

The guardian paradox: DNA damage response overactivation as an integrative driver of aging – a tumor suppressor–negative regulator framework

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ABSTRACT

Aging is multi-causal, yet its molecular hallmarks may converge on a few upstream integrating nodes. We advance the hypothesis that chronic, largely mutation-independent overactivation of the DNA damage response (DDR) is one such node, dysregulating the cell's intact guardian pathways—the tumor-suppressor networks and their negative regulators—to induce senescence, deplete stem-cell pools, and drive inflammaging. Unlike cancer, where mutations inactivate these guardians, aging more often reflects functional dysregulation of wild-type pathways, either chronically overactivated (p53, p16INK4a) or epigenetically silenced (SIRT1, FOXO3, NRF2, Klotho). That the very programs guarding against cancer can, when chronically engaged, come to drive aging we term the guardian paradox.

We organize 16 candidate axes—which we term the aging axis—across three evidence tiers, mapped onto the hallmarks of aging, outlining for each a conceptual diagnostic signature and an illustrative restoration strategy, with all numeric biomarker bands and vector details confined to the Supplementary Information as non-clinical placeholders. We set this DDR-integrator hypothesis against mitochondria-, proteostasis-, and reprogramming-first models and pre-specify longitudinal, mediation, and head-to-head tests that could support, demote, or refute it. No integrated human interventional data yet exist; we therefore present a falsifiable research agenda, not a therapeutic protocol.

INTRODUCTION: THE DDR HYPOTHESIS OF AGING

The DNA damage response as a candidate integrating node

The DNA Damage Response (DDR) represents the cell's surveillance system for detecting and repairing genomic damage. In young, healthy tissues, the DDR activates transiently in response to acute DNA damage—double-strand breaks, replication stress, oxidative lesions—and resolves once repair is complete. With advancing age, a different pattern emerges: the DDR becomes chronically activated even in the absence

of new driver mutations. We hypothesize that this chronic, largely mutation-independent DDR overactivation acts as a major—though not exclusive—driver of biological aging by dysregulating the cell's guardian pathways (the structurally intact tumor-suppressor networks and their negative regulators that normally restrain growth and preserve genome integrity), with somatic-mutational processes, most notably clonal hematopoiesis, acting as accelerating cofactors rather than the initiating lesion (the Limitations section) [1–5] (Graphical Abstract).

Multiple converging lines of evidence are consistent with this paradigm. Telomere attrition generates

persistent DDR signaling at chromosome ends through ATM/ATR pathway activation without any coding mutation. Epigenetic erosion exposes cryptic transcription start sites and repetitive elements, triggering innate immune sensors (cGAS-STING) and DDR activation without sequence alterations. Mitochondrial dysfunction and reactive oxygen species accumulation create oxidative DNA lesions that, while repaired, leave epigenetic scars that perpetuate DDR signaling. Derepression of transposable elements activates both DDR and interferon pathways. In each case, the DDR fires not because of mutations but because of structural and epigenetic changes that mimic damage signals [2, 5]. We note that these same observations are also compatible with models in which DDR activation is a consequence rather than a cause; adjudicating between these orderings is the purpose of the research agenda set out in the research-agenda section.

The downstream consequence is a self-amplifying cascade: chronic DDR activation stabilizes p53, induces p21 and p16INK4a, arrests the cell cycle, and triggers the senescence-associated secretory phenotype (SASP) [6, 7]. SASP factors—IL-6, IL-8, TNF- α , matrix metalloproteinases—create a pro-inflammatory micro-environment that spreads senescence to neighboring cells (paracrine senescence), depletes stem cell pools, disrupts tissue homeostasis, and drives systemic inflammation (Figure 1). Importantly, this cascade can occur with intact tumor suppressor genes—the

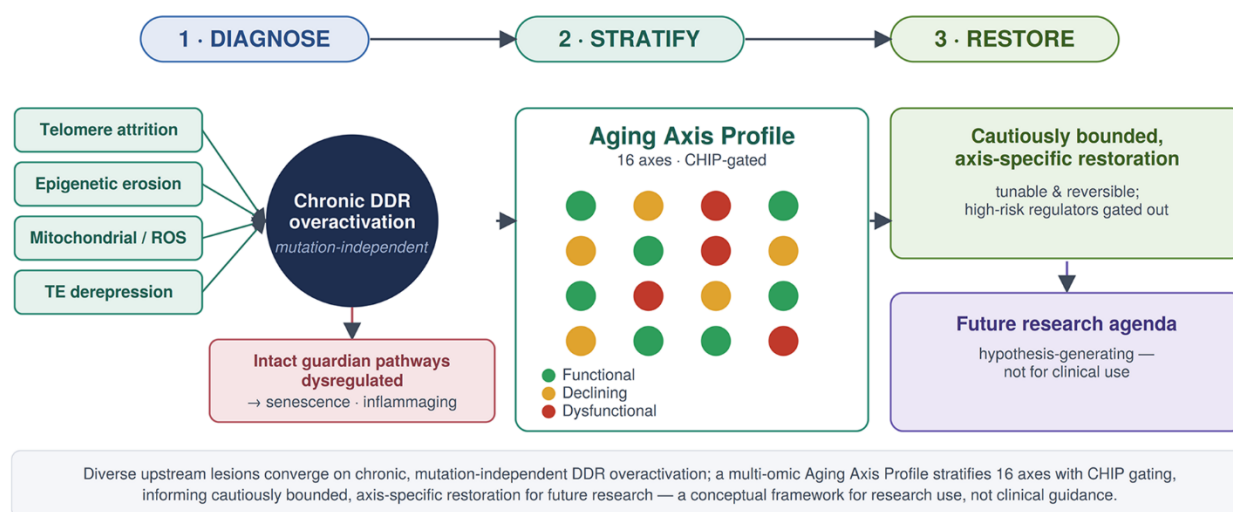
pathways are overactivated or dysregulated, not mutated [1, 8–12].

Scope of the thesis and reconciliation with somatic mutation

A largely mutation-independent model must be reconciled with clonal hematopoiesis of indeterminate potential (CHIP), in which age-acquired somatic mutations in DNMT3A, TET2, and ASXL1 expand clonally—affecting an estimated 10–20% of individuals over 70—and causally promote inflammaging and cardiovascular disease [13–15]. We therefore frame DDR overactivation as a major and broadly druggable driver of aging, while explicitly recognizing somatic-mutational processes such as CHIP as genuine accelerating cofactors. The framework accommodates this directly: candidates are screened for CHIP, and CHIP-positive individuals would be managed under a mutation-aware approach that engages the DNMT3A/TET2 axis and excludes the high-risk oncogenic-regulator axes (the safety and oncogenesis section). This reframing is intended to strengthen rather than weaken the underlying logic: the intact-pathway axes remain restorable in principle regardless of whether DDR overactivation arose from telomeric, epigenetic, or clonal-mutational triggers [16].

Competing integrative models of aging

A DDR-centric account must be weighed honestly against other integrative models of aging, each of which



Graphical Abstract. Schematic overview of the DDR-integrator hypothesis and the diagnose → stratify → restore paradigm.

Diverse upstream aging lesions (telomere attrition, epigenetic erosion, mitochondrial dysfunction, transposable-element derepression) converge on chronic, largely mutation-independent DNA damage response (DDR) overactivation, which dysregulates structurally intact tumor-suppressor networks and drives senescence and inflammaging. A conceptual multi-omic “Aging Axis Profile” then stratifies individuals by the functional status of 16 tumor suppressor–negative regulator axes, gating high-risk oncogenic regulators and, in principle, informing cautiously bounded, axis-specific restoration strategies for future mechanistic and translational studies rather than immediate clinical use.

can claim substantial independent support. We summarize the strongest form of each, together with the evidence that is most difficult to reconcile with DDR-primacy, before stating the bounded claim we actually defend.

Mitochondria-first models hold that bioenergetic decline, mitochondrial DNA damage, and altered redox and retrograde signaling are the proximal drivers of aging, with nuclear DDR activation a downstream readout of oxidative stress rather than its cause. The tight coupling of mitochondrial dysfunction to senescence—and the demonstrated capacity of mitochondrial perturbation to drive cytoplasmic-chromatin formation and inflammatory signaling [17]—is difficult to reconcile with a strictly DDR-first ordering, and would predict that bioenergetic rescue should attenuate DDR signaling rather than the reverse.

Proteostasis-first models place the collapse of protein quality control—chaperone insufficiency, impaired autophagy and mitophagy, and aggregate accumulation—at the apex, positing that loss of proteostasis generates the cellular stress that secondarily engages the DDR.

The centrality of autophagy to aging, and evidence that the DDR itself can drive senescence by inhibiting autophagy of selected substrates [18], give this model standing as either an upstream cause or a co-equal partner of the DDR.

Epigenetic-drift and reprogramming-centric views, exemplified by information-theoretic accounts of aging and partial-reprogramming experiments, argue that progressive loss of epigenetic information is the fundamental and at least partly reversible cause, with DNA-damage signaling acting as one upstream trigger among several. The most direct challenge to DDR-primacy comes from this quarter: cyclic expression of reprogramming factors can restore youthful epigenetic patterns and function—including visual function after optic-nerve injury—apparently without first correcting the underlying genomic lesions [19–21]. If aging phenotypes can be reversed while DNA damage persists, DDR overactivation cannot be the sole or final common cause.

These models are not mutually exclusive, and the DDR intersects all of them: telomeric and oxidative lesions feed mitochondrial and epigenetic dysfunction, and the

Diverse upstream lesions converge on chronic, largely mutation-independent DDR overactivation, propagating through structurally intact tumor-suppressor (guardian) pathways to cellular senescence and inflammaging.

UPSTREAM “DAMAGE MIMICS”

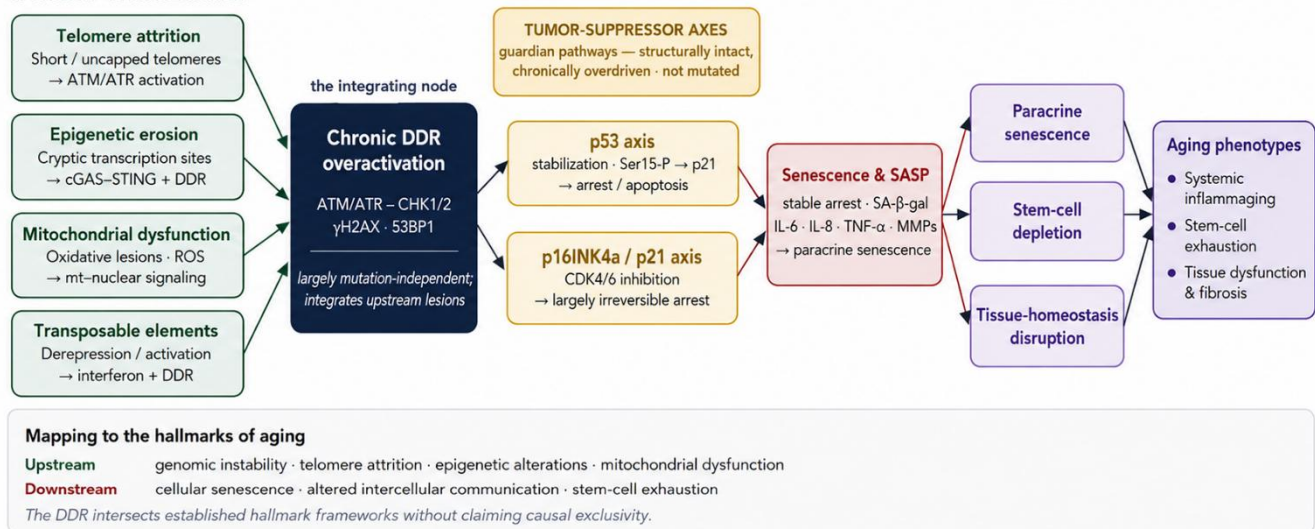


Figure 1. The DNA damage response (DDR) cascade as a candidate integrating node in aging. Diverse upstream lesions—including telomere attrition, epigenetic erosion, mitochondrial dysfunction/ROS, and transposable-element derepression—converge on chronic DDR overactivation (ATM/ATR–CHK1/2, γH2AX, 53BP1) largely independent of new driver mutations. This persistent DDR stabilizes p53 and induces p21 and p16INK4a, driving senescence and a senescence-associated secretory phenotype (SASP) that propagates paracrine senescence, depletes stem-cell pools, disrupts tissue homeostasis, and promotes systemic inflammaging. The mapped hallmarks (genomic instability, telomere attrition, epigenetic alterations, mitochondrial dysfunction, cellular senescence, altered intercellular communication) illustrate how the DDR intersects established aging frameworks without claiming causal exclusivity.

reverse also holds [5]. Our claim is therefore deliberately bounded. We advance DDR overactivation not as the sole cause of aging, nor necessarily as the earliest, but as a useful integrating node—broadly upstream of senescence and inflammaging, mechanistically connected to the competing models, and unusually tractable because it acts through structurally intact regulatory pathways. Whether it holds quantitative primacy over the mitochondrial, proteostatic, and epigenetic alternatives is unresolved; the predictions and experiments that would adjudicate this question are set out in the research-agenda section. In relation to the field's established syntheses—the hallmarks framework [9] and contemporary senescence reviews [12, 22]—our intended contribution is narrow and additive: to reorganize tumor suppressor–negative regulator signaling into a tiered set of candidate axes, and to pre-commit to the causal and refutation tests by which a DDR-centric reading should be judged.

It is equally important to distinguish this proposal from the established conceptual frameworks that organize the field, because our contribution is intended to be additive rather than a replacement. The Hallmarks of Aging catalogues the cellular and molecular features of aging without asserting a causal hierarchy among them [8, 9]. Systems- and network-biology accounts model aging as an emergent property of interactions among many robust molecular processes, with no single dominant driver. Damage-based (damage-repair) paradigms locate the primary cause in the accumulation of unrepaired molecular damage [5]. Our framework does not supplant these. It selects the DDR as one candidate integrating node within the damage-based tradition, reorganizes the tumor-suppressor and negative-regulator machinery downstream of it into a tiered, individually falsifiable axis architecture, and adds an explicit diagnose-and-restore translational layer. Table 1 summarizes these relationships and the framework's specific points of departure and overlap.

Aging vs. cancer: the critical distinction

Cancer and aging can be viewed as mirror-image pathologies of tumor suppressor networks (Figure 2). In cancer, somatic mutations directly inactivate tumor suppressors (TP53 loss, RB1 deletion, PTEN mutation), removing brakes on proliferation. In aging, the same pathways are typically structurally intact but functionally dysregulated by chronic DDR signaling. p53 becomes chronically stabilized rather than lost; p16INK4a accumulates inexorably rather than being deleted; PTEN remains present but is functionally suppressed by PI3K/AKT hyperactivation driven by inflammatory signaling. In this view the tumor suppressor genes are not broken—they are held in a

state of chronic overactivation or epigenetic silencing mediated by DDR-driven negative regulators [23–27].

This distinction carries a clear therapeutic implication, which we present as a hypothesis to be tested rather than an established route. In cancer, restoring tumor suppressor function requires replacing lost genes—a formidable challenge against a background of genomic instability. In aging, if the relevant genes are indeed intact, the therapeutic task would instead be to rebalance those pathways by modulating the negative regulators that have become overexpressed or dysregulated, restoring youthful homeostasis through precision modulation rather than wholesale replacement.

