazard ratio for MACE-plus was 1.07 (95% CI 0.94 to 1.21).

The FDA advisory committee's concerns were amplified by the disclosure that FibroGen had altered parameters in its pooled cardiovascular safety analyses (specifically, modifications to the coding of cardiovascular events and to the analytic time window) in a manner that made the cardiovascular profile appear more favorable than initial analyses had suggested [10]. This data integrity concern was a substantial factor in the committee's negative vote.

7.5 Thrombotic events

A safety signal for thrombotic events was identified in the Phase 3 program. In dialysis-dependent populations, roxadustat was associated with increased rates of vascular access thrombosis (relative risk approximately 1.5 versus epoetin alfa) and deep venous thrombosis (relative risk approximately 3.9 versus epoetin alfa) [10]. In the non-dialysis-dependent population, increased rates of venous thromboembolic events were also observed relative to placebo. The mechanism is not fully characterized but may relate to HIF-dependent induction of pro-coagulant factors (tissue factor, plasminogen activator inhibitor-1) and to erythropoiesis-associated changes in blood viscosity and platelet activation.

7.6 Chinese clinical program

The Chinese Phase 3 studies (conducted by FibroGen and AstraZeneca at 29 sites across China) were among the first to report positive results and supported the 2018 NMPA approval. Roxadustat demonstrated superiority to placebo in non-dialysis-dependent patients and non-inferiority to epoetin alfa in hemodialysis and peritoneal dialysis patients. Safety findings included hyperkalemia (increase in serum potassium) and metabolic acidosis as notable adverse events in the Chinese population [31].

7.7 Beyond CKD anemia

Exploratory clinical studies have evaluated roxadustat for chemotherapy-induced anemia and for anemia in myelodysplastic syndromes. An open-label Phase 3 study in China evaluated roxadustat for chemotherapy-induced anemia, though concerns

have been raised regarding the potential effect of HIF stabilization on tumor biology (HIF activation is a hallmark of the tumor microenvironment and may promote angiogenesis and tumor progression in some contexts) [32]. No registrations beyond CKD anemia have been granted.

8. Sourcing and Quality Verification

Pharmaceutical-grade roxadustat is available as marketed tablets (Evrenzo brand, Astellas Pharma) in jurisdictions where the compound is registered: Japan, European Union member states, China, South Korea, and Chile. Tablet strengths include 20 mg, 50 mg, 70 mg, 100 mg, and 150 mg, supplied as film-coated tablets for oral administration.

Research-grade roxadustat is supplied by multiple chemical and pharmaceutical research suppliers (Selleck Chemicals, MedChemExpress, Cayman Chemical, Sigma-Aldrich/MilliporeSigma, Tocris, BocSci, Xcess Biosciences, AbMole BioScience, and others) at greater than 98 percent purity by HPLC. Supply is typically in milligram to gram quantities as the free acid. Pricing is moderate, in the range of \$50 to \$300 per gram of research-grade material depending on quantity and supplier.

Investigators should confirm compound identity and purity on every research-grade lot through:

- High-performance liquid chromatography area-percent purity, targeting greater than 98 percent
- High-resolution mass spectrometry confirmation of molecular weight (353.1137 [M+H]⁺ for the protonated free acid)
- Nuclear magnetic resonance spectroscopy (¹H and ¹³C) for structural confirmation
- Karl Fischer or loss-on-drying analysis for residual water content
- Residual solvent analysis by gas chromatography

Since roxadustat is achiral, enantiomeric purity determination is not required. Certificate of analysis from the supplier should be independently verified, particularly for in vivo research applications where dose-response precision is important. Third-party analytical laboratories (Janoshik Analytical, Anabolic Lab, others) provide independent confirmation services.

9. Reconstitution and Handling

Roxadustat free acid is sparingly soluble in water at neutral pH but freely soluble in dimethyl sulfoxide (DMSO). For in vitro research applications, stock solutions are typically prepared at 10 to 50 mM in DMSO, stored in single-use aliquots at minus 20 degrees Celsius, and diluted into culture medium or assay buffer immediately before use. The final DMSO concentration in cell-based assays should be maintained below 0.1 to 0.5 percent (v/v) to avoid solvent toxicity artifacts.

