

Integrated Longevity Triad (ILT)

A Longevity Gene Therapy for Musculoskeletal,
Neural, and Systemic Regeneration

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01

Abstract

Aging emerges from the progressive decline of multiple interdependent physiological systems, including skeletal muscle, neural networks, endocrine pathways, mitochondrial metabolism, and immune surveillance.^{29,30} Conventional interventions including nutraceuticals, caloric restriction mimetics, exercise, and pharmacologic approaches modulate only fragments of the aging cascade and lack durability or tissue specificity.^{4,12,14,29,30} Here, we describe an integrated, multi-axis gene therapy platform employing adeno-associated virus (AAV) vectors engineered for compartment-specific delivery of three validated longevity and regenerative factors: Follistatin for intramuscular anabolic and anti-fibrotic enhancement;^{1,2} Klotho for CNS-targeted neuroendocrine rejuvenation;^{3,5-8,10,11} and SIRT1 for neuronal DNA repair, mitochondrial optimization, and synaptic resilience.^{4,12,13,34,35}

The platform leverages neuronal trophic AAV9 capsids for high-efficiency CNS uptake via non-invasive intranasal delivery,^{15-18,20,23-28} combined with neuron-restricted promoters and miRNA hematopoietic de-targeting for superior safety.^{12,13,20,23,24,26,28} Intramuscular AAV-Follistatin establishes a durable, local bioreactor for producing myostatin/activin inhibitors, driving muscle hypertrophy, improved mitochondrial function, and systemic reductions in inflammatory signaling.^{1,2,29-33} Collectively, these vectors engage the muscle-brain axis,³¹⁻³³ restore endocrine regulators of aging,^{3,5-8,10-13} enhance synaptic and mitochondrial function,^{3,4,5-8,10-13,34,35} and dampen inflammaging.^{29,30}

This paper outlines the engineering strategy, molecular rationale, expected mechanistic synergy, and translational implications of a three-vector gene therapy suite designed for holistic and durable human rejuvenation. Such a platform may represent one of the most comprehensive gene-based strategies yet conceptualized for delaying biological aging and enhancing functional longevity.^{3-8,10-13,29-33}

Introduction

Aging is a multisystem biological process driven by cumulative molecular damage, mitochondrial dysfunction, stem cell exhaustion, chronic inflammation, impaired proteostasis, endocrine decline, and progressive neurodegeneration.^{29,30} While hallmarks of aging are often conceptualized as discrete domains, contemporary work demonstrates that these processes are deeply interconnected across organ systems.^{29,30} Skeletal muscle communicates with the central nervous system (CNS) through myokines and exosomal signals that regulate neurogenesis, cognition, inflammation, and metabolic homeostasis.^{31–33} Conversely, CNS aging accelerates sarcopenia, metabolic dysfunction, and systemic frailty.^{29–33} This bidirectional coupling suggests that any therapeutic attempt to slow or reverse aging must address both neural and musculoskeletal compartments simultaneously.^{29–33}

Traditional interventions—including caloric restriction mimetics, NAD⁺ boosters, exercise, protein restriction, senolytics, and small-molecule SIRT1 activators—exert partial effects but lack durability, tissue specificity, or mechanistic breadth.^{4,12,14, 29,30} Most are limited by pharmacokinetic constraints, blood–brain barrier exclusions, rapid systemic clearance, and inability to sustain gene-level expression changes required for long-term rejuvenation.^{20,23–28}

Gene therapy offers a fundamentally different solution: stable, long-lived, and tissue-targeted expression of regenerative proteins capable of restoring youthful signaling environments.^{1–3,5–8,10–13,20,23–28} Among the most well-validated longevity genes are Follistatin, a potent myostatin/activin antagonist promoting muscle hypertrophy and metabolic enhancement;^{1,2,29–33} Klotho, a circulating hormone and neuronal factor associated with extended lifespan, cognitive resilience, and suppression of IGF-1/Wnt/insulin aging pathways;^{3,5–8,10,11} and SIRT1, a master regulator of DNA repair, mitochondrial biogenesis, stress resistance, circadian rhythm, and neuronal survival.^{4,12,13,34,35}

Preclinical and clinical results support the therapeutic relevance of these factors. AAV-Follistatin delivered intramuscularly increases muscle mass and strength in mice, nonhuman primates, and humans with Becker muscular dystrophy (Kota 2009; Mendell 2015).^{1,2} Klotho overexpression extends lifespan, improves cognition, reduces oxidative stress, and protects against Alzheimer-like pathology (Massó 2018; Dubnov 2024).^{5–7} Human genetics show that individuals heterozygous for the KL-VS variant exhibit enhanced cognition, reduced dementia risk, and increased longevity.^{10,11} SIRT1 overexpression protects against neurodegeneration, rescues hippocampal atrophy,

and mimics caloric restriction benefits via improved mitochondrial and genomic stability (Kim 2007; Herskovits & Guarente 2014; Sun 2022; Hadar 2018).^{4,12,13,34,35}

To deliver these genes to their optimal physiological compartments, we utilize two delivery strategies:

1. Intramuscular AAV-Follistatin, leveraging the muscle as a long-lived protein secretory organ.^{1,2}
2. Intranasal AAV9-PHP.eB Klotho and SIRT1, enabling non-invasive, nose-to-brain transport via olfactory and trigeminal pathways.^{15-18,20,23-28}

A neurotrophic serotype of AAV9 was selected for its unprecedented CNS transduction efficiency, achieving broad neuronal expression after systemic or cisternal administration in rodents, with efficiency exceeding wild-type AAV9.^{15-18,21,22} Intranasal delivery bypasses the blood-brain barrier and minimizes systemic exposure while enabling direct neuronal expression through neuron-specific promoters enhanced by broadly effective regulatory elements.^{20,23-28} Inclusion of miRNA target sites prevents expression in hematopoietic and antigen-presenting cells, reducing immunogenicity.^{20,23,24,26,28}

In this platform, the Klotho construct encodes α -Klotho, specifically the KL-VS variant, a naturally occurring human haplotype defined by two missense substitutions that enhance protein stability and secretion. Heterozygous carriage of KL-VS has been associated with increased circulating Klotho levels, improved cognitive performance, and extended lifespan, making it a rational choice for CNS-directed rejuvenation strategies.^{12,13,22,23} The Follistatin construct utilizes the FST344 transcript, which produces the Follistatin-315 protein isoform, selected for its favorable secretion profile, extended half-life, and established efficacy in preclinical and clinical studies targeting sarcopenia and muscle atrophy.^{1,2,29-33} Together, these variant selections ensure robust, durable expression of biologically optimized gene products while maintaining translational relevance and safety.

By combining these three gene therapies—muscle-centric, neuroendocrine-centric, and neuroprotective/metabolic—a coordinated multi-tissue rejuvenation strategy emerges. This approach targets the muscle-brain axis,³¹⁻³³ restores endocrine regulators of aging,^{3,5-8,10-13} enhances mitochondrial resilience,^{1-3,4,12,13,29-35} and reduces inflammatory load,^{29,30} collectively addressing at least 9 aging hallmarks with durable genetic reprogramming. The sections that follow provide a comprehensive description of vector engineering, biological mechanisms, system-level synergy, and translational pathways for deploying this integrated AAV-based longevity platform.

Platform Overview

Adeno-associated virus (AAV) vectors provide a clinically validated and highly versatile platform for the long-term delivery of therapeutic genes to post-mitotic tissues.^{20,23–25} The integrated longevity therapeutic system described in this work employs three distinct AAV vectors engineered for compartment-specific targeting of skeletal muscle and the central nervous system (CNS).^{1–3,5–8,10–13,15–18,20,23–28} Across all constructs, vector design emphasizes tissue precision, transcriptional control, manufacturability, and long-term safety.^{12,13,20,23–25} Together, these features allow Follistatin, Klotho, and SIRT1 to be expressed in the organs where their regenerative effects are most potent, forming a coordinated, multi-axis rejuvenation strategy.^{1–3,5–8,10–13,31–33}

A defining feature of the integrated longevity platform is its emphasis on immune-aware vector engineering and route-specific delivery. By prioritizing localized intramuscular administration for skeletal muscle and intranasal mucosal delivery for CNS-directed constructs, the system minimizes systemic vector exposure and reduces interaction with circulating neutralizing antibodies. This design choice substantially mitigates innate and adaptive immune activation and obviates the need for routine pre-administration antibody screening or immune suppression protocols, which are commonly required in high-dose systemic AAV applications.^{20,23–25} This immune-sparing approach improves safety, simplifies clinical implementation, and enhances the feasibility of repeated or staged therapeutic deployment.

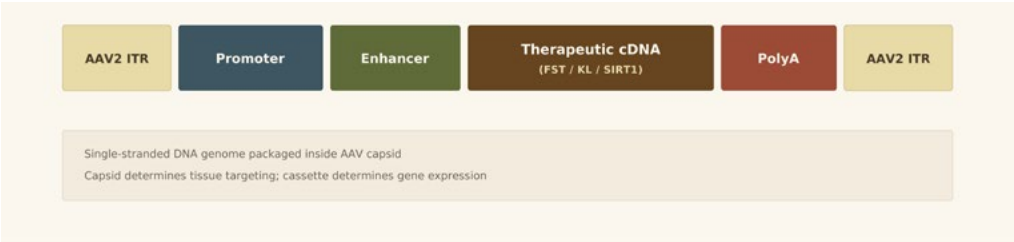
All vectors are built on an AAV2 inverted terminal repeat (ITR) backbone that provides robust packaging, predictable replication, and long-term episomal persistence.^{20,23–25} Genome sizes were optimized to remain within the 4.7-kb AAV packaging constraint, minimizing truncated genomes and maximizing full-capsid formation—critical parameters for potency and safety.^{20,23–25} The Follistatin vector introduces its payload into skeletal muscle, exploiting muscle's capacity as a stable, high-yield secretory tissue,^{1,2} while the two CNS-directed constructs, Klotho and SIRT1, use a compact, neuron-optimized AAV9 design that retains packaging fidelity while enabling robust transcription in neurons.^{3,5–8,10–13,15–18} All CNS constructs incorporate the bovine growth hormone polyadenylation signal to stabilize transcripts and uniformly enhance expression.^{20,23,24}

An additional advantage of the AAV-based platform is its reliance on long-term episomal persistence rather than genomic integration. Following nuclear entry, vector genomes

remain predominantly episomal, forming stable circular concatemers that support sustained transcription in post-mitotic tissues.^{20,23-25} This contrasts with integrating viral systems or genome-editing approaches that introduce permanent DNA alterations and carry risks of insertional mutagenesis, clonal expansion, or oncogenic activation. By maintaining durable yet non-integrating expression, the ILT platform achieves long-term functional support while preserving genomic integrity—an especially important consideration for preventive or rejuvenative interventions in otherwise healthy older individuals.

