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Review

Targeting oxidized sGC in calcific aortic valve stenosis: a narrative review of ataciguat

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Abstract

Objectives: No pharmacologic treatment has been shown to slow the course of calcific aortic valve stenosis (CAVS), an active fibrocalcific disease. A downstream signaling bottleneck in the NO–sGC–cyclic guanosine monophosphate (cGMP) pathway is created when oxidative stress transforms soluble guanylate cyclase (sGC) into nitric oxide (NO)–insensitive, oxidized/heme-free states. With an emphasis on the NO-independent sGC activator ataciguat (HMR-1766), this narrative review aims to highlight the molecular, translational, and clinical evidence supporting oxidized/heme-free sGC as a therapeutic target in CAVS.

Methods: We conducted a structured narrative literature search across PubMed/MEDLINE, Embase, Web of Science, Scopus, Cochrane Central, and trial registries (ClinicalTrials.gov/WHO ICTRP) through October 19, 2025. We prioritized original mechanistic/structural studies, preclinical pharmacology, valve-biology investigations, and human translational/clinical studies evaluating ataciguat, sGC redox biology, and disease-modification endpoints (e.g., CT-derived aortic valve calcium [CT-AVC] and ^18F-NaF PET).

Results: Across structural and biochemical studies, heme-mimetic sGC activators selectively bind oxidized/heme-free sGC and restore cGMP signaling by occupying the heme pocket, thereby bypassing NO insensitivity. Valve-biology studies indicate that NO–sGC–cGMP signaling and NO-dependent S-nitrosylation/NOTCH pathways function as complementary anti-calcific mechanisms linked to shear stress and endothelial integrity. Imaging biomarkers such as CT-AVC and ^18F-NaF PET provide sensitive readouts of calcification burden and activity. Early randomized clinical evidence in moderate CAVS suggests oral ataciguat is generally well tolerated and is associated with a directional slowing of CT-AVC progression over six months.

Conclusions: All currently available information suggests that oxidized/heme-free sGC is a biologically reasonable and treatable target in CAVS. Ataciguat exhibits early human signs of delayed calcific development and redox-selective restoration of NO–cGMP signaling. To verify long-term structural advantages and clinical impact, however, longer-term, well-powered clinical investigations are needed.

Keywords: Calcific aortic valve stenosis, soluble guanylate cyclase, oxidized sGC, heme-free sGC, ataciguat (HMR-1766), cyclic GMP

Introduction

The most common valvular condition in older people is calcific valve stenosis (CAVS), which affects approximately 1-2% of those aged ≥ 65, and ~12% to ≥75. Despite significant procedural advancements, observation and eventual valve replacement are the usual course of treatment. Meanwhile, no medication has been shown to alter the course of the condition. Pathway-targeted strategies that can prevent valve damage and reduce leaflet calcification are encouraged by this therapeutic gap (1, 2). CAVS has been reframed from "degeneration" to an active fibro-calcific condition because of endothelial damage, inflammation, lipid/lipoprotein deposition (particularly Lp (a), and osteogenic reprogramming of valvular interstitial cells (VICs) throughout the last ten years. Early in the course of the disease, lipoprotein-initiated biology is highlighted by genetic and epidemiological studies that support the causal involvement of Lp (a) in quicker hemodynamic development (3-5). Hemodynamically, progressive leaflet calcification increases transvalvular gradients, decreases effective orifice area, and stiffens cusps. In natural-history investigations and early-phase trials, CT-derived aortic valve calcium (CT-AVC), which measures the calcium burden underlying this physiology, has become a reliable progression surrogate that offers complementary and more reproducible assessment compared with echocardiography (6, 7). The NO-sGC-cGMP-PKG axis is a crucial homeostatic brake on valvular calcification. Endothelial NO signaling inhibits VIC activation and osteogenic programming. However, pro-calcific pathways speed up when NO signaling is compromised. Enhancing NO-cGMP signaling can help prevent calcification, as suggested by various experimental models, including tissue studies, single-cell transcriptomics, and cellular analyses (8, 9). Furthermore, the sGC heme is changed to ferric or "heme-free" states by oxidative stress in diseased valve tissue, resulting in NO-insensitive (oxidized) sGC and attenuated cGMP production. This redox bottleneck offers a mechanistic justification for the direct activation of oxidized/heme-free sGC, which may limit the efficacy of NO donors or sGC stimulators that need reduced-heme sGC (9-11). Pharmacologically, sGC activators (e.g, ataciguat/HMR-1766 and cinaciguat) bind and activate oxidized or heme-free sGC, restoring cGMP production under oxidative conditions. However, sGC stimulators (e.g., riociguat and vericiguat) sensitize the reduced enzyme to NO. This mechanistic distinction is consistently supported by structural, biochemical, and pharmacologic data (10, 11). An oral, NO-independent sGC activator that preferentially acts on oxidized/heme-free sGC is ataciguat (HMR-1766). Current clinical studies have identified ataciguat as a primary drug for targeting this pathway. Early mechanistic work has shown that HMR-1766 stimulates ferric/heme-free sGC and resists tolerance, creating a suitable fit to the redox-high milieu of calcified valves (11, 12). Now, translational evidence transcends mechanism. The proof-of-concept that reactivating oxidized sGC can alter a known disease surrogate in humans was demonstrated by a Phase II randomized, placebo-controlled trial in moderate CAVS, which found that 6 months of ataciguat slowed CT-AVC progression compared to placebo, with supportive signals on valve function and an acceptable safety profile. The results support the route theory and pave the way for more extensive studies (1, 2). Strategies for biomarkers and imaging are still essential to the development of new treatments. While 18F-NaF PET detects early microcalcification activity and is standardized for use as a research endpoint, CT-AVC gives quantifiable progression readouts across months. Imaging is complemented through putative soluble biomarkers and echo- hemodynamics (valve area/gradients) to characterize underlying pathophysiology and functional outcomes (6, 8, 12). Practical and safety factors are pertinent to the class. Blood pressure can be lowered by sGC activation; previous IV programs (such as cinaciguat in acute HF) highlight the importance of cautious dosage and patient selection in older CAVS patients. On the other hand, preliminary research has demonstrated that oral ataciguat regimens in moderate CAVS are tolerable (2, 7, 11). Overall, the drug-disease match is strong: ataciguat directly targets oxidized/heme-free sGC and has shown a human signal on the course of CT-AVC; oxidative inactivation of sGC represents a therapeutically targetable molecular lesion in CAVS. We provided a summary of the molecular underpinnings, translational and clinical evidence, and practical implications for ataciguat's placement within a disease-modifying approach for CAVS in this review (2, 9, 10, 13). Consequently, this review poses the following five queries: (1) In calcific aortic valve stenosis (CAVS), what mechanistic evidence supports oxidized/heme-free soluble guanylate cyclase (sGC) as a druggable lesion? (2) What novel preclinical and translational evidence is there for ataciguat (HMR-1766) as a sGC activator in aortic valve-relevant oxidizing environments? (3) Which

human clinical indicator safety, imaging/hemodynamics, and efficacy have been linked to ataciguat in CAVS thus far? (4) How do outcomes like echocardiographic measurements, 18F-NaF PET, and CT-derived aortic valve calcium (CT-AVC) relate to disease change and trial design for this pathway? (5) How can wevtarget oxidized sGC in CAVS? What are the main gaps, restrictions, and future directions (e.g., disease stage, length of treatment, combinations with Lp (a)-lowering)? Our objective is to compile the original research on ataciguat, from the bench to the bedside, give a clear explanation of the selection and identification of the literature, and discuss the useful implications for further research and clinical use.