For in vivo research applications in rodents, roxadustat can be formulated as a suspension in 0.5 percent carboxymethylcellulose sodium with 0.1 percent Tween 80 in water, or dissolved in polyethylene glycol 400/water mixtures for oral gavage. The compound has been administered by oral gavage in the majority of published preclinical studies.

The solid compound should be stored at minus 20 degrees Celsius in a desiccated, light-protected container. Stability studies indicate the compound is stable for at least 24 months under these conditions. DMSO stock solutions are stable at minus 20 degrees Celsius for at least 6 months; repeated freeze-thaw cycles should be avoided by aliquoting.

For clinical-research applications, the marketed tablet formulations (Evrenzo) are supplied as film-coated tablets in blister packs and should be stored at controlled room temperature (15 to 30 degrees Celsius) per the approved label.

10. Stack Interactions and Combinations

Roxadustat interacts with several drug classes through its metabolic profile and through its inhibition of drug transporters. Investigators conducting research with roxadustat, particularly in chronic dosing regimens or in combination with other compounds, should consider:

CYP2C8 inhibitors: Gemfibrozil (a strong CYP2C8 and OATP1B1 inhibitor) increased roxadustat AUC by approximately 2.3-fold [26]. Other CYP2C8 inhibitors (clopidogrel glucuronide, trimethoprim) may produce smaller but measurable increases in roxadustat exposure. Dose reduction or enhanced hemoglobin monitoring is recommended with concurrent CYP2C8 inhibitors.

UGT1A9 inhibitors: Probenecid increased roxadustat exposure by approximately 2.3-fold through UGT and OAT1/OAT3 inhibition [26]. Concurrent use requires monitoring.

Phosphate binders: Sevelamer carbonate and calcium acetate substantially reduce roxadustat absorption (AUC reduced to 59 percent and 69 percent, respectively) [26]. Administration should be separated by at least 1 hour. Other multivalent cation-containing compounds (aluminum-containing antacids, iron supplements) may similarly reduce absorption through chelation.

Statins (BCRP and OATP1B1 substrates): Roxadustat inhibits the breast cancer resistance protein (BCRP) and organic anion transporting polypeptide 1B1 (OATP1B1) drug transporters, which mediate intestinal and hepatic disposition of statins [33]. Co-administration produced the following increases: rosuvastatin C_{max} 4.47-fold and AUC 2.93-fold; simvastatin C_{max} 1.87-fold and AUC 1.75-fold; simvastatin acid C_{max} 2.76-fold and AUC 1.85-fold; atorvastatin C_{max} 1.34-fold and AUC 1.96-fold. Given the high prevalence of statin use in CKD populations, this interaction is clinically significant. Statin dose reduction should be considered, and monitoring for statin-related adverse events (myopathy, hepatotoxicity) is recommended.

Erythropoiesis-stimulating agents: Concurrent use of roxadustat with injectable ESAs (epoetin alfa, darbepoetin alfa) is not recommended owing to the risk of excessive erythropoiesis and polycythemia. The clinical trials required ESA washout before roxadustat initiation.

Iron supplementation: Oral iron supplements containing ferrous salts may chelate roxadustat and reduce absorption; temporal separation is recommended. Intravenous iron can be administered concurrently without pharmacokinetic interaction.

Other HIF-PHI inhibitors: Concurrent use with other members of the HIF-PHI class (daprodustat, vadadustat, molidustat) is pharmacologically redundant and has not been studied. The combination would not be expected to produce additive efficacy and would increase the risk of excessive HIF activation.

Anticoagulants and antiplatelet agents: Given the thrombotic safety signal observed in clinical trials, concurrent use with agents that affect hemostasis should be approached with awareness of the potential additive or opposing effects on thrombotic risk.

11. Adverse Events and Safety

11.1 Common adverse events

The principal adverse events at approved doses, as characterized across the Phase 3 clinical program, include:

- Hypertension (10 to 15 percent; the most frequently reported adverse event, possibly related to EPO-mediated effects on vascular reactivity and endothelin production)
- Nausea (5 to 10 percent)
- Diarrhea (5 to 8 percent)
- Peripheral edema (5 to 8 percent)
- Hyperkalemia (3 to 7 percent; particularly in the Chinese population)
- Vascular access thrombosis (in dialysis-dependent patients; see thrombotic events below)
- Headache (2 to 5 percent)
- Fatigue (2 to 4 percent)
- Urinary tract infection (2 to 4 percent)

Adverse-event rates were generally comparable to those observed with epoetin alfa in the dialysis-dependent active-comparator trials. In the non-dialysis-dependent placebo-controlled trials, the overall incidence of adverse events was modestly higher with roxadustat than with placebo.

