

Review

A Biological Alignment Framework for Interpreting Anti-Inflammatory Cardiovascular Trials in Atherosclerosis

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ABSTRACT: The inflammatory hypothesis of atherosclerosis has generated large-scale clinical trials with heterogeneous outcomes—from landmark success (CANTOS) to informative neutral results (CIRT). Understanding why anti-inflammatory therapies produce inconsistent outcomes requires a structured translational framework. We conducted a narrative review of randomized controlled trials, Mendelian randomization studies, and mechanistic investigations in anti-inflammatory cardiovascular therapy, searching PubMed, EMBASE, and the Cochrane database through December 2024. Trial outcomes were analyzed through four alignment domains: pathway specificity, target engagement, patient selection, and endpoint matching. Clinical trial outcomes can be systematically characterized by the degree of coherence—or *biological alignment*—across these four domains. High alignment (CANTOS, Total Alignment Score [TAS] 8/8) is associated with robust clinical benefit; partial alignment (COLCOT, LoDoCo2, TAS 6/8) yields consistent but modest effects; and low alignment (CIRT, TAS 4/8) is associated with neutral results. TAS is calculated as the additive sum of four domain scores (0–8); the "multiplicative" characterization in prior descriptions refers to a conceptual metaphor for rate-limiting failure, not a formal mathematical model. Aging-related processes (inflammaging, immunosenescence, clonal hematopoiesis of indeterminate potential [CHIP]) modify each alignment domain and require explicit incorporation into trial design. The Biological Alignment Model provides a mechanistically grounded, extensible framework for interpreting divergent anti-inflammatory cardiovascular trial outcomes. It enables iterative trial refinement and supports the design of precision immunotherapy strategies in atherosclerosis.

Keywords: atherosclerosis, biological alignment, anti-inflammatory therapy, inflammaging, CHIP, precision cardiovascular medicine

Introduction

Despite major advances in lipid-lowering therapies, a substantial burden of residual cardiovascular risk persists. Even among patients achieving stringent LDL-C targets, recurrent atherosclerotic events remain frequent, with approximately 30–40% of risk unexplained by lipid pathways alone [1–5]. This observation has fuelled investigation of non-lipid pathogenic mechanisms, among which innate immune activation—specifically macrophage-driven vascular inflammation—has emerged as a causally relevant therapeutic target.

Large-scale population studies have demonstrated that circulating inflammatory biomarkers, most notably high-sensitivity C-reactive protein (hsCRP), predict cardiovascular risk independently of LDL-C [6–14]. The failure of Lp-PLA₂ inhibition with darapladib in two large trials [14, 15] underscored that not all inflammatory pathways are equally tractable and foreshadowed the need for a more structured approach to anti-inflammatory target selection.

The CANTOS trial demonstrated that selective interleukin-1 β (IL-1 β) inhibition reduces cardiovascular events independently of lipid lowering [16]. However, the

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CIRT trial failed to reduce inflammatory biomarkers or cardiovascular events with low-dose methotrexate [17], indicating a target engagement failure rather than an invalid biological concept. Colchicine-based strategies demonstrated modest but consistent benefit [18, 19]. These divergent findings motivate the concept of biological alignment, defined as the coherence among therapeutic mechanism, target engagement, patient phenotype, and endpoint selection. Of particular relevance to the readership of *Aging and Disease*, the biology of aging intersects profoundly with each alignment domain. Inflammaging, immunosenescence, CHIP, and senescence-associated secretory phenotype (SASP) accumulation modify the inflammatory landscape in ways that fundamentally alter the optimal targets, required intensities, and expected outcomes of anti-inflammatory interventions.

Inflammatory Pathways in Atherosclerosis: Causality, Aging, and Biological Alignment

Hierarchical view of causality

Atherosclerosis is characterized by a complex inflammatory milieu; not all inflammatory pathways contribute equally to disease progression. A critical distinction must be made between causal drivers, amplifiers, and epiphenomenal responses [20–22]. Therapeutic success depends on targeting the appropriate level within the causal hierarchy. The CIRT trial and darapladib trials illustrate the consequences of failing to do so.

The IL-1 β –IL-6–CRP axis

The NLRP3 inflammasome–IL-1 β –IL-6–CRP cascade represents the most consistently validated inflammatory axis in atherosclerosis. NLRP3 activation is triggered by lipid-derived danger signals including cholesterol crystals and oxidized LDL [23, 24], directly linking lipid accumulation to innate immune activation. The causal relevance of this pathway is supported by Mendelian randomization analyses of the IL-6 receptor [25, 26], large prospective studies linking IL-6 to coronary heart disease [27], and CANTOS [16, 28].

Residual inflammatory risk

Residual inflammatory risk, defined by elevated hsCRP despite optimal lipid control, represents a clinically actionable phenotype. The JUPITER trial demonstrated that hsCRP ≥ 2 mg/L identifies individuals who derive substantial benefit from targeted intervention [13]. IL-6 has emerged as a more proximal mediator; Mendelian

randomization data [25, 26], and the RESCUE trial [29] support a causal role for IL-6 signaling.

Interplay between innate and adaptive immunity and implications for target selection

While the NLRP3–IL-1 β –IL-6 axis represents the central innate immune pathway, adaptive immunity plays an important modulatory role [30]. Nonspecific immunomodulatory approaches may fail through insufficient pathway specificity or off-target immunosuppression. Plaque phenotype—the balance between rupture-prone and erosion-prone lesions [31–33]—may further determine which inflammatory pathways are most therapeutically relevant [34, 35]. These observations motivate the concept of biological alignment. Figure 1 provides a structural overview of the Biological Alignment Model.

Clinical Trial Landscape: Interpreting Success and Failure Through Biological Alignment

Full mechanistic alignment: CANTOS

The CANTOS trial provides the most compelling example of full biological alignment. Selective IL-1 β inhibition with canakinumab produced downstream suppression of IL-6 and hsCRP without affecting lipid levels, yielding a significant reduction in recurrent cardiovascular events (HR 0.85, 95% CI 0.74–0.98) [16]. Clinical benefit correlated with the degree of hsCRP reduction [28], and IL-6 pathway modulation was confirmed as a mediating mechanism [36]. Plaque imaging substudy data provided further mechanistic validation [37]. Important caveats include a dose-dependent increase in infection risk (principally fatal infections and sepsis), substantial cost limiting clinical adoption, and absence from cardiovascular prevention guidelines to date [38].