Materials and Methods

Design and reporting

This article presents a narrative literature review that follows a predetermined strategy for searching and selecting relevant studies. In the context of calcific aortic valve disease and stenosis, we focus on original research, including mechanistic and structural studies, cell and animal research, human translational studies, and clinical trials, all of which are directly related to oxidized/heme-free sGC and ataciguat (HMR-1766). The methods used in this review are transparently disclosed in accordance with SANRA's good-practice guidelines.

Data sources and time frame

We looked through the following databases through October 19, 2025:

- PubMed/MEDLINE
- Elsevier's Embase
- Core Collection of the Web of Science
- Scopus
- Cochrane Central (to reveal any studies that are randomized)
- WHO ICTRP (trial identification; only included if findings were published in peer-reviewed journals) and ClinicalTrials.gov

Additionally, we supplemented our database searches with targeted Google Scholar searches for hard-to-find original studies, as well as backward and forward citation tracking.

Eligibility Criteria

Original research in the following areas was included: a) oxidized/heme-free sGC biology or pharmacology; b) ataciguat (HMR-1766) (any model), with special attention to studies that explicitly link to CAVS or valve biology; c) clinical ataciguat data in CAVS; and d) imaging/biomarker endpoints (CT-AVC, 18F-NaF PET, Aortic valve area (AVA)/gradients) when used to evaluate disease modification relevant to ataciguat's mechanism. Non-sGC targets; studies unrelated to oxidized/heme-free sGC activation; narrative reviews, editorials, and comments without new data; conference abstracts without full articles; preprints not subsequently published; and non-English (unless an English abstract explicitly indicated original data directly on ataciguat/oxidized sGC and an English full text was available) are all excluded.

Search Strategy across Databases

We merged free-text keywords with regulated vocabulary (MeSH/Emtree). With medication and endpoint words, the fundamental ideas were (CAVS/valve calcification) AND (sGC/oxidation/activator).

Study selection and data extraction

After screening titles and abstracts for eligibility, we conducted a full-text review of each included study, charting the following: setting/model, design, sample size, exposure (drug/construct; dose/duration), comparators, primary outcomes (e.g., cGMP/PKG signaling, BMP/NOTCH readouts, VIC calcification, valve function; for human studies: CT-AVC, 18F-NaF, AVA/mean gradient, safety/AEs/BP), and key findings. we setteled any discrepancies by re-examination against the criteria, and where needed, related papers (methods, companions/appendices) were retrieved to elucidate endpoints.

Synthesis

We synthesized findings across ten strands (A–J), grouped under five core questions: (1) mechanistic rationale (oxidized/heme-free sGC); (2) ataciguat pharmacology (cell/animal); (3) valve biology links (VIC/ Valvular endothelial cell (VEC) anti-osteogenic signaling); (4) imaging/biomarker endpoints for disease modification; and (5) human clinical signals and safety. There was no attempt at quantitative pooling.

Results

We arrange the evidence in the following section according to the five questions posed by the review. Before analyzing the preclinical pharmacology of ataciguat under oxidizing circumstances, including its effects on sGC activation and durability, we evaluate mechanistic studies demonstrating that

oxidized/heme-free sGC is a druggable lesion. We then connect these processes to disease-modification readouts (echocardiographic hemodynamics, CT-AVC, and 18F-NaF PET) and valve biology (VIC/VEC anti-osteogenic signaling). We conclude by noting gaps and implications for trial design and potential combinations (e.g., Lp(a)-lowering) by synthesizing human clinical signals for ataciguat in CAVS, including efficacy surrogates, safety/tolerability, and hemodynamics.

A. Mechanistic evidence that oxidized/heme-free sGC is a druggable lesion

We start by providing the mechanistic evidence that oxidized/heme-free sGC is a lesion that can be treated with medication in CAVS. We describe how heme-mimetic activators re-engage cGMP signaling, how oxidative stress causes sGC to shift toward ferric/apo states, and how activation kinetics, including irreversibility, manifest at the enzyme and tissue levels. Table 1 compiles these findings.

Table 1. Mechanistic Proof that Oxidized/Heme-Free sGC Is a Druggable Lesion

Study (year)	System/Model	sGC redox/ligand state interrogated	Core methods	Key mechanistic findings	Implications for druggable lesion	Notable limitations
Kollau et al., (2018)(14)	Intact vessels (porcine coronaries, rat aorta), cultured endothelial cells, purified bovine-lung sGC	Apo-sGC (heme-free) favored; ferric (ODQ-oxidized) probed; native ferrous as control	Organ bath vasorelaxation ± washout; tissue cGMP RIA; purified sGC activity ± Tween-20 (heme removal) and ODQ; dilution/"washout" irreversibility tests	Cinaciguat produces time- and concentration-dependent vasorelaxation with non-reversible cGMP elevation; potently activates heme-free sGC (EC50 ≈ 0.2 μM with heme removed), only modestly affects ferric sGC; enzyme activation persists after dilution (irreversible binding/stabilization)	Demonstrates that apo/oxidation-shifted pools can be selectively and durably reactivated, explaining efficacy under oxidative stress and class hypotension risk	Detergent-induced heme removal (Tween-20) is non-physiologic; ferric activation judged "modest" vs apo, leaving open in-cell ferric→apo conversion dynamics
Surmeli & Marletta, Angew (2012) (15)	Purified full-length mammalian sGC and H-NOX truncations	Ferric (ODQ-oxidized) vs ferrous vs apo	UV–vis heme-loss kinetics with apomyoglobin trap; ± cinaciguat; parallel cGMP activity assays over time	Ferric sGC loses heme faster than ferrous; cinaciguat accelerates ferric-heme dissociation ~5× and activity increases in step with oxidized-heme displacement early in time course	Establishes mechanism: activator facilitates ferric-heme exit → occupies pocket → restores activity; validates oxidized/heme-free sGC as a bona fide target	In vitro kinetics; later plateau in activity despite ongoing heme loss suggests stability/turnover nuances not captured by the assay
Martin et al., (2010) (16)	Crystallography (2.3 Å) with bacterial H-NOX	Heme-depleted pocket mimicked;	X-ray co-crystal of cinaciguat in the heme pocket; mapping Y-x-S-	Cinaciguat displaces heme and binds as a heme-mimetic, engaging	Provides structural blueprint for ligandable	Uses homologous H-NOX (not full human sGC);

Study (year)	System/Model	sGC redox/ligand state interrogated	Core methods	Key mechanistic findings	Implications for druggable lesion	Notable limitations
	homolog (35% identity to sGC β1 H-NOX) + mutagenesis in mammalian sGC	activation-competent conformation	x-R motif contacts; αF-helix shift analysis; β1-H-NOX mutagenesis (R40A, I111A, R116A)	Y-x-S-x-R motif; induces αF-helix rotation consistent with activation; mutations in the putative communication surface reduce NO-stimulated activity	site in oxidized/heme-depleted sGC and a conformational pathway that links pocket occupancy to activation	static snapshot—no kinetics or cellular redox context

These three papers, when combined, provide a coherent mechanistic arc from structure to biochemistry to tissue physiology, confirming oxidized/heme-free sGC as a viable therapeutic target. As a heme mimic, cinaciguat sits in the vacant heme pocket, activating the conserved Y-x-S-x-R motif and generating a αF-helix rotation, presumably sending an activation signal to the catalytic domains, according to the crystal structure (16). The idea that the pocket remains ligandable and conformationally connected to function even in the event of heme loss or oxidation is anchored by this structural "fit." The biochemical kinetics (15) then show that the ferric (oxidized) enzyme is primed to lose heme, and that cinaciguat speeds up this heme loss by about five times. During the early phase, cGMP-forming activity also increases in tandem, providing direct evidence that functional rescue is mechanistically linked. During the early phase, cGMP-forming activity also increases in tandem, providing direct evidence that functional rescue is mechanistically linked to activator displacement of ferric heme. By helping to generate apo-sGC from the ferric state and reactivating the enzyme, cinaciguat bridges the gap between oxidation state and ligand action. Finally, intact-tissue physiology (14) demonstrates that cinaciguat causes time-dependent, washout-resistant vasorelaxation in native vascular rings and endothelial cells, along with prolonged cGMP accumulation and—most importantly—strong activation of heme-free sGC, while only slightly influencing ferric sGC directly. In addition to confirming that apo-sGC is the high-affinity functional target under oxidative conditions, the irreversibility at the enzyme level (lasting after dilution) explains the hypotension liability and prolonged pharmacodynamics seen with IV activators. In comparison, Surmeli & Marletta (15) present the causative kinetic mechanism (oxidation → heme displacement → activity rescue); Martin et al. (16) present the atomic reasoning (where and how the drug binds and signals); and Kollau et al. (14) show physiological consequences and durability in tissues. Collectively, they support the main idea of the review section: Oxidized/heme-free sGC is a druggable lesion that heme-mimetic activators selectively re-engage—exactly the biological niche that ataciguat fills. It is not a dead end of NO biology.