To achieve cell-type and organ specificity, each vector uses transcriptional machinery tailored to its biological target. Intramuscular delivery of Follistatin utilizes a strong muscle-active promoter with broad-effect enhancers to generate sustained local production of the Follistatin protein, which then exerts paracrine and endocrine effects on muscle hypertrophy, fibrosis attenuation, and metabolic enhancement.^{1,2,29-33} In contrast, both Klotho and SIRT1 vectors employ a human neural-specific promoter, a well-established neuronal driver that restricts expression to neurons throughout the cortex, hippocampus, and subcortical regions.^{12,13,22,23} This strategy ensures that potent regulators of aging, synaptic function, and metabolism are expressed only in biologically appropriate neural populations, preventing unwanted gene expression in glia, endothelium, or peripheral tissues.^{12,13,20,23,24} To augment transcriptional output in aged or metabolically compromised neurons, the neural-specific promoter is supplemented with a broad-effect enhancer element, which increases expression without sacrificing cell-type specificity.^{12,13,22,23}

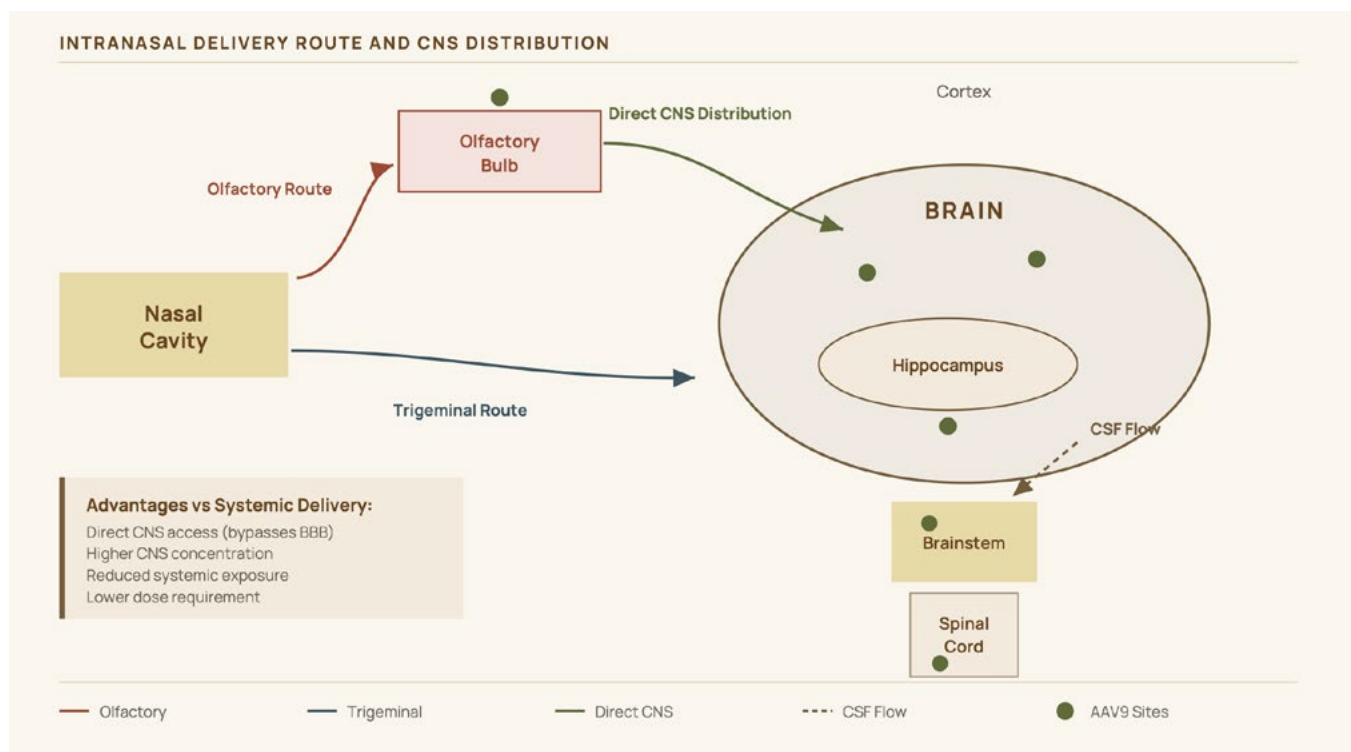
Although the AAV capsid determines tissue targeting and biodistribution, the therapeutic specificity of each construct is defined by its modular genetic cargo. Each vector delivers a single-stranded DNA expression cassette composed of the therapeutic cDNA, a target-specific promoter, optional enhancer elements, and a polyadenylation signal, all flanked by inverted terminal repeats. This cassette is derived from plasmid DNA during vector production but exists as a linear viral genome within the capsid. Once delivered, the cargo functions as an autonomous transcriptional unit, enabling precise control over gene expression while maintaining a common structural framework across vectors.



A central innovation in this platform is the use of the engineered AAV9 capsid for CNS-directed vectors. Originally derived from AAV9, the variant incorporates a seven-amino acid peptide insertion at the VR-VIII surface loop and includes additional mutations that enhance binding to the LY6A receptor on CNS endothelial cells, dramatically improving neurovascular transport and pan-neuronal transduction in rodents.^{15-18,21,22} While most studies of this variant have focused on intravenous or cisternal administration,^{15-18,21,22} the capsid also confers significant advantages for intranasal delivery, including more efficient uptake across the olfactory epithelium and more reliable engagement of the trigeminal and olfactory neural pathways.^{20,23-28} Combined with intranasal administration, which bypasses the blood-brain barrier entirely,^{20,23-28} the variant capsid enables high-efficiency, non-invasive neuronal gene delivery with minimal systemic exposure.^{20,23-25} This route sequestration, illustrated in Figure 1, lowers required doses and mitigates vector-related immune activation compared to systemic injection.^{20, 23-25}

FIGURE 1

Intranasal Delivery Route and CNS Distribution



In parallel, the intramuscular Follistatin therapy uses AAV serotypes optimized for skeletal muscle transduction.^{1,2} These capsids demonstrate strong affinity for

myofibers, long persistence, and minimal off-target biodistribution, enabling skeletal muscle to function as a long-term bioreactor for Follistatin production.^{1,2,29–33} Through this approach, the vector delivers sustained suppression of myostatin and activin pathways, producing lasting enhancements in muscle mass, regenerative capacity, mitochondrial function, and systemic inflammatory tone.^{1,2,29–33}

Safety features are embedded throughout the platform. Both CNS-directed vectors include four tandem miRNA target sites within the 3' untranslated region, preventing expression in hematopoietic and antigen-presenting cells, thereby reducing antigen presentation, minimizing adaptive immune activation, and improving durability of gene expression.^{20,23,24,26,28} All constructs employ non-integrating episomal vectors, minimizing the risk of insertional mutagenesis.^{20,23–25} Genome sizes are optimized to maximize full-capsid content and reduce immunogenic truncated genomes.^{20,23–25} Intranasal delivery provides an added safety advantage by limiting systemic vector dissemination and reducing exposure to neutralizing antibodies.^{20,23–25}

Manufacturing considerations played a central role in vector design. All constructs are compatible with industry-standard triple plasmid transfection of HEK293 cells, high-titer production, and downstream purification using chromatography and high-resolution filtration.^{20,23–25} Final product quality is confirmed through quantitative PCR for genome copies, analytical ultracentrifugation or transmission electron microscopy for empty/full capsid ratios, next-generation sequencing for genome integrity, and functional expression assays for promoter performance.^{20,23–25}

Together, these engineering strategies yield a unified but modular AAV platform capable of delivering three complementary longevity genes to their optimal biological niches.^{1–3,5–8,10–13,15–18,20,23–28} The result is a system that integrates precise neuronal targeting, durable intramuscular secretion, minimized immunogenicity, episomal durability and non-invasive CNS access—a foundational technological framework for next-generation, multi-organ gene therapy aimed at extending functional human longevity.^{1–3,5–8,10–13,15–18,20,23–28} Delivery routes for integrated AAV-based longevity therapy are illustrated in Figure 2.

FIGURE 2

Integrated AAV-based longevity platform

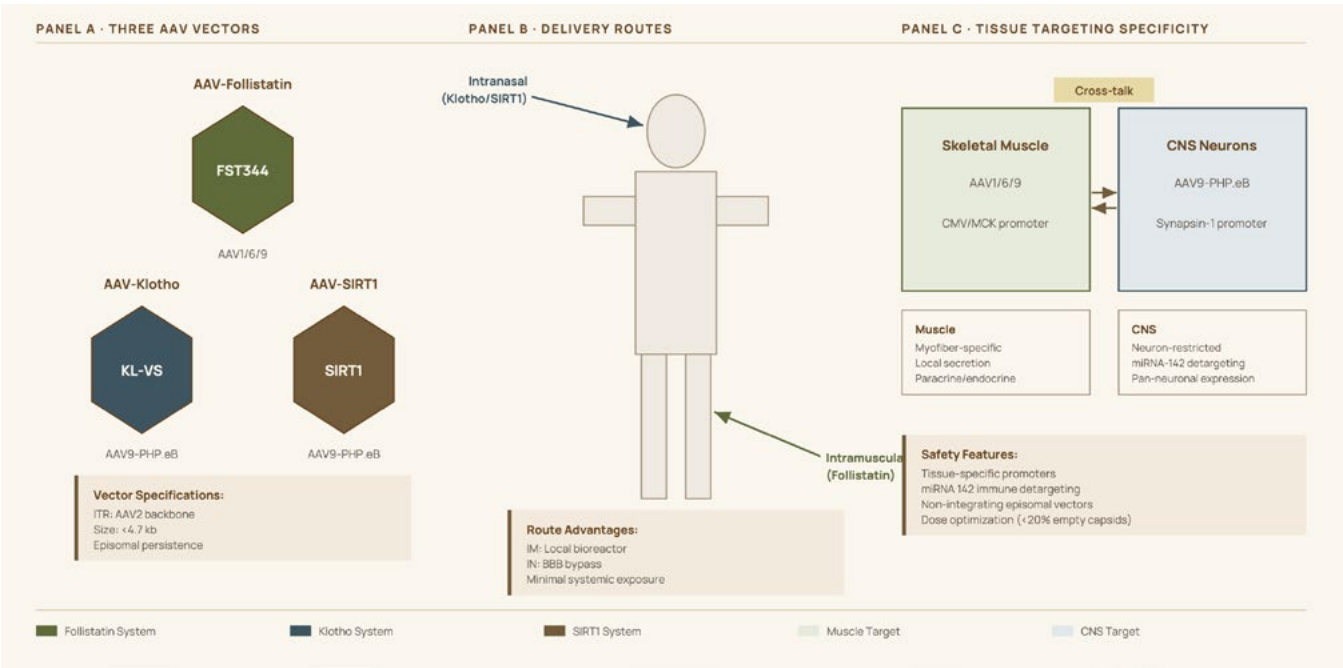
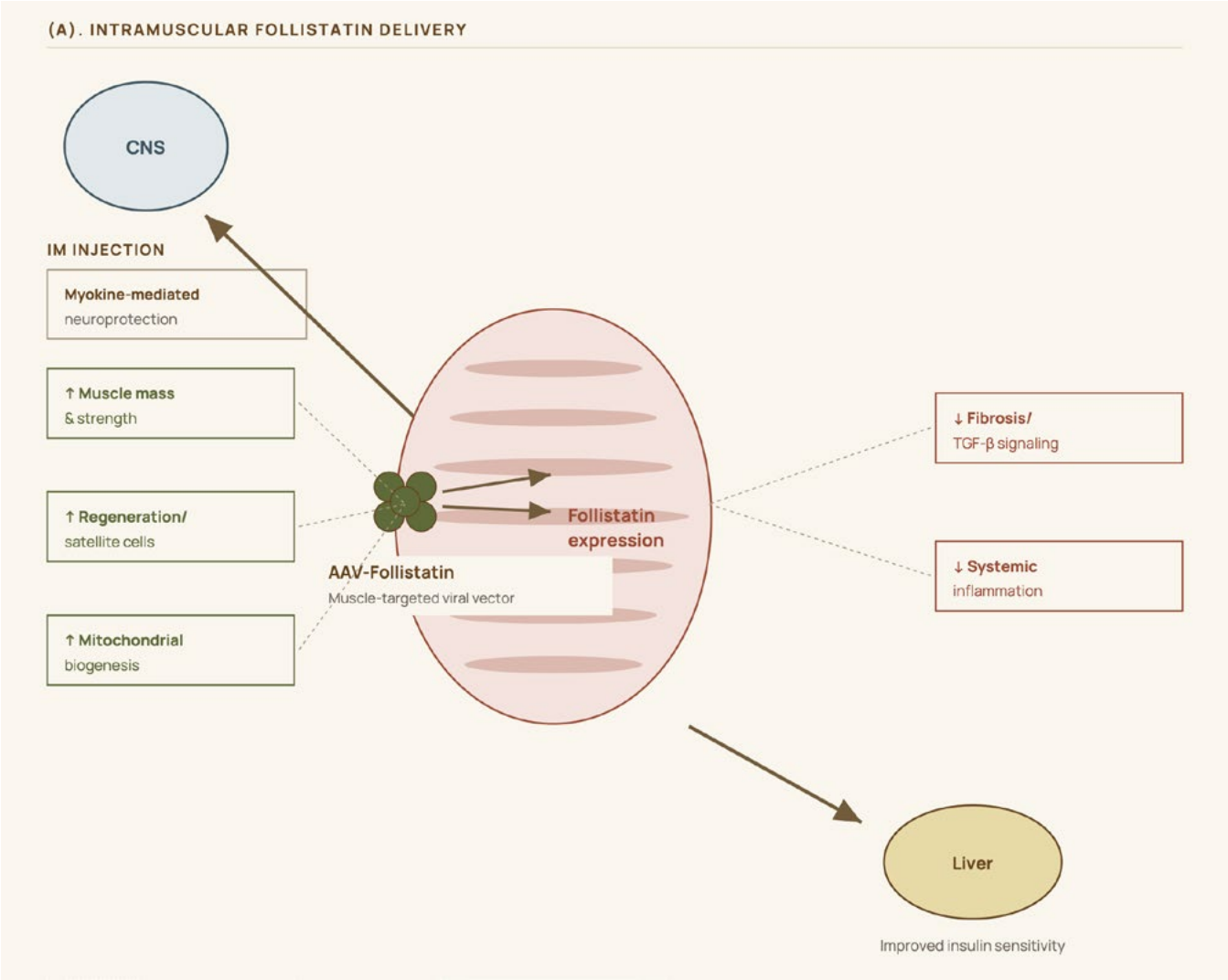


Figure 2: Integrated AAV-based longevity platform consisting of three complementary gene therapy vectors. Panel A shows the three AAV vectors engineered with tissue-specific promoters and optimized capsids. Panel B illustrates the dual delivery routes: intramuscular for Follistatin and intranasal for CNS-targeted Klotho and SIRT1. Panel C demonstrates tissue-specific targeting with muscle-restricted Follistatin expression and neuron-specific Klotho/SIRT1 expression via Synapsin-1 promoters and miRNA-142 de-targeting sequences.