Partial alignment: colchicine trials

COLCOT (HR 0.77, 95% CI 0.61–0.96) and LoDoCo2 (HR 0.69, 95% CI 0.57–0.83) demonstrate partial biological alignment [18, 29]. Although colchicine inhibits the NLRP3 inflammasome and microtubule assembly, its precise causal pathway in cardiovascular protection remains incompletely established: pleiotropic effects on neutrophil function and endothelial activation may contribute independently of inflammasome inhibition [39–42]. The safety profile warrants acknowledgment: gastrointestinal intolerance affects approximately 10–20% of patients [43]; a non-cardiovascular mortality signal was observed in LoDoCo2

(HR 1.56, 95% CI 0.96–2.52) [19, 44]; and pharmacokinetic interactions with CYP3A4 inhibitors introduce complexity in elderly patients.

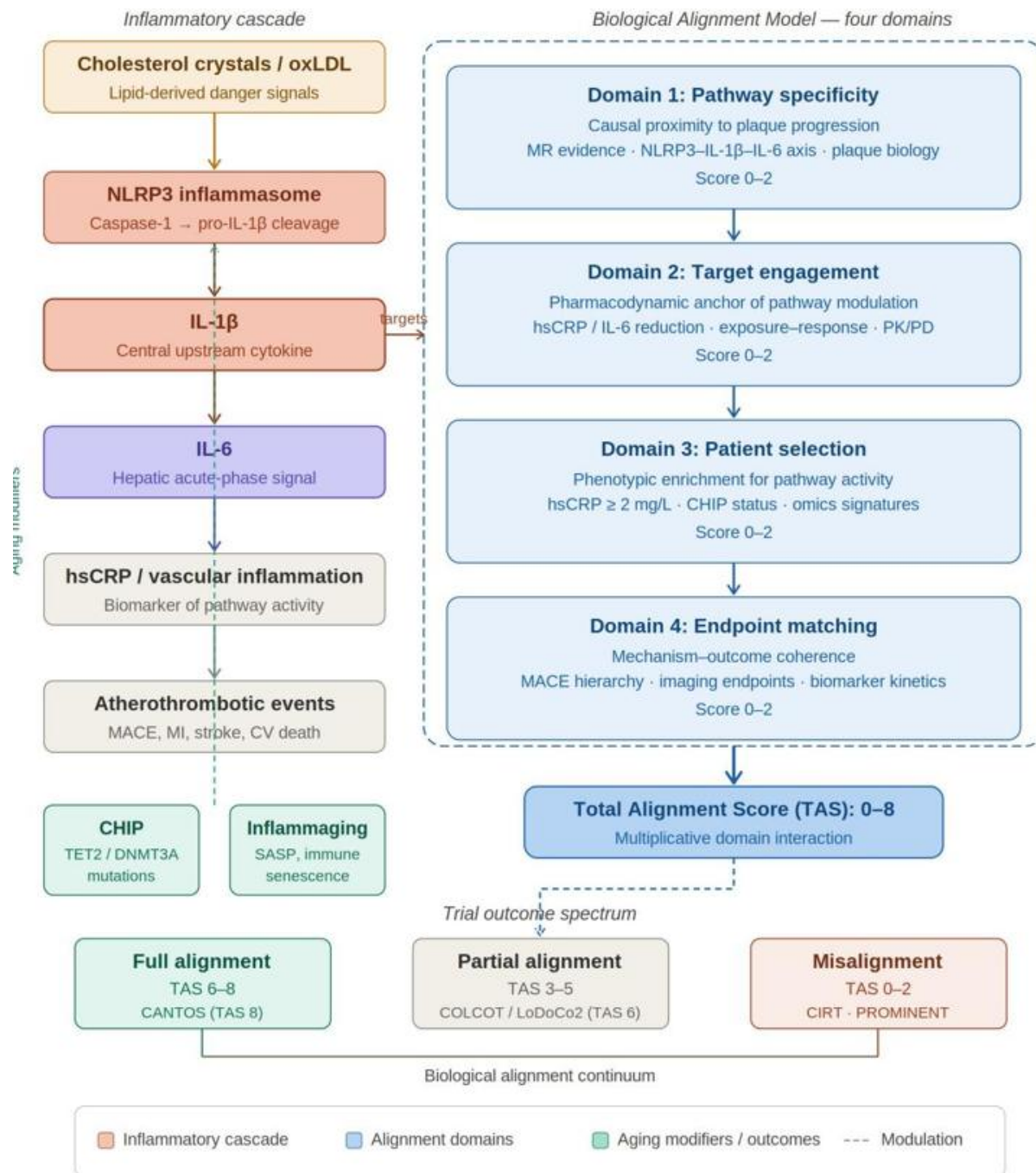


Figure 1. The Biological Alignment Model integrating lipid-driven inflammation and immune networks in atherosclerosis. Left column: the NLRP3 inflammasome–IL-1β–IL-6–CRP cascade linking lipid-derived danger signals to atherothrombotic events. Right column: the four alignment domains (pathway specificity, target engagement, patient selection, endpoint matching), each scored 0–2 and summed to a Total Alignment Score (TAS) of 0–8. Aging-related modifiers (CHIP, inflammaging/SASP) feed into the cascade, illustrating the age-dependent shift in alignment baseline. TAS is an additive score; the multiplicative language used elsewhere in this framework refers to a rate-limiting-step metaphor, not a formal mathematical model. TAS scores are retrospectively assigned and exploratory. CHIP = clonal hematopoiesis of indeterminate potential; SASP = senescence-associated secretory phenotype.

Table 1. Total Alignment Score and primary outcome of four randomized controlled trials.

Trial	Pathway specificity	Target engagement	Patient selection	Endpoint matching	TAS† (0–8)	Primary outcome
CANTOS	2	2	2	2	8	HR 0.85 (95% CI 0.74–0.98); significant
COLCOT	2	1	1	2	6	HR 0.77 (95% CI 0.61–0.96); significant
LoDoCo2	2	1	1	2	6	HR 0.69 (95% CI 0.57–0.83); significant
CIRT	1	0	1	2	4	HR ≈1.0; neutral

† TAS = Total Alignment Score (0–8); calculated as the additive sum of four domain scores. Scores were assigned retrospectively after trial outcomes were known (see Section 4 and Table 2 for scoring rubric and per-trial justification). Score criteria: 0 = no/opposing evidence; 1 = indirect evidence (animal models, observational associations, or mechanistic plausibility without direct human interventional data); 2 = strong direct human evidence (Mendelian randomization, RCT sub-analyses, or prospectively confirmed biomarker–outcome associations). HR = hazard ratio.

Target engagement failure: CIRT

CIRT demonstrated no reduction in IL-1 β , IL-6, or CRP with low-dose methotrexate, and no clinical benefit [17]. Target engagement failure is the primary mechanistic explanation: methotrexate does not effectively engage the dominant NLRP3–IL-1 β –IL-6 axis in atherosclerosis.

Suboptimal patient phenotyping (selection based on diabetes or metabolic syndrome rather than inflammatory biomarker elevation), insufficient anti-inflammatory dosing, and population-level mismatch may have contributed independently or synergistically [45] (see Table 1 footnotes and Table 2 for per-domain score justification).