B. Ataciguat (HMR-1766) pharmacology & preclinical originals

We provide a summary of ataciguat's actions in oxidizing, NO-desensitized biology in both cell and whole-animal models, including redox dependence, tissue-level physiology, durability/tolerance, and target engagement (cGMP/PKG readouts). Our research covers pulmonary vascular remodeling in hypoxic conditions, platelet modulation in diabetes, and vascular smooth muscle signaling. Together, these studies outline the pharmacological framework that we later apply to calcific aortic valve stenosis (CAVS), recent evidence, and applicability. The same disease framework, calcific aortic valve stenosis (CAVS), is caused by endothelial dysfunction and oxidative stress that impair nitric oxide (NO) signaling and downstream cyclic guanosine monophosphate (cGMP) biology, and more recent valve-focused and translational literature support this; however, early pharmacology studies of ataciguat predate the most recent era of valve-disease modeling. Current imaging studies further validate computed tomography-derived aortic valve calcium (CT-AVC) and ^18F-sodium fluoride positron emission tomography (^18F-NaF

PET) as sensitive progression and activity endpoints. Recent mechanistic studies in valve cells and developmental models support NO-related anti-calcific signaling and its interaction with NOTCH and mechanotransduction pathways. All of these recent findings support the further clinical testing of heme-independent sGC activation techniques in CAVS and bolster the translational significance of focusing on oxidized/heme-free soluble guanylate cyclase. (sGC) (1, 2, 6, 27, 31, 32). The retrieved details from each study are shown in Table 2.

Table 2. Ataciguat (HMR-1766) Pharmacology & Preclinical Originals

Study (year)	Model / system	Oxidative / redox context	sGC state / target features	Dosing / exposure (index)	Key endpoints	Principal findings	Notes for CAVS relevance
Zhou et al., (2008) (17)	Rat aortic SMCs; COS-7 expressing WT or heme-deficient sGC	Exogenous ROS (H_2O_2, SIN-1, menadione); endogenous ROS (rotenone); ODQ	HMR-1766 more effective on oxidized/heme-free sGC; requires β1 N-terminus; ROS potentiation is heme-dependent	H_2O_2 500 µM; SIN-1 500 µM; menadione 40 µM; rotenone 10 µM; HMR-1766 0.01–10 µM	cGMP (EIA), EC_{50} shift on H105F; ODQ/H_2O_2 modulation; tolerance assays	cGMP↑ under ROS with HMR-1766; no tolerance or cross-tolerance vs SNP; β1 N-terminus required; heme-deficient mutants show higher sensitivity	Mechanistic confirmation that ataciguat preferentially rescues NO-insensitive-sGC expected in calcified valves
Schäfer et al., (2006) (18)	Streptozotocin-diabetic rats, chronic PO HMR-1766	Diabetes (systemic oxidative stress; NO bioavailability↓)	Platelet NO/cGMP axis readouts (VASP Ser157/Ser239)	10 mg/kg PO, bid (chronic); acute in vivo & in vitro tests	Platelet VASP-P↑; P-selectin↓, fibrinogen binding↓, microparticles↓; ADP aggregation ↓; NO sensitivity restored after in vivo dosing	Chronic sGC activation improves platelet NO/cGMP signaling and attenuates platelet activation in diabetes	Systemic confirmation that ataciguat restores cGMP signaling under oxidative stress without relying on NO
Weissmann et al., (2009) (19)	Chronic hypoxia PH mice; isolated perfused lungs; PASMC	Hypoxia (oxidative milieu; NADPH oxidase activity↑)	Left-shifted HMR-1766 sensitivity under hypoxia; PASMC cGMP rescue	10 mg/kg/day (s.c./PO per protocol) from day 21–35; 0.1–10 µM in isolated lung	HPV inhibition; telemetry RVSP↓; RV hypertrophy↓; vascular muscularization↓; SAP unchanged; PASMC cGMP↑	Demonstrates hemodynamic and anti-remodeling effects in oxidized-sGC context; pulmonary selectivity (no systemic hypotension)	Shows tissue-level benefit of sGC activation in oxidizing disease; supports safety signal (SAP preserved)

Study (year)	Model / system	Oxidative / redox context	sGC state / target features	Dosing / exposure (index)	Key endpoints	Principal findings	Notes for CAVS relevance
Oberwittler et al., (2007) (20)	Oberwittler (human)	Healthy volunteers	CYP2C9 DDI	HMR-1766 + single-dose warfarin	PK/PD of (S)-warfarin	↑(S)-warfarin exposure and anticoagulant effect	Safety/PK caution for future trials (anticoagulant co-meds in CAVS)

Abbreviations. ODQ, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one; HPV, hypoxic pulmonary vasoconstriction; PASMC, pulmonary artery smooth muscle cells; SAP, systemic arterial pressure; VASP, vasodilator-stimulated phosphoprotein.

When combined, these investigations provide a redox-tuned, cohesive pharmacology for ataciguat. Ataciguat specifically restores cGMP signaling in cells where oxidative stress transforms sGC toward NO-insensitive states (17). In line with binding in the heme pocket, the effect is heme-dependent and linked to the N-terminus of the β1-subunit. Crucially, unlike NO donors, ataciguat does not cause tolerance or cross-tolerance, which is essential for any therapy aimed at changing chronic diseases. Despite upstream NO deficits, ataciguat restores downstream function at the whole-organism level. Chronic dosage in diabetic rats (18) decreases many aspects of platelet activation and re-engages the platelet NO/cGMP/PKG pathway (VASP-P↑), which is consistent with an anti-thrombo-inflammatory profile that should be advantageous in vascular calcification milieus. A favorable treatment window exists when oxidized soluble guanylate cyclase (sGC) is abundant, which is evident from ataciguat's ability to suppress hypoxic pulmonary vasoconstriction (HPV), reduce right ventricular systolic pressure (RVSP), and partially reverse vascular remodeling in cases of hypoxic pulmonary hypertension (PH). Observations across various models indicate a trend: the pharmacodynamic benefits, such as left-shifted responses and enhanced restoration of cyclic guanosine monophosphate (cGMP), improve in an oxidizing environment. This redox selectivity is particularly relevant for calcific aortic valve stenosis (CAVS), where fibro-calcific remodeling occurs alongside oxidative stress and a weakened nitric oxide (NO) - sGC signaling pathway. Furthermore, the interaction of CYP2C9 with warfarin highlights the importance of careful management of anticoagulant co-medication in future ataciguat trials for CAVS, drawing on valuable insights from human pharmacokinetic research.

C. Valve-relevant NO–sGC–cGMP biology (anti-calcific signaling in VIC/VEC)

With significant side-specific (fibrosa vs. ventricularis) hemodynamic control, this subsection summarizes the evidence that valve endothelial nitric oxide (NO) inhibits valve interstitial cell (VIC) calcification through complementary mechanisms, including sGC/cGMP signaling, S-nitrosylation-dependent NOTCH activation, and maintenance of eNOS coupling/redox homeostasis. Key design elements and conclusions from four typical studies that serve as the foundation for this framework are taken from Table 3.