11.2 Thrombotic and thromboembolic events

The most clinically significant safety signal was an increased rate of thrombotic and thromboembolic events. In dialysis-dependent populations, roxadustat was associated with approximately 1.5-fold increased risk of vascular access thrombosis and approximately 3.9-fold increased risk of deep venous thrombosis compared to epoetin alfa [10]. Pulmonary embolism and arteriovenous access thrombosis were also reported at increased frequency. The thrombotic signal was present regardless of dialysis status and was a primary basis for the FDA advisory committee's nega-

tive recommendation. The mechanism is incompletely understood but may involve HIF-dependent upregulation of tissue factor and plasminogen activator inhibitor-1 (PAI-1), increased blood viscosity from rising hematocrit, and platelet activation associated with erythropoiesis.

11.3 Cardiovascular events

As discussed in the Clinical Evidence Base section, the pooled non-dialysis-dependent cardiovascular analysis showed a numerical (but not statistically significant) increase in MACE (HR 1.10; 95% CI 0.96 to 1.27) relative to placebo. The dialysis-dependent analysis showed no increase relative to epoetin alfa. The incident dialysis subgroup showed a favorable MACE reduction. The heterogeneity of cardiovascular findings across populations and the data integrity concerns have prevented definitive characterization of the cardiovascular risk profile.

11.4 Seizures and infections

The FDA advisory committee noted increased frequencies of seizures and serious infections in roxadustat-treated patients relative to placebo in the non-dialysis-dependent population [10]. The seizure signal was modest but was identified as a potential concern requiring further characterization. The infection signal was of uncertain mechanism and clinical significance.

11.5 Metabolic effects

Hyperkalemia and metabolic acidosis were reported, particularly in the Chinese Phase 3 program [31]. These metabolic disturbances are common in the CKD population and may be exacerbated by the iron and erythropoietic changes induced by roxadustat.

11.6 Hepatic safety

Liver enzyme elevations were reported infrequently. Unlike the first-generation FibroGen PHD inhibitor FG-2216 (which was associated with a fatal hepatic necrosis event), roxadustat has not produced a clinically significant hepatotoxicity signal across the Phase 3 program. Hepatic monitoring is not routinely required in approved labels.

11.7 Oncologic considerations

The theoretical concern that HIF-PHI-mediated HIF stabilization could promote tumor angiogenesis, growth, or metastasis through activation of VEGF and other oncogenic HIF target genes has been raised but not substantiated in the clinical program [32]. Long-term cancer incidence data from the Phase 3 program have not demonstrated an increased malignancy rate with roxadustat relative to comparators. Ongoing post-marketing surveillance will continue to monitor this theoretical risk. The European Medicines Agency's risk management plan includes long-term malignancy monitoring.

11.8 Pregnancy and lactation

Animal reproductive toxicity studies demonstrated embryo-fetal toxicity (reduced fetal weight, skeletal variations) at doses producing maternal exposures above the human therapeutic range. The compound is not recommended during pregnancy and lactation in approved labels. Effective contraception is recommended for women of childbearing potential during treatment and for one week after the last dose.

12. Comparative Assessment

This section presents a structured comparative assessment of five HIF-PHI class members against roxadustat, evaluated on five competency standards: novelty, effect size, promising potential (current clinical and regulatory state), side-effect profile, and overall validation. The five comparators were selected as the principal HIF-PHI compounds with substantive clinical development programs for CKD anemia.

12.1 Daprodustat (GSK1278863)

Mechanism: Orally bioavailable HIF-PHI; inhibits PHD1, PHD2, and PHD3 with similar potency. Structural class is distinct from roxadustat (pyrimidine-trione scaffold versus isoquinoline glycinamide) [34].

Development trajectory: GlaxoSmithKline advanced daprodustat through Phase 3 trials (ASCEND program) in dialysis-dependent and non-dialysis-dependent CKD populations. The Phase 3 ASCEND-D trial in dialysis-dependent patients demonstrated non-inferiority to ESA for hemoglobin maintenance. The ASCEND-ND trial in non-dialysis-dependent patients demonstrated non-inferiority to darbepoetin alfa [35]. Daprodustat (marketed as Jesduvroq) received FDA approval in February 2023 for CKD anemia in adult patients on dialysis, making it the first HIF-PHI approved in the United States.