Table 2. Per-trial justification of Total Alignment Score (TAS) domain scores.

Trial	Domain 1: Pathway specificity	Domain 2: Target engagement	Domain 3: Patient selection	Domain 4: Endpoint matching	TAS
CANTOS	Score 2. IL-1 β is causally supported by Mendelian randomization of the IL-6 receptor pathway and confirmed as the central upstream mediator of NLRP3-driven atherothrombotic inflammation.	Score 2. Canakinumab produced pre-specified, dose-dependent reductions in hsCRP ($\geq 50\%$) and IL-6, with clinical benefit correlating with magnitude of hsCRP reduction.	Score 2. Patients selected by prior MI and hsCRP ≥ 2 mg/L—direct biomarker-based enrichment for residual inflammatory risk.	Score 2. MACE composite (MI, stroke, CV death) directly aligned with the atherothrombotic mechanism of IL-1 β –mediated plaque instability.	8
COLCOT	Score 2. Colchicine inhibits the NLRP3 inflammasome and microtubule assembly, indirectly targeting the IL-1 β axis with established mechanistic plausibility in vascular inflammation.	Score 1. No direct pharmacodynamic confirmation of IL-1 β or hsCRP reduction in COLCOT; pleiotropic anti-inflammatory mechanism assumed rather than prospectively measured.	Score 1. Recent MI patients, but not selected by hsCRP or inflammatory biomarkers; population enrichment was clinical (ACS) rather than biological (biomarker-based).	Score 2. MACE composite well-aligned with post-ACS atherothrombotic risk reduction; primary endpoint mechanistically coherent.	6
LoDoCo2	Score 2. Same mechanistic rationale as COLCOT: colchicine targets NLRP3 inflammasome and microtubule dynamics, indirectly engaging the IL-1 β pathway.	Score 1. No direct PD confirmation; anti-inflammatory mechanism assumed based on prior mechanistic studies rather than measured in this trial.	Score 1. Stable chronic coronary disease patients; no biomarker-based enrichment; population is clinically but not biologically stratified for inflammatory pathway activity.	Score 2. MACE composite appropriate; endpoint hierarchy mechanistically consistent with anti-inflammatory strategy in stable atherosclerosis.	6
CIRT	Score 1. Methotrexate has established anti-inflammatory properties in rheumatoid arthritis, conferring indirect	Score 0. No reduction in IL-1 β , IL-6, or CRP was observed, confirming target engagement failure	Score 1. Patients selected on the basis of diabetes or metabolic syndrome rather than elevated inflammatory	Score 2. MACE composite appropriate; endpoint mismatch was not the primary	4

mechanistic plausibility; however, it does not directly target the NLRP3–IL-1 β axis operative in atherosclerosis.	in the atherosclerotic context.	biomarkers; pathway activity in the enrolled population was not confirmed.	explanation for the neutral result.
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The role of biological enrichment

JUPITER demonstrated that hsCRP ≥ 2 mg/L substantially enhances treatment benefit [13]. Even within CANTOS, greater hsCRP reduction correlated with greater clinical benefit [28], confirming that patient selection is a central determinant of biological alignment. Lack of phenotypic enrichment generates population dilution that systematically underestimates treatment effects in biologically responsive subgroups.

Constructive failure and the role of aging

As illustrated in Figure 2, neutral outcomes can be deconstructed into four diagnostic categories—engagement failure, pathway mismatch, population dilution, and endpoint mismatch—each pointing toward distinct corrective actions. Inflammaging [46, 47] may attenuate therapeutic responsiveness by establishing a constitutively elevated inflammatory baseline that resists single-node cytokine blockade.