Table 3. Valve NO–sGC–cGMP / S-nitrosylation / NOTCH axis in anti-calcific VIC/VEC signaling

Study (year)	Model / system	NO axis manipulation	Principal readouts	Key anti-calcific findings	Pathway evidence / notes
Bosse et al., (2013) (21)	Porcine Aortic valve interstitial cell (AVICs) ± VEC co-culture; transwells with WT vs Nos3−/− EC; mouse genetics (Nos3−/−;Notch	NO donor (DETA-NONOate) or NOS inhibition (L-NAME); endothelial source tested with	Nodule counts; Alizarin/Von Kossa; pSMAD1/5/8; HEY1 WB; NICD nuclear localization; mouse echo/histology	Endothelial-derived NO inhibits AVIC calcification; Nos3−/− EC lose protection; NICD nuclear localization ↑ with NO; compound	NO→NOTCH1/HEY1 signaling in AVICs; genetic interaction NOS3↔Notch1; positions NOTCH downstream of endothelial NO

Study (year)	Model / system	NO axis manipulation	Principal readouts	Key anti-calcific findings	Pathway evidence / notes
	1+/−)	Nos3−/− EC		Nos3−/−;Notch1+/− mice develop aortic valve disease	
Richards et al., (2013)(22)	3D anchored porcine VIC hydrogels ± VEC; ex vivo porcine cusps with side-specific exposure; human valve eNOS IHC	NO donor (DETA-NO); NOS blocker (L-NAME); sGC inhibitor (ODQ) & activator (BAY); shear waveforms	ARS/von Kossa; α-SMA, RUNX2, osteocalcin; cGMP levels; eNOS expression (side-specific)	VEC suppress VIC calcification and activation; DETA-NO mimics protection; L-NAME abrogates it; ODQ increases calcification, BAY reduces; ventricularis shear → higher cGMP; calcified human valves show reduced eNOS, fibrosa-biased	Strong support for NO→sGC/cGMP as anti-calcific axis; hemodynamic/side-specific regulation of NO signaling
Majumdar et al., (2021)(8)	Porcine AVICs; scRNA-seq; BST-MS proteomics; HEK293 mechanistic assays; Usp9x Tie2-Cre mice; human valves	NO donor vs cGMP analog (8-Br-PET-cGMP) vs S-nitrosylator (GSNO); USP9X inhibition/siRNA	Nodule formation; RUNX2; protein S-nitrosylation; MIB1 ubiquitination; NICD; mouse echo/histology; human valve IP/WB	NO and GSNO prevent calcification; cGMP analog does not in vitro; USP9X S-nitrosylation → deubiquitinates/stabilizes MIB1 → NOTCH1 activation; Usp9x EC-lineage KO → AV stenosis/calcification; human CAVD: ↓S-NO-USP9X, ↓MIB1/NICD	Defines NO→S-nitrosylation(USP9X)→MIB1→NOTCH1 anti-calcific axis; cGMP-independent in their assays
Farrar et al., (2015)(23)	Porcine VEC on 3D gels; ex vivo porcine cusps; human calcified valves	TNF-α to induce endothelial stress; BH4 (recouple eNOS), peg-SOD, apocynin; L-NAME	Superoxide (DHE), H2O2, mtROS; NO (Griess); VE-cadherin/eNOS/VCAM-1; α-SMA; ECM histology; ARS/von Kossa; osteogenic genes	Calcified human valves: fibrosa endothelial superoxide ↑, SOD1 ↓. TNF-α causes VEC oxidative stress, ↓NO, eNOS/VE-cadherin loss, ↑VCAM-1, ↑VIC activation/calcification; BH4 and peg-SOD rescue endothelial functionand reducecalcification	Highlights eNOS coupling/redox as gatekeeper of NO's protective effects; supports targeting BH4 / ROS to restore NO signaling

A consistent theme emerges from these investigations: valve endothelial NO is a master anti-calcific cue for VICs, and the delivery and transduction of NO are important. Richards et al. (22) described a traditional pathway regulated by hemodynamics, where NO activates soluble guanylate cyclase (sGC) to produce cyclic guanosine monophosphate (cGMP). Increased unidirectional shear stress, resembling that in the ventricularis, enhances cGMP levels and helps prevent calcification. However, inhibiting sGC with ODQ results in an increase in osteogenic markers and calcium deposition, indicating that NO signaling is vital in this process.This study positions cGMP signaling and shear-regulated eNOS as a specific mechanism that prevents calcification. Bosse adds to this by demonstrating that endothelial NO also feeds into NOTCH1 within AVICs, increasing NICD nuclear localization and HEY1 and interacting genetically with Notch1 in vivo, thereby solidifying a paracrine VEC→VIC pathway that inhibits osteogenic conversion (21). Additionally, Majumdar expands on the NOTCH narrative by identifying S-nitrosylation as a second, cGMP-independent pathway. This process stabilizes MIB1, which activates NOTCH1 in nearby cells and inhibits RUNX2 and calcification by S-nitrosylating USP9X. Notably, a cGMP analog did not replicate the protective effects observed in their in vitro system, demonstrating a context-dependent response. For instance, S-nitrosylation predominated in rigid 2D VIC cultures used to test post-translational regulation, while cGMP proved significant in 3D/ex vivo conditions and under physiological shear forces. Farrar et al. conclude their findings by explaining the challenges of achieving protection in a disease context, particularly in the fibrosa layer. They note that inflammation (TNF-α) uncouples eNOS, shifting its function from producing NO to generating superoxide. However, scavenging reactive oxygen species (ROS) using peg-SOD or recoupling eNOS with BH4 can restore endothelial function and reduce calcification and matrix derangement.

D. Imaging & biomarker endpoints that quantify disease modification

In trials for calcific aortic stenosis (AS), imaging biomarkers that indicate active calcification and structural burden or progression can help shorten the duration of studies and reduce the required sample sizes. However, CT-AVC effectively assesses the structural load and its changes with high repeatability. And 18F-NaF PET captures microcalcification activity, which is a key indicator of growth. When these two imaging techniques are accompanied by optimal PET capture and processing, they provide both operational support and insights into the mechanisms resulting in Phase 2–3 outcomes. Table 4 presents design elements, endpoints, quantitative findings, and implications for powering in detail.

Table 4. Imaging endpoints for disease-modification trials in calcific AS

Study (year)	Imaging endpoint(s)	Design / N	Population	Key methods / optimization	Principal quantitative findings	Trial design implications
Dweck et al., (2014)(24)	18F-NaF PET (activity), 18F-FDG; CT-AVC follow-up	Histology validation (n≈10/ tracer) + 1-yr progression (n=18)	AS spectrum incl. sclerosis–moderate; AVR tissue for histology	PET TBR vs TNAP & osteocalcin; baseline PET vs 1-yr CT-AVC change; voxel mapping (PET+ / CT−)	NaF correlated with TNAP (r=0.65) & osteocalcin (r=0.68); baseline NaF predicted 1-yr CT-AVC increase (r=0.66; up to 0.75 in PET+/CT− areas); FDG not predictive (r=−0.11)	Establishes biologic & predictive validity: NaF is a PD biomarker of active calcification likely to respond over short windows
Pawade et al., (2016)(25)	18F-NaF PET-CT methodology & reproducibility	Scan–rescan (n=15), ~4 weeks	Mild–severe AS	Contrast CT, ECG-gated PET, right-	Improved scan–rescan to ±10% error	Demonstrates trial-ready precision and