This mirror-image framing is reinforced by the cancer side of the same axis. In a substantial fraction of human cancers, the TP53 gene remains wild type, and p53 is instead functionally silenced by overexpression or amplification of its negative regulators MDM2 and MDMX (MDM4), achieving the effective equivalent of TP53 mutation without altering the gene [28]. Cutaneous melanoma is a clear example: TP53 mutation is rare, yet MDM4 is upregulated in roughly two-thirds of tumors to overcome p53, and inhibiting the MDM4-p53 interaction restores p53 function [29]. That cancer and aging can both dysregulate structurally intact p53 through its negative regulators, silencing it in the one case and, we argue, chronically overactivating it in the other, strengthens the central premise that these are problems of restoring regulation on intact pathways rather than of gene replacement.

The two-step conceptual paradigm: diagnose → restore

The framework is organized around a two-step logic that mirrors the diagnostic structure of precision oncology but inverts its therapeutic vector. We describe it as a conceptual paradigm; the specific analytes, thresholds, and constructs referenced below are illustrative and are tabulated in the Supplementary Information.

Step 1 — Precision diagnostics

Multi-omic biomarker panels would assess the functional status of each tumor suppressor–negative regulator axis. Unlike cancer diagnostics that search for mutations, aging diagnostics would measure pathway activity—protein levels, phosphorylation states, epigenetic modifications, transcriptomic signatures, and senescence markers—to generate a personalized “Aging Axis Profile” indicating which pathways are dysfunctional and to what degree.

Table 1. Relationship of the DDR-integrator framework to existing conceptual frameworks of aging.

Framework	Core premise	Stance on causal hierarchy	Primary output	Relation to / distinction from this framework
Hallmarks of Aging	Enumerates the cellular and molecular hallmarks that jointly contribute to aging	Deliberately non-hierarchical; hallmarks are interconnected	A catalogue and organizing checklist of aging processes	Shared substrate; this work selects the DDR as an integrating node and adds causal-ordering tests and a restoration layer the hallmarks do not specify
Systems / network biology of aging	Aging emerges from interactions across robust molecular networks	Distributed causation; no single dominant driver	Computational and network models of resilience and failure	Complementary; this work is a testable reductionist subset positing one tractable node and uses network logic in its synergy architecture (Section 7)
Damage-repair paradigm	Aging is the accumulation of unrepaired molecular, especially DNA, damage	Damage is the primary cause	Mechanistic account centered on the damage/repair balance	Sits within the damage tradition but shifts emphasis from damage per se to chronic DDR signaling on structurally intact pathways
DDR-integrator framework (this work)	Chronic, mutation-independent DDR overactivation dysregulates intact tumor-suppressor networks	DDR proposed as a major integrating node, explicitly falsifiable, not the sole cause	Tiered, per-axis falsifiable architecture plus a diagnose-and-restore research agenda	Reference framework of the present review

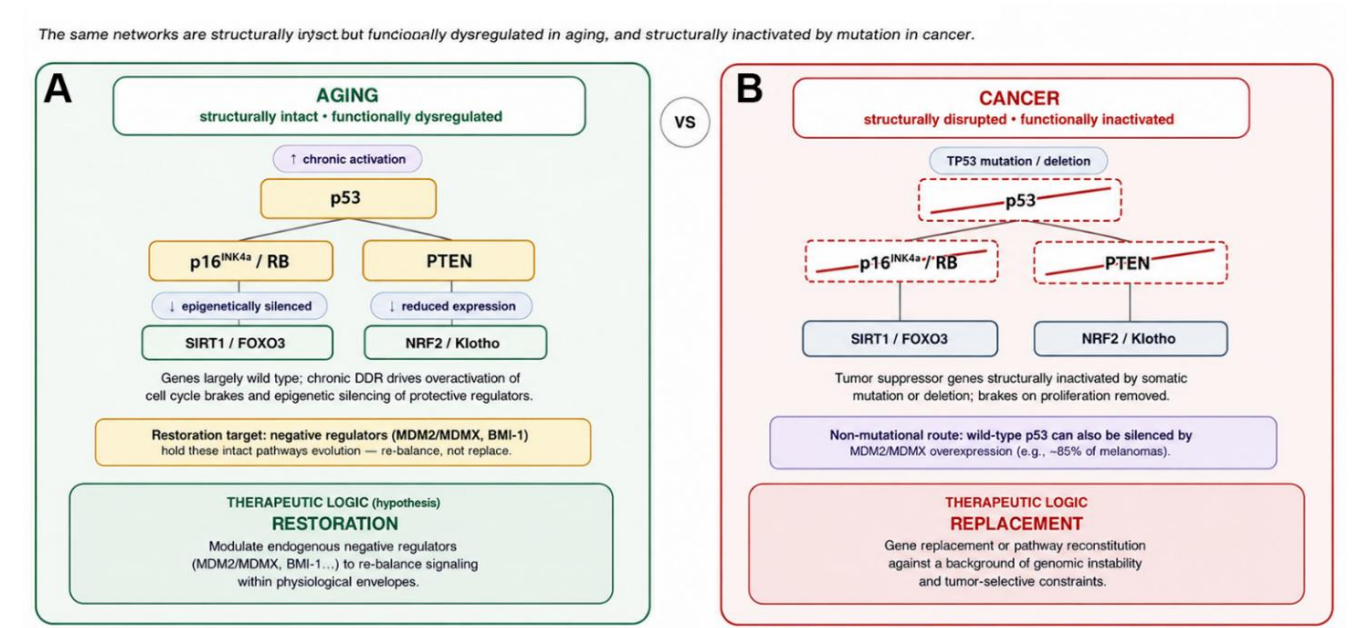


Figure 2. Aging versus cancer as mirror-image dysregulation of tumor-suppressor networks and the resulting therapeutic logic. (A) In aging, tumor-suppressor pathways such as p53, p16INK4a/RB, and PTEN are typically structurally intact but functionally dysregulated by chronic DDR signaling, with chronic overactivation of cell-cycle brakes and progressive epigenetic silencing of protective regulators (e.g., SIRT1, FOXO3, NRF2, Klotho); the therapeutic logic is restoration — modulating endogenous negative regulators (MDM2/MDMX, BMI-1) to re-balance signaling within physiological envelopes. (B) In cancer, the same networks are structurally disrupted by somatic mutation or deletion (TP53 loss, RB1 deletion, PTEN mutation), removing proliferative brakes; the therapeutic logic is replacement — gene replacement or pathway reconstruction against a background of genomic instability. This mirror-image framing suggests that aging is, in principle, better approached as a problem of restoring physiological regulation of intact pathways, whereas cancer requires gene replacement or alternative strategies against a background of genomic instability.

Step 2 — Endogenous regulator restoration

For each dysfunctional axis, targeted gene therapy would deliver endogenous negative regulators to restore homeostatic balance. Where tumor suppressors are chronically overactivated (p53, p16, p21), negative-regulator restoration (e.g., MDM2, BMI-1) would aim to dampen the response toward physiological levels; where protective pathways are epigenetically silenced (SIRT1, FOXO3, NRF2, Klotho), gene therapy would aim to restore their expression. The goal is explicitly not to eliminate tumor suppressor function—which would risk cancer—but to restore the dynamic, responsive regulation characteristic of young tissues.

This framing is, in principle, well matched to gene-therapy delivery because the targets are endogenous genes requiring expression modulation rather than replacement; AAV vectors can provide sustained, tissue-specific expression; the intended therapeutic window is restoration of normal function rather than supraphysiological activation; and multi-gene platforms could, in principle, address several axes together. Each of these propositions is a hypothesis requiring empirical test, and each is bounded by the safety, delivery, and validation constraints detailed in the later sections.

Aims of this review

Accordingly, we (i) synthesize the evidence linking DDR overactivation with the canonical and emerging

hallmarks of aging, distinguishing human from animal and *in vitro* support; (ii) organize key tumor suppressor–negative regulator pairs into a tiered, field-organizing framework intended as a reference scaffold; (iii) set the DDR-integration hypothesis explicitly against competing causal models and specify the observations that would distinguish or refute it; and (iv) lay out a prioritized research agenda spanning diagnostics, mechanism, and cautiously bounded translation. This article is a narrative, hypothesis-driven review rather than a systematic review, and focuses on conceptual integration, falsifiability, and research prioritization in a rapidly evolving field.

A CONCEPTUAL DIAGNOSTIC FRAMEWORK: THE AGING AXIS PROFILE

Central to the framework is the concept of an Aging Axis Profile (AAP)—a multi-omic panel that would quantify the functional status of each tumor suppressor–negative regulator axis across several molecular levels. We describe the AAP as a conceptual diagnostic architecture; it has not been analytically or clinically validated, and the evidentiary standard it would have to meet is set out in the biomarker-validation section. The AAP integrates the following layers (Figure 3).

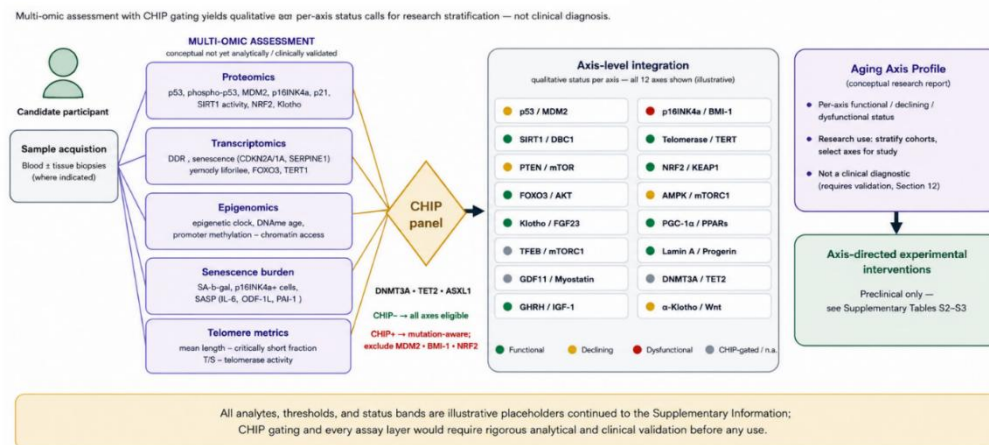


Figure 3. The Aging Axis Profile (AAP) diagnostic workflow: a conceptual multi-omic framework for DDR-resolved aging.

Candidate participants provide blood and, where indicated, tissue samples that feed into five assay modules: proteomics (e.g., p53, phospho-p53, MDM2, p16INK4a, p21, SIRT1 activity, NRF2 nuclear localization, Klotho), transcriptomics (DDR, senescence, longevity, metabolic panels), epigenomics (epigenetic clocks and locus-specific promoter methylation), senescence burden (cellular and circulating SASP markers), and telomere metrics (mean length, critically short fraction, telomere dysfunction-induced foci, telomerase activity). CHIP testing (DNMT3A, TET2, ASXL1) gates high-risk oncogenic-regulator axes (MDM2, BMI1, NRF2) into or out of consideration. Integrated per-axis readouts yield qualitative functional/declining/dysfunctional status calls, which are intended for research stratification and hypothesis testing, not clinical diagnosis; all illustrative analytes and placeholder thresholds are confined to the Supplementary Information and would require rigorous analytical and clinical validation.

Proteomic assessment

Quantitative measurement of key pathway proteins, their phosphorylation states, and interaction status (ELISA, mass spectrometry, proximity ligation), e.g., p53 and phospho-Ser15/Ser20, MDM2 and p53–MDM2 complex formation, p16INK4a and p21, SIRT1 activity, NRF2 nuclear localization, FOXO3 phosphorylation, and Klotho levels.

Transcriptomic profiling

RNA-seq and targeted panels quantify the DDR transcriptome (ATM, ATR, CHK1/2, BRCA1, 53BP1), the senescence transcriptome (CDKN2A/p16, CDKN1A/p21, SERPINE1, SASP factors), the longevity transcriptome (sirtuins, FOXOs, TERT, NRF2 targets, Klotho), and the metabolic transcriptome (AMPK subunits, PGC-1 α , TFEB, mTOR components).

Epigenomic analysis

DNA-methylation arrays, ATAC-seq, and ChIP-seq assess epigenetic age and pathway-specific status. Epigenetic clocks (Horvath, GrimAge, DunedinPACE) provide biological-age estimates [30–32], while locus-specific methylation at tumor suppressor and longevity promoters identifies epigenetically silenced pathways.

Senescence biomarkers

Circulating and tissue-based markers index senescent-cell burden and SASP intensity (SA- β -galactosidase, lipofuscin, senescence-associated heterochromatic foci, circulating IL-6/GDF-15/PAI-1, p16INK4a-positive cells) [12, 33, 34].

Telomere assessment

Q-FISH, terminal restriction fragment analysis, and STELA measure telomere length, critically short telomere frequency, and telomerase activity, linking telomere status to DDR activation [35, 36].

Axis status calls: a deliberately qualitative scheme

Each axis is conceived as having a diagnostic signature that integrates several biomarkers into a status call. We deliberately use a simple three-state scheme—functional, declining, dysfunctional—rather than numeric cut-points, because no validated prognostic thresholds yet exist for these axes:

Functional

Axis operating within youthful reference ranges; routine monitoring.

Declining

Measurable deviation from youthful homeostasis; a candidate state for closer monitoring or preventive study.

Dysfunctional

Substantial departure from homeostasis with functional consequences; the state at which axis-directed intervention would, hypothetically, be evaluated.

To make the operationalization concrete without overstating its maturity, Box 1 illustrates how a single axis might be parameterized. Fully illustrative, non-validated parameterizations for all 16 axes—including the specific analytes, assays, and placeholder numeric boundaries—are provided in Supplementary Table 1. We stress that every numeric value in the Supplement is a conceptual placeholder, not a clinical recommendation: each would require empirical derivation and prospective qualification (the biomarker-validation section) before any use. A diagnosis-first logic of this kind is what would, in principle, allow interventions to be targeted to genuinely dysfunctional axes rather than applied indiscriminately.

Tissue- and cell-type-resolved aging and its diagnostic implications

A central question for any diagnose-and-restore framework is whether tissues and cell types age in synchrony or independently. The most defensible answer is that both occur, and that aging is a mixture of communal and cell-autonomous processes rather than either alone. On the communal side, the senescence-associated secretory phenotype (SASP) propagates senescence from cell to neighboring cell through paracrine signaling, so that aging within a tissue niche is partly a coordinated community effect rather than a sum of independent events [6, 7, 22]. On the cell-autonomous side, epigenetic aging is distributed unevenly: epigenetic-clock and methylation measures differ between tissues, and between regions and cell types within a single organ, indicating independent variation superimposed on any shared trajectory [30]. At the organ level, plasma-proteomic organ clocks show frank asynchrony between organs of the same individual, with roughly one in five adults a pronounced single-organ ager and organ-specific age predicting organ-specific disease and mortality [37, 38]. Aging is therefore best modeled as a partly coordinated mosaic of tissue- and cell-type-specific trajectories rather than a single organismal clock, and further evidence of both coordinated and independent dynamics is likely to emerge as tissue-resolved longitudinal data accumulate.