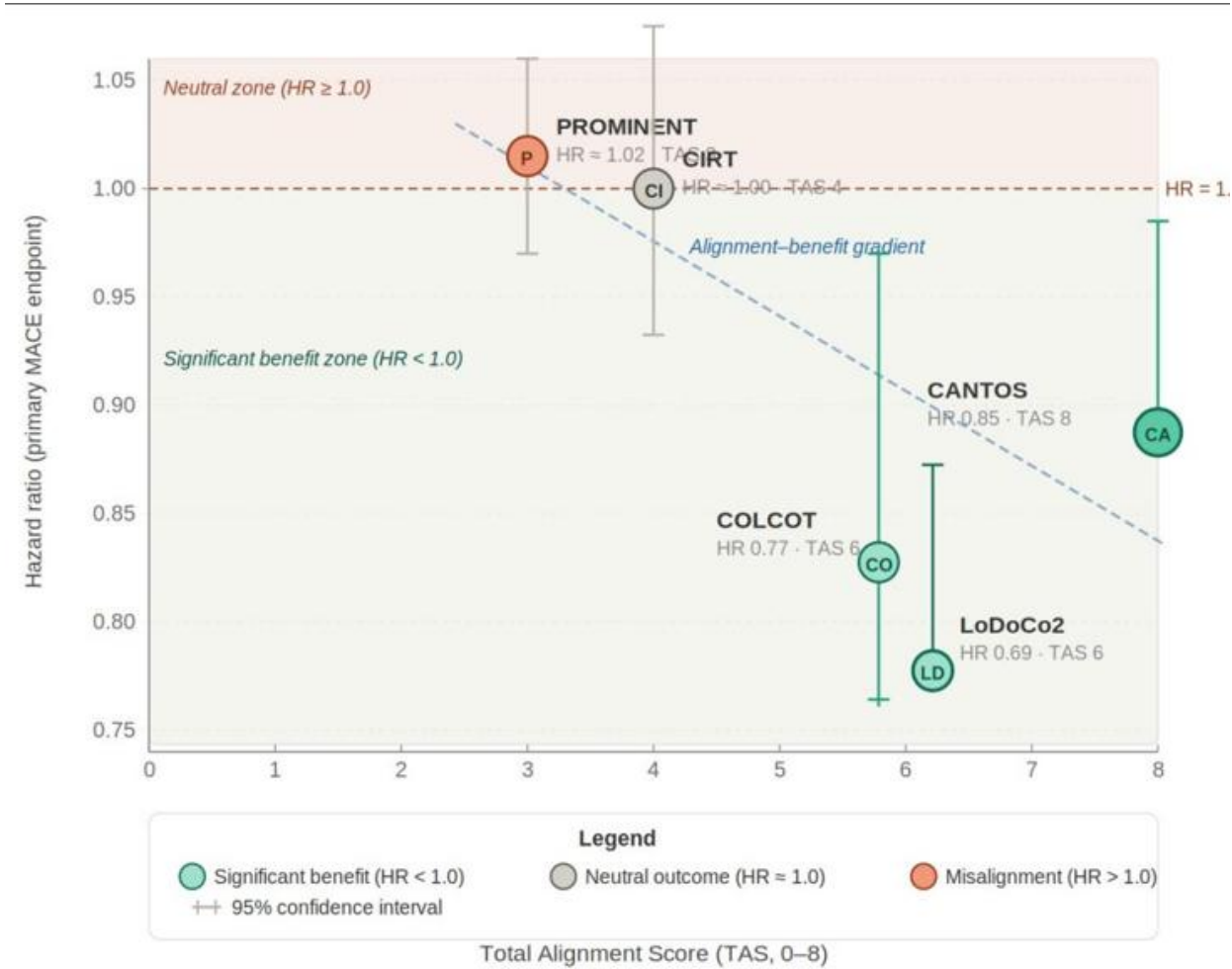


Figure 2. Conceptual concordance between biological alignment scores and observed trial outcomes in directly anti-inflammatory trials. Each trial is plotted by Total Alignment Score (TAS, x-axis) and hazard ratio for the primary MACE endpoint (y-axis) with 95% confidence intervals. TAS scores were assigned retrospectively after outcomes were known; this figure illustrates conceptual concordance, not prospective predictive validation. Only trials directly testing anti-inflammatory mechanisms are plotted in the core comparison (CANTOS, COLCOT, LoDoCo2, CIRT). CA = CANTOS; CO = COLCOT; LD = LoDoCo2; CI = CIRT.

Quantitative synthesis: TAS and trial outcomes

Important methodological note on TAS scoring. The following details address the scoring process. (1) Score assignment: TAS scores were assigned by the authors following independent review of the published trial reports, with final scores determined by consensus discussion. (2) Blinding: Scorers were not blinded to trial outcomes; scores were assigned retrospectively with full knowledge of results. This is the framework's most significant limitation and is explicitly acknowledged. (3) Disagreement resolution: disagreements were resolved through consensus discussion without a formal adjudication procedure. (4) Scoring order: scoring was performed after trials had already been categorized by outcome, introducing potential circularity that limits the interpretive value of TAS as a predictive tool. (5) Inter-rater reliability: formal inter-rater reliability has not been assessed; this is a priority for future validation. (6) Equal domain weighting: all four domains are weighted equally (each 0–2) as a deliberate simplification for transparency and reproducibility; differential weighting based on empirical evidence remains a priority for future development. (7) 0–2 scale: the three-level scale was chosen to reflect a clinically meaningful gradation of evidence strength (absent/indirect/direct human evidence) without implying a finer granularity that the available data cannot support. (8) Additive vs. multiplicative: the TAS is calculated additively (sum of four domain scores, range 0–8). The term “multiplicative” used in prior descriptions of this framework refers exclusively to a conceptual metaphor for rate-limiting failure—the observation that deficiency in a single domain can disproportionately attenuate the overall treatment effect, analogous to a rate-limiting step in a biochemical pathway. This is not a formal mathematical model; whether the true relationship between domain scores and clinical outcomes is additive, multiplicative, or non-linear cannot be determined from retrospective analysis of a small number of trials.

Boundary case: PROMINENT as metabolic-inflammatory pathway mismatch

PROMINENT is presented as a boundary case rather than a core test of the biological alignment framework. Pemafibrate is a selective PPAR α modulator whose primary action is triglyceride and VLDL reduction via the lipid-metabolic axis; it was not designed to test the inflammatory hypothesis and does not directly engage the IL-1 β –IL-6 cascade [48]. Its neutral outcome [48] is therefore an illustration of metabolic-inflammatory pathway mismatch at the boundary of the framework rather than a direct test of it. Including PROMINENT in the core TAS comparison risks creating a straw-man

comparison—explaining a trial that was never intended to satisfy the model's criteria. Accordingly, PROMINENT has been removed from Figure 2 and Table 1 and is discussed here separately as an instructive boundary case. This contrasts with icosapentaenoic acid (REDUCE-IT) [49], where proposed anti-inflammatory pleiotropic effects may have contributed to cardiovascular benefit beyond triglyceride lowering—illustrating that the metabolic-inflammatory boundary warrants ongoing investigation.

A Unified Framework: The Biological Alignment Model

Conceptual foundation and the four-domain model

The Biological Alignment Model defines the degree of coherence between therapeutic mechanism, target engagement, patient phenotype, and endpoint matching. This is a hypothesis-generating framework requiring prospective validation, not a proven causal model.

Domain 1: Pathway specificity (causal proximity).

The selected target should lie on a pathway causally proximal to plaque progression. The IL-1 β –IL-6–CRP axis is supported by Mendelian randomization evidence [25, 26], plaque biology [23], and CANTOS [16, 28]. Operationalization: genetic support via Mendelian randomization; plaque-level biology (macrophage activation, NLRP3 inflammasome activity, foam cell formation); prior human intervention signals linking biomarker modulation to outcome reduction.

Domain 2: Target engagement (biological execution). Demonstrable pathway modulation is necessary to test biological hypothesis. Reductions in IL-6 and hsCRP serve as pharmacodynamic anchors of engagement [16, 28, 36]. Operationalization: pre-specified biomarker thresholds (≥ 30 –40% hsCRP reduction); exposure–response relationships; time-course concordance between early biomarker changes and later clinical events.

Domain 3: Patient selection (phenotypic enrichment). Therapeutic benefit depends on enrolling patients in whom the targeted pathway is biologically active. Residual inflammatory risk (elevated hsCRP despite LDL-C control) is the primary enrichment phenotype [50]. CHIP status [51, 52] and IL-6 levels [26, 30] offer complementary enrichment dimensions.

Domain 4: Endpoint matching (mechanism–outcome coherence). MACE composites aggregate mechanistically heterogeneous events [31, 32, 53]. Endpoints must be matched to the specific mechanistic subtype targeted. Table 3 provides a detailed guide to mechanistically aligned versus less-aligned endpoints.

Additive TAS scoring and the rate-limiting-step metaphor

The TAS is the additive sum of four domain scores (each 0–2), yielding a range of 0–8. The score criteria are: 0 = no/opposing evidence; 1 = indirect evidence (animal models, observational data, or mechanistic plausibility without direct human interventional data); 2 = strong direct human evidence (Mendelian randomization, RCT sub-analyses, or prospectively confirmed biomarker–outcome associations).

The description of a “multiplicative interaction” in the context of this framework refers specifically to a conceptual metaphor for rate-limiting failure: the empirical observation that deficiency in a single alignment domain can substantially attenuate the overall treatment effect, regardless of how well other domains are optimized. This is analogous to a rate-limiting step in a

biochemical pathway where one slow reaction controls the overall flux. This metaphor is not a formal mathematical model; the TAS is calculated additively, and whether the true relationship between domain scores and clinical outcomes is linear, multiplicative, or non-linear cannot be determined from retrospective analysis of a small number of trials. Future prospective validation will be needed to determine the optimal mathematical relationship.