Study (year)	Imaging endpoint(s)	Design / N	Population	Key methods / optimization	Principal quantitative findings	Trial design implications
				atrium blood-pool, Most-Diseased-Segment (MDS)	(TBR_MDS mean); leaflet-level localization to tips/commissures; sample-size example ≈57/arm to detect 10% NaF change @80% power	feasible N for NaF as a short-horizon PD endpoint
Doris et al., (2020)(6)	CT-AVC (Agatston) vs echocardiography progression	Reproducibility (n=33) + progression (n=81; 1–2 yrs)	Broad AS spectrum	Bland-Altman, ICC; annualized change; Cohen's d; power models	CT-AVC ICC 0.99, LoA −12% to +10%; median +152 AU/yr; d=3.12 vs echo (Vmax d=0.71). To detect a 20% slowing: CT-AVC ~43 vs Vmax ~787 (80% power)	High SNR structural endpoint → order-of-magnitude smaller trials than echo-based designs
Murtazaliev a et al., (2022)(26)	18F-NaF & 18F-FDG PET/CT; CT-AVC; echo follow-up	Prospective cohort (n=71), ~17 months; TAV vs BAV	Mild–severe asymptomatic AS	Multivariable models by phenotype	TAV: baseline NaF TBR + Vmax independently predicted hemodynamic progression; FDG not predictive. BAV: Vmax (±age) predicted; NaF less informative	Supports NaF as prognostic (esp. TAV) → enrich phase 2 with high-risk TAV by NaF; FDG not useful for progression
Lassen et al., (2022)(27)	18F-NaF PET with triple motion correction (3×MC)	Test–retest (n=14), two 30-min scans	AS patients	Correct cardiac + respiratory + patient motion; right-atrium background	TBRmax ↑ ~33%, SUVmax ↑ ~26% with 3×MC; SNR ↑ (largest with 3×MC); better correlation to AVC (R^2 up to 0.46); repeatability preserved (~14% TBRmax RC)	Technical optimization boosts effect-size without sacrificing repeatability → smaller N and more reliable PD reads

Abbreviations. TBR, target-to-blood ratio; TNAP, tissue non-specific alkaline phosphatase; MDS, most-diseased segment; ICC, intraclass correlation coefficient; LoA, limits of agreement; Vmax, peak aortic jet velocity; TAV/BAV, tricuspid/bicuspid aortic valve; SNR, signal-to-noise ratio; RC, repeatability coefficient.

According to Dweck et al., 18F-NaF exhibits the traditional leading-indicator behavior of a disease-modification PD biomarker by tracking osteogenic activity (TNAP/osteocalcin) and forecasting future calcium increase on CT within a year (24). Pawade et al. transform NaF PET into a trial-ready tool. Uptake can be localized to leaflet stress zones, exactly the point where

medicines should act, and test-retest error drops to around 10% when combined with gated PET, contrast CT, right-atrium backgrounding, and MDS quantification (25). Lassen's three-fold motion correction enhances the detectable effect size without increasing noise levels by further amplifying the signal and signal-to-noise ratio (SNR), while also strengthening its correlation to CT-AVC, all while maintaining repeatability. Doris et al. demonstrate that CT-AVC requires more than ten times fewer samples to identify the same proportion of treatment impact compared to echo metrics. Additionally, it shows exceptional consistency and a significantly larger standardized annual change. Consequently, NaF PET provides an early pharmacodynamic readout within 3 to 6 months, allowing for validation of target engagement and a decision on whether to proceed with treatment. In contrast, CT-AVC serves as an effective endpoint for assessing structural progression over a 12 to 24-month period. Murtazalieva et al. note that NaF is less effective in patients with bicuspid aortic valves (BAV), where maximum velocity, adjusted for age, is more significant; however, it does predict hemodynamic changes in patients with trileaflet aortic valves (TAV).To optimize event yield and PD sensitivity, practically, enrich Phase 2 with TAV patients who show high NaF uptake; cautiously incorporate BAV or stratify by phenotype. FDG doesn't need to be given priority because it consistently performs poorly in AS advancement. Combine CT-AVC (medium-horizon structural progression) with NaF PET (short-horizon PD) using improved acquisition/analysis (MDS, right-atrium background, motion correction). Conventional echo is still necessary in the clinical context and for safety. However, this two-tier approach increases biologic credibility, lowers N, and speeds up timelines.

E.Human clinical signals with ataciguat in CAVS(safety, imaging/hemodynamics, efficacy)

This subsection provides an overview of human clinical signals related to ataciguat in moderate calcific aortic valve stenosis (CAVS), including hemodynamics (AVA/gradients), imaging (CT-AVC), safety/orthostatic tolerance, and early effectiveness. Table 5 presents key design features and results from the published I/IIa RCTs (reported in Circulation 2025) and the ongoing pivotal 3 KATALYST-AV trial (NCT07001800), highlighting how endpoints and exposure/duration are changing to address previous limitations.

Table 5. Human clinical signals with ataciguat in CAVS: study extraction and key outcomes

Study / Year	Design & Phase	Population (moderate CAVS unless noted)	Intervention & Duration	Primary/Key Endpoints (safety, imaging, hemodynamics, efficacy)	Top-line Findings	Registration / Pub. status
Zhang et al., (2025)(2)	Two RCTs: Phase I 14-day safety/tolerability; Phase IIa 6-month efficacy; randomized, double-blind, placebo-controlled	Adults with moderate CAVS; echo-confirmed; phase IIa enriched for higher CT-AVC	Ataciguat 100–200 mg QD (phase I); 200 mg QD (phase IIa)	Safety/orthostasis: seated→standing & tilt tests; Imaging: CT-AVC; Hemodynamics: AVA/velocity/gradients; LV function: EF, SV, diastolic indices	Well-tolerated; minor BP reductions at rest without orthostatic intolerance. CT-AVC progression reduced ≈70% vs placebo over 6 mo (borderline p≈0.051). Trends toward less AVA decline and better LV	Peer-reviewed publication (Circulation 2025). Trial IDs reported: NCT02049203 (phase I) and NCT02481258 (phase IIa).

Study / Year	Design & Phase	Population (moderate CAVS unless noted)	Intervention & Duration	Primary/Key Endpoints (safety, imaging, hemodynamics, efficacy)	Top-line Findings	Registration / Pub. status
					systolic/diastolic function; exploratory greater benefit in men.	
Mayo Clinic-NCT02049203- Clinical Trial	Phase I(b) randomized, double-blind, placebo-controlled; 14 days	Moderate CAVS; n≈44	Ataciguat 50/100/200 mg QD for 14 days vs placebo	Primary: orthostatic intolerance and BP responses (seated→standing; head-up tilt)	No increase in orthostatic symptoms; small BP reductions at rest; supports hemodynamic tolerability. Note: these results are the same dataset summarized in Zhang et al. 2025.	Completed; registry record; results published within Zhang et al. 2025. (ClinicalTrials)
KATALYST-AV-NCT07001800-Clinical Trial	Phase 3, randomized, double-blind, placebo-controlled; Part A + Part B	Adults ≥50 y with moderate CAVS; AVA 1.0–1.5 cm²; AVC thresholds by sex; EF ≥45%	Ataciguat QD up to 156 weeks vs placebo; planned n≈1410	Part A: ΔCT-AVC (24 wk) and correlation with peak VO_2; Part B dual-primary: %ΔAVA (48 wk) and Δpeak VO_2 (48 wk); secondary: ΔLVMI, ΔAVC (48 wk), progression to AVA <1.0 cm², time to TAVR/SAVR/death	Ongoing—no results yet. Design directly tests structural and functional efficacy with longer exposure and clinical-trajectory readouts.	Recruiting (US, multi-center). Registry record only (no publication yet). (ClinicalTrials)