Novelty: Low relative to roxadustat. Same mechanism class; structural differentiation but pharmacological convergence.

Effect size: Comparable to roxadustat for hemoglobin correction and maintenance in Phase 3 trials. Network meta-analyses suggest daprodustat may show advantages in iron mobilization (greater hepcidin reduction and ferritin reduction, higher transferrin saturation improvement) compared to roxadustat [36].

Promising potential: High. FDA-approved in the United States (the only HIF-PHI with US approval). The ASCEND cardiovascular safety program produced results that satisfied the FDA, in contrast to the roxadustat program.

Side-effect profile: The ASCEND trials demonstrated cardiovascular non-inferiority to ESAs. Gastrointestinal adverse events (diarrhea, constipation) were reported at modestly higher rates than with ESAs. The thrombotic signal observed with roxadustat was not prominently identified with daprodustat, though class-level concerns remain [36].

Overall validation: High. The most validated HIF-PHI in the United States regulatory context, with successful Phase 3 completion and FDA approval.

Comparison to roxadustat: Daprodustat shares the core HIF-PHI mechanism with roxadustat but achieved the US regulatory milestone that roxadustat did not. Roxadustat has broader global approval (China, Japan, EU, South Korea, Chile) and a larger clinical dataset. The two compounds appear broadly comparable in erythropoietic efficacy, with daprodustat potentially superior in iron parameter improvement and roxadustat potentially superior in the incident dialysis cardiovascular subgroup. The thrombotic safety signal that limited roxadustat's US regulatory path was less pronounced with daprodustat, though whether this reflects genuine pharmacological differences or differences in trial design and analysis remains uncertain.

12.2 Vadadustat (AKB-6548)

Mechanism: Orally bioavailable HIF-PHI; pyridine carboxamide structural class. Inhibits PHD enzymes with selectivity for PHD2 and PHD3 [37].

Development trajectory: Akebia Therapeutics advanced vadadustat through the Phase 3 PRO2TECT (non-dialysis-dependent) and INNO2VATE (dialysis-dependent) trials. The PRO2TECT trials in non-dialysis-dependent CKD patients met their primary efficacy endpoint (non-inferiority to darbepoetin alfa for hemoglobin correction) but failed the key secondary cardiovascular safety endpoint, with a hazard ratio for first MACE of 1.17 (95% CI 1.01 to 1.36), which did not satisfy the pre-specified non-inferiority margin [38]. The INNO2VATE trials in dialysis-dependent patients demonstrated non-inferiority to darbepoetin alfa for both efficacy and cardiovascular safety. The FDA issued a Complete Response Letter in March 2022. Vadadustat has been approved in Japan (as Vafseo, marketed by Mitsubishi Tanabe Pharma).

Novelty: Low. Same mechanism class as roxadustat with no clinically distinguishing pharmacological feature.

Effect size: Comparable to roxadustat and daprodustat for hemoglobin correction.

Promising potential: Moderate. Japanese approval achieved; US approval denied; European application not filed as of the most recent revision.

Side-effect profile: The cardiovascular safety failure in PRO2TECT non-dialysis-dependent patients mirrors (and potentially exceeds) the concerns raised for roxadustat in the same population. The thrombotic event profile has not been separately characterized with the same granularity as for roxadustat.

Overall validation: Moderate. Extensive Phase 3 data but failed the US cardiovascular safety benchmark in the non-dialysis-dependent population.

Comparison to roxadustat: Vadadustat and roxadustat share similar regulatory fates in the United States. Both compounds demonstrated erythropoietic efficacy but encountered cardiovascular safety concerns that prevented FDA approval. Roxadustat has broader global approval and a larger published clinical dataset.

12.3 Molidustat (BAY 85-3934)

Mechanism: Orally bioavailable HIF-PHI; triazine structural class. Developed by Bayer AG [39].

Development trajectory: Bayer advanced molidustat through Phase 3 trials (MIYABI program) in Japan. The Phase 3 program demonstrated non-inferiority to darbepoetin alfa in non-dialysis-dependent CKD and to ESAs in dialysis-dependent CKD. Molidustat was approved in Japan in 2021 (marketed as Masyfas).

Novelty: Low. Same mechanism class.