Aging-adjusted biological alignment: domain-specific modifications

Aging represents a critical modifier of each alignment domain requiring explicit recalibration of scoring and trial design criteria. Table 3 summarizes how principal age-related inflammatory processes modify each of the four domains, with age-adjusted scoring rules.

Table 3. How aging biology modifies the four alignment domains.

Aging biology	Primary domain	Mechanism of modification	Implication for trial design	Age-adjusted scoring	Example
Inflammaging	Domain 3: Patient selection	Chronically elevated hsCRP/IL-6 reduces specificity of enrichment criteria	Dynamic biomarker thresholds; consider hsCRP ≥ 3 mg/L in cohorts aged ≥ 70 years	Higher or dynamic enrichment thresholds may be required	JUPITER trial
Immunosenescence	Domain 2: Target engagement	Blunted pharmacodynamic response to IL-1 β inhibition; altered drug PK	Age-stratified PK/PD in Phase I; elderly-specific PD thresholds	Acceptable hsCRP reduction threshold may need adjustment in elderly patients	CANTOS sub-analyses
CHIP (TET2/DNMT3A)	Domain 1: Pathway specificity	NLRP3 inflammasome hyperactivation amplifies pathway activity; clonal expansion increases with age	CHIP status as enrichment criterion; elderly with CHIP may be preferentially responsive to IL-1 β blockade	CHIP-positive status may upgrade Domain 1 score by +1	Jaiswal et al. 2017
SASP (senescent cell burden)	Domains 1+2	Persistent SASP-driven IL-1 α , IL-6, and MMP secretion may compensate for targeted cytokine blockade	Multi-pathway targeting; senolytics as potential adjunct (investigational); SASP markers in biomarker panels	High SASP burden may lower Domain 2 score in elderly patients	Childs et al. 2017
Altered PK (hepatic/renal decline)	Domain 2: Target engagement	Drug accumulation or reduced bioavailability alters exposure–response	Mandatory age-stratified dosing cohorts in Phase I	Age-stratified dosing required before assigning Domain 2 score	General geriatric pharmacology

Inflammaging [46, 47] establishes a constitutively elevated inflammatory baseline in older patients. CHIP-mediated NLRP3 hyperactivation [51, 54] may render elderly patients preferentially responsive to IL-1 β blockade, suggesting CHIP status as an independent enrichment criterion. SASP burden [55] and immunosenescence may limit target engagement,

requiring age-stratified PK/PD characterization before Phase II progression.

Informative misalignment: a diagnostic taxonomy

The model enables decomposition of neutral outcomes into four diagnostic categories: engagement failure $\rightarrow$ refine dosing regimen, PK/PD profile, or target selection;

pathway mismatch → re-center on causally proximal mechanisms; population dilution → adopt biomarker-driven inclusion criteria; endpoint mismatch → redesign

endpoint hierarchy (Table 4). This taxonomy allows neutral trial results to be interpreted mechanistically and used to guide the design of subsequent studies.

Table 4. Mechanistically aligned versus less-aligned endpoints for anti-inflammatory atherosclerosis trials.

Endpoint type	Alignment	Rationale	Recommended role	Example
Plaque rupture-driven MI (Type 1)	High	Directly driven by plaque inflammation and NLRP3 activation	Primary endpoint	CANTOS primary MACE
Ischaemic stroke (atherothrombotic)	High	Shares atherosclerotic mechanistic substrate with plaque-driven MI	Primary endpoint	CANTOS secondary
Plaque erosion-driven MI	Intermediate	Inflammatory contribution less well established	Secondary endpoint; consider MI subtype adjudication	Plaque imaging studies
Cardiovascular death	Intermediate	Multifactorial; includes non-atherothrombotic CV deaths	Include in MACE composite; sensitivity analysis excluding non-atherothrombotic deaths	All major CV outcome trials
Cardioembolic stroke (AF-related)	Low	Driven by atrial thrombus, not plaque inflammation	Exclude from primary composite; retain in sensitivity analysis	Relevant in elderly trials (high AF prevalence)
Heart failure hospitalization	Low–intermediate	Partially inflammation-related; heterogeneous and less directly linked to atherothrombosis	Exploratory; exclude from primary composite in atherosclerosis trials	Non-CV mortality signal in LoDoCo2
All-cause mortality	Low	Predominantly driven by non-cardiovascular causes; dilutes anti-inflammatory signal	Sensitivity analysis only	Standard regulatory requirement
Coronary CTA high-risk plaque features	High	Directly captures plaque inflammation and vulnerability	Co-primary or key secondary in Phase II proof-of-concept trials	Recommended for Phase II alignment-guided trials
FDG-PET/CT vascular inflammation	High	Directly quantifies arterial wall inflammatory activity; mechanistically specific	Surrogate endpoint in early-phase trials; requires prospective biomarker qualification	CANTOS substudy

Limitations of the Biological Alignment Model

Key limitations: (1) inflammatory signaling involves non-linear, context-dependent network interactions not fully captured by a four-domain structure [56]; (2) the four domains are not independent (e.g., hsCRP-based enrichment in Domain 3 may influence apparent pharmacodynamic response in Domain 2); (3) alignment parameters are dynamic, shifting with disease progression and aging, which a static TAS cannot capture; (4) all TAS scores were assigned retrospectively with full knowledge of trial outcomes—the framework’s most significant limitation; (5) inter-rater reliability has not been formally tested; (6) equal domain weighting is a simplifying assumption that may not reflect true mechanistic importance; and (7) prospective validation against pre-registered TAS predictions in future trials has not yet been performed.

From Alignment to Action: Designing Next-Generation Learning Trials

From outcome trials to learning trials

The Biological Alignment Model supports a transition toward learning trials: studies structured to generate mechanistic insight alongside clinical outcomes, forming an iterative, self-correcting knowledge-generating system [57–59] particularly suited to anti-inflammatory cardiovascular therapy.

Operationalizing biological alignment in trial design

Future trials should implement four alignment-guided design features: (1) pathway-guided target selection, prioritizing the IL-1 β –IL-6–CRP axis [25, 26, 60] with pathway specificity confirmed at the plaque microenvironment level; (2) prospective confirmation of target engagement, with pre-specified pharmacodynamic

thresholds (≥ 30 –40% hsCRP/IL-6 reduction) and adaptive go/no-go decision points [16, 28]; (3) biomarker-driven patient enrichment, incorporating multi-biomarker panels, imaging-based vascular inflammation assessment, and CHIP status as an additional enrichment dimension [51, 52]; and (4) mechanism-aligned endpoint selection,

guided by Table 3 with pre-specified MI subtype adjudication, hierarchical win-ratio composite analyses, and coronary CTA-derived plaque phenotype data as co-primary or key secondary endpoints in proof-of-concept trials.