This combination of communal and cell-autonomous dynamics has direct consequences for the Aging Axis Profile. The axis or axes that are dysfunctional, and the degree of dysfunction, may differ between blood, vascular wall, myocardium, brain, and skin within the same individual. They may differ again between cell types within one tissue, for example between dermal

Box 1. Conceptual example — operationalizing one axis (p53/MDM2)

For the p53/MDM2 axis, a youthful “functional” state would correspond to low basal p53 with preserved acute inducibility and physiological MDM2-mediated turnover. A “dysfunctional” state would correspond to chronically elevated basal p53 with constitutive Ser15 phosphorylation and downstream p21 induction, together with an elevated senescent-cell burden. The transition between these states is what an Aging Axis Profile would attempt to detect.

The specific analytes and assays for this axis are listed in Supplementary Table 1. The numeric boundaries shown there are illustrative and unvalidated; they are included only to demonstrate that the scheme is, in principle, measurable, and they must not be read as thresholds for clinical action.

fibroblasts and epidermal stem cells in skin, even as paracrine SASP signaling partially coordinates the surrounding niche. A profile built solely from an accessible compartment such as peripheral blood cannot be assumed to represent the functional state of a post-mitotic tissue such as brain or heart.

The framework accommodates this in three ways, each of which adds complexity that we make explicit rather than conceal. First, the AAP is conceived as a tissue-resolved rather than a single systemic readout: accessible surrogates (circulating proteins, PBMC-based assays, cell-free DNA methylation, and senescence or SASP markers) are paired, where a specific tissue is implicated and clinically justified, with targeted tissue sampling or emerging imaging surrogates. Second, axis status calls are made per compartment, so that intervention is considered only for the tissue or tissues in which an axis is genuinely dysfunctional. Third, restoration is matched to that resolution through tissue-restricted vectors and promoters (the safety and delivery-considerations sections), so that a systemically delivered agent does not act on compartments that are still functional.

We regard tissue- and cell-type resolution as one of the framework’s principal unsolved problems rather than a settled feature. Deconvolving accessible biomarkers into tissue-of-origin signals, establishing which peripheral readouts faithfully report inaccessible tissues, and defining when tissue sampling is warranted are prerequisites for the validation program of the biomarker-validation section. The added interventional complexity of targeting only specific compartments is one further reason the framework is presented as a research agenda rather than a clinical protocol.

Artificial intelligence and multi-omic integration in profile construction

Constructing an Aging Axis Profile from proteomic, transcriptomic, epigenomic, senescence, and telomere layers is fundamentally a high-dimensional integration problem, and machine-learning methods are increasingly

central to it. Deep-learning aging clocks trained on large multi-omic and clinical datasets already match or outperform hand-curated feature sets for chronological- and biological-age prediction, and the organ-specific models noted above are themselves machine-learning constructs [37, 39, 40].

Within this framework, artificial intelligence has four specific and complementary roles. First, profile construction: multi-modal models can integrate heterogeneous, differently scaled assays into per-axis functional, declining, or dysfunctional calls more robustly than fixed thresholds. Second, biomarker prioritization: feature-attribution and causal-inference methods can identify the smallest analyte panel that captures each axis, reducing assay cost and complexity. Third, patient stratification: unsupervised clustering can define molecular sub-phenotypes and match them to the axes most likely to benefit, operationalizing the heterogeneity discussed in the Limitations section. Fourth, therapeutic decision support: models that jointly weigh axis status, CHIP and oncogenic-risk gating, sex, and comorbidity could rank candidate axes and flag safety contraindications before any intervention is considered. A consolidated per-axis view of these elements is given in Table 2.

These applications are enabling rather than validating. Machine-learning outputs inherit the limitations of their training data, can encode cohort- and ancestry-specific biases, and remain correlational. They are therefore subject to the same analytical and clinical qualification required of any AAP component (the biomarker-validation section), and none substitutes for the causal-ordering tests of the research-agenda section.

TIER 1 AXES: BEST-SUPPORTED TARGETS

Tier 1 axes are the tumor suppressor–negative regulator pairs with the strongest experimental evidence for roles in aging and the most developed restoration strategies. For each axis we summarize the mechanism, the diagnostic signature (analytes and assays, illustrative numeric parameters in Supplementary Table 1), the

Table 2. Aging Axis Profile: consolidated per-axis summary of biological rationale, evidence level, representative biomarkers, and proposed restoration strategy for all 16 candidate axes.

Axis (suppressor / regulator)	Tier	Biological rationale	Evidence level	Representative biomarker(s)	Proposed restoration strategy
p53 / MDM2	1	Chronic DDR stabilizes p53, driving senescence, SASP, and stem-cell depletion	Robust mechanistic and rodent; no human interventional	Serum p53, phospho-p53(Ser15), p53-MDM2 complex ratio, p21	AAV MDM2 restoration to normalize p53 turnover
p16INK4a / BMI-1	1	p16 accumulates as BMI-1/PRC1 silencing of CDKN2A declines, enforcing irreversible arrest	Strong rodent genetic; no human	p16 mRNA (T cells), p16 IHC, BMI-1/p16 ratio	AAV BMI-1 restoration to re-silence CDKN2A
SIRT1 / DBC1	1	NAD ⁺ decline and DBC1 inhibition lower SIRT1, driving epigenetic and inflammatory aging	Extensive invertebrate/rodent; human unverified	SIRT1 activity, NAD ⁺ /NADH, SIRT1-DBC1 ratio	SIRT1 + NAMPT restoration, optional DBC1 knockdown
Telomerase / TRF1	1	Telomere attrition triggers mutation-independent DDR at chromosome ends	Reproducible AAV9-TERT rodent; human untested; species caveat (Sec 3.4)	Telomere length, critically short fraction, telomerase activity (TRAP)	AAV TERT, species-appropriate and surveilled
PTEN / PI3K-AKT-mTOR	1	PTEN loss yields mTORC1 hyperactivation, suppressed autophagy, and SASP	Transgenic rodent; no human	PTEN activity, phospho-AKT(Ser473), phospho-S6K1, HOMA-IR	AAV PTEN restoration
NRF2 / KEAP1	1	Antioxidant-response collapse despite rising oxidative burden	Strong mechanistic/rodent; no human; context-dependent oncogenicity	NRF2 targets (NQO1, HMOX1), GSH/GSSG, 8-OHdG	AAV KEAP1-resistant NRF2 or KEAP1 knockdown, expression-limited
FOXO3 / AKT	2	AKT excludes FOXO3 from the nucleus; FOXO3 is the leading human-longevity gene	Human association and model organism; no human gene therapy	FOXO3 nuclear localization and phosphorylation	Constitutively nuclear FOXO3 variant, or AKT suppression
AMPK / mTORC1	2	AMPK decline shifts metabolism toward growth and away from autophagy	Robust pharmacological and genetic rodent; conceptual gene therapy	AMPK activity, phospho-ACC	Constitutively active AMPK-alpha1 or LKB1
Klotho / FGF23	2	Circulating Klotho declines, accelerating vascular, renal, and cognitive aging	Rodent overexpression/knockout and human observational; no human gene therapy	Serum alpha-Klotho	AAV alpha-Klotho restoration
PGC-1alpha / PARIS	2	PARIS represses PGC-1alpha, driving mitochondrial decline that feeds DDR	Preclinical, context-dependent	PGC-1alpha level, mitochondrial density	PGC-1alpha restoration with PARIS knockdown
TFEB / mTORC1	2	mTORC1 keeps TFEB inactive, impairing autophagy and lysosomal clearance	Preclinical autophagy data; no human	TFEB localization, LC3-II/I	mTORC1-resistant TFEB variant
Lamin A / Progerin	2	Low-level progerin accumulation disrupts nuclear architecture with age	Strong in progeroid models; partial in normative aging	Progerin, nuclear morphology	Enhance prelamin A processing (ZMPSTE24) plus splice-blocking ASO
GDF11 / Myostatin	3	Putative systemic rejuvenation factor (contested)	Contested; key findings not reproduced	GDF11/myostatin balance (LC-MS/MS)	Myostatin antagonism (follistatin), not GDF11 supplementation
DNMT3A / TET2	3	Methylation-landscape dysregulation; point of contact with CHIP	Emerging; CHIP-gated	Methylation clocks, locus-specific methylation, CHIP status	DNMT3A restoration and targeted demethylation, CHIP-gated
CISD2 / BCL-2	3	CISD2 decline impairs ER-mitochondrial calcium transfer and mitochondrial integrity	Emerging rodent	CISD2 level	AAV CISD2 restoration
alpha-Klotho / Wnt	3	Klotho decline permits Wnt hyperactivation and stem-cell exhaustion	Emerging	Klotho, Wnt target activity	Klotho restoration with tissue-restricted Wnt dampening

proposed restoration strategy at a conceptual level, and an explicit evidence-level statement. Detailed vector and construct specifications, where given, are confined to Supplementary Table 3.

The p53/MDM2 axis: senescence master switch

Mechanism in aging

p53 is the central mediator of the DDR-aging cascade [24, 41]. In aging tissues, chronic DDR activation stabilizes p53 through ATM/ATR-mediated phosphorylation, disrupting the p53–MDM2 interaction that normally maintains p53 at low levels. The result is chronic p53 accumulation that drives permanent cell-cycle arrest, senescence, SASP activation, and stem-cell depletion. TP53 typically remains wild-type in aging—the protein is overactivated, not mutated. Mouse models with constitutively active p53 show accelerated aging phenotypes and reduced lifespan, demonstrating that p53 hyperactivation, without any somatic mutation, can be sufficient to drive aging [42, 43].

Diagnostic signature

Primary analytes: serum p53 protein (ELISA), phospho-p53(Ser15) in PBMCs (flow cytometry), p53–MDM2 complex ratio (proximity ligation). Secondary analytes: p21 in skin biopsies (immunohistochemistry), BAX/BCL-2 ratio, PUMA/NOXA expression. Functional readout: clonogenic capacity in patient-derived progenitor cells. Illustrative status bands are given in Supplementary Table 1.

Proposed restoration strategy: MDM2 modulation

The conceptual approach is AAV-mediated MDM2 restoration to re-establish physiological p53 turnover. The goal is not to eliminate p53 (which would create cancer risk) but to restore the dynamic MDM2-mediated cycle that maintains youthful basal p53 with preserved acute responsiveness. Preclinical data in aged mice indicate that restoring MDM2 expression can reduce p53 stabilization, clear senescent cells, and improve tissue regeneration [44, 45]. Evidence level: robust mechanistic and rodent senescence data for p53/MDM2 dynamics; MDM2 restoration as an anti-aging therapy has no human interventional data, and MDM2 is a proto-oncogene subject to the safety constraints in the safety and oncogenesis section.

The MDM2-MDMX module and a more selective lever. The negative-regulatory arm of this axis is not MDM2 alone. MDMX (MDM4), a structural homolog of MDM2, is a non-redundant second regulator of p53: MDM2 principally controls p53 abundance through ubiquitin-dependent degradation, whereas MDMX principally restrains p53 transcriptional output, and their loss-of-function phenotypes are correspondingly distinct,

biased toward apoptosis and toward cell-cycle arrest respectively [28]. This distinction bears directly on the restoration strategy. Because the aging phenotype the axis targets is chronic p53-driven arrest and senescence rather than apoptosis, restoring MDMX, rather than or alongside MDM2, could in principle dampen the chronic transcriptional-arrest output while preserving p53 abundance and its acute, apoptotic genome-surveillance function. Framing the target as the MDM2-MDMX module, with MDMX as an output-selective knob, may therefore offer a more precise route to youthful p53 dynamics than degradation-based MDM2 restoration alone; the molecular regulation and therapeutic manipulation of this module are reviewed in detail elsewhere [28].

The p16INK4a/BMI-1 axis: irreversible senescence gatekeeper

Mechanism in aging

p16INK4a (encoded by CDKN2A) is among the most robust biomarkers of biological aging and a primary enforcer of irreversible senescent arrest [46, 47]. Its expression rises with age across tissues as Polycomb (PRC1) occupancy at CDKN2A declines. BMI-1 maintains CDKN2A silencing through H2A ubiquitination in young tissues; with aging, BMI-1 declines, derepressing CDKN2A and allowing p16INK4a to accumulate, inhibit CDK4/6, and lock cells in arrest. Unlike p53-mediated senescence, p16-mediated arrest is considered largely irreversible under physiological conditions [48, 49].

Diagnostic signature

Primary analytes: p16INK4a mRNA in peripheral blood T cells (RT-qPCR), p16INK4a protein in skin biopsies (immunohistochemistry), BMI-1/p16 ratio. Secondary analytes: SA- β -galactosidase-positive cells, H2AK119ub at CDKN2A (ChIP-qPCR), CDK4/6 activity. Illustrative bands in Supplementary Table 1.

Proposed restoration strategy: BMI-1 modulation

The conceptual approach is AAV-mediated BMI-1 restoration to re-establish Polycomb-mediated CDKN2A silencing. In preclinical models, BMI-1 overexpression preserves stem-cell self-renewal and reduces p16INK4a accumulation, with reported lifespan effects in mice [50–53]. The intent is to restore dynamic regulation—so that p16 can still be induced by genuine oncogenic signals—rather than to abolish its tumor-suppressive function. Evidence level: strong rodent genetic and senescent-cell data; BMI-1 gene therapy for aging is preclinical with no human data, and BMI-1 is a proto-oncogene subject to the constraints of the safety and oncogenesis section.

The SIRT1/DBC1 axis: epigenetic aging clock

Mechanism in aging

SIRT1 is the mammalian ortholog of yeast Sir2, an NAD⁺-dependent deacetylase linked to longevity across species [54, 55]. It deacetylates p53, FOXO3, PGC-1 α , NF- κ B/p65, and histones. With aging, SIRT1 activity declines through NAD⁺ depletion (CD38 upregulation, NAMPT decline) and through inhibition by DBC1, whose binding to SIRT1 increases with age and DNA damage. Chronic DDR activation promotes the DBC1–SIRT1 interaction via PARP1-mediated NAD⁺ consumption, linking DDR overactivation to epigenetic aging [56, 57].

Diagnostic signature

Primary analytes: SIRT1 activity in PBMCs (fluorometric deacetylation), NAD⁺/NADH ratio (mass spectrometry), DBC1 levels and SIRT1–DBC1 co-immunoprecipitation ratio. Secondary analytes: acetyl-p53(K382), acetyl-NF- κ B/p65(K310), H3K9ac/H3K9me3 ratio, GrimAge residual. Illustrative bands in Supplementary Table 1.

Proposed restoration strategy: SIRT1 + NAD⁺ support

The conceptual approach combines SIRT1 restoration with NAMPT co-expression to raise both enzyme and cofactor, optionally with DBC1 knockdown to liberate endogenous SIRT1. SIRT1-transgenic mice show delayed aging features; NAD⁺-pathway agents have entered human testing, but SIRT1 gene therapy in humans is untested [58–63]. Evidence level: extensive invertebrate and rodent data; epigenetic-age effects unverified in humans.

The telomerase/TRF1 axis: replicative aging clock

Mechanism in aging

Telomere attrition is a primary hallmark of aging and a direct trigger of mutation-independent DDR activation. As telomeres shorten, the shelterin complex can no longer suppress DDR recognition of chromosome ends; critically short telomeres are read as double-strand breaks, activating ATM/ATR and p53 and driving senescence. TRF1 negatively regulates telomere length by limiting telomerase access; with aging, declining somatic TERT shifts the balance toward shortening [64–67].