Effect size: Comparable to other HIF-PHIs for hemoglobin correction. Network meta-analyses suggest molidustat may have a more favorable iron metabolism profile and lower risk of hyperkalemia compared to roxadustat [36].

Promising potential: Moderate. Japanese approval only; no US or EU development.

Side-effect profile: Phase 3 data suggest a generally favorable safety profile within the Japanese population studied. No prominent thrombotic signal, though the Japanese trial population may differ in baseline thrombotic risk from Western

populations.

Overall validation: Moderate. Japanese Phase 3 completion and approval with limited global reach.

Comparison to roxadustat: Molidustat has narrower regulatory approval and a smaller clinical dataset than roxadustat. The two compounds appear comparable in efficacy. The more limited geographic development of molidustat reduces its relevance as a global comparator.

12.4 Enarodustat (JTZ-951)

Mechanism: Orally bioavailable HIF-PHI developed by Japan Tobacco/Torii Pharmaceutical [40].

Development trajectory: Enarodustat completed Phase 3 trials in Japan for CKD anemia in both dialysis-dependent and non-dialysis-dependent populations. The compound was approved in Japan in 2020 (marketed as Enaroy).

Novelty: Low. Same mechanism class with Japan-only development.

Effect size: Comparable to other HIF-PHIs in the Japanese Phase 3 program.

Promising potential: Low to moderate. Japanese approval only; no global development.

Side-effect profile: Limited published safety data outside the Japanese regulatory filing. No prominent safety signals distinguishing it from the class.

Overall validation: Low to moderate. Japanese approval with very limited international data.

Comparison to roxadustat: Enarodustat is a Japan-only HIF-PHI with substantially less clinical data and narrower regulatory approval than roxadustat. It serves as a local therapeutic alternative in the Japanese market rather than a global competitor.

12.5 Desidustat (ZYANI)

Mechanism: Orally bioavailable HIF-PHI developed by Cadila Healthcare (Zydus Lifesciences) in India [41].

Development trajectory: Desidustat completed Phase 3 trials in India (DREAM-ND and DREAM-D trials) in non-dialysis-dependent and dialysis-dependent CKD populations. The compound received approval in India in 2022 (marketed as Oxemia).

Novelty: Low. Same mechanism class with India-focused development.

Effect size: Comparable to other HIF-PHIs for hemoglobin correction in the Indian Phase 3 program.

Promising potential: Low to moderate. Indian approval only; no global regulatory development.

Side-effect profile: The Phase 3 program in India reported a generally acceptable safety profile. Limited comparative data with other HIF-PHIs from network meta-analyses due to smaller trial sizes.

Overall validation: Low to moderate. Indian approval with limited international exposure.

Comparison to roxadustat: Desidustat is a regional (India-focused) HIF-PHI with substantially less clinical data and a narrower regulatory footprint than roxadustat. It serves as a local therapeutic option rather than a global competitor.

12.6 Comparator summary

The five HIF-PHI class members occupy distinct positions in the global regulatory and clinical landscape. **Daprodustat** is the most validated comparator, having achieved US FDA approval and produced the most favorable cardiovascular safety profile among the class, making it the principal competitive alternative to roxadustat globally. **Vadadustat** shares a similar regulatory trajectory to roxadustat, with US rejection and limited approvals, and confirmed the class-level cardiovascular concern in non-dialysis-dependent populations. **Molidustat**, **enarodustat**, and **desidustat** are regionally focused compounds (Japan, Japan, and India, respectively) with narrower clinical datasets and limited global relevance.

Roxadustat retains a unique position as the first-in-class HIF-PHI and the compound with the broadest global regulatory approval and the largest clinical dataset. Its principal advantages are the breadth of approved markets (China, Japan, EU, South Korea, Chile), the extensive Phase 3 evidence base (more than 9,600 patients

across seven trials), the demonstrated iron-mobilizing and hepcidin-lowering effects, and the growing body of preclinical evidence for pleiotropic benefits (renoprotection, anti-inflammatory activity, lipid modulation). Its principal disadvantages are the unresolved US regulatory status, the thrombotic and cardiovascular safety signals, and the data integrity concerns that emerged during the FDA review process. Among the HIF-PHI class, the choice of agent in clinical practice is increasingly guided by regional availability, cardiovascular risk profile, and insurance coverage rather than by clinically distinguishing differences in erythropoietic efficacy.

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