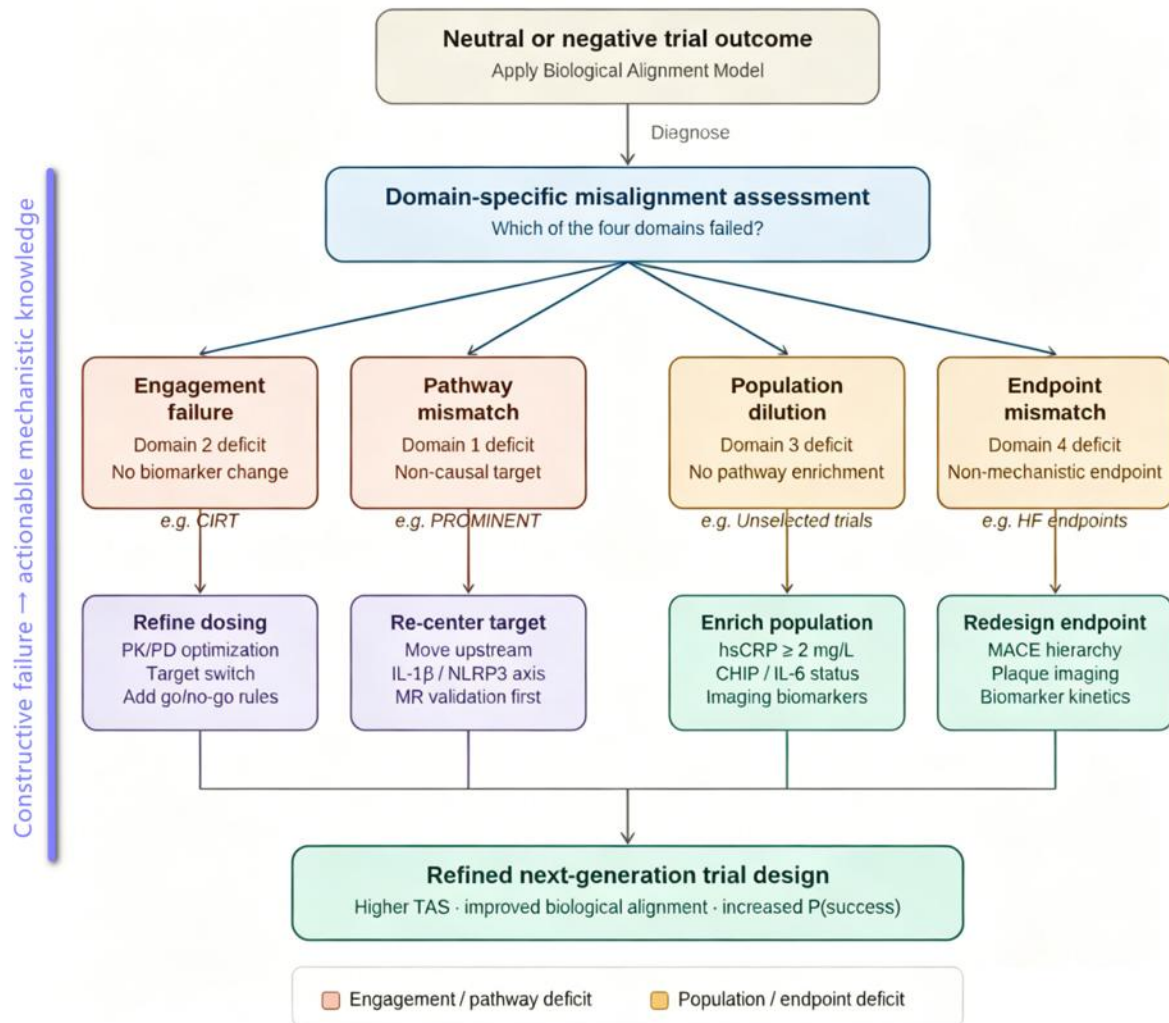


Figure 3. Diagnostic taxonomy of biological misalignment: from neutral trial outcome to corrective action and three-phase trial roadmap. Upper panel: neutral or negative trial outcomes are decomposed into four diagnostic categories, each pointing to distinct corrective actions that feed into refined next-generation trial designs. Lower panel: three-phase alignment-guided roadmap. Phase I (target and pathway validation) establishes pharmacodynamic biomarker panels and PK/PD across age strata. Phase II (alignment-guided proof of concept) confirms target engagement in enriched cohorts and enables prospective TAS validation. Phase III (enriched learning outcome trial) tests efficacy in fully aligned cohorts with adaptive elements. Each phase is gated by a formal Biological Alignment Review. TAS = Total Alignment Score.

Adaptive architectures, systems biology, and the three-phase roadmap

Key features of adaptive learning trials include: adaptive randomization; seamless phase II/III transitions; pre-specified biomarker-guided subgroup analyses; and Bayesian statistical frameworks [58, 59, 61]. High-

dimensional molecular integration [62, 63] and spatially resolved single-cell transcriptomics [64, 65] enable lesion-level patient stratification. Digital twin frameworks enable simulation of alignment parameters, although cardiovascular applications remain in early conceptual development and should be regarded as a distant future direction [66]. Figure 3 illustrates the diagnostic

misalignment taxonomy and three-phase roadmap linking neutral trial outcomes to iterative next-generation trial design.