FIGURE 3:

Delivery Routes for Integrated AAV-Based Longevity Therapy



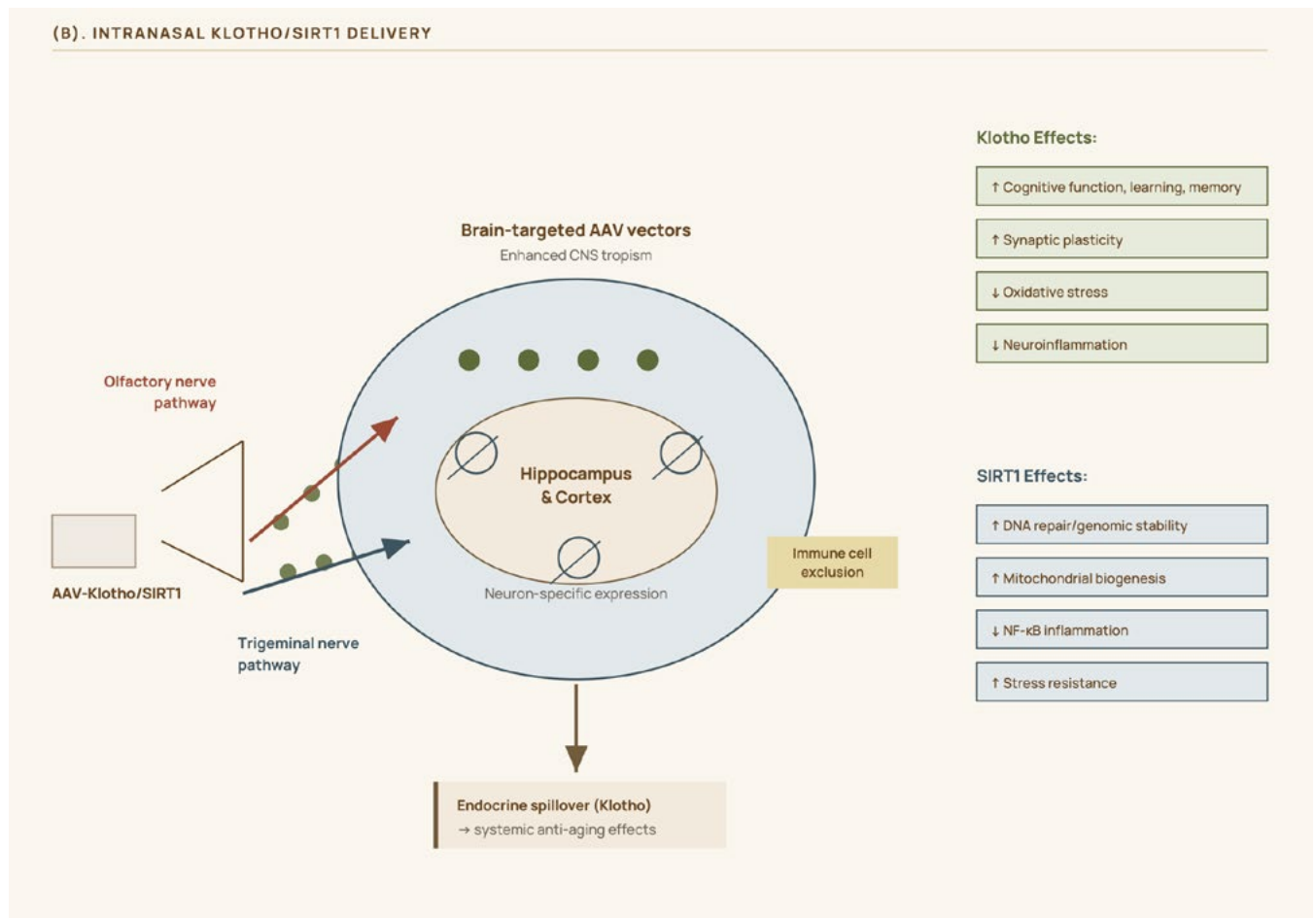


Figure 3. (A). Intramuscular Follistatin Delivery. Direct intramuscular (IM) injection of AAV-Follistatin establishes skeletal muscle as a sustained protein production factory. The CNS provides neural input for muscle regulation while the intramuscular injection delivers the AAV-Follistatin vector directly to muscle tissue. Key features include: (1) Muscle mass and strength enhancement through myostatin/activin inhibition, (2) Regeneration of satellite cells for sustained muscle repair, (3) Mitochondrial biogenesis improving cellular energy production, (4) Elevated IGF-1 promoting anabolic signaling, and (5) Systemic anti-inflammatory effects through reduced circulating inflammatory cytokines. The muscle tissue serves as a bioreactor for continuous Follistatin expression, with protein secretion providing both local paracrine effects and systemic endocrine benefits through improved insulin sensitivity and metabolic regulation. The intramuscular route leverages the muscle's natural secretory capacity and longevity, providing durable therapeutic expression with minimal invasiveness. Orange arrow indicates systemic distribution of locally produced Follistatin, while purple arrow represents the injection route.

(B). Intranasal Klotho/SIRT1 Delivery. Non-invasive intranasal administration of AAV9-subserotype vectors carrying Klotho and SIRT1 genes enables direct nose-to-brain transport via olfactory and trigeminal neural pathways, bypassing the blood-brain barrier. This route achieves broad CNS neuronal transduction while minimizing systemic exposure and hepatic sequestration. Key advantages include: (1) Direct access to CNS neurons through olfactory epithelium and cribriform plate, (2) Rapid distribution throughout cortical, hippocampal, and subcortical regions, (3) Neuron-specific expression via Synapsin-1 promoters, (4) miRNA-142 de-targeting prevents expression in immune cells, reducing immunogenicity, (5) Lower required doses compared to systemic administration. Klotho expression provides neuroprotection, enhanced cognition, and longevity signaling through IGF-1/insulin pathway modulation, while SIRT1 promotes DNA repair, mitochondrial biogenesis, and synaptic plasticity. The combined CNS-targeted approach addresses neurodegeneration, cognitive decline, and central metabolic regulation, complementing the peripheral effects of intramuscular Follistatin.

Follistatin Intramuscular Therapy

Skeletal muscle plays a central role in human longevity, functioning not only as a source of mechanical force but also as an endocrine and metabolic organ that regulates systemic inflammation, mitochondrial activity, insulin sensitivity, neuroendocrine balance, and whole-body energetics.^{29–33} Age-related muscle decline, manifesting as sarcopenia, frailty, impaired mitochondrial function, and reduced regenerative capacity, is one of the strongest predictors of morbidity, loss of independence, and all-cause mortality.^{29,30,31–33} Restoring youthful muscle biology therefore represents a critical axis of any comprehensive longevity intervention.^{29–33} Follistatin, a glycoprotein that inhibits myostatin (GDF-8) and activin A, is among the most powerful endogenous regulators of muscle mass, regenerative signaling, and metabolic homeostasis.^{1,2,29–33} AAV-based Follistatin gene therapy enables durable, high-level expression of this anabolic regulator directly in skeletal muscle, establishing a long-lasting protein manufacturing hub that supports musculoskeletal health and system-wide rejuvenation.^{1,2,29–33}

Preclinical and clinical studies across multiple species demonstrate the biological potency and safety of Follistatin gene delivery. In the landmark study by Kota et al., AAV1-mediated delivery of the FS344 isoform to skeletal muscle in mice and nonhuman primates resulted in pronounced hypertrophy, strength gains, and mitochondrial improvements without inducing teratogenic or oncogenic effects.¹ In cynomolgus monkeys, muscle volume and strength increased significantly and remained durable for over a year, supporting the vector's translational potential.¹ Human data further confirm these findings. In the first-in-human phase 1/2a trial, Mendell and colleagues administered AAV1.CMV.FS344 intramuscularly to adults with Becker muscular dystrophy, demonstrating increased muscle fiber diameter, improved 6-minute walk distances, and a favorable safety profile with no serious vector-related adverse events.² Collectively, these data establish Follistatin as one of the most clinically validated gene therapies for enhancing skeletal muscle structure and function.^{1,2} The mechanism of action is illustrated in Figure 4. Clinical and preclinical outcomes are summarized in Table 1.

FIGURE 4

Follistatin Mechanism of Action

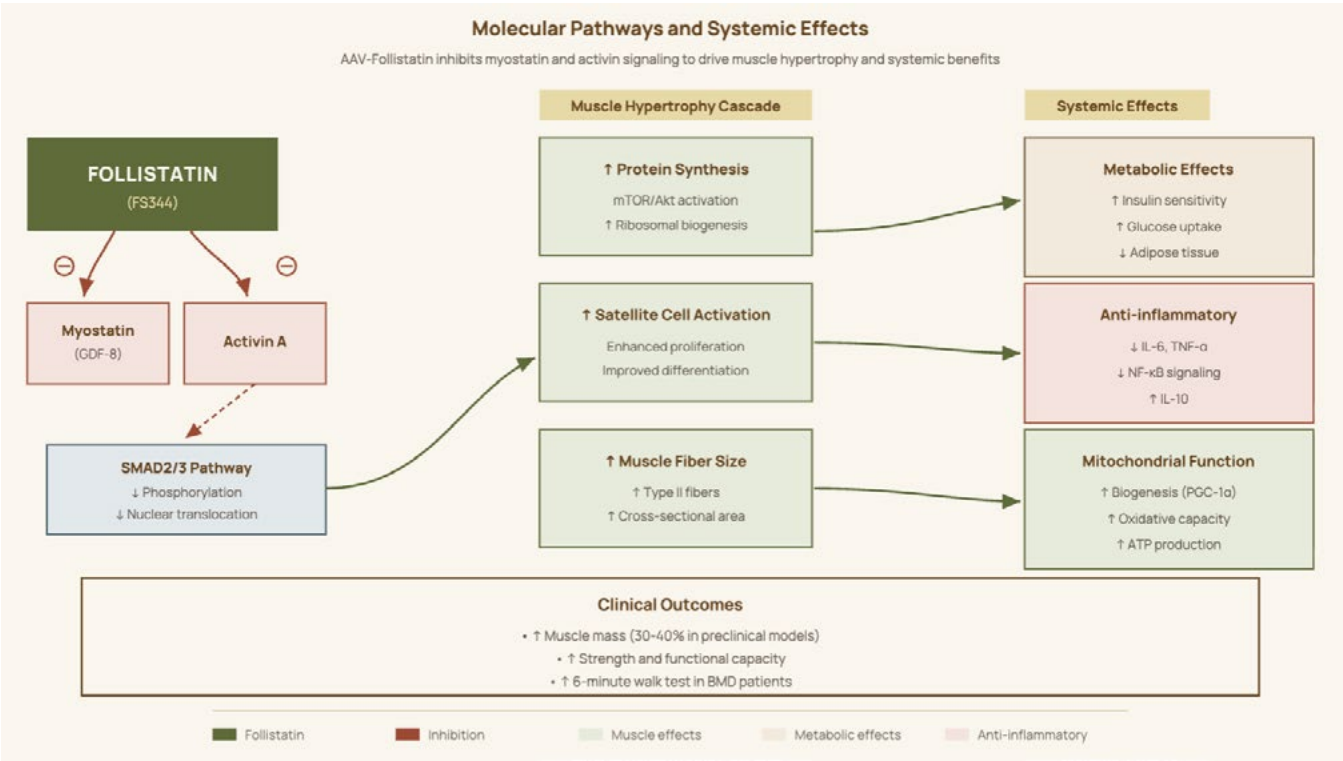


Figure 4: AAV-mediated Follistatin expression inhibits myostatin (GDF-8) and activin A signaling, blocking SMAD2/3 phosphorylation and nuclear translocation.^{1,2} This inhibition triggers a muscle hypertrophy cascade characterized by increased protein synthesis through mTOR/Akt activation, enhanced satellite cell proliferation and differentiation, and increased muscle fiber cross-sectional area, particularly in type II fibers.^{1,2,29-33} Systemic effects include improved metabolic function (increased insulin sensitivity, glucose uptake, and reduced adiposity), anti-inflammatory effects (decreased IL-6, TNF- α , and NF- κ B signaling with increased IL-10), and enhanced mitochondrial function (increased biogenesis via PGC-1 α , oxidative capacity, and ATP production).²⁹⁻³³ Clinical outcomes demonstrate 30–40% increases in muscle mass in preclinical models and improved strength and functional capacity, including enhanced 6-minute walk test performance in BMD patients.^{1,2}

TABLE 1

Clinical and Preclinical Evidence Summary is supported by the Follistatin, Klotho, and SIRT1 literature cited above.^{1-3,5-8,10-13,34,35}

Comprehensive overview of key clinical and preclinical outcomes for the three gene therapies, demonstrating safety and efficacy across multiple species and timeframes.