Diagnostic signature

Primary analytes: mean telomere length (Q-FISH/Flow-FISH), critically short telomere fraction (STELA), telomerase activity (TRAP). Secondary analytes: TRF1 levels, telomere dysfunction-induced foci, shelterin integrity. Illustrative bands in Supplementary Table 1.

Proposed restoration strategy: TERT gene therapy

The reference preclinical result is AAV9-TERT in mice, which extended median lifespan without increasing cancer incidence and improved multiple aging biomarkers [68–70]. Evidence level: reproducible AAV9-TERT rodent data; human telomerase gene therapy for aging is untested and carries a theoretical neoplastic risk that must be weighed explicitly.

Species caveat (human versus rodent telomere biology). The rodent data that anchor this axis must be interpreted against a fundamental difference in telomere biology between mice and humans. Laboratory mice have long telomeres and constitutive somatic telomerase activity, whereas humans have short telomeres and repress telomerase in most somatic tissues, so that replicative senescence contributes to human but not routine murine aging [71]. Comparative analyses across more than sixty mammalian species indicate that short telomeres with repressed telomerase are the ancestral, human-like state, and that laboratory-mouse telomere biology is a derived exception [71]. Two consequences follow. First, the lifespan and biomarker benefits of AAV9-TERT in mice may translate poorly, or differently, to a human context in which telomerase is normally restrained. Second, the neoplastic risk of reactivating telomerase is plausibly greater in humans, where telomere-based replicative senescence remains an active tumor-suppressive barrier, than in mice, where it is largely absent. Human-relevant models, together with species-appropriate dosing and surveillance, are therefore prerequisites for this axis, and the murine results should be read as proof of mechanism rather than as quantitative predictions for humans.

The PTEN/PI3K-AKT-mTOR axis: metabolic aging hub

Mechanism in aging

The PI3K/AKT/mTOR cascade is among the most validated pro-aging pathways, with inhibition extending lifespan across species. PTEN is its master negative regulator. In aging, PTEN function is compromised through epigenetic silencing, oxidative inactivation, and increased degradation, yielding mTORC1 hyperactivation that suppresses autophagy, inhibits FOXOs, promotes SASP, and suppresses TFEB. PTEN-overexpressing mice show modest lifespan extension with improved metabolism [72–76].

Diagnostic signature

Primary analytes: PTEN protein and phosphatase activity, phospho-AKT(Ser473)/total AKT, phospho-

S6K1(Thr389), and a metabolic correlate (fasting insulin/HOMA-IR). Secondary analytes: PTEN promoter methylation, phospho-4E-BP1, LC3-II/I, FOXO3 localization. Illustrative bands in Supplementary Table 1.

Proposed restoration strategy: PTEN modulation

The conceptual approach is AAV-mediated PTEN restoration to re-establish PI3K/AKT/mTOR homeostasis. The “Super-PTEN” mouse shows modest lifespan extension with improved metabolic health and reduced cancer incidence [77–79]; whether comparable effects occur in humans is unknown. Evidence level: transgenic rodent data; no human interventional evidence.

The NRF2/KEAP1 Axis: Oxidative Stress Defense

Mechanism in aging

NRF2 is the master transcription factor of the antioxidant response [80], regulating cytoprotective genes; KEAP1 targets it for degradation. With aging, this protective mechanism fails through increased KEAP1, GSK-3 β -driven KEAP1-independent degradation, Bach1 accumulation, and epigenetic silencing of NRF2 targets, yielding progressive collapse of antioxidant defenses despite rising oxidative burden [81–83].

Diagnostic signature

Primary analytes: NRF2 target panel (NQO1, HMOX1, GCLC, GCLM, TXNRD1), GSH/GSSG ratio, 8-OHdG, NRF2 nuclear localization. Secondary analytes: KEAP1 levels, Bach1/NRF2 ratio, protein carbonyls, F2-isoprostanes, 4-HNE adducts. Illustrative bands in Supplementary Table 1.

Proposed restoration strategy: NRF2/KEAP1 modulation

The conceptual approach is AAV-mediated expression of a KEAP1-resistant NRF2 variant and/or KEAP1 knockdown, with expression-limiting design to prevent excessive, potentially pro-tumorigenic NRF2 activation [84]. Evidence level: strong mechanistic and rodent data; NRF2 gene therapy for aging is untested, and NRF2 carries well-documented context-dependent oncogenic activity (the safety and oncogenesis section).

TIER 2 AXES: ADVANCED PRECLINICAL TARGETS

Tier 2 axes have strong mechanistic evidence and emerging restoration strategies, but less direct *in vivo* aging-reversal data than Tier 1. They are summarized more briefly; analytes, illustrative bands, and any construct detail appear in Supplementary Tables 1, 3.

FOXO3/AKT: longevity gene network

FOXO3 is the gene most consistently associated with human longevity across populations [85], coordinating DNA repair, antioxidant defense, autophagy, and cell-cycle control. AKT phosphorylation excludes FOXO3 from the nucleus; chronic PI3K/AKT hyperactivation in aging keeps it inactive. The conceptual approach restores a constitutively nuclear FOXO3 variant or wild-type FOXO3 with upstream AKT suppression. Evidence level: human longevity-association and model-organism data; no human gene-therapy data.

AMPK/mTORC1: nutrient-sensing regulator

AMPK is the cellular energy sensor mediating much of caloric restriction’s benefit, inhibiting mTORC1 and activating autophagy and mitochondrial biogenesis. AMPK activity declines with age, shifting metabolism toward growth. The conceptual approach restores AMPK activity (constitutively active AMPK α 1 or LKB1) [86–89]. Evidence level: robust pharmacological and genetic rodent data; AMPK-directed gene therapy for aging is conceptual.

Klotho/FGF23: anti-aging hormone

α -Klotho disruption produces a premature-aging syndrome in mice [90]; circulating Klotho declines with age through epigenetic silencing and reduced shedding [91, 92]. The conceptual approach restores α -Klotho expression; rodent overexpression extends lifespan and improves vascular, cognitive, and renal aging, and human observational associations are supportive [93–95]. Evidence level: rodent overexpression/knockout and human observational data; no human gene-therapy data.

PGC-1 α /PARIS: mitochondrial aging

PGC-1 α is the master regulator of mitochondrial biogenesis; PARIS (ZNF746) represses it and accumulates as Parkin activity declines with age, contributing to mitochondrial decline that feeds back on DDR activation [96–98]. The conceptual approach restores PGC-1 α with PARIS knockdown. Evidence level: largely preclinical with context-dependent effects; no human gene-therapy data.

TFEB/mTORC1: autophagy–lysosomal aging

TFEB is the master regulator of autophagy and lysosomal biogenesis; chronic mTORC1 hyperactivation in aging keeps it inactive, impairing clearance of aggregates and dysfunctional organelles [99–102]. The conceptual approach expresses an mTORC1-resistant TFEB variant. Evidence level: preclinical autophagy data; no human interventional data.

Lamin A/Progerin: nuclear architecture aging

Lamin A maintains nuclear architecture; in Hutchinson-Gilford progeria a cryptic splice product, progerin, disrupts it. Normal aging also accumulates low-level progerin as ZMPSTE24 activity declines and cryptic splicing increases [103–106]. The conceptual approach enhances prelamin A processing (ZMPSTE24) and blocks the cryptic splice site. Evidence level: strong in progeroid models; relevance to normative aging is partial and preclinical.

TIER 3 AXES: EMERGING TARGETS

Tier 3 axes have mechanistic rationale and early preclinical support but the least direct evidence; GDF11 in particular rests on contested data and is retained only with explicit caveats.

GDF11/Myostatin: systemic rejuvenation factor (contested)

GDF11 emerged from parabiosis experiments as a putative circulating rejuvenation factor [107–109].

Scientific caveat

The GDF11 rejuvenation hypothesis is contested. Independent groups have failed to reproduce key parabiosis and cardiac findings, and specific LC–MS/MS assays that distinguish GDF11 from myostatin report no consistent age-related decline in humans—with some data indicating GDF11 can inhibit muscle regeneration [110, 111]. GDF11 is therefore retained only as a Tier 3 candidate, advanced solely where axis-specific assays confirm an unfavorable GDF11/myostatin balance, and prioritized for myostatin antagonism (follistatin), where evidence is stronger, over GDF11 supplementation.

DNMT3A/TET2: epigenetic drift control

DNMT3A and TET2 control the methylation landscape underlying epigenetic clocks; with aging, their balance is dysregulated and TET2/DNMT3A loss-of-function contributes to CHIP [19–21, 112, 113]. The conceptual approach restores DNMT3A capacity and uses targeted epigenetic editing to demethylate silenced longevity promoters. This axis is the platform's point of contact with the somatic-mutation problem and is gated by CHIP screening.

CISD2/BCL-2: calcium–mitochondrial homeostasis

CISD2 maintains ER–mitochondrial calcium transfer and mitochondrial integrity; it declines with age, and

overexpression extends lifespan in mice [114–117]. The conceptual approach restores CISD2 expression.

α -Klotho/Wnt: stem-cell exhaustion

Beyond its endocrine functions, α -Klotho antagonizes Wnt signaling, whose hyperactivation drives stem-cell exhaustion; Klotho decline permits Wnt hyperactivation across hematopoietic, intestinal, follicular, and satellite-cell compartments [118]. The conceptual approach combines Klotho restoration with tissue-restricted Wnt dampening.

ILLUSTRATIVE MULTI-AXIS RESEARCH DIRECTIONS

The combinations in this section are illustrative research directions, not clinical protocols. They are intended to show how the framework's systems-level logic could be explored experimentally for major aging phenotypes; the specific axis groupings, vectors, and sequencing are unvalidated and would require empirical derivation. Representative construct compositions are tabulated in Supplementary Table 2; the discussion below names the axes engaged and the conceptual rationale rather than prescribing constructs.

Neuroaging (cognitive decline, neurodegeneration)

The brain is vulnerable to DDR-driven aging given post-mitotic dependence on DNA repair, high metabolic demand, and limited regeneration. A candidate direction would coordinate senescence control (p53/MDM2), neuroprotective deacetylation (SIRT1), oxidative defense (NRF2), aggregate clearance (TFEB), and Klotho support, delivered with neuron- or glia-restricted tropism [119].

Cardiovascular aging

A candidate direction would combine cardiomyocyte senescence control (p53/MDM2), metabolic rebalancing (PTEN), telomere protection (TERT), and vascular protection (Klotho), with cardiac-restricted delivery [120].

Metabolic rejuvenation

A candidate direction would target insulin signaling (PTEN), nutrient sensing (AMPK), NAD⁺-dependent regulation (SIRT1/NAMPT), mitochondrial biogenesis (PGC-1 α), and stress resistance (FOXO3) across liver and muscle [121].

Musculoskeletal rejuvenation

A candidate direction would combine satellite-cell senescence control (p16/BMI-1), myostatin antagonism (follistatin), mitochondrial support (PGC-1 α), telomere protection (TERT), and bone-mineral support (Klotho) [122, 123].

Immune rejuvenation (immunosenescence, inflammaging)

A candidate direction would reduce senescent-T-cell accumulation (p16/BMI-1), restore memory and stress responses (FOXO3), dampen NF- κ B-driven inflammation (SIRT1), extend replicative lifespan (TERT), and restrain HSC myeloid skewing (Klotho/Wnt), with optional thymic-regeneration support [124, 125].

Skin and integumentary rejuvenation

A candidate direction would address dermal fibroblast senescence (p53/MDM2), epidermal stem-cell renewal (p16/BMI-1), photo-oxidative defense (NRF2), epigenetic integrity (SIRT1), and replicative capacity (TERT), with localized delivery [126].

CROSS-AXIS SYNERGIES AND FEEDBACK ARCHITECTURE

The 16 axes do not operate in isolation; they form an interconnected network with cross-talk, feedback, and amplification. Understanding these synergies is relevant both for designing multi-axis experiments and for anticipating emergent properties of combinations. We describe four principal loops (Figure 4).

DDR–senescence–SASP amplification loop

DDR activation $\rightarrow$ p53/p21 stabilization $\rightarrow$ senescence $\rightarrow$ SASP $\rightarrow$ paracrine DDR activation in neighbors $\rightarrow$ expanded senescence. Intervention at any node (MDM2, BMI-1, SIRT1) would be predicted to dampen the loop;

Four principal feedback loops interlock through shared nodes (DDR, SIRT1) and converge on the senescence / inflammaging program; a gut–inflammaging input feeds the same pathway.

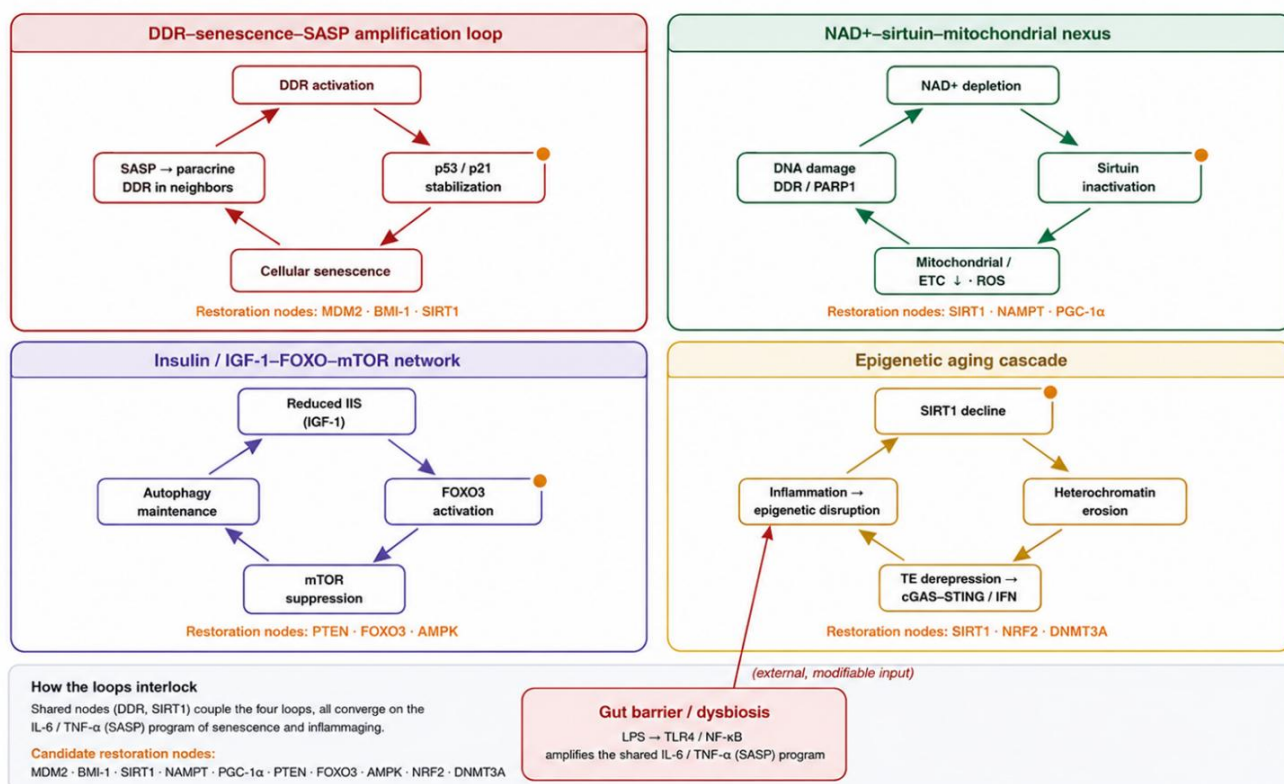


Figure 4. Cross-axis synergy and feedback architecture. Four principal feedback loops interlock through shared nodes and converge on the senescence/inflammaging program. Loop ① (DDR–senescence–SASP amplification): chronic DDR activation stabilizes p53 and p21, driving senescence and a senescence-associated secretory phenotype (SASP) that propagates paracrine DDR to neighboring cells; candidate restoration nodes are MDM2, BMI-1, and SIRT1. Loop ② (NAD⁺–sirtuin–mitochondrial nexus): NAD⁺ depletion inactivates sirtuins, impairing mitochondrial function and generating reactive oxygen species that feedback as DNA damage (SIRT1, NAMPT, PGC-1 α). Loop ③ (insulin/IGF-1–FOXO–mTOR network): reduced insulin/IGF-1 signaling activates FOXO3 and suppresses mTOR to sustain autophagy (PTEN, FOXO3, AMPK). Loop ④ (epigenetic aging cascade): SIRT1 decline and heterochromatin erosion derepress transposable elements and trigger cGAS–STING/interferon signaling (SIRT1, NRF2, DNMT3A). Shared nodes (DDR and SIRT1) couple the loops, which converge on the IL-6/TNF- α SASP program of senescence and inflammaging; a gut-barrier/dysbiosis input (LPS $\rightarrow$ TLR4/NF- κ B) acts as an external, modifiable amplifier of the same program. All candidate restoration nodes are illustrative and non-validated.

whether multi-node intervention is synergistic is an explicit, testable prediction rather than an established result [22, 127].