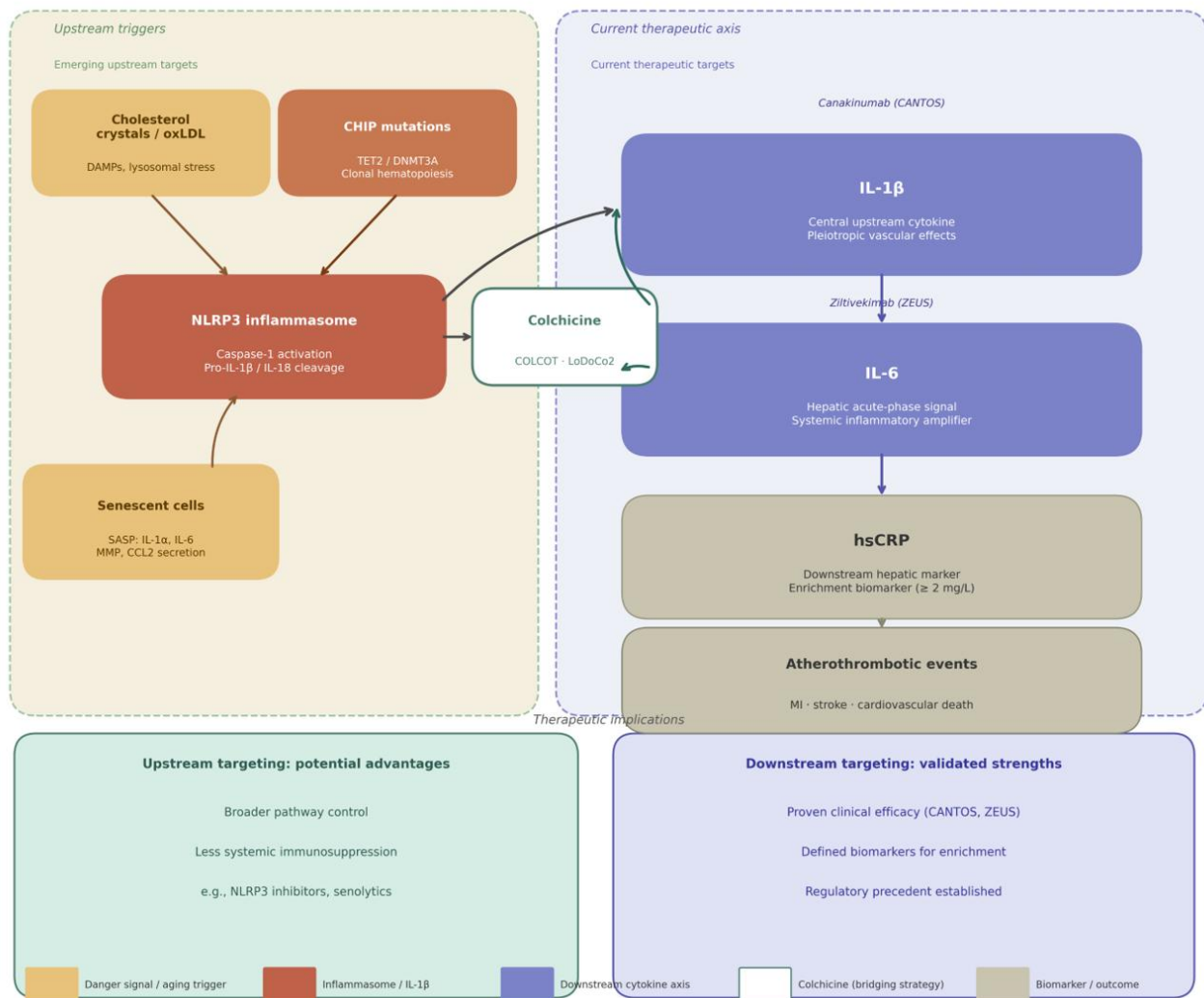


Figure 4. From downstream cytokine targeting to upstream inflammatory triggers in atherosclerosis. Left panel (upstream target zone): cholesterol crystals/oxLDL and CHIP mutations activate the NLRP3 inflammasome; senescent cells further amplify inflammation through SASP. Colchicine bridges both levels through partial inflammasome suppression. Right panel (current therapeutic targets): the validated downstream effector axis IL-1 β –IL-6–hsCRP–atherothrombotic events, with canakinumab (CANTOS) and ziltivekimab (ZEUS) annotated at their respective target levels. CHIP = clonal hematopoiesis of indeterminate potential; SASP = senescence-associated secretory phenotype.

Regulatory implications

Surrogate endpoint qualification requires rigorous causal evidence per the BEST framework [67, 68]. Real-world evidence [69] and platform trials [70, 71] offer complementary mechanisms for generating generalizable evidence.

Discussion

Rethinking heterogeneity and revisiting causality

Heterogeneity across anti-inflammatory trials directly testing the inflammatory hypothesis—CANTOS, COLCOT, LoDoCo2, and CIRT—should be interrogated as a signal of incomplete understanding rather than treated as evidence against the inflammatory hypothesis itself [72]. Available evidence most closely supports a hybrid model: inflammation is causally relevant in a biologically defined subset of patients, functioning primarily as an amplifier of lipid-driven pathology rather than an independent initiator [16, 60]. Population-level trials enrolling unselected patients systematically

underestimate treatment effects in the responsive subgroup.

Challenging the centrality of the IL-6 axis

The NLRP3 inflammasome acts as the critical upstream sensor activated by cholesterol crystals and oxidized lipoproteins [23, 73], initiating caspase-1-mediated IL-1 β secretion. Small-molecule NLRP3 inhibitors have demonstrated anti-atherosclerotic effects in experimental systems [74]. A shift toward upstream inflammatory triggers may provide more durable strategies. Figure 4 illustrates the mechanistic hierarchy from upstream triggers to downstream effectors and current therapeutic intervention points.

Biomarker reductionism and trial design distortions

hsCRP is an indirect and nonspecific systemic marker. Biomarker reduction does not necessarily equate to local plaque modification, and patients with CHIP-associated inflammasome activation may have active plaque inflammation not reflected in circulating hsCRP [51, 54]. The therapeutic window problem—applying intervention to patients with advanced, structurally irreversible disease [75, 76]—and the endpoint dilution problem—aggregating mechanistically heterogeneous events in a single MACE composite [31, 32]—represent additional sources of biological distortion in trial design (Table 4). A neutral outcome is a domain-specific diagnostic enabling the next iteration of experimental design through constructive failure [72, 77].

Unresolved questions

Key unresolved questions include multi-pathway targeting strategies and their safety profiles; optimal therapeutic timing relative to disease stage; dynamic alignment recalibration across the lifespan; prospective TAS validation against pre-registered predictions in future trials; and adaptation of enrichment criteria for diverse global populations and acute-phase settings.

Clinical Implications

The alignment framework supports reserving anti-inflammatory intervention for patients with demonstrably elevated pathway activity [16, 67]. Persistently elevated hsCRP (≥ 2 mg/L) despite statin therapy remains the most rigorously validated enrichment criterion [50]. Current cardiovascular prevention guidelines [78, 79] do not yet incorporate inflammatory biomarkers as indications for specific anti-inflammatory therapy. Barriers include

absence of approved indications for IL-1 β or IL-6 inhibitors in atherosclerosis in most jurisdictions; safety concerns (infection risk with canakinumab; non-cardiovascular mortality signal with colchicine [19, 43]); limited cost-effectiveness data; and underdeveloped implementation pathways.

Treatment monitoring through serial hsCRP and IL-6 measurement during the first three to six months may identify early non-responders. Failure to achieve $\geq 30\%$ hsCRP reduction should prompt reassessment of dosing, adherence, and pathway compensation rather than automatic escalation [16, 28, 80, 81]. Rationally designed combination strategies targeting complementary mechanistic axes [72, 82]—high-intensity lipid-lowering combined with targeted anti-inflammatory therapy—represent the most immediately actionable approach. The ZEUS trial of ziltivekimab [83] and emerging IL-6 inhibition data [84] suggest promise in patients with elevated hsCRP and established ASCVD or CKD.