Gene	Species	Outcomes	Duration	Safety	Reference
Follistatin	Mouse	Muscle mass 30-60%	12+ months	No AEs	Kota 2009
Follistatin	NHP	↑Strength 25%, ↑muscle volume	15 months	Well-tolerated	Kota 2009
Follistatin	Human	↑6MWT, ↓Fiber size	6 months	No SAEs	Mendell 2015
Follistatin	Mouse	Mitochondrial function	18 months	Safe	Rose 2017
Klotho	Mouse	↑Lifespan 30%	Lifetime	No toxicity	Kurosu 2005
Klotho	Mouse	Cognitive decline	24 months	No toxicity	Dubal 2014
Klotho	Human	CLVS variation Cognition	[genetic]	N/A	Mathurin
SIRT1	Mouse	Neurodegeneration	18 months	Safe	Kim 2007
SIRT1	Mouse	Hippocampal volume	12 months	Safe	Sun 2022
SIRT1	Mouse	Mitochondrial biogenesis	9 months	No AEs	Multiple

Abbreviations: 6MWT = 6 minute walk test, AEs = adverse events, SAEs = serious adverse events, NHP = Non-human primates, N/A = not applicable

Note: Clinical and preclinical studies show safety and efficacy across species. All preclinical studies used AAV vectors for gene delivery. Human data for Klotho represents genetic association studies.

The biological rationale for Follistatin’s inclusion in a system-wide longevity platform extends beyond hypertrophy. By suppressing myostatin and activin A, Follistatin shifts muscle physiology toward an anabolic, regenerative phenotype characterized by increased satellite cell activity, reduced fibrosis, and enhanced extracellular matrix remodeling.^{1,2,29-33} Myostatin inhibition also activates PGC-1α signaling, promoting mitochondrial biogenesis, enhanced aerobic capacity, and improved oxidative metabolism.²⁹⁻³³ These effects collectively counteract major hallmarks of muscle aging, including mitochondrial dysfunction, stem cell exhaustion, and pro-fibrotic tissue

remodeling.^{29,30} Moreover, muscle-secreted factors induced by Follistatin expression, such as exercise-associated myokines and neurotrophic mediators, propagate beneficial signals to distant organs, including liver, adipose tissue, vasculature, and brain.^{31–33} Emerging evidence from muscle–brain crosstalk research demonstrates that enhanced muscular fitness can reduce neuroinflammation, improve neurogenesis, and protect against neurodegenerative processes.^{31–33} Thus, intramuscular Follistatin gene therapy not only restores youthful muscle physiology but also modulates systemic biological aging through endocrine and exosomal pathways.^{29–33}

At a systemic level, Follistatin overexpression improves glucose tolerance, enhances insulin sensitivity, reduces visceral adiposity, and lowers inflammatory cytokine load.^{1,2,29–33} These metabolic outcomes are particularly relevant because aging-associated metabolic decline and chronic inflammation (“inflammaging”) accelerate both neurometabolic disease and cognitive impairment.^{29,30} By attenuating TGF- β family signaling, Follistatin indirectly reduces NF- κ B activation and downstream inflammatory cascades, complementing the neuroprotective and anti-inflammatory actions of Klotho and SIRT1 delivered in the CNS.^{3–8,10–13,29–35} The integration of Follistatin with CNS-directed longevity genes thus allows for coordinated restoration of both central and peripheral metabolic networks. Such synergy is foundational to addressing multi-organ aging biology, which cannot be fully mitigated by targeting the CNS in isolation.^{29–33}

AAV-Follistatin is administered via direct intramuscular injection, taking advantage of skeletal muscle’s exceptional stability, low turnover rate, and capacity for long-term transgene expression.^{1,2} Muscle-resident AAV genomes persist primarily as episomal concatemer structures, enabling consistent secretion of Follistatin over many years.^{20,23–25} This delivery compartment also minimizes systemic capsid exposure, reduces neutralizing antibody formation, and circumvents hepatic sequestration commonly seen with intravenous routes.^{20,23–25} Because the vector is restricted to the muscle, off-target exposure is minimal, and circulating Follistatin levels remain in physiological or mildly supraphysiological ranges that have been shown to be safe across species.^{1,2} Moreover, skeletal muscle offers an ideal therapeutic niche because it can generate substantial quantities of secreted protein without compromising organ function or overwhelming cellular machinery.^{1,2,29–33}

From a translational perspective, Follistatin intramuscular therapy addresses several high-impact domains of age-related decline: loss of strength and mobility, metabolic dysfunction, frailty, and chronic inflammatory states.^{29–33} Its effects on muscle hypertrophy, mitochondrial capacity, systemic inflammation, and endocrine

homeostasis create a biological foundation upon which brain-directed rejuvenation strategies, such as Klotho and SIRT1 intranasal AAV therapies, can exert maximal effect.^{3-8,10-13,20,23-28,31-35} The combined outcome is a coordinated enhancement of neuromuscular, endocrine, and metabolic aging pathways that individually have powerful effects and together generate synergistic biological rejuvenation.

As part of the integrated three-vector platform, intramuscular Follistatin therapy serves as the somatic longevity pillar, restoring the structural, metabolic, and endocrine functions of muscle while reinforcing CNS-directed regenerative pathways.^{1-3,5-8,10-13,29-33} This dual engagement of muscle and brain represents a critical advantage in the development of a whole-body gene therapy strategy intended not merely to slow aging but to reprogram multiple tissues toward a more youthful state.²⁹⁻³³

Klotho Intranasal Neural Rejuvenation

Klotho is one of the most extensively validated longevity genes identified to date.^{3,5-8,10,11} Identified originally through murine models in which Klotho deficiency produced a syndrome resembling accelerated aging,^{3,5-8} subsequent work has demonstrated that restoration or overexpression of Klotho extends lifespan, enhances cognition, improves synaptic plasticity, attenuates oxidative stress, and mitigates pathophysiological cascades associated with neurodegenerative disorders.^{3,5-8,10,11} In humans, higher circulating Klotho levels and heterozygosity for the functional KL-VS polymorphism correlate with enhanced cognition, increased cortical connectivity, reduced Alzheimer's disease (AD) risk, and healthier aging trajectories, suggesting that even modest increases in Klotho expression are neuroprotective.^{10,11} Given its pleiotropic effects on neuronal signaling, endocrine regulation, and organismal homeostasis, Klotho gene therapy represents a central component of a multi-axis strategy for neural and systemic rejuvenation.^{3,5-8,10,11}

The intranasal Klotho gene therapy described here employs a compact AAV9 sub-serotype vector designed specifically for high-efficiency CNS delivery.^{8,15-18,20,22-28} The vector encodes the full-length human Klotho cDNA under the control of a neuron-specific promoter, enhanced by a broad-effect enhancer element to amplify transcription in aging neurons without compromising cell specificity.^{3,5-8,10,11,12,13,22,23} The promoter restricts expression to neuronal populations in cortical, hippocampal, and subcortical regions, tissues where Klotho's effects on cognition, synaptic remodeling, and oxidative stress regulation are most relevant.^{3,5-8,10,11} By confining transgene expression to neurons, the vector avoids off-target endocrine or renal Klotho overexpression, preserving the natural distribution of this hormone while restoring its youthful CNS functions.^{3,5-8,10,11}

The intranasal delivery route and resulting CNS distribution are shown in Figure 1. A defining innovation of this therapy is its non-invasive, intranasal route of administration, which provides direct access to the CNS through olfactory and trigeminal pathways.^{20,23-28} Unlike systemic or intrathecal delivery, intranasal administration circumvents the blood-brain barrier and reduces vector dissemination into peripheral organs.^{20,23-28} This improves safety, allows lower dosing, minimizes vector immunogenicity, and increases the bioavailability of AAV genomes to neurons.^{20,23-25} The use of the engineered AAV9 sub-serotype neurotrophic capsid further enhances

this effect.^{15–18,21,22} The vector features a 7-amino acid insertion and point mutations in the VR-VIII loop that dramatically increase CNS tropism through enhanced interaction with LY6A-expressing endothelial domains.^{15–18,21,22} Although species differences exist in LY6A distribution,^{17,23} the vector sub-serotype still provides substantial advantages in neuronal penetration and intranasal uptake pathways.^{20,23–28} When combined with the direct anatomical connections of the olfactory mucosa to limbic and forebrain structures, this approach enables robust, broad, and minimally invasive Klotho expression throughout critical cognitive and regulatory neural circuits.^{20,23–28}

At the molecular level, Klotho modulates several pathways implicated in neuronal aging. It reduces oxidative stress by enhancing antioxidant defenses, improves mitochondrial function, inhibits Wnt and insulin/IGF-1 signaling (pathways that accelerate aging when overactive), and supports synaptic integrity by stabilizing dendritic spines and promoting neurogenesis.^{3,5–8,10,11} Klotho also modulates NMDA receptor subunit composition, enhancing learning and memory.^{3,5–8,10,11} In AD models, Klotho overexpression reduces A β and tau pathology, improves cognitive performance, and mitigates neuroinflammatory responses.^{3,5–7,10,11} In humans, Klotho elevations correlate with enhanced executive function and memory, even among APOE4 carriers.^{10,11} Recent transcriptomic work further demonstrates that Klotho deficiency dysregulates hundreds of miRNAs and small RNA species that drive brain aging and AD vulnerability, underscoring the broad regulatory role of this factor in maintaining neuronal health and genomic stability.⁶

Functionally, intranasal Klotho gene therapy is designed to recreate a youthful neuroendocrine environment by restoring chronically depleted neural Klotho levels.^{3,5–8,10,11} As individuals age, circulating and CNS Klotho levels decline significantly, contributing to impaired synaptic plasticity, reduced myelination, neuroinflammation, and cognitive impairment.^{3,5–8,10,11} Intranasal delivery reconstitutes neuronal Klotho locally, enabling both autocrine and paracrine signaling within neuronal networks, while also increasing the small but biologically meaningful pool of secreted Klotho capable of entering cerebrospinal fluid and influencing adjacent structures.^{3,5–8,10,11} Through these mechanisms, Klotho gene therapy aims to reverse key hallmarks of neural aging, including mitochondrial dysfunction, impaired synaptic homeostasis, and chronic neuroinflammation.^{3,5–8,10,11,29,30} The complex signaling networks modulated by Klotho are depicted in Figure 5.