NAD⁺–sirtuin–mitochondrial nexus

NAD⁺ depletion inactivates sirtuins; sirtuin loss drives epigenetic erosion and reduced ETC efficiency; resulting ROS damages DNA, activating DDR and PARP1, which further consumes NAD⁺. Coordinated SIRT1 + NAMPT + PGC-1 α support is hypothesized to break this cycle at multiple points.

Insulin/IGF-1–FOXO–mTOR network

Reduced IIS extends lifespan across species via FOXO activation, mTOR suppression, and autophagy enhancement. Coordinated PTEN + FOXO3 + AMPK restoration is hypothesized to recapitulate features of the low-IIS state seen in long-lived individuals.

Epigenetic aging cascade

SIRT1 decline $\rightarrow$ heterochromatin erosion $\rightarrow$ transposable-element derepression $\rightarrow$ cGAS-STING activation $\rightarrow$ interferon response $\rightarrow$ inflammation $\rightarrow$ further epigenetic disruption [17, 18, 128–131]. Coordinated SIRT1 + NRF2 + DNMT3A support is hypothesized to address deacetylation fidelity, oxidative protection, and methylation maintenance together.

Dysbiosis and the gut–inflammaging input

Among the twelve hallmarks, dysbiosis-associated signaling links the network’s intracellular loops to an external, modifiable input: age-related loss of gut-barrier integrity increases translocation of microbial products (e.g., LPS), engaging TLR4/NF- κ B and amplifying the same IL-6/TNF- α program that constitutes the SASP [132]. The framework does not target the microbiome directly; the immune-rejuvenation direction would only partially attenuate this input, and fuller coverage would require complementary microbiome-directed strategies, identified as a future direction (the knowledge-gaps section).

FRAMEWORK SUMMARY TABLES

The following tables consolidate the field-organizing content of the framework: the 16 axes and their tiering (Table 3), representative preclinical evidence (Table 4), and positioning against existing geroscience interventions (Table 5) [133]. Illustrative diagnostic parameters, multi-axis construct compositions, and AAV vector/packaging specifications are provided in the Supplementary Information (Supplementary Tables 1–3) to keep the main text non-prescriptive.

Two further consolidated tables summarize the framework at a glance: a per-axis view of the Aging

Axis Profile, including biological rationale, evidence level, representative biomarkers, and proposed strategy (Table 2), and the framework’s relationship to existing conceptual models of aging (Table 1).

SAFETY AND ONCOGENESIS RISK MANAGEMENT

Several of the negative regulators proposed for restoration are bona fide proto-oncogenes, and their therapeutic overexpression would carry oncogenic risk that must be explicitly bounded. This section defines the principal hazards, the exposure limits that would govern every high-risk axis, and the engineered containment that clinical translation would require. We treat this residual oncogenic risk as the framework’s principal safety liability. The detailed construct-level specifications and placeholder diagnostic bands referenced here are confined to the Supplementary Information for the sole purpose of informing experimental design; none is a proposed clinical protocol. For the proto-oncogenic axes in particular (MDM2, BMI-1, NRF2), pre-defined expression envelopes, orthogonal suicide switches, CHIP gating, and long-term oncogenicity surveillance are treated as prerequisites to any consideration of these axes, not as afterthoughts.

Oncogenic potential of restored regulators

MDM2 is amplified in a meaningful fraction of human cancers and has p53-independent oncogenic activities; constitutive supraphysiological MDM2 could blunt legitimate p53 responses. BMI-1 is an established driver in leukemia and several solid tumors and is itself an oncology drug target, so unchecked overexpression is pro-tumorigenic. NRF2, though cytoprotective in normal tissue, supports chemoresistance and tumor-cell anabolism when constitutively activated in established malignancy. The framework therefore designates MDM2, BMI-1, and NRF2 as high-surveillance axes subject to the tightest expression control and the most conservative eligibility criteria.

The oncogenic case against unbounded restoration of this axis is particularly well documented. MDM2 and MDMX are established drivers whose amplification or overexpression inactivates wild-type p53 across breast carcinoma, retinoblastoma, melanoma, hepatoblastoma, and acute myeloid leukemia, and correlates with poor prognosis and therapeutic resistance [28, 29]. Restoring these regulators in otherwise healthy aged tissue therefore reproduces, at the molecular level, the very lesion these malignancies exploit; the manipulation is defensible only within a strictly bounded expression envelope and under the

Table 3. The 16 candidate tumor suppressor–negative regulator axes, by tier.

#	Axis (driver / suppressor)	Restored regulator / gene	Tier	Development stage
1	p53 / MDM2	MDM2 (restore p53 turnover)	1	Validated target; preclinical
2	p16INK4a / BMI-1	BMI-1	1	Validated target; preclinical
3	SIRT1 / DBC1	SIRT1 (+ NAMPT)	1	Validated target; preclinical
4	Telomerase / TRF1	TERT	1	Validated (AAV9-TERT mouse)
5	PTEN / PI3K-AKT-mTOR	PTEN	1	Validated (Super-PTEN mouse)
6	NRF2 / KEAP1	NRF2 Δ Neh2	1	Validated target; preclinical
7	FOXO3 / AKT	FOXO3-TM (nuclear)	2	Advanced preclinical
8	AMPK / mTORC1	AMPK α 1(T172D) / LKB1	2	Advanced preclinical
9	Klotho / FGF23	α -Klotho	2	Advanced preclinical
10	PGC-1 α / PARIS	PGC-1 α (+ miR-PARIS)	2	Advanced preclinical
11	TFEB / mTORC1	TFEB(S211A)	2	Advanced preclinical
12	Lamin A / Progerin	ZMPSTE24 (+ ASO)	2	Advanced preclinical
13	GDF11 / Myostatin	GDF11 + follistatin	3	Emerging (contested)
14	DNMT3A / TET2	DNMT3A (+ dCas9–TET2)	3	Emerging
15	CISD2 / BCL-2	CISD2	3	Emerging
16	α -Klotho / Wnt	α -Klotho + sFRP1/DKK1	3	Emerging

Representative serotype and promoter proposals for each axis are given in Supplementary Table 3; tissue-restricted and senescence-responsive promoters are specified for the high-surveillance axes (1, 2, 6) per the safety and oncogenesis section.

Table 4. Representative preclinical evidence for selected axes.

Axis / intervention	Model	Key effect	Effect size	Reference
TERT (AAV9-TERT)	Adult/old mice	Delayed aging, no $\uparrow$ cancer	+24% median (1-yr)	Bernardes de Jesus 2012
α -Klotho overexpression	Mice	Lifespan; vascular protection	+20–30%	Kuro-o 1997; Bian 2015
PTEN (Super-PTEN)	Mice	Improved metabolism, $\downarrow$ cancer	+12%	Ortega-Molina 2012
BMI-1 overexpression	Preclinical	Anti-aging, self-renewal	Qualitative	Bruggeman 2005
SIRT1 transgenic	Mice	Delayed aging features	Qualitative	Satoh 2013
p16+ cell clearance	INK-ATTAC mice	Delayed age disorders	Healthspan $\uparrow$	Baker 2011/2016
FOXO3-TM	Model organisms	Stress resistance, longevity	+15–30% (model)	Willcox 2008
Rapamycin (comparator)	Mice	Lifespan extension late in life	+ mouse lifespan	Harrison 2009
Partial reprogramming	Mice (RGC/tissues)	Youthful epigenome; vision	Functional	Lu 2020; Ocampo 2016
GDF11 (contested)	Mice (parabiosis)	Reported; not reproduced	Contested	Loffredo 2013; Harper 2016

Table 5. Comparison with existing geroscience interventions.

Intervention	Mechanism	Evidence	Durability	Key risks	Ref.
DDR-axis framework	Diagnosis-guided axis-specific restoration	Hypothesis / preclinical	Durable (AAV)	Oncogenesis, delivery, cost	This work
Senolytics (D+Q, fisetin)	Clearance of senescent cells	Early human trials	Intermittent	Off-target, transient	133, 134
Rapamycin / rapalogs	mTOR inhibition	Robust animal; human emerging	Chronic dosing	Immunosuppression, metabolic	65
NAD ⁺ boosters (NMN/NR)	Restore NAD ⁺ cofactor	Mixed clinical	Chronic dosing	Inconsistent efficacy	49
Metformin	AMPK / senotherapeutic	Epidemiologic; TAME pending	Chronic dosing	GI, B12 depletion	135
Partial reprogramming (OSK)	Epigenetic rejuvenation	Preclinical	Potentially durable	Teratoma, identity loss	20, 19

containment and surveillance requirements below. Conversely, oncology experience with pharmacologic inhibitors of this axis, where p53 reactivation produces mechanism-based hematologic toxicity in normal progenitors, shows how narrow the tolerance of normal tissue is to perturbation of p53 in either direction, and provides an empirical calibration point for the therapeutic-window analysis that follows [28].

Quantitative safety thresholds and the therapeutic window

The governing principle is restoration to youthful physiological set-points, not supraphysiological gain. For each high-risk axis a target expression envelope would be defined relative to the young-adult tissue mean, with a hard ceiling below the lowest expression associated with transformation in the relevant tissue; NRF2 restoration would target the young-adult range and be contraindicated where premalignant lesions or field cancerization are detected. Dose would be titrated using vector-genome ranging studies anchored to per-tissue expression readouts. Individuals with active malignancy, prior radiotherapy fields, or detectable CHIP clones would be excluded from the MDM2, BMI-1, and NRF2 axes. Formal dose-response and therapeutic-window modeling for these regulators is a prerequisite deliverable before any first-in-human dosing. (Illustrative numeric envelopes are given in Supplementary Table 3.)

Engineered safety switches and failure-mode analysis

All high-risk constructs would incorporate layered containment: tissue-restricted and senescence-responsive promoters; microRNA detargeting; drug-dependent expression control (riboswitch or destabilizing-domain systems); and an inducible suicide cassette (e.g., iCasp9/AP1903) enabling ablation of transduced cells if a neoplastic signal emerges. Because no single switch is fail-safe, the framework would require at least two orthogonal containment mechanisms per high-risk construct and a pre-specified failure-mode response: rising tumor-associated biomarkers or new imaging findings would trigger pharmacological transgene suppression, suicide-switch activation, and reliance on AAV non-integration so that expression wanes with cell turnover. These mitigations remain unvalidated in long-term primate studies and shape eligibility, monitoring, and endpoint design throughout.

Restoring physiological set-points without oncogenic overshoot

The framework's guiding principle, restoration to youthful physiological set-points rather than supra-

physiological gain, raises an obvious and still-unsolved question: how can the function of an intact tumor-suppressor or negative-regulator pathway be returned to normal with enough precision that cancer risk is not increased? We treat this precision-restoration problem as a central limitation, not a solved design detail.

Several features of the proposal are intended to help, though none is sufficient by itself. Because the targets are structurally intact endogenous genes, the therapeutic aim is to rebalance a dynamic circuit rather than to force a static level. Restoring MDM2 to re-establish the p53-MDM2 autoregulatory loop, for example, is intended to preserve the acute inducibility of p53 rather than to clamp it low. Restoration is bounded above by a hard expression ceiling set below the lowest level associated with transformation in the relevant tissue (the therapeutic-window subsection), and is delivered through tissue-restricted, senescence-responsive, and drug-regulatable expression systems so that output can be titrated toward a target window and withdrawn if that window is exceeded (the engineered safety-switch subsection).

Even so, decisive obstacles remain. Normal is a distribution rather than a point, and youthful set-points vary by tissue, sex, and ancestry, so the target itself must first be defined empirically (the biomarker-validation section). Current AAV expression is difficult to titrate precisely and cannot yet be closed-loop-controlled *in vivo*. Regulatable, feedback-controlled expression systems that sense a pathway's activity and adjust transgene output accordingly are, in our view, a prerequisite technology for any high-surveillance axis rather than an optional refinement. Until such control is demonstrated, the safest applications are axes for which the youthful range is well characterized and the consequences of modest overshoot are least dangerous. The proto-oncogenic axes (MDM2, BMI-1, NRF2) should remain gated behind the containment and surveillance requirements of the therapeutic-window subsection and the engineered safety-switch subsection, and, where precise restoration cannot be assured, restraint in advancing an axis is itself a safety decision.

Precision has three components, which the available toolbox addresses unequally. Spatial precision—restricting expression to the intended tissue and cell type—is comparatively mature: tissue- and cell-type-specific promoters and enhancers, synthetic and senescence-responsive promoters, engineered capsids and peptide-display retargeting for tropism, and microRNA detargeting to silence expression in off-target tissues are all established tools [134–136]. Quantitative and temporal precision—how much

regulator is expressed, and when—is only partially solved: small-molecule-inducible systems (for example tetracycline-, tamoxifen-, or dimerizer-regulated), riboswitches, and destabilizing-domain systems provide reversible on–off or coarse dose control, and vector-genome dose-ranging sets a population-level average, but none yet delivers a quantitatively predictable per-cell set-point [136]. Closed-loop control—sensing a pathway’s activity and auto-adjusting transgene output toward a physiological set-point—essentially does not exist *in vivo* and is the genuine technological gap for the high-surveillance axes. Representative construct-level choices for each axis are confined to Supplementary Table 3, consistent with the non-prescriptive intent of this review.