Some published randomized controlled trials (RCTs) suggest that patients with moderate calcific aortic valve stenosis (CAVS) can tolerate ataciguat. There was no significant orthostatic hypotension observed when standing or during head-up tilt maneuvers, and only minor decreases in resting blood pressure were noted. This aligns with the selectivity of the soluble guanylate cyclase (sGC) activator for oxidized and heme-free sGC present in diseased tissue. The primary aim of restoring nitric oxide (NO)–sGC–cyclic guanosine monophosphate (cGMP) signaling in the heart valve, while avoiding systemic vasodepression, is supported by these human results, including findings from a phase 3 longer-term dosing study. In the 6-month Phase IIa RCT, the progression of calcific aortic valve disease (CT-AVC) decreased by nearly 70% compared to a placebo group. However, this finding was borderline significant due to the small sample size and short follow-up period. Additionally, there was a tendency for AVA to decrease more slowly and for LV systolic/diastolic indices to improve; Zhang et al.'s exploratory analysis revealed greater effects in men, which reflected sex-phenotypes observed in CAVS calcific burden (2). These results, which are biologically congruent with the preclinical program, are the first randomized human data to show a pharmacologic agent-induced directional slow-down of CAVS calcification. The ongoing Phase 3 KATALYST-AV trial (ClinicalTrials.gov NCT07001800) extends exposure (up to 156 weeks), increases power (n≈1410), and raises functional/hemodynamic endpoints (peak VO_2, %ΔAVA) to co-primary status while maintaining

CT-AVC as an early structural readout to address the durability and clinical-meaningfulness issues brought up by the smaller phase IIa. If ataciguat replicates (or scales) the Phase IIa signals, it could be the first disease-modifying medication to shorten the duration of mild CAVS and potentially delay the time to TAVR/SAVR (2). Until the data are read out, claims should be cautious and grounded in the peer-reviewed Phase I/IIa evidence.

F. Class-level clinical safety signals (oxidized-sGC activators) to contextualize hypotension risk

The class-defining safety risk with oxidized-sGC activators is hypotension, as they cause strong venous and arterial vasodilation. To set expectations for this class and guide risk mitigation when considering sGC activation tactics in valve disease populations, Table 6 lists the human safety signals (blood pressure changes and hypotension/orthostasis events) in IV cinaciguat in ADHF.

Table 6. Human clinical safety signals with oxidized-sGC activators (focus: hypotension)

Agent (class)	Setting & population	Design	N	Dose / duration	Hemodynamic effects (during infusion)	Hypotension / orthostasis signal	Other safety notes	Take-home for hypotension risk
Lapp et al., (2009)(28)	Acute decompensated HF (PCWP ≥ 18 mmHg)	Multicenter, phase II, non-randomized (dose-finding + proof-of-concept)	60 (safety); 30 (hemodynamics in part B)	50–400 µg/h IV for 6 h (titrated)	↓PCWP −7.9 mmHg, ↓SVR −597 dyn·s·cm^{-5}, ↓MAP −14.3 mmHg, ↑CO +1.68 L/min, modest ↑HR +4.4 bpm	Most common drug-related AE = hypotension; symptomatic hypotension in 6/60; mean SBP −13.9 mmHg during infusion; some discontinuations	Renal function preserved (no creatinine rise); neurohormonal activation consistent with vasodilation	Demonstrates clear hypotension liability when sGC is activated aggressively IV in acutely ill, vasodilator-sensitive patients

According to Lapp et al.'s trial, hypotension is the primary safety signal for all oxidized-sGC activators (28). Patients receiving IV cinaciguat through profound venous and arterial vasodilation achieved the intended unloading (PCWP −7.9 mmHg; SVR −597 dyn·s·cm^{-5}; CO +1.68 L/min) in ADHF; however, this was associated with clinically significant BP reductions (mean MAP −14 mmHg, SBP −14 mmHg) and symptomatic hypotension, the most common drug-related adverse event. Increases in renin and noradrenaline are neurohormonal alterations that mimic a potent vasodilatory stimulus. The signal is constant, instantaneous (during infusion), and frequent enough to necessitate dose adjustment and, occasionally, cessation.This trend suggests that route, acuity, and dosage intensity play a major role in determining hypotension liability with sGC activators, which is important for contextualizing risk beyond ADHF. Tight titration, invasive monitoring, and blood pressure are crucial because IV treatment in decompensated situations shortens the therapeutic window, and benefit and hypotension occur simultaneously. In contrast, published data indicate a more controllable profile with lower BP effects and minimal orthostatic intolerance in stable outpatient populations and with oral dosing. Practically speaking, when transferring classroom knowledge to CAVS, expect blood pressure to drop, check for low baseline blood pressure or concurrent vasodilators, and keep an eye on supine and orthostatic vitals. However, keep in mind that the greatest risk of hypotension occurs with IV activators in patients with acute heart failure rather than with long-term oral use in stable patients.

G. Additional ataciguat originals (PK/PD; systems relevance)

The translational profile of ataciguat depends on its

pharmacokinetic behavior, oxidative stress-induced modulation of sGC, and organ/system-level manifestation of characteristics. To demonstrate the intersections of exposure-response, redox biology, and whole-organ hemodynamics—as well as the importance of clinical guardrails (such as CYP2C9 interactions) Table 7 compiles the original ataciguat studies covering human PK/PD (warfarin DDI), cellular mechanisms under ROS, and in vivo pulmonary hypertension.

Table 7. Ataciguat (HMR-1766) originals—PK/PD and systems relevance

Study (year)	Context & model	Design / N	Regimen / exposure	Key PK findings	Key PD / mechanistic findings	Systems relevance & signals	Safety/operational notes
Oberwittler et al., (2013)(20)	Human PK/PD DDI (healthy men)	Randomized, crossover-style DDI; n=18	Ataciguat (HMR-1766) steady-state + single warfarin 20 mg vs placebo	(S)-warfarin AUCinf ↑ from ~33,148 to 106,471 h·µg/L; t½ ↑ from ~31.7 h to 82.9 h (CYP2C9 inhibition)	Anticoagulant effect ↑: max ↓ in prothrombin time 58.8% vs 39.9%	Clinically meaningful CYP2C9-mediated DDI; dose adjustment/INR monitoring required when co-administered	First human PD signal tying ataciguat to DDI risk management rather than intrinsic hypotension
Zhou et al., (2008)(29)	Cellular PD / mechanism (RASMCs, COS7; oxidative stress)	In-vitro cGMP assays; mutagenesis (β1-H105F/H105C), truncations	HMR-1766 up to µM range; ROS via H_2O_2, SIN-1, menadione, rotenone	—	ROS potentiates HMR-1766 cGMP; prefers oxidized/heme-free sGC; β1-N-terminus required; no tolerance, no cross-tolerance vs SNP	Explains disease-targeting under oxidative stress (e.g., valve disease, PH); supports combination logic with NO donors without cross-tolerance	Mechanistic basis for selectivity in diseased tissue; suggests stable PD over time
Weissmann et al., (2009)(30)	In-vivo PD / systems (mouse chronic hypoxia PH)	Isolated lung & telemetry; chronic dosing	10 mg/kg/day SC (days 21–35 after PH established); plasma ~0.6 µg/mL	—	↓RVSP (to ~29 mmHg), ↓RV hypertrophy, ↓vascular muscularization; left-shifted dose–response in hypoxic lungs; SAP unchanged	Demonstrates organ-level efficacy and pulmonary selectivity under oxidative milieu; supports therapeutic window with limited systemic hypotension	Systemic BP not reduced, implying lower hypotension liability in this context

Abbreviations. AUCinf, area under the curve to infinity; t½, half-life; DDI, drug–drug interaction; RASMC, rat aortic smooth-muscle cells; SNP, sodium nitroprusside; RVSP, right-ventricular systolic pressure; RV, right ventricle; SAP, systemic arterial pressure.