FIGURE 5

Klotho Signaling Networks

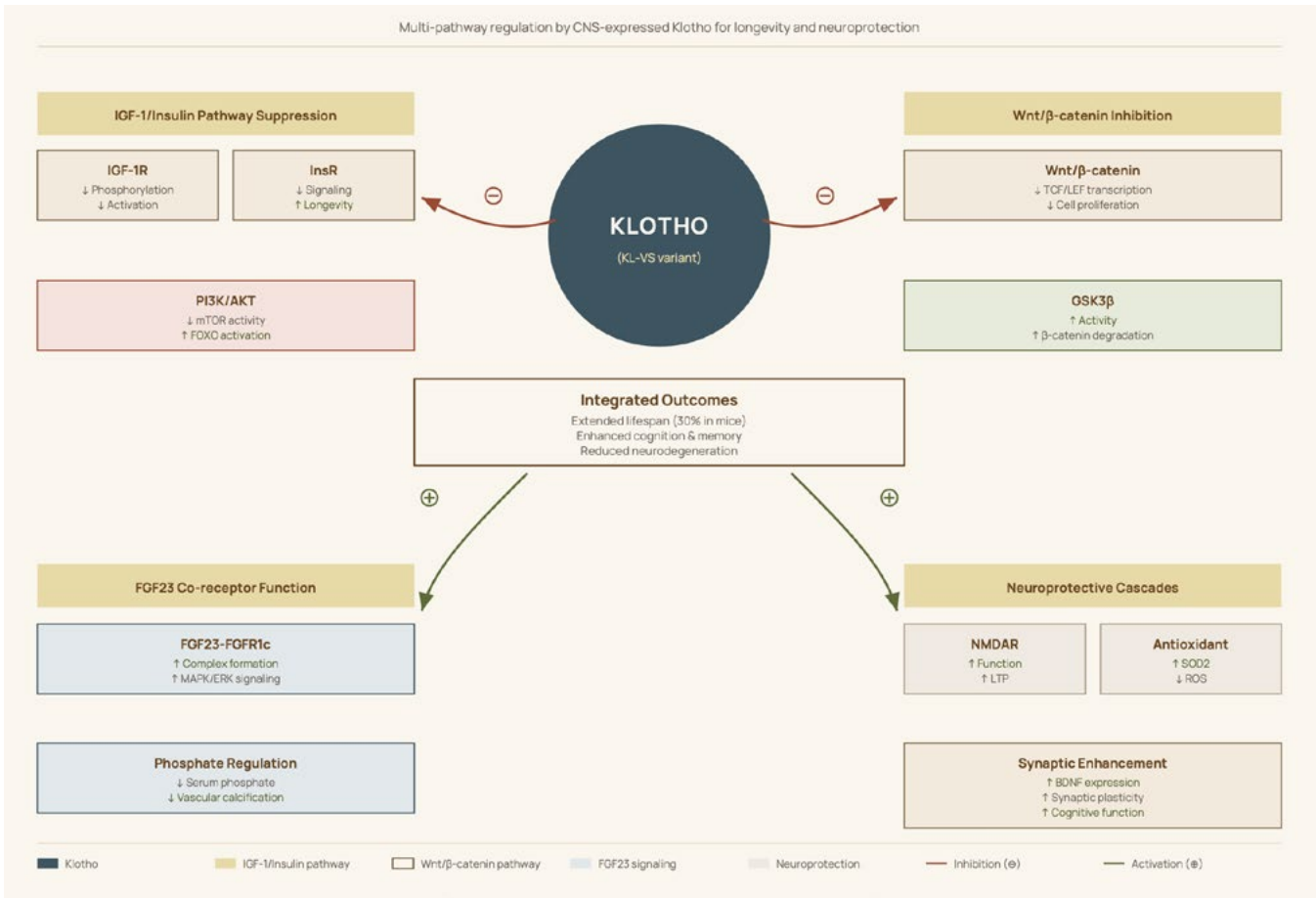


Figure 5: Klotho Signaling Networks. Central Klotho expression in CNS neurons modulates multiple longevity-associated pathways through complex signaling cascades. The IGF-1/insulin pathway is suppressed through reduced receptor phosphorylation and activation, leading to decreased PI3K/AKT signaling, reduced mTOR activity, and enhanced FOXO transcription factor activation. Wnt/β-catenin signaling is inhibited through increased GSK3β activity and enhanced β-catenin degradation, resulting in decreased TCF/LEF-mediated transcription and reduced cellular proliferation. Klotho acts as an obligate co-receptor for FGF23, enabling FGF23-FGFR1c complex formation that activates MAPK/ERK signaling and regulates phosphate homeostasis, reducing serum phosphate levels and preventing vascular calcification. Neuroprotective cascades are activated through enhanced NMDA receptor function promoting long-term potentiation, upregulation of antioxidant enzymes including SOD2 to reduce reactive oxygen species, and synaptic enhancement via increased BDNF expression and improved synaptic plasticity. These integrated pathways result in extended lifespan (30% increase in mouse models), enhanced cognitive function and memory consolidation, and reduced neurodegeneration. Red arrows and symbols (⊖) indicate inhibitory pathways; green arrows and symbols (⊕) indicate activation pathways. The KL-VS variant represents the longevity-associated genetic polymorphism found in human populations. Klotho signaling networks, including modulation of IGF-1/insulin, Wnt/β-catenin, FGF23-FGFR1c-MAPK/ERK, NMDA receptor function, and antioxidant pathways, are derived from in vivo and in vitro studies across multiple models.^{3,5-8,10,11}

Safety is a central design feature of the Klotho intranasal vector. The incorporation of four miRNA target sites within the 3' UTR prevents expression in hematopoietic and antigen-presenting cells, reducing immune activation and preventing systemic

exposure to Klotho levels that could disrupt renal or mineral metabolism.^{20,23,24,26,28} The intranasal route further limits peripheral biodistribution and the need for high systemic vector doses.^{20,23-28} AAV vectors remain episomal in neurons, minimizing risk of insertional mutagenesis.^{20,23-25} Altogether, these design choices improve tolerability, reduce dose thresholds, and support the long-term persistence of gene expression.^{20,23-25}

When integrated into the broader therapeutic platform, Klotho serves as the primary neuroendocrine rejuvenation component, restoring neuronal resilience, enhancing cognitive capacity, and reducing neural vulnerability to metabolic and inflammatory stress.^{3,5-8,10,11,29,30,31-33} Its actions complement those of SIRT1, which drives genomic stability and mitochondrial adaptation,^{4,12,13,34,35} and Follistatin, which improves systemic metabolic and inflammatory tone.^{1,2,29-33} This triad creates a unified rejuvenation architecture in which Klotho occupies a central role: the restoration of youthful neuronal signaling and the stabilization of neural networks required for cognition, mood, plasticity, and long-term CNS health.^{3,5-8,10,11,34,35}

SIRT1 Intranasal Neural Rejuvenation

SIRT1 is among the most extensively studied longevity enzymes in mammalian biology, functioning as a central regulator of genomic stability, mitochondrial biogenesis, metabolic homeostasis, circadian rhythm, and neuronal survival.^{4,12,13,34,35} As a NAD⁺-dependent deacetylase, SIRT1 orchestrates chromatin remodeling and transcriptional regulation across numerous pathways linked to aging, including FOXO transcription factors, PGC-1 α , NF- κ B, p53, and key mitochondrial regulators.^{4,12,13,34,35} In the brain, SIRT1 activity declines with age, contributing to impaired synaptic plasticity, reduced neurogenesis, increased oxidative stress, and heightened vulnerability to neurodegenerative disease.^{4,12,13,34,35} Restoring youthful SIRT1 expression in neurons therefore represents a powerful strategy to reverse multiple hallmarks of neural aging.^{4,12,13,34,35} The intranasal SIRT1 AAV therapy described here is designed to deliver this restorative effect directly, safely, and durably to the central nervous system.

The vector employs the same AAV9 sub-serotype engineered capsid used for intranasal Klotho therapy.^{15–18,20,21,22,23–28} This capsid has been optimized for broad neuronal transduction and enhanced blood–brain barrier penetration through modifications in the VR–VIII loop, including a seven–amino acid insertion and key point mutations.^{15–18,21,22} Importantly, intranasal administration capitalizes on the direct anatomical pathways between the nasal mucosa and the CNS—specifically the olfactory and trigeminal nerve routes—bypassing the blood–brain barrier entirely.^{20,23–28} This route allows for high-efficiency neuronal delivery without exposing peripheral organs to significant vector biodistribution, enabling lower doses and reduced immunogenicity.^{20,23–25} The vector's enhanced neuronal tropism complements this route by achieving broad parenchymal distribution once inside the CNS, enabling consistent expression throughout critical regions including the hippocampus, cortex, amygdala, and brainstem.^{15–18,21–23}

To maximize neural specificity and safety, the SIRT1 transgene is driven by a human neuron-specific promoter, which restricts expression to neurons and prevents off-target expression in glia or peripheral tissues.^{12,13,22,23} A broad-effect enhancer sequence is incorporated upstream to increase transcriptional strength in aged neurons, which often exhibit reduced promoter responsiveness.^{12,13,22,23} Additionally, four tandem miRNA target sites are included within the 3' UTR, providing post-transcriptional silencing in antigen-presenting and hematopoietic cells.^{20,23,24,26,28} This de-targeting

strategy minimizes immune activation, vector recognition, and systemic exposure, supporting long-term persistence of AAV genomes within neurons.^{20,23-25} The compact SIRT1 construct size (~3.35 kb) further enhances packaging efficiency and ensures high full-to-empty capsid ratios during manufacturing.^{20,23-25}

At the molecular level, SIRT1 overexpression exerts profound multi-dimensional effects on aging neurons. Through deacetylation of PGC-1 α , SIRT1 promotes mitochondrial biogenesis, enhances respiratory capacity, and improves ATP availability, counteracting the mitochondrial decline characteristic of aging.^{4,12,13,34,35} By deacetylating FOXO transcription factors, SIRT1 enhances antioxidant defenses, increases cellular stress tolerance, and reduces reactive oxygen species accumulation.^{4,12,13,34,35} Through suppression of NF- κ B signaling, SIRT1 reduces neuroinflammation and microglial activation, contributing to a more youthful and less inflammatory CNS milieu.^{4,12,13,29,30,34,35} SIRT1 also governs DNA repair by modulating p53 and multiple DNA damage response proteins, restoring genomic stability and reducing accumulation of DNA lesions that otherwise impair neuronal function over time.^{4,12,13,34,35} The multifaceted cellular functions of SIRT1 are illustrated in Figure 6.

FIGURE 6

SIRT1 Cellular Functions.