A natural existence proof for bounded restoration. That such a therapeutic window can exist in principle is illustrated by multilineage-differentiating stress-enduring (Muse) cells, endogenous pluripotent-like stem cells resident in mesenchymal tissues [137, 138]. Muse cells express core pluripotency factors and differentiate into all three germ layers from a single cell, yet they are non-tumorigenic and do not form teratomas, in contrast to embryonic and induced pluripotent stem cells expressing the same factors, and they exhibit low telomerase activity with a bias toward spontaneous, context-appropriate differentiation rather than unchecked proliferation [137]. They thus demonstrate that a cell can hold the expression of oncogenic-capable programs within a repair-competent but non-malignant range through endogenous restraint rather than engineered control, the very property that axis restoration seeks to reproduce.

Two caveats bound the analogy. Muse cells correspond most closely to the reprogramming and epigenetic modality (the comparative-positioning section) rather than to single-axis tumor-suppressor restoration, so the parallel holds at the level of principle rather than mechanism. In addition, the molecular basis of their restraint is incompletely defined, which makes its elucidation a research priority in its own right, since a defined natural restraint mechanism would directly inform the regulatable, feedback-controlled expression systems identified above as prerequisites for the high-surveillance axes.

Physiological roles of senescence and the risks of over-suppression

Senescence is not solely pathological, but its benefits are a property of a regulated program rather than of the senescent state itself. Under homeostatic conditions a persistent senescent cell confers little benefit on its neighbors, and through chronic low-grade SASP

signaling it may impose a net liability. Its documented advantages are transient and context-specific: developmental patterning [139], wound healing, and the limitation of fibrosis, in each case delivered as a bounded deployment whose function is coupled to the cell’s subsequent removal. This reframes the relevant biology: the useful unit is not the senescent cell but its controlled induction, function, and clearance.

Wound healing is the clearest model of this control loop. Senescent fibroblasts and endothelial cells are induced at the wound, where they accelerate closure through PDGF-AA secretion [140] and, through CCN1-driven fibroblast senescence, switch to an antifibrotic secretome that restrains scarring [141]. The same logic limits fibrosis in other organs, as when senescent hepatic stellate cells curb liver fibrosis before being cleared by natural killer cells [142]. Crucially, the benefit depends on timing and disposal: a transient pulse of senescence promotes cellular plasticity and regeneration, whereas prolonged exposure converts the identical signal into paracrine senescence arrest [143]. Utilization and clearance are two halves of one program, and the senescent cell has value chiefly in the act of being spent.

Two opposite failures define the hazard. When senescence is not induced, damaged fibroblasts continue to proliferate and deposit matrix, producing excess scar and disorganized repair. When senescence is induced but not cleared, the cells persist, their SASP becomes chronic, and they act as toxic neighbors that propagate senescence and drive inflammaging, the tissue-level equivalent of a chronic, non-healing wound [144]. Aging resembles the second failure more than the first: induction remains active while resolution fails, so a normally self-terminating program becomes permanent. On this reading, restoring the clearance step is a first-class therapeutic target rather than an afterthought.

This reframing sharpens the therapeutic logic and argues against indiscriminate action. Because senescent cells carry no standing benefit, only two coherent strategies exist: senolytic removal, or reconstruction of the induce-function-clear cycle so that any transient benefit is realized and then withdrawn. The framework pursues the second. It intends to restore the dynamic, self-terminating regulation of these pathways rather than to abolish them, preserving inducibility in response to genuine oncogenic or wound signals; it gates intervention to axes and tissues scored as dysfunctional rather than merely declining (the diagnostic-framework section); and it excludes individuals with active malignancy, premalignant lesions, or detectable CHIP (the therapeutic-window subsection). The net effect of any axis intervention on beneficial senescence, and on

the clearance step in particular, is unknown and must be a pre-specified safety endpoint in the preclinical program of the intervention-testing subsection, with explicit assessment of wound healing, tissue repair, and tumor surveillance.

DELIVERY CONSIDERATIONS: AAV CAPACITY, IMMUNOGENICITY, AND MANUFACTURING

Transgene size and the 4.7 kb packaging limit

AAV has a practical packaging capacity of roughly 4.7 kb inclusive of ITRs, promoter, transgene, and polyadenylation signal. Several multi-axis constructs exceed this ceiling once regulatory elements are counted. The framework therefore specifies, for every direction, one of three solutions: (a) single-transgene vectors co-administered as a cocktail; (b) dual-AAV systems using split-intein trans-splicing or overlapping-homology reconstitution; or (c) lipid-nanoparticle (LNP) mRNA for large or transient payloads, accepting repeat dosing in exchange for effectively unlimited cargo and non-integration. Coding sizes, an explicit oversize flag for each construct, and the designated delivery solution are enumerated in Supplementary Table 3 [134, 135, 145].

Pre-existing immunity and immunogenicity

Pre-existing neutralizing antibodies against common AAV serotypes are present in a substantial fraction of adults, and capsid-directed T-cell responses can curtail durability. Because a geroscience population is older and frequently seropositive, the framework incorporates pre-treatment NAb screening, serotype selection or capsid engineering, transient peri-dosing immunomodulation, and serotype switching or mRNA-LNP delivery for re-administration. Where pre-existing titers would otherwise preclude dosing, reported strategies for transiently lowering circulating anti-capsid antibodies to open a delivery window include IgG-cleaving endopeptidases such as imlifidase (IdeS) [146], therapeutic plasma exchange (plasmapheresis) combined with B-cell-depleting or T-cell immunosuppression [147], and serotype-specific antibody removal by immuno-adsorption or hemapheresis [148]. A distinct risk is immunity against the transgene product itself when a restored regulator is expressed at levels or in tissues that breach tolerance; this is a further reason to restrict expression to physiological set-points [149]. Transgene-directed immunity can be further limited at the cassette level by confining expression to target tissues with cell-type-specific promoters and by de-targeting antigen-presenting cells with microRNA target sequences (e.g., miR-142), reducing the immune visibility of the transgene [150].

Manufacturing and CMC scalability

A 16-axis platform raises chemistry, manufacturing, and controls demands beyond a single gene therapy, since each axis is, in principle, a distinct AAV product. The framework proposes a modular strategy—shared ITRs, a small set of validated capsids from common cell banks, and interchangeable cassettes swapped into a qualified backbone—so that effort concentrates on cassette-specific attributes; multi-axis directions would be formulated from individually released single-transgene lots, with LNP-mRNA produced on a separate platform. Early development would proceed through CDMOs, with dedicated manufacturing reserved for lead axes reaching pivotal trials. The comparability and cost burden of many parallel products remains a material development consideration (the knowledge-gaps section).

Why gene therapy? rationale, advantages, and limitations of the AAV-based approach

We have presented gene therapy, and AAV in particular, as the illustrative delivery modality for axis restoration. The choice warrants explicit justification, because it is a hypothesis about the best-matched technology rather than a settled conclusion. The rationale is that the framework's targets are endogenous regulators whose expression must be modulated, durably and in a tissue-restricted manner, to counter a chronic process, and that requirement maps naturally onto gene delivery.

The advantages are concrete. AAV can provide sustained, single-administration expression that matches the chronic nature of aging, avoiding the daily-dosing burden of small molecules. It can restrict expression to relevant tissues through capsid tropism and cell-type-specific promoters. It can express payloads such as constitutively active or dominant-negative variants, deacetylases, and transcription factors that small molecules cannot readily mimic. It remains predominantly episomal, which reduces, though it does not eliminate, insertional-mutagenesis risk relative to integrating vectors. And it is a clinically validated modality, with several approved AAV products establishing manufacturing and regulatory precedent [132, 134, 135, 145, 149].

The limitations are equally concrete and, for an aging indication, severe. The approximately 4.7 kb packaging limit constrains construct design and multi-axis payloads (the packaging-limit subsection). Pre-existing and dose-induced anti-capsid immunity restricts the redosing that a durable but not permanent intervention would require (the pre-existing-immunity subsection,

which also outlines strategies to mitigate this). Expression, once delivered, is difficult to titrate or reverse, which is precisely why the layered safety switches set out earlier are mandatory. Biodistribution is imperfectly controlled, and per-patient cost is currently very high (the cost-and-accessibility subsection).

For these reasons the framework does not treat AAV as the only or final modality. Lipid-nanoparticle mRNA offers tunable, non-integrating, redosable expression for large or transient payloads, at the cost of durability. Engineered next-generation capsids and non-viral delivery may relax the immunity and targeting constraints. Small molecules remain appropriate where a druggable node and episodic action suffice. The proper reading is that gene therapy is the best-matched current tool for durable, tissue-restricted restoration of endogenous regulators, not that it is uniquely correct, and the delivery modality for each axis should be selected empirically against the constraints above.

Clinical cost, scalability, and patient accessibility

Translational feasibility depends not only on efficacy and safety but on cost, scalability, and access, and here the framework faces its steepest practical challenges. Approved one-time AAV gene therapies are currently among the most expensive medicines ever launched, with list prices ranging from roughly one to more than three million US dollars per patient. A framework that contemplates multiple axis-specific products, potentially in combination and tailored to an individual profile, would compound this cost unless manufacturing economics change substantially.

Scalability is constrained by manufacturing. Producing many distinct AAV products at population scale strains vector-production capacity, comparability testing, and quality control. The modular, shared-backbone strategy proposed in the manufacturing and CMC subsection is intended to mitigate this burden but cannot eliminate it. An aging indication, by definition addressing a very large population rather than a rare-disease cohort, magnifies the problem relative to current gene-therapy precedents.

Accessibility and equity follow directly. At present costs, axis-restoration therapy would be available only to the wealthiest, raising the prospect that a putative anti-aging intervention could widen rather than narrow health disparities. We regard cost reduction, through platform manufacturing, improved yields, and, where adequate, lower-cost modalities such as mRNA-LNP or small molecules, as a translational objective on par with efficacy and safety. Diagnosis-guided targeting, by restricting intervention to genuinely dysfunctional axes,

is intended in part to avoid the cost and risk of untargeted treatment. These realities reinforce that any near-term value of the framework lies in research rather than clinical deployment.

COMPARATIVE POSITIONING AMONG GEROSCIENCE INTERVENTIONS

The framework should be judged against leading geroscience modalities rather than in isolation (Table 5). Senolytics clear senescent cells episodically and have entered early human trials, but they remove cells rather than restoring pathways and show mixed durability. Pharmacological mTOR inhibition is among the most reproducible lifespan-extending interventions in mice yet is constrained in humans by immunosuppressive and metabolic effects; the framework instead proposes to modulate the mTOR network at the node level (PTEN, AMPK, FOXO3) with tissue restriction. NAD⁺ boosters raise a key cofactor but have produced inconsistent clinical endpoints and are treated here as adjunctive to SIRT1 restoration. Partial epigenetic reprogramming is the most mechanistically ambitious comparator and can reverse epigenetic age but carries teratoma and identity-loss risks that promoter-restricted, single-axis restoration is designed to avoid [72, 151–153].

The framework's differentiator is diagnosis-guided, axis-specific restoration to physiological set-points; its principal disadvantages—greater manufacturing complexity, the delivery constraints above, and the oncogenic-surveillance burden—are the explicit costs of that specificity. In several scenarios the alternatives may be preferable: where rapid, low-cost, episodic benefit is the goal, senolytics or mTOR modulation are simpler and already in human testing; where broad epigenetic rejuvenation is sought, partial reprogramming is mechanistically more comprehensive; and in frail or heavily immunized older adults, the immunogenicity and re-dosing limits of AAV may make axis-restoration impractical relative to small-molecule or systemic-factor strategies. Strategies to mitigate pre-existing immunity are discussed in the pre-existing-immunity subsection, though they do not fully remove this constraint. The framework is therefore best viewed as complementary to—not a replacement for—these modalities.

Multilineage-differentiating stress-enduring (Muse) cells sharpen this comparison from the opposite direction. They represent an endogenous state of multilineage plasticity that is nonetheless non-tumorigenic and teratoma-free [137, 138], indicating that the tumorigenic and identity-loss risks associated with reprogramming-based rejuvenation are not inevitable. If the restraint mechanisms that keep Muse-cell plasticity

non-malignant can be defined, they could inform safer design for both reprogramming approaches and the axis-restoration strategy proposed here (the set-point restoration subsection).

BIOMARKER VALIDATION AND ENDPOINT STRATEGY

The Aging Axis Profile would be held to the evidentiary standard required of any clinical diagnostic, proceeding through analytical validation (accuracy, precision, limits of detection, reproducibility), clinical validation (association of each axis readout with the intended physiological state across independent cohorts), and demonstration of clinical utility (evidence that acting on the profile changes outcomes). Epigenetic clocks merit specific caution as trial endpoints: although first- and second-generation clocks and pace-of-aging measures correlate with morbidity and mortality, they are correlational, sensitive to cell-type composition and pre-analytical variables, not qualified by regulators as validated surrogate endpoints, and can shift without corresponding functional benefit. The framework therefore pairs molecular surrogates with functional and clinical endpoints and treats clock movement as supportive rather than primary evidence until formal qualification is achieved.

Specific discordances underscore this caution. Different clocks disagree with one another and are differentially sensitive to cell-type composition, and methylation age can be moved by an intervention without a demonstrated change in function or disease incidence—for example, transient reprogramming and some *in vitro* protocols reset methylation-based age [19, 20] while the durability and clinical meaning of the change remain unproven [21, 112]. Pace-of-aging measures such as DunedinPACE behave differently again from cross-sectional clocks [32]. Any clock movement is therefore treated as a hypothesis-generating signal requiring orthogonal molecular, functional, and clinical corroboration before it is credited as evidence of biological-age reversal.

FALSIFIABILITY AND A PRIORITIZED RESEARCH AGENDA

Because the central claim of this review is a causal-ordering hypothesis—that DDR overactivation is an integrating node broadly upstream of senescence and inflammation—it must be stated in a form that competing models could defeat. This section sets out the predictions that distinguish a DDR-integration account from the mitochondria-first, proteostasis-first, and epigenetic-drift alternatives, the observations that would refute or demote the hypothesis, and a ranked set

of experiments capable of adjudicating between them. We regard this as the most important section of the review: the framework earns its place only insofar as it generates discriminating, falsifiable tests.

Discriminating predictions

Temporal precedence

If DDR overactivation is upstream, then in longitudinal, tissue-resolved human and animal cohorts, durable rises in DDR markers (persistent γ H2AX/53BP1 foci, sustained ATM/ATR signaling) should, on average, precede the onset of bioenergetic decline and proteostatic collapse in the same cells or tissues. A mitochondria-first or proteostasis-first ordering predicts the reverse sequence.

Intervention asymmetry

If DDR overactivation is upstream, then attenuating chronic DDR signaling (including via the negative-regulator restoration proposed here) should blunt downstream senescence, SASP, and inflammation even when mitochondrial or proteostatic status is held constant. Conversely, if mitochondria-first is correct, bioenergetic rescue should reduce DDR signaling more than DDR-axis restoration reduces mitochondrial dysfunction. The relative magnitude of these cross-effects is directly measurable in matched preclinical designs.

Mediation

In causal-mediation analyses applied to existing and new multi-omic datasets, the effect of canonical upstream lesions (telomere attrition, transposable-element derepression, oxidative load) on aging phenotypes should be substantially mediated through DDR/p53 nodes if the hypothesis holds, and only weakly so if the DDR is a parallel readout.