All issues considered, the data point to ataciguat as a redox-tuned sGC activator whose clinical use is primarily influenced by CYP2C9-mediated drug–drug interactions rather than intrinsic hypotension: the human DDI study by Oberwittler et al. (20) revealed a ~3× increase in (S)-warfarin exposure and increased anticoagulation, necessitating dose/INR management. Zhou et al. (17) provided a mechanistic explanation for the long-lasting pharmacodynamics in oxidizing disease beds by showing that reactive oxygen species enhance ataciguat's cGMP signaling by favoring oxidized/heme-free sGC without tolerance or cross-tolerance. Organ-selective advantages under oxidative stress were supported by Weissmann et al. (30), who observed decreased RV pressures and remodeling in hypoxic PH with unchanged systemic arterial pressure. Oberwittler et al. (20) corroborate these findings by confirming that ataciguat inhibits CYP2C9 and significantly increases exposure to (S)-warfarin, which is the primary PK/PD warning when co-prescribing narrow-therapeutic-index substrates. This co-medication treatment poses a greater operational risk to stable outpatients than class-intrinsic hypotension. In the meantime, ataciguat is strongly recommended for diseases like calcific aortic valve disease and pulmonary hypertension due to its oxidative targeting, lasting PD, and organ-level selectivity, as reported by Zhou et al. (17) and Weissmann et al. (30).

H. Complementary valve-oxidative stress/NO pathway originals (context for Discussion)

In addition to oxidative-stress tales, this subsection places nitric oxide (NO) biology as a supplementary lens for valve disease mechanisms. Using a single-cell, mechanistic investigation in valve interstitial cells and an in-vivo developmental study of eNOS depletion, we demonstrate how NO availability interacts with NOTCH and integrin/focal-adhesion processes that influence valve calcification and structure. To serve as an anchor for the subsequent synthesis, Table 8 (at the end) captures key design elements, readouts, and constraints.

Table 8. Core features of NO-pathway studies informing valve biology

Study (year)	Biological system & design	NO pathway leverage	Primary valve/arch findings	Key signaling readouts	Outcome measures	Main limitations
Eley et al., (2024)(31)	In vivo mouse eNOS−/−; fetal → adult histology & lineage tracing	Genetic NO deficiency (Nos3 null)	BAV in subset; ventricular trabeculation defects; aortic arch tortuosity/shortening; peri-ductal chondroid metaplasia; baroreceptor loss	↓Notch1ICD/Jag1 in ductus/arch & ventricles; expanded Sox9 in periductal region	Survival; gross & histologic valve/aorta features; elastin analyses; response to Ang II challenge	Mouse strain/background effects; developmental lethality enriches for survivors; indirect readouts of oxidative stress
Majumdar et al., (2022)(32)	In vitro porcine VICs ± NO donor/GSNO; scRNA-seq; in vivo mouse valve IHC	Pharmacologic NO/S-nitrosylation (detaNONOate; GSNO)	NO suppresses spontaneous/osteogenic calcification; down-regulates ITGA8/VCL; NO effect is transient without continued exposure	Pathway over-representation: integrin, Rho-GTPase, Wnt, TGF-β; prior linkage to NOTCH1 activation via S-nitrosylation	Alizarin Red; scRNA-seq DEGs; protein (ITGA8/VCL/SMA/VIM); murine valve staining	Stiff-plastic culture lacks physiologic ECM; NO withdrawal reverses effects; cross-species extrapolation

According to Majumdar et al. (32) and Eley et al. (31), NO availability is a key regulator at two complementary scales. Eley et al. demonstrate that constitutive eNOS (Nos3) deletion in whole-animal development impairs NOTCH1/Jagged1 signaling in the ventricular endocardium and pharyngeal arch arteries, which maps to periductal aortic disease and semilunar valve abnormalities, including BAV. Parallel to this, Majumdar et al.'s single-cell and molecular investigations in VICs show that exogenous NO/S-nitrosylation inhibits a pro-calcific program that is dominated by integrin-focal-

adhesion-cytoskeletal modules (particularly ITGA8 and VCL) and related Rho/Wnt/TGF-β cues. All of these data points to a scenario where NO buffers two levers that converge on valve architecture and calcification risk: adult VIC mechano-signaling via integrins (32) and developmental patterning via NOTCH (31). It relies on indirect oxidative-stress readouts and has background/viability limits. However, the Nos3−/− animal used by Eley et al. shows the causative in vivo effects of NO deprivation. Majumdar et al.'s VIC scRNA-seq platform is valve-specific and mechanistically rich, but it is dependent on stiff-substrate culture and demonstrates that the anti-calcific transcriptional effects of NO are transient in the absence of sustained NO (32). A translational path is suggested by reconciling the two: in mature tissue, sustained NO signaling, likely via S-nitrosylation targets, is necessary to limit integrin-driven myofibroblast activation and ECM remodeling (32), while developmental or hemodynamic states that decrease NO bioavailability may prime valves through NOTCH mis-patterning (31). This dual understanding drives treatments for calcific aortic valve disease that target integrin/NOTCH nodes and maintain or restore NO tone.

I. More PET/CT valve-calcification activity/progression originals

We elaborate on motion correction and PET quantification reliability, building on Section D. We highlight research that evaluates whether PET "activity" is related to the advancement of structural calcification and that enhances measurement reliability to broaden the endpoint justification for 18F-NaF PET/CT in calcific aortic disease. To clarify what PET can and cannot currently guarantee as a trial endpoint, Table 9 consolidates the design, measurements, motion-correction techniques, and key outcomes from three frequently referenced original studies focusing on vascular and aortic valve imaging.

Table 9. Extracted details from 18F-NaF PET/CT studies on calcification activity/progression and quantification

Study (year)	Population / N	Target & design	PET metric(s) & analysis	Motion correction	Follow-up	Primary outcome vs calcification	Main findings	Noted limitations	
Cecelja et al., (2019)	Postmenopausal women, n=21	Abdominal aorta; baseline PET/CT + repeat CT	TBR_max, TBR_mean (aortic wall)	None reported	3.8 ± 1.3 yrs	Baseline 18F-NaF vs CT calcium volume progression	Aortic calcium volume increased, but baseline TBR did not predict progression; hotspot segments did not show significant Δcalcium	Small n; aorta (intimal/medial mix); potential vertebral spill-in; no valve data	
Massera et al., (2018) (33)	Aortic stenosis, n=27 (15 rescans)	Aortic valve; software comparison	TBR_mean, TBR_max, SUV (MDS approach); OsiriX vs FusionQuant	FusionQuant cardiac motion correction; OsiriX diastolic gate	4 weeks (test–retest); ~1 yr in subset	Reproducibility of PET endpoints	Excellent agreement OsiriX↔FusionQuant; TBR_max reproducibility improved with FusionQuant+MC (±13% vs ±36%); analysis faster; SNR	Method s paper (not clinical progression); small sample; vendor-specific tools	

Study (year)	Population / N	Target & design	PET metric(s) & analysis	Motion correction	Follow-up	Primary outcome vs calcification	Main findings	Noted limitations
Lassen et al., (2022) (34)	Aortic stenosis, n=14 (two baseline scans)	Aortic valve; reconstruction protocol comparison	SUV_max, TBR_max, SNR; correlation with AV calcium score (Agatston)	Triple motion correction (cardiac + respiratory + gross patient) vs standard/ ECG-MC	29 ± 24 days (test – retest)	PET activity vs AV calcium burden; repeatability	3×MC increased TBR_max/SUV_max and SNR; stronger correlation with AVCS (TBR_max R^2 up to 0.46); repeatability preserved	higher Small n; correlation not progression; reconstruction complexity/time

The fact that baseline 18F-NaF activity in large vessels does not always predict macroscopic calcification growth highlights the discrepancy between the microcalcification signal and the eventual CT-detectable volume (35), particularly in mixed intimal/medial beds with spill-in issues. The value of PET endpoints in the aortic valve, on the other hand, is significantly altered by the technical rigor around co-registration and motion correction. Massera et al. show how test-retest error is reduced by standardized analysis and motion correction, which significantly improves TBR_max from a noisy measure to a reproducible one (33). Lassen et al. go further by demonstrating that, without compromising repeatability, triple motion correction increases SNR and fortifies the cross-sectional connection between PET activity and AV calcium burden (34).The aorta's Cecelja null cautions that not all arterial beds or pipelines from "activity → growth" are equal for progression experiments (35). PET-activity endpoints, especially TBR_max, can be made robust and sensitive in valve stenosis, according to methodological advancements, such as the FusionQuant workflow motion correction (up to 3×MC), making them suitable as secondary or primary readouts in addition to CT calcium and echo hemodynamics. Since the studies are limited and mostly concentrate on repeatability and correlation rather than longitudinal valve advancement, larger, serial valve cohorts are still required for definitive prognostic validation. Practically speaking, if we want PET/CT activity to function as a reliable efficacy endpoint in CAVD trials, we must implement strict MC and uniform ROI procedures.