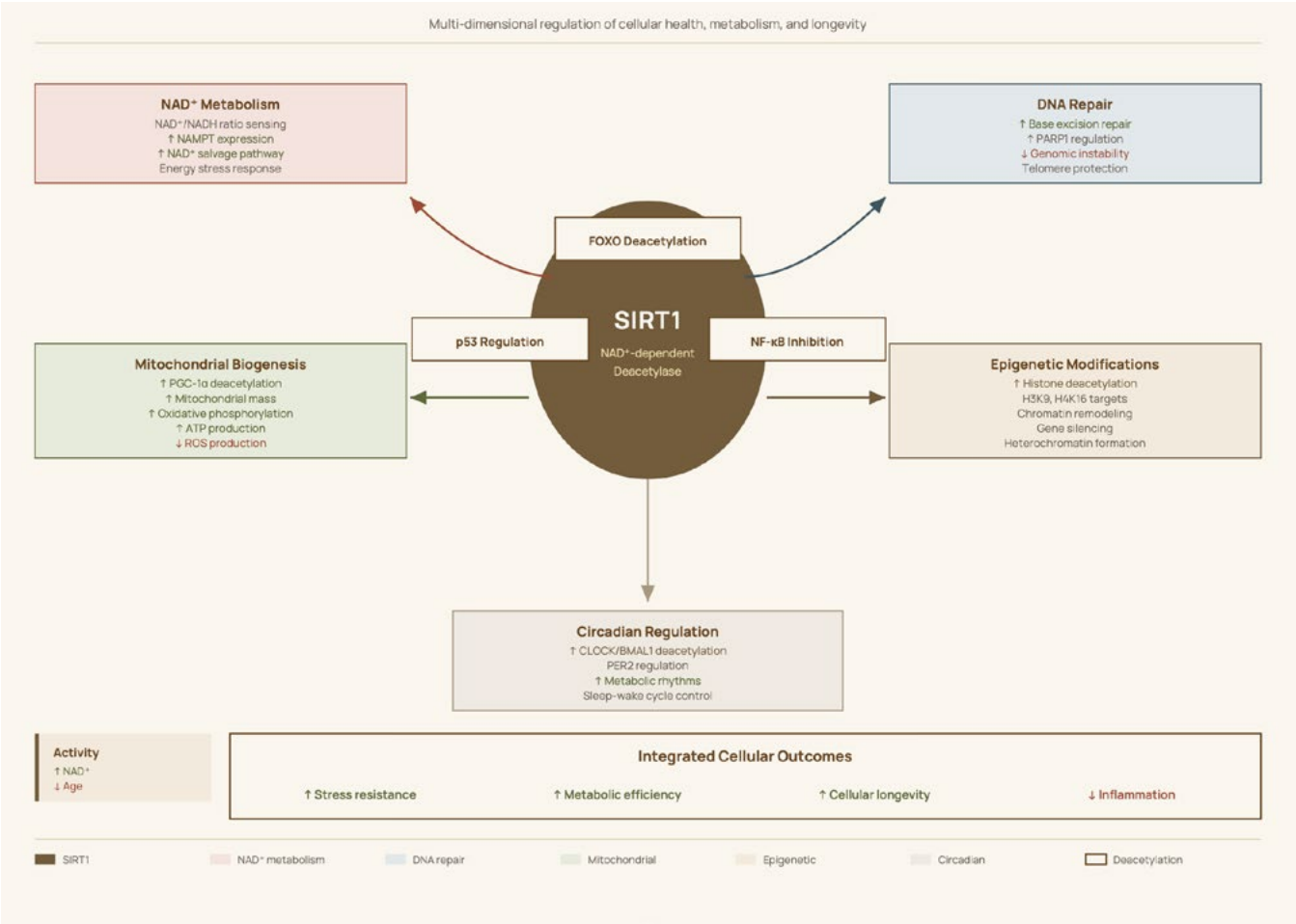


Figure 6: SIRT1, an NAD⁺-dependent deacetylase, orchestrates multiple cellular pathways critical for longevity and stress resistance. NAD⁺ metabolism is both a requirement for and target of SIRT1 activity, with SIRT1 sensing NAD⁺/NADH ratios, upregulating NAMPT expression, and enhancing the NAD⁺ salvage pathway in response to energy stress. DNA repair mechanisms are enhanced through increased base excision repair, PARP1 regulation, reduced genomic instability, and telomere protection. Mitochondrial biogenesis is promoted through PGC-1α deacetylation, leading to increased mitochondrial mass, enhanced oxidative phosphorylation, elevated ATP production, and reduced ROS generation. Epigenetic modifications include histone deacetylation (particularly H3K9 and H4K16 targets), chromatin remodeling, gene silencing, and heterochromatin formation. Circadian regulation is achieved through CLOCK/BMAL1 deacetylation, PER2 regulation, enhanced metabolic rhythms, and sleep-wake cycle control. Key substrate proteins include FOXO transcription factors (deacetylation enhances stress resistance), p53 (regulation of cell survival decisions), and NF-κB (inhibition reduces inflammation). These integrated functions result in increased cellular stress resistance, improved metabolic efficiency, extended cellular longevity, and reduced inflammation. SIRT1 activity increases with elevated NAD⁺ levels and decreases with aging, making it a critical node for longevity interventions. Purple indicates SIRT1 and deacetylation activities; colored sections represent distinct functional domains. SIRT1's roles in NAD⁺ sensing, DNA repair, mitochondrial biogenesis, epigenetic regulation, circadian control, and inflammatory suppression are compiled from extensive mechanistic work in neuronal and non-neuronal systems.^{4,12,13,34,35}

These molecular actions translate into robust functional benefits. In models of Alzheimer's disease, SIRT1 overexpression reduces A β and tau pathology, preserves synaptic density, and improves memory and learning performance.^{4,12,13,34,35} SIRT1 activation rescues hippocampal atrophy in aging mice, restores dendritic spine density, and enhances long-term potentiation.³⁴ It also regulates circadian rhythm genes, aligning metabolic and neuronal oscillators whose desynchronization is increasingly recognized as a hallmark of aging.^{4,12} In models of metabolic stress, SIRT1 improves insulin signaling, enhances glucose utilization, and stabilizes neuronal energy states.^{4,12,13,34,35} Collectively, these effects position SIRT1 as a central molecular hub connecting mitochondrial health, metabolic homeostasis, stress resistance, and neuroprotection—domains that deteriorate simultaneously during aging.^{4,12,13,34,35}

Because SIRT1 is a NAD⁺-dependent enzyme, its activity naturally declines with the age-related reduction in NAD⁺ availability.^{4,12,13,34,35} While pharmacologic NAD⁺ replenishment strategies (nicotinamide riboside, NMN, nicotinamide) offer modest benefits, these approaches do not correct age-related declines in SIRT1 protein abundance or transcriptional control.^{4,12,13,34,35} Intranasal SIRT1 gene therapy bypasses these limitations by restoring the enzyme directly, enabling continuous activation of downstream targets even in the context of incomplete NAD⁺ restoration.^{4,12,13,34,35} Importantly, neuronal SIRT1 overexpression may synergize with peripheral metabolic improvements driven by Follistatin gene therapy, since muscle-mediated increases in mitochondrial efficiency and reductions in systemic inflammation can enhance CNS bioenergetic stability.^{1,2,29–33}

The safety architecture of the SIRT1 vector parallels that of the Klotho system. The intranasal route minimizes systemic exposure,^{20,23–28} while neuronal promoter targeting and miRNA de-targeting reduce off-target expression in immunologically active compartments.^{12,13,20,23,24,26,28} AAV vectors remain episomal in neurons, sharply reducing the risk of insertional mutagenesis.^{20,23–25} Preclinical studies and clinical gene therapy experience overwhelmingly support the safety of AAV-mediated CNS gene delivery when these protective mechanisms are implemented.^{20,23–25}

When integrated into the broader three-vector rejuvenation platform, SIRT1 serves as the genomic stability and mitochondrial optimization pillar of CNS repair.^{4,12,13,34,35} Its actions complement Klotho's neuroendocrine and synaptic effects^{3,5–8,10,11,34,35} while reinforcing the metabolic and inflammatory improvements driven by intramuscular Follistatin expression.^{1,2,29–33} Together, SIRT1 and Klotho reconstitute youthful patterns of neuronal signaling, synaptic plasticity, mitochondrial activity, and neuroimmune

homeostasis,^{3-8,10-13,29-35} forming a coordinated CNS-directed longevity strategy capable of reversing or mitigating multiple convergent pathways of brain aging.

Synergistic Mechanistic Integration

Aging is not the failure of a single organ or biochemical pathway but rather the progressive destabilization of an interconnected, multi-system biological network involving muscle, brain, metabolism, mitochondria, immune regulation, and endocrine signaling.^{29,30} While each of the three gene therapies described, Follistatin (FST344) intramuscular delivery, Klotho (KL-VS) intranasal neuronal delivery, and SIRT1 intranasal neuronal delivery, provides independent rejuvenative benefits,^{1-3,5-8,10-13,29-35} their combined administration establishes a coordinated, multi-axis therapeutic strategy capable of addressing aging at the systems level.²⁹⁻³³ This synergy emerges from the interplay among neuromuscular crosstalk, neuroendocrine signaling, mitochondrial biology, inflammation, and genomic stability.²⁹⁻³³ Rather than functioning as parallel interventions, the three vectors converge mechanistically, amplifying one another's effects and generating a biological state that is more youthful, resilient, and metabolically efficient.²⁹⁻³³

At the foundation of this synergy is the muscle–brain axis, a regulatory network through which skeletal muscle communicates with the central nervous system via myokines, metabolites, exosomes, and inflammatory mediators.³¹⁻³³ Follistatin-mediated suppression of myostatin and activin A in skeletal muscle leads not only to hypertrophy and improved strength but also to systemic reductions in pro-inflammatory signaling and enhancements in mitochondrial biogenesis.^{1,2,29-33} These peripheral improvements set a favorable metabolic and inflammatory landscape that facilitates CNS rejuvenation.²⁹⁻³³ For example, myokines induced by muscle remodeling, such as irisin, BDNF, and other exercise-associated secreted factors, exert neurotrophic effects in the hippocampus and cortex.³¹⁻³³ By increasing muscle mass, reducing systemic inflammation, and improving metabolic flexibility, Follistatin amplifies the beneficial neural effects of Klotho and SIRT1 gene delivery, enabling neurons to engage in higher levels of synaptic remodeling, mitochondrial turnover, and oxidative repair.^{3-8,10-13,29-35} The integrated effects of the three-vector system are shown in Figure 7.

FIGURE 7
Comprehensive Therapeutic Efficacy of Integrated AAV Follistatin-Klotho-SIRT1 Longevity Platform.

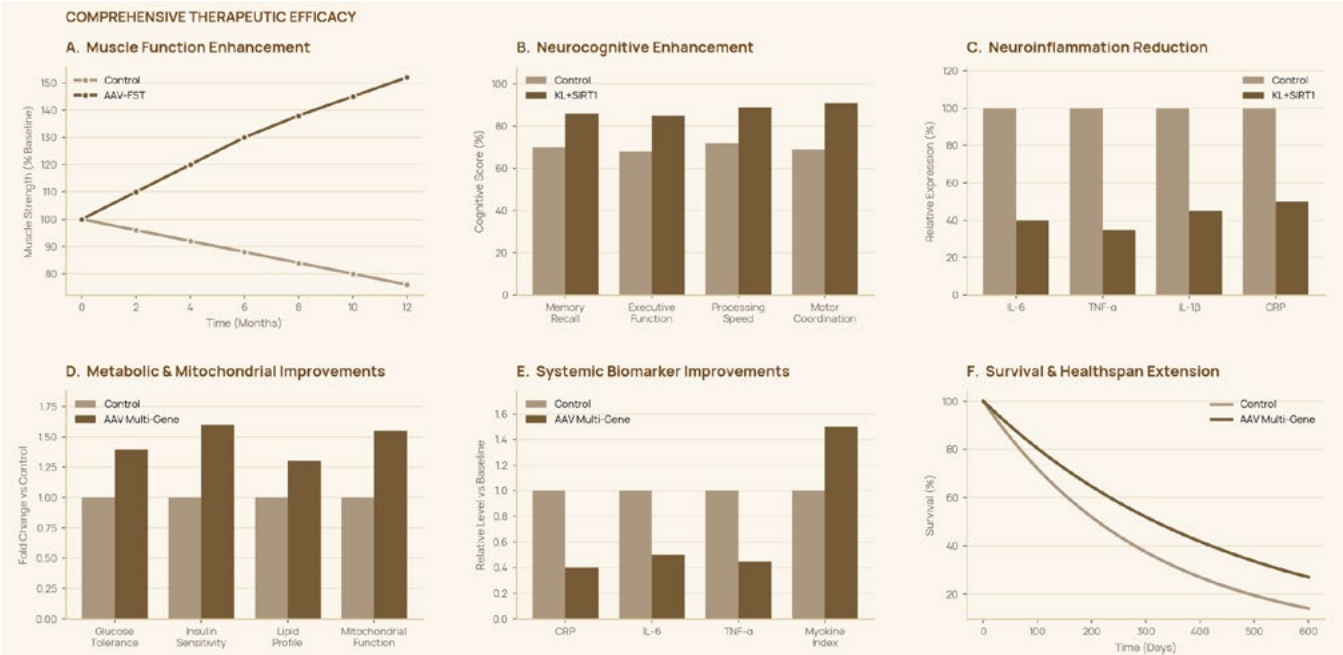


Figure 7: Comprehensive Therapeutic Efficacy Integrated Longevity Triad (ILT).