Reversibility dissociation

If aging phenotypes can be durably reversed by epigenetic reprogramming without normalization of DDR signaling [20, 21], then DDR overactivation is, at most, one of several sufficient drivers and cannot be the final common cause; the framework would then be demoted from “integrating node” to “one tractable axis among several.”

Refutation criteria

The DDR-integration hypothesis should be considered refuted, or materially demoted, if any of the following are robustly established across tissues and models: (1) DDR markers consistently lag, rather than lead, mitochondrial and proteostatic markers in longitudinal data; (2) restoration of the proposed negative

regulators corrects axis biomarkers without producing functional or histological benefit; (3) interventions that reverse aging phenotypes do so without reducing DDR signaling, while DDR-directed interventions fail to move the same phenotypes; or (4) formal mediation analyses assign the DDR a small share of the causal effect of upstream lesions. We commit to these criteria in advance to avoid the post hoc reinterpretation that weakens many integrative theories of aging.

Prioritized experiments

1. Longitudinal, tissue-resolved human multi-omic cohort. Repeated sampling with DDR-resolved markers alongside mitochondrial, proteostatic, and epigenetic readouts, powered for the temporal precedence and mediation tests above. This is the single most informative and most resource-intensive study, and its design should precede any therapeutic program.

2. Head-to-head preclinical comparison of upstream rescue. In shared models and endpoints, compare single-axis DDR restoration against mitochondrial and proteostatic rescue to quantify the intervention-asymmetry prediction. This is the most decisive near-term experiment and is feasible with existing tools.

3. Causal-mediation reanalysis of existing datasets. Apply mediation methods to published longitudinal multi-omic and reprogramming datasets to obtain an early, low-cost estimate of the DDR's mediating share before new cohorts mature.

4. Analytical and clinical validation of the Aging Axis Profile. Establish accuracy, precision, and youthful, sex- and ancestry-stratified reference ranges for the highest-value axes, converting the illustrative parameters of Supplementary Table 1 into qualified measurements.

5. Safety and therapeutic-window modeling for oncogenic regulators. Dose-response and containment studies for MDM2, BMI-1, and NRF2, including suicide-switch performance and long-term oncogenicity surveillance, as the gating deliverable for any first-in-human consideration.

Experiments 2 and 3 can begin immediately and would, on their own, materially raise or lower confidence in the hypothesis; experiment 1 is the definitive arbiter; experiments 4 and 5 are prerequisites for any translational step and are deliberately placed downstream of the causal-ordering tests.

From hypothesis tests to intervention tests: preclinical requirements before human application

The experiments of the prioritized-experiments subsection are designed to test the DDR-integration hypothesis. A separate and equally demanding body of work would be required to test the interventions themselves before any human application, and we set it out here to avoid conflating mechanistic validation with therapeutic readiness.

For single-axis interventions, the minimum preclinical program includes biodistribution and durability studies for each vector and promoter; dose-ranging against the youthful expression window (the therapeutic-window subsection); demonstration of on-target efficacy in aged, human-relevant models rather than in progeroid or knockout systems alone; and long-term oncogenicity surveillance, with validated performance of the suicide switches and expression-control systems on which safety depends (the engineered safety-switch subsection). For the telomerase and proto-oncogenic axes, species-appropriate models are essential, given the telomere-biology differences noted in the Telomerase/TRF1 axis.

Combination interventions raise a further and steeper barrier. Because the axes interact through the cross-axis feedback architecture, simultaneous modulation of several pathways is likely to produce non-additive and potentially unforeseen effects and cannot be assumed to equal the sum of its parts. Establishing safety and efficacy for combinations would require factorial preclinical designs that test axes individually and in defined groupings; explicit study of sequence and timing, that is, simultaneous versus staged intervention; dose de-escalation to account for interaction; and extended observation for emergent toxicity, all in appropriately aged large-animal models before any human exposure.

These requirements are substantial, and we consider them prerequisites rather than parallel activities. Single-axis safety and window definition precede combination testing, which in turn precedes any first-in-human consideration (the translational-pathway section). The scale of this program is itself an argument for the diagnosis-first, single-axis-first posture the framework adopts.

LIMITATIONS

This work is a hypothesis-generating synthesis, and most proposed interventions remain preclinical or theoretical; the framework has not been tested as an

integrated system in humans, and individual axes vary widely in supporting evidence. Aging is multi-causal, and the DDR-overactivation thesis is best understood as identifying a candidate dominant, druggable driver rather than a sole cause.

Clonal hematopoiesis of indeterminate potential (CHIP)—somatic DNMT3A, TET2, and ASXL1 mutations in 10–20% of individuals over 70—causally promotes inflammaging and cardiovascular disease and is a genuine counterexample to a strictly mutation-independent model; the framework addresses this by reframing somatic mutation as an accelerating cofactor, screening for CHIP before deploying the DNMT3A/TET2 axis or any high-risk oncogenic regulator, and managing CHIP-positive individuals under a distinct, mutation-aware approach.

We make the principal risks explicit. (1) Oncogenic risk: several proposed interventions restore or overexpress proto-oncogenic regulators (MDM2, BMI-1, NRF2) in otherwise healthy individuals, where even a small absolute increase in cancer risk may be unacceptable; this is the central safety liability and motivates the engineered switches and surveillance above. (2) Immunological barriers: pre-existing anti-AAV immunity and the response to a first dose constrain the repeat administration an aging indication may require. (3) Heterogeneity: aging trajectories differ markedly between individuals, so a DDR-centric intervention may benefit only certain molecular sub-phenotypes—axis selection and stratification, not a universal protocol, are essential. (4) Ethical and regulatory status: gene therapy directed at aging rather than a defined disease raises distinct risk–benefit, consent, equity, and regulatory questions, since aging is not currently a recognized indication and long-term follow-up obligations are substantial.

The GDF11 axis rests on contested evidence and is retained only at Tier 3 with explicit caveats, and sex-specific differences in axis dysfunction are not yet incorporated into axis status calls. Additional constraints include the long-term follow-up burden of gene-therapy regulation, the delivery limitations above, and the cost and accessibility challenges intrinsic to multi-vector personalized gene therapy. Finally, the framework may simply be wrong in its emphasis: if mitochondrial dysfunction or systemic metabolic derangement proves dominant and only partially mediated by the DDR, targeting DDR-centric axes may have limited impact on the key phenotypes of aging. The falsifiability tests in the research-agenda section are intended precisely to detect this case.

Sex-dimorphic aging trajectories

Aging is sex-dimorphic, and the framework’s diagnostic and therapeutic logic must reflect this. In women, the menopausal transition produces an abrupt, coordinated acceleration across multiple axes: estrogen withdrawal lowers NRF2 tone and mitochondrial efficiency, coincides with accelerated telomere attrition and a pro-inflammatory immune shift, and disrupts the Klotho/FGF23–vitamin D–mineral axis governing bone and vascular aging. In men, the more gradual decline of testosterone preferentially affects musculoskeletal and metabolic axes (myostatin/GDF11 balance, PGC-1 α , AMPK–insulin sensitivity). X-linked dosage of several immune- and longevity-associated genes and documented sex differences in immunosenescence further shape these trajectories.

The framework accommodates sex-dimorphism by using sex-stratified reference ranges; by making axis prioritization sex- and life-stage-aware (peri- and post-menopausal women prioritized for NRF2, telomerase, Klotho, and immune-rejuvenation axes, with explicit coordination with hormone-therapy decisions; musculoskeletal and metabolic axes taking precedence in aging men); and by recognizing the menopausal transition as a defined window for pre-emptive, diagnostically guided study. Formal sex-stratified reference ranges and sex-specific outcome studies are required to validate these distinctions (the knowledge-gaps section).

Inter-individual heterogeneity: genetic, lifestyle, environmental, and comorbidity factors

Beyond the sex-dimorphism discussed above, aging trajectories vary widely between individuals, and this variability bears directly on how an Aging Axis Profile would be interpreted and prioritized. Genetic background shapes baseline axis function. FOXO3 and Klotho longevity-associated variants, APOE genotype, and polymorphisms in DNA-damage-response and antioxidant-response genes are among the determinants, so that youthful reference ranges and intervention thresholds may need to be genotype-aware rather than universal.

Lifestyle and environment modulate the same axes through partly modifiable routes. Physical activity, diet and caloric intake, smoking, sleep, and psychosocial stress, together with exposures such as ultraviolet radiation, air pollution, and other genotoxics, influence DDR tone, oxidative burden, senescent-cell accumulation, and epigenetic-clock measures. Recent organ-clock analyses show that diet, lifestyle, and medications measurably shift organ-specific aging rates

[38]. A profile is therefore a snapshot of a modifiable trajectory, and low-risk lifestyle and environmental modification should logically precede any consideration of gene-therapy intervention.

Comorbidities further complicate prioritization. Age-related diseases, including cardiovascular disease, type 2 diabetes, chronic kidney disease, neuro-degeneration, and cancer or a history of its treatment, both alter axis readouts and constrain which interventions are safe, most obviously through the oncogenic-risk and CHIP gating of the safety and oncogenesis section. Realistic implementation would thus require integrating genetic, lifestyle, environmental, and comorbidity data with the molecular profile. This multi-dimensional stratification problem is well suited to the machine-learning approaches of the AI and multi-omics subsection, and it further argues against anyone-size-fits-all protocol.

KNOWLEDGE GAPS AND FUTURE DIRECTIONS

Beyond the causal-ordering tests of the research-agenda section, several questions are genuinely unresolved and define the framework's broader research agenda. The optimal composition, sequence, and timing of multi-axis intervention remain unknown: whether axes should be addressed simultaneously or in a defined order, and at what life stage. So do the durability of restoration and the corresponding re-dosing or re-induction strategy.

Integration of additional hallmark inputs, in particular the microbiome and the metabolomic and proteostasis networks, is incomplete. Sex- and ancestry-stratified reference ranges must be generated empirically before the Aging Axis Profile can be deployed broadly.

Finally, the field lacks regulator-qualified surrogate endpoints for biological aging. Advancing endpoint qualification, and testing whether axis restoration can be layered with, or eventually superseded by, partial epigenetic reprogramming, are priorities that will determine how far a diagnose-and-restore paradigm can go.

A POSSIBLE TRANSLATIONAL PATHWAY

Translating a diagnose-and-restore framework of this kind would proceed through the generic stages common to any gene-therapy program rather than a fixed timetable, and only after the causal-ordering questions of the research-agenda section have been addressed (Figure 5). Proof-of-concept work would first need to

establish, in appropriate model systems, that restoring a given negative-regulator axis produces durable, on-target physiological benefit without loss of tumor-suppressor surveillance. In parallel, the Aging Axis Profile would require analytical and clinical validation, including youthful reference ranges across diverse populations; the thresholds discussed earlier are illustrative and would have to be empirically derived before any clinical use.

Subsequent stages would mirror established gene-therapy development: biodistribution and safety characterization, carefully bounded first-in-human studies of single axes in well-stratified participants, and only then any consideration of multi-axis combinations—each step contingent on the preceding one and on the safety and delivery constraints above. Because aging is not presently a recognized regulatory indication and validated surrogate endpoints do not yet exist, the regulatory path remains undefined; the sequence outlined here is a high-level possibility, not a development plan.

Long-term follow-up

Because AAV vectors persist in transduced cells, FDA guidance recommends long-term follow-up for delayed adverse events—extended observation for persistent vectors and up to 15 years for integrating vectors [154]. Any clinical translation would therefore entail prolonged pharmacovigilance, including periodic vector-shedding and integration-site monitoring and oncogenicity surveillance, extending the realistic timeline well beyond conventional pharmaceutical programs.

CONCLUSIONS

In this review we have advanced a deliberately bounded thesis: that chronic, largely mutation-independent DDR overactivation is a major, but not exclusive, integrating driver of aging, acting through dysregulation of structurally intact tumor-suppressor networks. Within this frame we propose a tumor suppressor–negative regulator axis architecture and an illustrative Aging Axis Profile as tools to organize mechanistic data and translational thinking, not as ready-made clinical instruments.

Three features distinguish this framework from existing hallmarks-based syntheses. It sharpens the contrast between aging and cancer by emphasizing intact but overdriven tumor-suppressor pathways and the resulting bias toward restoration rather than replacement—understood as restoring the dynamic, self-terminating regulation of these intact pathways, including the induction–function–clearance cycle of senescence, rather than abolishing them; it links DDR signaling

explicitly to mitochondria-first, proteostasis-first, and epigenetic-drift/reprogramming accounts and, in the research-agenda section, specifies the causal-ordering and mediation tests that could validate, demote, or refute a DDR-integrator role; and it translates these ideas into per-axis, falsifiable signatures while keeping all numeric thresholds and vector designs quarantined in the Supplementary Information as non-validated, preclinical scaffolds.

The limitations are equally clear and are detailed in the Limitations section: most axes rest on rodent or *in vitro* evidence, integrated human datasets with DDR-resolved longitudinal phenotyping do not yet exist, the Aging Axis Profile lacks analytical and clinical validation, and restoring proto-oncogenic regulators would face stringent safety and dosing-window hurdles. We therefore suggest that the framework's primary value, at present, lies in research design: it

provides tractable, testable hypotheses and points to the biomarker panels, experimental comparators, and safety questions that should be prioritized before any consideration of human intervention. If subsequent work shows that restoring selected negative regulators can durably improve aging phenotypes while satisfying the refutation criteria set out here, DDR overactivation may justifiably be regarded as a central, druggable node in human aging; if not, the same studies will have clarified its place among the broader constellation of aging mechanisms. Either way, we offer this axis-based framing—what might be called aging-axis geroscience—as a lens for organizing which of these mechanisms are measurable, and potentially rebalanced toward youthful set-points, in a given individual.