Future Perspectives

Inflammaging [46, 47] is substantially mediated by the SASP of senescent cells [55], and senolytic therapy represents a fundamentally different intervention logic: intermittent pulsatile courses aim to eliminate the cellular source of inflammatory amplification [85]. However, the clinical evidence base for senolytics in atherosclerotic cardiovascular disease remains limited to preclinical models and early-phase feasibility studies [86, 87]. Cardiovascular-specific senolytic trials adequately powered for MACE endpoints have not commenced [43]. The inclusion of senolytics in the alignment framework should be regarded as a prospective investigational hypothesis, not an established or near-term therapeutic approach. Figure 5 illustrates the mechanistic cascade from aging to SASP to atherosclerosis and the theoretical position of senolytic intervention.

Future trials should stratify by plaque phenotype using coronary CTA characterization [88] and integrate multi-omics [62, 63] and spatially resolved single-cell transcriptomics [64, 65] for tissue-informed patient stratification. The core alignment principles are applicable across chronic inflammatory disease domains [89–91]. The alignment framework is applicable beyond atherosclerosis: in HFpEF, alignment-guided IL-1 β inhibition trials informed by CANTOS and the DHART2 program [92, 93] are already underway. The framework offers a transferable methodological template for any domain where biological complexity and translational attrition have produced informative-if-neutral clinical trials.

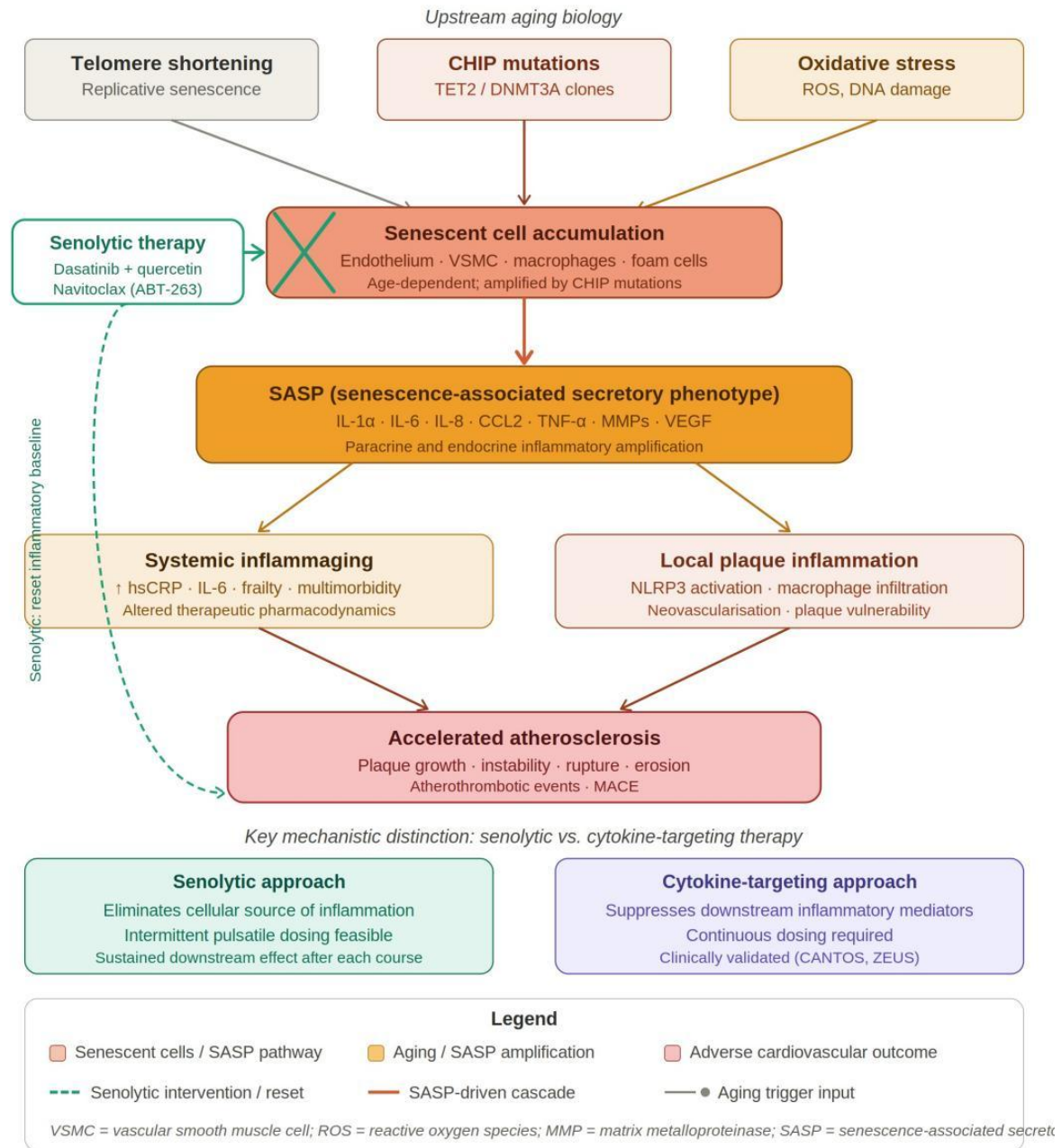


Figure 5. Inflammaging and senolytic intervention in atherosclerosis. Three upstream aging inputs converge to drive senescent cell accumulation. Senolytic therapy (green box) acts at this stage by selectively eliminating senescent cells, a mechanism that remains investigational in cardiovascular disease. SASP output bifurcates into systemic inflammaging and local plaque inflammation, both converging on accelerated atherosclerosis. The curved green arrow represents the theorized senolytic reset of the inflammatory baseline, currently supported only by preclinical and early feasibility data. VSMC = vascular smooth muscle cell; SASP = senescence-associated secretory phenotype; CHIP = clonal hematopoiesis of indeterminate potential.

Conclusion

The heterogeneous landscape of anti-inflammatory cardiovascular trials—encompassing successes such as CANTOS and COLCOT, and informative neutral results such as CIRT—reflects the inherent translational

challenge of preserving biological intent from mechanistic discovery to clinical outcome. The Biological Alignment Model provides a structured framework for understanding this heterogeneity by requiring explicit coherence across four domains: pathway specificity,

target engagement, patient selection, and endpoint matching.

The TAS is calculated additively; the multiplicative characterization refers to a rate-limiting-step metaphor, not a formal mathematical model. TAS scores are retrospectively assigned and not yet externally validated. Prospective validation through pre-registered TAS predictions in future trials is the essential next step.

Advances in precision medicine, adaptive trial design, multi-omics profiling, and aging biology create an opportunity to develop more precise anti-inflammatory strategies for cardiovascular disease. The Biological Alignment Model is not a final answer but an invitation to structured, self-correcting scientific inquiry.

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