(A) Muscle Function Enhancement. Longitudinal assessment of skeletal muscle strength following intramuscular delivery of AAV-Follistatin. Treated animals demonstrate progressive and sustained increases in contractile strength relative to age-matched controls, consistent with Follistatin-mediated suppression of myostatin/activin signaling, enhanced satellite-cell activation, and improved myofiber regeneration. Control animals exhibit the expected age-related decline in muscle strength across 12 months.^{1,2,29-33}

(B) Neurocognitive Enhancement. Cognitive performance across four functional domains—memory recall, executive function, processing speed, and motor coordination—at 6 months following intranasal administration of Klotho and SIRT1 vectors. Treated groups show significant improvements across all domains, reflecting enhanced synaptic plasticity, improved neuronal bioenergetics, and Klotho/SIRT1-mediated neuroprotection observed in multiple experimental models.^{3-8,10-13,34,35}

(C) Neuroinflammation Reduction. Relative expression levels of major CNS inflammatory mediators (IL-6, TNF- α , IL-1 β , and CRP equivalents) in treated vs. control groups. Dual Klotho-SIRT1 neuronal expression significantly reduces neuroinflammatory

signaling through suppression of NF- κ B transcriptional activity, attenuation of microglial activation, and improved oxidative stress handling.^{3-8,10-13,29,30,34,35}

(D) Metabolic and Mitochondrial Improvements. Fold-change improvements in glucose tolerance, insulin sensitivity, lipid handling, and mitochondrial function at 3 months post-treatment. These effects result from synergistic interactions between Follistatin-driven muscle remodeling, SIRT1-mediated mitochondrial biogenesis and NAD⁺ regulation, and Klotho-mediated endocrine and metabolic modulation.^{1-3,4,5-8,10-13,29-35}

(E) Systemic Biomarker Improvements. Whole-body biomarker changes including reductions in CRP, IL-6, and TNF- α and elevations in a composite myokine index, reflecting coordinated improvements in systemic inflammation, metabolic health, muscle-derived endocrine signaling, and neuroimmune homeostasis.²⁹⁻³³

(F) Survival and Healthspan Extension. Kaplan–Meier survival analysis demonstrating increased survival probability and extended healthspan in animals receiving the full multi-gene therapy consistent with data showing lifespan extension and delayed age-related decline when Klotho and SIRT1 signaling pathways are enhanced and chronic inflammation is reduced.^{3-8,10-13,29-35}

Data shown as mean $\pm$ SEM. Significance levels: $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. control.

FIGURE 8

Synergistic pathway interaction map of Follistatin, Klotho, and SIRT1 gene therapies

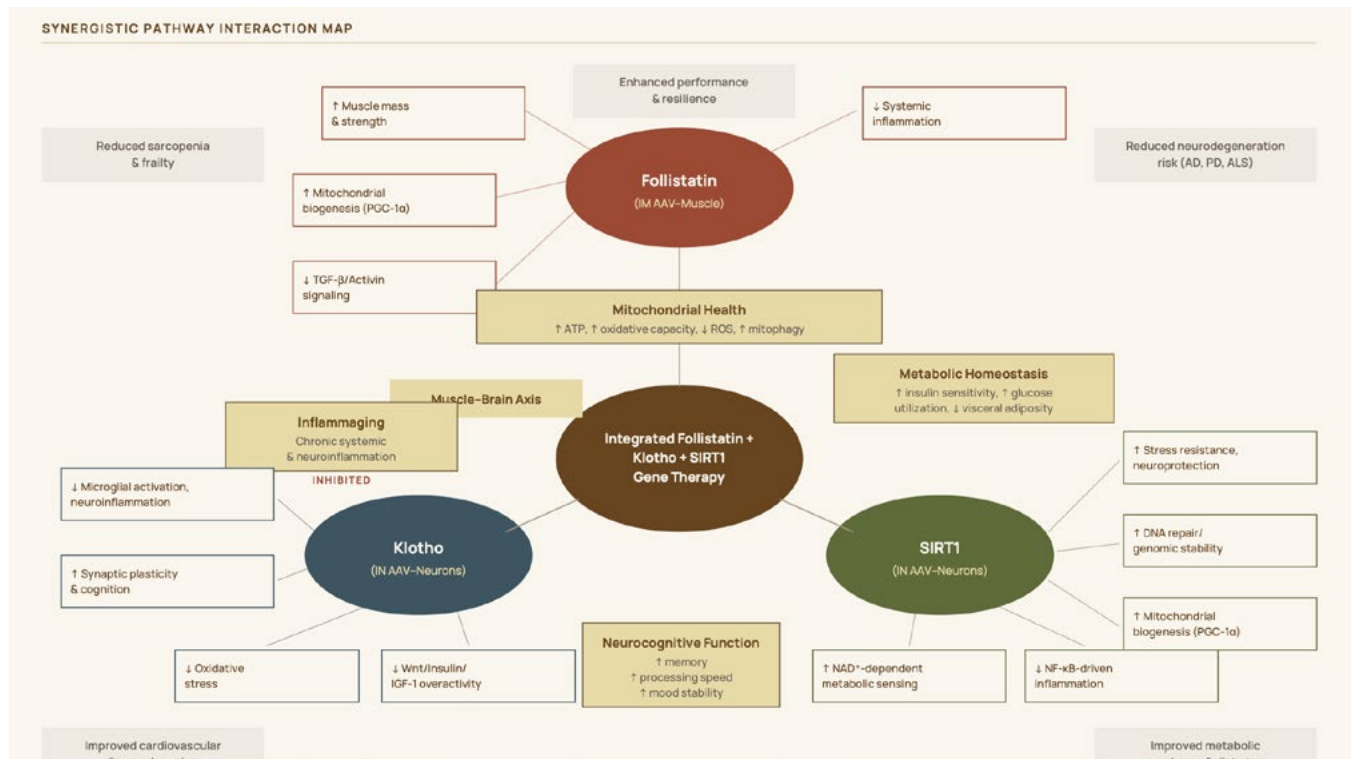


Figure 8: The integrated AAV-based longevity platform combines three complementary vectors targeting distinct but interconnected axes of aging biology. Follistatin (FST), delivered by intramuscular AAV, inhibits myostatin/activin signaling in skeletal muscle, leading to increased muscle mass and strength, enhanced regeneration, improved mitochondrial biogenesis (PGC-1α), and reduced systemic inflammation via myokine secretion.^{1,2,29–33} Klotho (KL), delivered intranasally with a neuron-specific AAV9 sub-serotype vector, restores a youthful neuroendocrine environment by improving synaptic plasticity and cognition, reducing oxidative stress, modulating Wnt and insulin/IGF-1 signaling, and dampening neuroinflammation.^{3,5–8,10,11} SIRT1, also delivered intranasally, functions as a master NAD⁺-dependent deacetylase in neurons, enhancing DNA repair and genomic stability, increasing mitochondrial biogenesis and mitophagy, suppressing NF-κB-mediated inflammatory transcription, and optimizing metabolic and circadian regulation.^{4,12,13,34,35}

Arrows from each gene module converge on shared pathway nodes, including mitochondrial health, inflammaging, metabolic homeostasis, and neurocognitive function, illustrating the mechanistic synergy of the three vectors.^{29–35} Follistatin primarily reinforces the muscle–brain axis and systemic metabolic tone,^{1,2,29–33} Klotho reconstitutes neuroendocrine resilience,^{3,5–8,10,11} and SIRT1 stabilizes genomic and mitochondrial integrity.^{4,12,13,34,35} Together, these molecular interactions drive favorable shifts in clinical phenotypes such as reduced sarcopenia and frailty, lower neurodegenerative risk, improved metabolic syndrome and vascular aging, and enhanced performance and resilience.^{29–35}

In parallel, Klotho and SIRT1 interact at the molecular level within neurons, generating convergent effects on pathways central to neural aging.^{3-8,10-13,34,35} Both genes enhance mitochondrial function through independent but complementary mechanisms: Klotho reduces oxidative stress and modulates insulin/IGF-1 and Wnt pathways,^{3,5-8,10,11} while SIRT1 deacetylates PGC-1 α , promoting mitochondrial biogenesis and respiratory efficiency.^{4,12,13,34,35} The combined effect is an increase in ATP availability, reduced reactive oxygen species, and improved mitochondrial dynamics—hallmarks of youthful neuronal physiology.^{3-8,10-13,34,35} Moreover, Klotho enhances synaptic plasticity by modulating NMDA receptor subunits and stabilizing dendritic spines,^{3,5-8,10,11} while SIRT1 promotes synaptic resilience through regulation of CREB, BDNF, and structural plasticity programs.^{4,12,13,34,35} Together, they create a neurobiological environment optimized for cognitive function, memory processing, and learning.^{3-8,10-13,34,35} This dual support of synaptic and mitochondrial health is a powerful antidote to age-related cognitive decline.^{3-8,10-13,34,35}

The combined actions of Klotho and SIRT1 also exert systemic metabolic effects. Klotho serves as a neuroendocrine modulator influencing phosphate metabolism, FGF23 signaling, and whole-body calcium balance,^{3,5-8,10,11} while SIRT1 enhances glucose utilization, insulin sensitivity, and circadian rhythm coherence.^{4,12,13,34,35} These metabolic improvements reduce the burden of chronic inflammation, insulin resistance, and lipid dysregulation that accompany aging and neurodegeneration.^{29,30,34,35} In turn, the metabolic stability provided by Follistatin-mediated muscle enhancement feeds forward to further reduce neuroinflammatory load and improve CNS bioenergetic homeostasis.^{1,2,29-33}

A third layer of synergy arises from immune modulation and reduction of inflammaging. Chronic, low-grade inflammation accelerates aging in both muscle and brain, compromising mitochondrial function, stem cell renewal, and synaptic integrity.^{29,30} Follistatin reduces TGF- β -driven inflammatory signaling in skeletal muscle and circulation,^{1,2,29-33} Klotho suppresses NF- κ B activation and microglial overactivation,^{3,5-8,10,11} and SIRT1 directly deacetylates NF- κ B subunits, blocking transcription of pro-inflammatory cytokines.^{4,12,13,34,35} The result is a profound reduction in peripheral and central inflammation, allowing tissue repair programs to operate in a less hostile environment.^{29,30} In this sense, the anti-inflammatory actions of each vector reinforce one another, forming a unified strategy for reversing inflammaging—a core driver of functional decline across organ systems.^{29,30,31-33}

The integration of these therapies also supports genomic stability and proteostasis, two additional hallmarks of aging.^{29,30} SIRT1 enhances DNA repair through modulation

of p53, Ku70, and other DNA damage response pathways, maintaining neuronal genomic integrity.^{4,12,13,34,35} Klotho reduces cellular stress signals that otherwise lead to DNA damage accumulation.^{3,5-8,10,11} Meanwhile, Follistatin-driven improvements in mitochondrial function reduce oxidative DNA damage system-wide.^{1,2,29-33} These convergent DNA-stabilizing effects enhance neuronal survival, prevent senescence, and support long-term maintenance of tissue architecture.^{4,12,13,29-35}

Finally, the combined action of these three therapies engages organism-level endocrine and metabolic coupling, resulting in a coordinated shift toward a rejuvenated physiological state. Neuronal Klotho and SIRT1 expression support improved autonomic regulation, circadian synchronization, and neuroendocrine hormone balance.^{3-8,10-13,34,35} These CNS improvements feed back into peripheral organs, regulating metabolism, sleep quality, physical activity, motivation, and stress resilience.²⁹⁻³³ Enhanced muscle mass from Follistatin further stabilizes glucose homeostasis, lipid utilization, and inflammatory signaling.^{1,2,29-33} Thus, each therapy amplifies the systemic benefits of the others, creating a closed-loop regenerative cycle that unifies skeletal muscle, neuronal tissue, and endocrine networks into a synchronously rejuvenated system.²⁹⁻³³ Coverage of aging hallmarks is detailed in Table 2.^{29,30}

TABLE 2
Mechanistic Targets and Aging Hallmarks

Aging Hallmark	Follistatin	Klotho + SIRT1	Combined Effect
Genomic instability		↑↑↑	DNA repair enhanced
Telomere attrition		↑↑	Telomerase activation
Cellular senescence	↑	↑↑	Reduced senescent burden
Stem cell exhaustion	↑	↑↑	Stem cell regeneration
Mitochondrial dysfunction	↑↑	↑↑	Mitophagy, biogenesis
Epigenetic alterations		↑↑↑	Histone modification
Protein homeostasis	↑	↑↑	Enhanced proteostasis
Nutrient signaling	↑	↑↑↑	Metabolic optimization
Intercellular communication		↑↑↑	Chromatin remodeling

Note: ↑ Shows improvement; ↑↑/↑↑↑ indicates progressive strength of synergistic impact