16 Axes • 6 Multi-Axis Research Directions • 12 Hallmarks Addressed • 3 Evidence Tiers

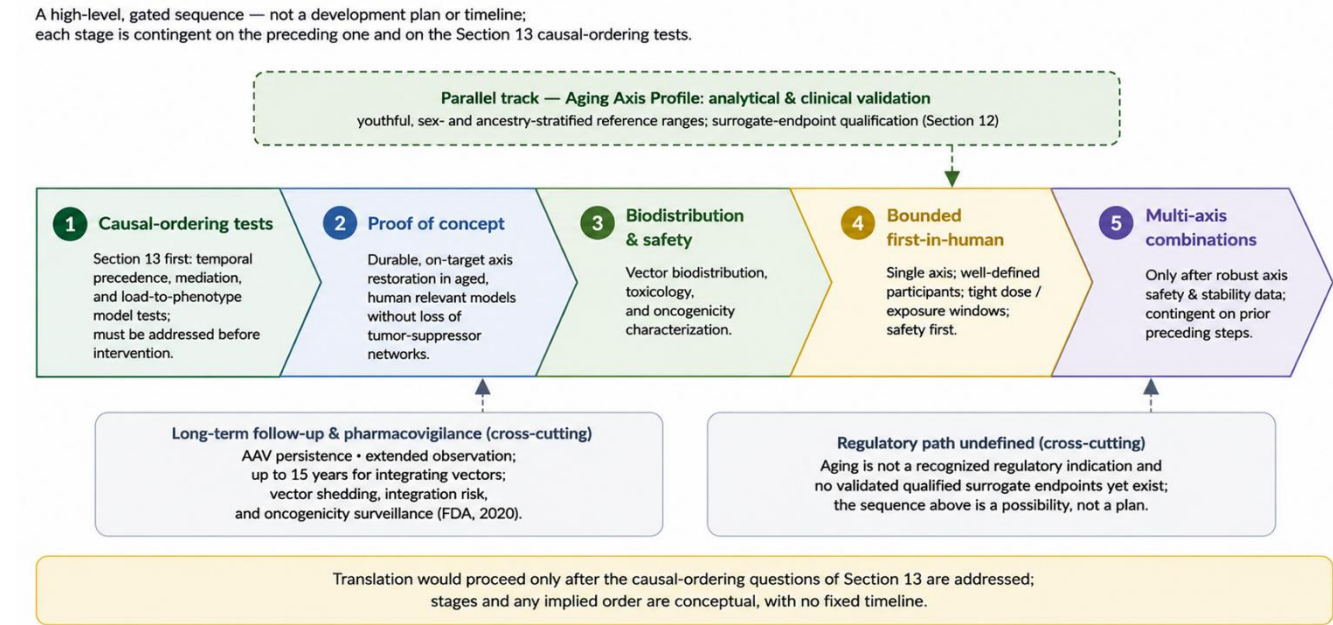


Figure 5. A high-level, illustrative schematic of a possible translational pathway. The pathway is a gated sequence — not a development plan or timeline — in which each stage is contingent on the preceding one and on the causal-ordering tests of the research-agenda section. Stage 1, causal-ordering tests (temporal-precedence, mediation, and load-to-phenotype tests), must be addressed before any intervention; Stage 2, proof of concept, requires durable, on-target axis restoration in aged, human-relevant models without loss of tumor-suppressor surveillance; Stage 3 covers vector biodistribution, toxicology, and oncogenicity characterization; Stage 4, bounded first-in-human studies, treats single axes in well-stratified participants under tight dose and expression envelopes; and Stage 5, multi-axis combinations, is considered only after robust single-axis safety and stability data and remains contingent on the preceding steps. In parallel, the Aging Axis Profile undergoes analytical and clinical validation, including youthful, sex- and ancestry-stratified reference ranges and surrogate-endpoint qualification. Two cross-cutting requirements span the pathway: long-term follow-up and pharmacovigilance (AAV persistence may require extended observation, up to ~15 years for integrating vectors, with vector-shedding, integration-site, and oncogenicity surveillance) and an as-yet-undefined regulatory path, since aging is not a recognized regulatory indication and no qualified surrogate endpoints yet exist. All stages and any implied order are conceptual, with no fixed timetable — a possibility, not a plan.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work the author used generative AI-based tools to assist with manuscript formatting, reference styling, figure drafting, and language editing. After using these tools, the author reviewed and edited the content as needed and takes full responsibility for the content of the published article.

Data availability

No new datasets were generated or analyzed in this work; it is a hypothesis-generating review based on previously published studies, all of which are cited.

AUTHOR CONTRIBUTIONS

Patrick E. Sewell: Conceptualization, Methodology, Investigation, Visualization, Writing – original draft, Writing – review and editing.

CONFLICTS OF INTEREST

The author declares the following financial interests/personal relationships which may be considered as potential competing interests: P.E.S. is the founder, Chief Executive Officer, and Chief Scientist of Triple Helix Science, Inc., a privately held gene-therapy and regenerative-medicine company developing technologies related to those described in this manuscript, and holds a financial interest in the company. In recognition of this interest, the manuscript has been written to give competing causal models their strongest form, to present all diagnostic and therapeutic content as explicitly conceptual and confined to illustrative status, and to specify in advance the observations that would refute the central hypothesis (the research-agenda section).

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SUPPLEMENTARY MATERIALS

Supplementary Tables

Supplementary Table 1. Illustrative per-axis diagnostic parameters for the Aging Axis Profile (all 16 axes).

Axis (suppressor / regulator)	Primary analytes and assays	Representative secondary analytes
1 p53 / MDM2	Serum p53 (ELISA); phospho-p53(Ser15) in PBMCs (flow cytometry); p53–MDM2 complex ratio (proximity ligation)	p21 (IHC, skin); BAX/BCL-2; PUMA/NOXA; clonogenic capacity (functional readout)
2 p16INK4a / BMI-1	p16INK4a mRNA in peripheral-blood T cells (RT-qPCR); p16INK4a protein (IHC, skin); BMI-1/p16 ratio	SA- β -gal-positive cells; H2AK119ub at CDKN2A (ChIP-qPCR); CDK4/6 activity
3 SIRT1 / DBC1	SIRT1 activity in PBMCs (fluorometric deacetylation); NAD ⁺ /NADH (mass spectrometry); DBC1 level and SIRT1–DBC1 co-IP ratio	acetyl-p53(K382); acetyl-NF- κ B/p65(K310); H3K9ac/H3K9me3; GrimAge residual
4 Telomerase / TRF1	Mean telomere length (Q-FISH/Flow-FISH); critically short fraction (STELA); telomerase activity (TRAP)	TRF1 level; telomere dysfunction-induced foci (TIFs); shelterin integrity
5 PTEN / PI3K-AKT-mTOR	PTEN protein and phosphatase activity; phospho-AKT(Ser473)/total AKT; phospho-S6K1(Thr389); fasting insulin / HOMA-IR	PTEN promoter methylation; phospho-4E-BP1; LC3-II/I; FOXO3 localization
6 NRF2 / KEAP1	NRF2 target panel (NQO1, HMOX1, GCLC, GCLM, TXNRD1); GSH/GSSG; 8-OHdG; NRF2 nuclear localization	KEAP1 level; Bach1/NRF2 ratio; protein carbonyls; F2-isoprostanes; 4-HNE adducts
7 FOXO3 / AKT	FOXO3 nuclear localization and phosphorylation status	AKT-pathway activity; FOXO3 target transcripts
8 AMPK / mTORC1	AMPK activity; phospho-ACC	phospho-S6K1; autophagy markers (LC3-II/I)
9 Klotho / FGF23	Serum α -Klotho	FGF23; vitamin D / mineral panel
10 PGC-1 α / PARIS	PGC-1 α level; mitochondrial density	PARIS (ZNF746); mtDNA copy number; OXPHOS markers
11 TFEB / mTORC1	TFEB nuclear localization; LC3-II/I	lysosomal (CLEAR-network) transcripts; p62/SQSTM1
12 Lamin A / Progerin	Progerin level; nuclear morphology	ZMPSTE24 activity; prelamin A
13 GDF11 / Myostatin	GDF11 / myostatin balance (specific LC-MS/MS)	folliculin; muscle-regeneration markers
14 DNMT3A / TET2	Methylation clocks; locus-specific promoter methylation; CHIP status	5mC/5hmC balance; DNMT3A/TET2 variant allele fraction
15 CISD2 / BCL-2	CISD2 level	ER–mitochondrial Ca ²⁺ transfer; mitochondrial-integrity markers
16 α -Klotho / Wnt	α -Klotho; Wnt target activity (e.g., AXIN2)	sFRP1 / DKK1; stem-cell-compartment markers

All analytes are illustrative and non-validated. Axis status is assigned on a deliberately qualitative three-state scheme — functional (within youthful reference range), declining (measurable deviation from youthful homeostasis), and dysfunctional (marked departure with functional consequences) — because no validated prognostic numeric thresholds yet exist (main text, the relevant section). Any numeric bands would be conceptual placeholders requiring empirical derivation and prospective qualification (the biomarker-validation section).

Supplementary Table 2. Representative multi-axis construct compositions for the illustrative research directions.

Research direction	Axes engaged	Representative restored regulators / genes	Delivery tropism
Neuroaging	p53/MDM2 · SIRT1 · NRF2 · TFEB · Klotho	MDM2; SIRT1; NRF2ΔNeh2; TFEB(S211A); α-Klotho	neuron-/glia-restricted
Cardiovascular aging	p53/MDM2 · PTEN · Telomerase · Klotho	MDM2; PTEN; TERT; α-Klotho	cardiac-restricted
Metabolic rejuvenation	PTEN · AMPK · SIRT1/NAMPT · PGC-1α · FOXO3	PTEN; AMPKα1/LKB1; SIRT1+NAMPT; PGC-1α; FOXO3-TM	liver / muscle
Musculoskeletal	p16/BMI-1 · Myostatin · PGC-1α · Telomerase · Klotho	BMI-1; follistatin; PGC-1α; TERT; α-Klotho	muscle / bone
Immune (immunosenescence)	p16/BMI-1 · FOXO3 · SIRT1 · Telomerase · Klotho/Wnt	BMI-1; FOXO3-TM; SIRT1; TERT; α-Klotho+sFRP1	hematopoietic; optional thymic support
Skin / integumentary	p53/MDM2 · p16/BMI-1 · NRF2 · SIRT1 · Telomerase	MDM2; BMI-1; NRF2ΔNeh2; SIRT1; TERT	localized / topical delivery

These are illustrative research directions, not clinical protocols; the specific axis groupings, regulators, and sequencing are unvalidated and would require empirical derivation (main text, the multi-axis research-directions section).

Supplementary Table 3. Illustrative AAV vector / packaging specifications (non-validated design placeholders).

Axis	Restored gene / product	Illustrative serotype	Promoter strategy	Approx. coding size	Oversize?	Delivery solution
1 p53/MDM2	MDM2	AAV9 / AAV6	tissue-restricted + senescence-responsive (high-surveillance)	~1.5 kb	No	single vector
2 p16/BMI-1	BMI-1	AAV9	tissue-restricted + senescence-responsive (high-surveillance)	~1.0 kb	No	single vector
3 SIRT1/DBC1	SIRT1 (+ NAMPT)	AAV9	tissue-restricted	~2.2 kb (+~1.6 kb)	Yes (combined)	dual-AAV or co-administered cocktail
4 Telomerase/TRF1	TERT	AAV9	regulatable; tightly controlled	~3.4 kb	Borderline	single vector (minimal regulatory elements) or mRNA-LNP
5 PTEN/mTOR	PTEN	AAV8 / AAV9	tissue-restricted	~1.2 kb	No	single vector
6 NRF2/KEAP1	NRF2 Δ Neh2 (KEAP1-resistant)	AAV9	expression-limited + senescence-responsive (high-surveillance)	~1.8 kb	No	single vector
7 FOXO3/AKT	FOXO3-TM (nuclear)	AAV9	tissue-restricted	~2.0 kb	No	single vector
8 AMPK/mTORC1	AMPK α 1(T172D) / LKB1	AAV8	tissue-restricted	~1.7 / ~1.3 kb	No	single vector
9 Klotho/FGF23	α -Klotho	AAV8 (hepatic secretion)	liver-restricted; secreted factor	~3.0 kb	Borderline	single vector
10 PGC-1 α /PARIS	PGC-1 α (+ miR-PARIS)	AAV9	tissue-restricted	~2.4 kb (+miR)	No	single vector
11 TFEB/mTORC1	TFEB(S211A)	AAV9	tissue-restricted	~1.5 kb	No	single vector
12 Lamin A/Progerin	ZMPSTE24 (+ ASO)	AAV9	tissue-restricted	~1.4 kb	No	single vector + splice-blocking ASO
13 GDF11/Myostatin	follistatin (preferred)	AAV8	muscle-restricted; secreted	~1.0 kb	No	single vector
14 DNMT3A/TET2	DNMT3A (+ dCas9-TET2)	AAV9 / dual	targeted; regulatable	DNMT3A ~2.7 kb; dCas9-TET2 >4.7 kb	Yes (dCas9-TET2)	dual-AAV or mRNA-LNP
15 Cisd2/BCL-2	Cisd2	AAV9	tissue-restricted	~0.4 kb	No	single vector
16 α -Klotho/Wnt	α -Klotho + sFRP1/DKK1	AAV8 / AAV9	tissue-restricted	~3.0 + ~1.0 kb	Yes (combined)	dual-AAV or cocktail

Every value is an illustrative, non-validated design placeholder, included only to demonstrate feasibility considerations (the ~4.7 kb packaging limit, promoter strategy, and delivery solution) and to inform experimental design; none is a proposed clinical construct. Coding sizes are approximate; serotype and promoter choices would require empirical optimization. High-surveillance axes (1, 2, 6) are shown with tissue-restricted and senescence-responsive promoters and would additionally carry the containment and suicide-switch elements of the engineered safety-switch subsection, which add to the effective cassette size.

Supplementary Information

Supplementary Note 1. Illustrative Multi-Axis Research Directions

The combinations below are illustrative research directions, not clinical protocols. They show how the framework's systems-level logic could be explored experimentally across major aging phenotypes; the specific axis groupings, vectors, and sequencing are unvalidated and would require empirical derivation. Representative construct compositions appear in Supplementary Table 2.

Neuroaging (cognitive decline, neurodegeneration). The brain is vulnerable to DDR-driven aging given post-mitotic dependence on DNA repair, high metabolic demand, and limited regeneration. A candidate direction would coordinate senescence control (p53/MDM2), neuroprotective deacetylation (SIRT1), oxidative defense (NRF2), aggregate clearance (TFEB), and Klotho support, delivered with neuron- or glia-restricted tropism [1].

Cardiovascular aging. A candidate direction would combine cardiomyocyte senescence control (p53/MDM2), metabolic rebalancing (PTEN), telomere protection (TERT), and vascular protection (Klotho), with cardiac-restricted delivery [2].

Metabolic rejuvenation. A candidate direction would target insulin signaling (PTEN), nutrient sensing (AMPK), NAD⁺-dependent regulation (SIRT1/NAMPT), mitochondrial biogenesis (PGC-1 α), and stress resistance (FOXO3) across liver and muscle [3].

Musculoskeletal rejuvenation. A candidate direction would combine satellite-cell senescence control (p16/BMI-1), myostatin antagonism (follistatin), mitochondrial support (PGC-1 α), telomere protection (TERT), and bone-mineral support (Klotho) [4, 5].

Immune rejuvenation (immunosenescence, inflamm-aging). A candidate direction would reduce senescent-T-cell accumulation (p16/BMI-1), restore memory and stress responses (FOXO3), dampen NF- κ B-driven inflammation (SIRT1), extend replicative lifespan (TERT), and restrain HSC myeloid skewing (Klotho/Wnt), with optional thymic-regeneration support [6, 7].

Skin and integumentary rejuvenation. A candidate direction would address dermal fibroblast senescence (p53/MDM2), epidermal stem-cell renewal (p16/BMI-1), photo-oxidative defense (NRF2), epigenetic integrity (SIRT1), and replicative capacity (TERT), with localized delivery [8].

Supplementary Note 2. Manufacturing and CMC Considerations

A 16-axis framework raises chemistry, manufacturing, and controls (CMC) demands beyond a single gene therapy, since each axis is, in principle, a distinct AAV product. A modular strategy—shared inverted terminal repeats, a small set of validated capsids from common cell banks, and interchangeable expression cassettes swapped into a qualified backbone—would concentrate development effort on cassette-specific attributes; multi-axis directions would be formulated from individually released single-transgene lots, with any LNP-mRNA components produced on a separate platform. Early development would proceed through contract development and manufacturing organizations (CDMOs), with dedicated manufacturing reserved for lead axes reaching pivotal trials. The comparability and cost burden of many parallel products remains a material development consideration. These considerations are provided to bound the feasibility discussion only; they do not constitute a development plan, and detailed process specifications are beyond the scope of the review.

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