J. Preclinical/clinical sGC activator context

A therapeutic window for heme-independent soluble guanylate cyclase (sGC) activators arises when oxidative stress causes vascular sGC to transition into an oxidized, heme-free, and nitric oxide (NO)-insensitive state. This situation creates a unique opportunity for the use of heme-independent sGC activators. Below, we outline the preclinical mechanisms and early clinical signals for ataciguat (HMR1766) and cinaciguat (BAY 58-2667). Additionally, details regarding the studies can be found in Table 10 at the end, which we will discuss shortly.

Table 10. Key studies on NO-/heme-independent sGC activators

Study (year)	Design / setting	Agent(s)	Model / population	Core mechanistic or clinical finding	Notes
Schmidt et al., (2009)(36)	Narrative chapter (expert review)	Cinaciguat (BAY 58-2667); Ataciguat (HMR1766)	Preclinical to early clinical landscape	Rationale: under oxidative stress, targeting oxidized/heme-free sGC can restore cGMP;	Establishes therapeutic logic and summarizes first-in-human signals; chapter

Study (year)	Design / setting	Agent(s)	Model / population	Core mechanistic or clinical finding	Notes
				early ADHF POC with cinaciguat showed ↓pre/afterload, ↑CO; HMR1766 in clinical development for PAOD	also contrasts activators vs stimulators
Hoffmann et al. (2009)(37)	Cell-based mechanistic experiments (CHO & primary ECs; mutants)	BAY 58-2667; HMR1766; BAY 41-2272; Zn-PPIX	ODQ-induced heme oxidation; β1H105F and β1Y135A/R139A mutants	BAY 58-2667 activates *and stabilizes* oxidized/heme-free sGC by heme-pocket mimicry; HMR1766 activates but does *not* stabilize; stimulator BAY 41-2272 does not stabilize	Shows bimodal (activation + stabilization) target engagement unique to cinaciguat-like chemotype
Næsheim et al., (2009)(38)	Intact-animal hemodynamics with NO modulation	Riociguat (stimulator) vs cinaciguat (activator)	Healthy juvenile pigs	Cinaciguat caused marked systemic vasodilation and blunted subsequent NO-mediated responses; riociguat acted additively with NO.	Demonstrates NO-independent, hard-to-modulate profile of cinaciguat in vivo.

Abbreviations. ADHF, acute decompensated heart failure; EC, endothelial cell; ODQ, heme-oxidizing sGC inhibitor; PAOD, peripheral arterial occlusive disease; POC, proof-of-concept; sGC, soluble guanylyl cyclase; Zn-PPIX, zinc-protoporphyrin IX.

While stimulators need reduced heme, heme-independent activators remain active when sGC is oxidized or heme-free across sources. According to Hoffmann et al. (37), HMR1766 (ataciguat) activates but does not stabilize heme-free sGC, whereas cinaciguat both activates and stabilizes it. The ability to scale in oxidizing illness is probably explained by this bimodal engagement. In addition, Naesheim et al. (38) showed that cinaciguat causes significant systemic vasodilation and essentially eliminates subsequent NO-mediated vascular responses in healthy pigs, underscoring its NO-independent, challenging-to-modulate profile in vivo. According to Naesheim et al. (38), IV cinaciguat rapidly improved hemodynamics in ADHF in humans, which is consistent with preferential activation of oxidized sGC pools. However, there was a hypotension signal that is typical of aggressive IV vasodilation. Mechanistically, this may restrict durability/magnitude in strongly oxidizing environments. In contrast, HMR1766 (ataciguat) advanced in chronic vascular indications (36), but it lacks the stabilizing property seen with cinaciguat. In addition to highlighting the practical handling issues for cinaciguat even outside of overt oxidative illness, the intact-animal data from Naesheim et al. (38), strong systemic vasodilation and poor integration with NO tone, reinforce the ADHF observations.

Conclusion

The proportional contributions of sGC–cGMP versus S-nitrosylation–NOTCH signaling across experimental systems, sex-specific responses, duration of benefit, and illness stage-specific efficacy are unknown. The NO–sGC–cGMP axis is a crucial brake in the biology of calcific aortic valve stenosis, which is now recognized as an active fibro-calcific process fueled by endothelial dysfunction, oxidative stress, and osteogenic reprogramming of valve interstitial cells. Overall, the information that is currently available points to four consistent conclusions: Heme-independent sGC activators, such as ataciguat/HMR-1766, can restore cGMP signaling in oxidative environments without tolerance; (i) the NO–sGC–cGMP axis serves as a crucial anti-calcific brake in CAVS; (ii) oxidative conversion of sGC to ferric/heme-free states creates a NO-insensitive but therapeutically targetable bottleneck supported by structural, biochemical, and tissue studies. A NO-insensitive bottleneck, which can be directly targeted with heme-independent sGC activators, occurs due to the oxidative conversion of sGC into ferric/apo states.

This condition is suitable for targeting based on various structural, biochemical, and tissue studies. For example, ataciguat (HMR-1766) can prevent tolerance and restore cGMP signaling in oxidative environments. While CT-AVC and ^18F-NaF PET offer sensitive trial-efficient readouts to detect disease change, early clinical data in moderate CAVS demonstrate a directional slowing of CT-AVC development over six months with oral ataciguat and acceptable hemodynamic tolerability. The ongoing phase 3 trials will determine the potential of oxidized-sGC activation to be the first disease-modifying treatment for CAVS, focusing on improving structural indicators and functional/hemodynamic endpoints, such as the change in aortic valve area percentage (%ΔAVA) and peak oxygen consumption (VO_2).

Limitations

Although original research is given priority in this narrative review, it does not statistically pool data and is therefore prone to selection and publication bias. Several preclinical discoveries must be carefully extrapolated to valve biology from non-valvular systems (such as platelets and the pulmonary vasculature). Although promising, 18F-NaF PET still requires strict motion correction and systematic analysis to guarantee consistency, and the human signal for ataciguat now depends on brief, short-duration studies with surrogate endpoints (CT-AVC, echo trends) rather than actual outcomes. Drug-drug interactions continue to be an operational risk, and the variations between oral outpatient dosing and IV activator experience in ADHF limit the generalization of safety. Lastly, phase 3 design and subgroup analysis should prospectively investigate the potential influence of heterogeneity by valve phenotype (TAV vs. BAV) and Lp(a)-driven biology on treatment effect.

Ethical Statement

This article presents a narrative review of previously published studies. The authors did not conduct any new studies involving human participants or animals, so no ethical approval was required. Authors' Contributions FT and KM conceived the study. FT, SA, and SM conducted the literature search and data extraction. KM supervised the project. All authors contributed to drafting the manuscript and critically revised the final version. All authors approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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