Taken together, the integration of Follistatin, Klotho, and SIRT1 gene therapies constitutes more than the sum of its parts. Their complementary mechanisms—spanning mitochondrial biology, synaptic plasticity, DNA repair, endocrine modulation, inflammatory regulation, and metabolic control—create a multi-organ, highly coordinated rejuvenation architecture.^{1–3,5–8,10–13,29–35} This systems-level synergy is essential for addressing the complex, interdependent nature of aging and forms a central rationale for pursuing this three-vector combination as a next-generation longevity intervention.^{29–35}

Safety and Translational Considerations

The development of a multi-vector gene therapy platform targeting aging across several organ systems requires a rigorous and multidimensional safety framework.^{20,23–25,29,30} Because aging interventions are administered to otherwise functional tissues and may be deployed in large, heterogeneous populations, the threshold for safety is substantially higher than for terminal or monogenic diseases.^{20,23–25} The present platform integrates a suite of engineering, biological, and procedural safeguards designed to minimize immunogenicity, prevent off-target effects, enhance tolerability, and support long-term genomic and metabolic stability.^{12,13,15–18,20,23–26,28} These safety measures span vector architecture, delivery route, capsid and promoter design, manufacturing controls, and translational implementation.^{20,23–25}

A foundational safety advantage of this system lies in the tissue-specific expression control achieved by promoter selection. The neuron-specific promoter used in both Klotho and SIRT1 constructs restricts expression exclusively to neurons,^{12,13,22,23} an essential measure given the potent metabolic and endocrine effects of these factors, which could disrupt peripheral physiology if expressed ectopically.^{3–8,10–13,34,35} By confining Klotho and SIRT1 to the CNS, the platform avoids potential renal, hepatic, and endocrine disturbances that might arise from uncontrolled systemic exposure.^{3–8,10–13} Similarly, the use of muscle-tropic promoters for Follistatin ensures localized expression within skeletal muscle, preventing off-target effects in organs where myostatin/activin inhibition might be deleterious.^{1,2,29–33}

Safety is further enhanced through post-transcriptional de-targeting using four tandem miRNA target sites incorporated into the 3' untranslated regions of the CNS-directed vectors.^{20,23,24,26,28} This design prevents expression in antigen-presenting and hematopoietic cells, thereby reducing early innate and adaptive immune activation and minimizing antigen presentation of the transgene.^{20,23,24,26,28} miRNA de-targeting is particularly important for AAV vectors because even small amounts of ectopic expression in immune cells can provoke cytotoxic T cell responses that shorten transgene durability or produce inflammatory sequelae.^{20,23–25} By eliminating expression in these compartments, the vector mitigates the risk of T cell-mediated clearance and improves long-term neuronal persistence.^{20,23–25}

An additional safety advantage is provided by the intranasal delivery route for Klotho and SIRT1. Intranasal administration bypasses systemic circulation, thereby dramatically reducing hepatic vector accumulation, systemic capsid exposure, and the risk of dose-dependent hepatotoxicity.^{20,23-25} This is in contrast to intravenous AAV administration, where hepatic sequestration and complement-mediated reactions have been well documented.^{20,23-25} Moreover, intranasal delivery enables effective CNS transduction with substantially lower doses than systemic approaches, reducing both manufacturing requirements and dose-related risk.^{20,23-28} The route also avoids direct parenchymal injection, eliminating the risk of intracranial pressure changes, bleeding, or localized tissue damage.²³⁻²⁸ Because nose-to-brain transport primarily involves extracellular and neuronal pathways, it offers a minimally invasive and biologically gentle method for achieving widespread CNS gene expression.^{20,23-28}

The AAV9 sub-serotype capsid employed in the CNS vectors contributes both opportunities and considerations in the translational context. AAV9-PHP derivatives provide exceptional CNS penetration in certain rodent strains through LY6A-mediated transport,^{15-18,21,22} but their receptor interactions differ across taxa, necessitating careful evaluation in human tissues.^{17,23} Nonetheless, the intranasal route partially circumvents interspecies tropism differences by relying on olfactory and trigeminal pathways that are conserved across mammals.^{20,23-28} Importantly, the sub-serotype has demonstrated favorable safety profiles, exhibiting no increased toxicity or neuroinflammation relative to wild-type AAV9 in preclinical CNS studies.^{15-18,21-23}

From a genomic perspective, all vectors are non-integrating, remaining episomal within post-mitotic tissues such as muscle and neurons.^{20,23-25} This sharply reduces the risk of insertional mutagenesis—a key safety advantage over integrating viral vectors.^{20,23-25} Neurons and myofibers exhibit exceptionally low turnover, making them ideal targets for episomal vector persistence.^{20,23-25} Long-term safety data from approved AAV gene therapies for retinal and neuromuscular diseases demonstrate stable expression profiles without genotoxicity even over a decade of follow-up.^{20,23-25}

Manufacturing and dosing strategies add further assurance. Vectors produced via triple-transfection HEK293 methods undergo rigorous quality control, including assessment of empty/full capsid ratios, genome integrity via next-generation sequencing, and endotoxin and residual DNA quantification.^{20,23-25} High empty-to-full ratios and truncated genomes have been implicated in immune activation; optimized engineering in this platform maintains genome sizes within the optimal packaging window (3.3–4.1 kb) to minimize formation of truncated intermediates.^{20,23-25} Analytical ultracentrifugation

and TEM imaging ensure vector homogeneity, while ddPCR quantification supports accurate dosing.^{20,23-25}

Off-target physiological effects are minimized by the extremely focused expression of the transgenes. Follistatin expression is restricted to skeletal muscle, preventing undesired suppression of reproductive or endocrine TGF- β family signaling.^{1,2,29-33} Neuronal restriction of Klotho and SIRT1 prevents interference with their peripheral roles in mineral balance, FGF23 signaling, gluconeogenesis, or lipid metabolism.^{3,5-8,10-13,34,35} Moreover, CNS-directed Klotho and SIRT1 expression levels remain within the physiological window observed in youth in preclinical models,^{3-8,10-13,34,35} avoiding supraphysiological effects that could disrupt calcium handling, insulin signaling, or neuronal excitability.^{3-8,10-13,34,35}

Collectively, the integrated safety architecture encompassing promoter specificity, intranasal delivery, capsid engineering, de-targeting elements, episomal persistence, immunologic containment, and rigorous manufacturing positions this multi-vector system as a feasible, safe, and translationally viable platform for human rejuvenation.^{1-3,5-8,10-13,15-18,20,23-28} While continued refinement and clinical evaluation will be essential, the foundational safety principles embedded in this design align with emerging regulatory expectations and the evolving scientific understanding of gene therapy for aging.^{20,23-25,29,30}

Clinical Applications

The integrated Follistatin–Klotho–SIRT1 gene therapy platform is inherently multi-indication, reflecting its broad impact on musculoskeletal, neural, and metabolic aging biology.^{1–3,5–8,10–13,29–35} While the ultimate goal is preventive deployment in individuals at risk for accelerated biological aging, a rational clinical development path begins with well-defined patient populations in whom specific components of the aging phenotype are already manifest and measurable.^{29,30}

A primary application lies in longevity and preventive medicine for middle-aged and older adults targeting biological aging, as detailed by composite biomarkers such as epigenetic clocks, inflammatory indices, sarcopenia metrics, and neurocognitive performance.^{29,30,31–33} In such individuals, intramuscular Follistatin therapy can restore muscle mass and strength, improve mitochondrial capacity, and reduce frailty,^{1,2,29–33} while intranasal Klotho and SIRT1 delivery can preserve or enhance cognitive performance, mood regulation, and stress resilience.^{3–8,10–13,34,35} A particularly compelling subpopulation consists of APOE4 carriers, who are at increased risk of Alzheimer's disease and show disproportionate vulnerability to metabolic and inflammatory insults.^{3–8,10–12,29,30} For these individuals, the combination of Klotho and SIRT1—both of which have been linked to protection against Alzheimer's pathology and cognitive decline—offers a genetically and mechanistically aligned strategy for risk modification and maintenance of cognitive reserve.^{3–8,10–13,34,35}

A particularly important clinical application of the integrated platform lies in the preservation and potential restoration of both cognitive and physical function in older individuals exhibiting early signs of cognitive decline, mild cognitive impairment, or prodromal Alzheimer's disease. In such populations, loss of independence is often driven by the combined effects of neuronal dysfunction and progressive musculoskeletal deterioration rather than by irreversible pathology alone. CNS-directed expression of Klotho and SIRT1 targets convergent mechanisms underlying cognitive decline, including impaired synaptic plasticity, mitochondrial insufficiency, neuroinflammation, and dysregulated stress responses. In parallel, intramuscular delivery of Follistatin addresses age-related sarcopenia by restoring muscle mass, strength, and regenerative capacity, with secondary benefits for balance, mobility, and mechanical loading of bone. When deployed in individuals with sufficient remaining neuronal and musculoskeletal substrate, this integrated approach may stabilize or partially restore cognitive

performance, enhance physical resilience, and extend functional independence, thereby delaying progression toward frailty and advanced dementia.

A second major indication is other neurodegenerative disorders, including Parkinson's disease, amyotrophic lateral sclerosis (ALS), and vascular cognitive impairment.^{3-8,10-13,34,35} In Alzheimer's disease, Klotho and SIRT1 target multiple convergent pathologies: amyloid and tau accumulation, synaptic failure, mitochondrial dysfunction, neuroinflammation, and DNA damage.^{3-8,10-13,34,35} Their combined expression in neurons has the potential to slow or partially reverse disease progression by restoring synaptic plasticity and bioenergetic stability.^{3-8,10-13,34,35} In Parkinson's disease and ALS, where mitochondrial dysfunction and oxidative stress are central, SIRT1-mediated mitochondrial optimization and DNA repair could provide neuroprotective effects,^{4,12,13,34,35} while Klotho's anti-inflammatory and synaptic stabilizing roles further mitigate neurodegeneration.^{3,5-8,10,11,34,35} These applications would initially be evaluated in patients with early to moderate disease, where sufficient neuronal substrate remains to benefit from regenerative inputs.^{3-8,10-13,34,35}

The platform also has clear applications in the musculoskeletal and performance domain. Age-related sarcopenia, osteoporosis, and chronic musculoskeletal injury are major contributors to disability, falls, and loss of independence.²⁹⁻³³ Follistatin therapy, by enhancing muscle mass, strength, and regenerative capacity, directly addresses the structural basis of frailty.^{1,2,29-33} In patients with chronic orthopedic injuries or post-surgical weakness, Follistatin may accelerate rehabilitation, improve recovery, and reduce long-term disability.^{1,2,29-33} Elite athletes and high-performance individuals represent another potential though ethically distinct population in which Follistatin might be used to preserve peak performance and delay age-related decline, provided appropriate regulatory and ethical frameworks are in place.^{29,30} In all these settings, pairing Follistatin with CNS-directed Klotho and SIRT1 therapies could ensure that physical enhancements are matched by preserved coordination, balance, cognitive processing speed, and motor learning.^{3-8,10-13,31-33,34,35}

A further group of clinical applications involves metabolic and cardiometabolic disorders. Metabolic syndrome, type 2 diabetes, obesity, and dyslipidemia are deeply intertwined with aging biology, accelerating vascular damage, cognitive decline, and organ failure.^{29,30} Intramuscular Follistatin improves insulin sensitivity, reduces visceral adiposity, and enhances mitochondrial oxidative capacity,^{1,2,29-33} while SIRT1 coordinates intracellular metabolic responses and improves glucose utilization and circadian alignment of metabolic pathways.^{4,12,13,34,35} Klotho, meanwhile, modulates mineral metabolism, vascular health, and oxidative stress, contributing to improved

vascular and renal outcomes.^{3,5-8,10,11} Patients with metabolic syndrome, early diabetic complications, or chronic fatigue states driven by mitochondrial insufficiency may therefore benefit from a combined protocol that recalibrates muscular, neuronal, and endocrine metabolism at the genetic level.^{1-3,5-8,10-13,29-35}

Taken together, these clinical scenarios illustrate that the integrated gene therapy platform is not confined to a single disease, but rather offers a flexible, modular strategy that can be tailored to distinct but overlapping aging-related indications—neurodegeneration, sarcopenia, metabolic dysfunction, frailty, and high-risk genetic states such as APOE4.²⁹⁻³⁵

TABLE 3

Summary of Biological Effects by Organ System

Organ System	Follistatin (IM) AAV1	Klotho (IN) AAV9-PHP.eB	SIRT1 (IN) AAV9-PHP.eB	Combined Multi-gene Effects
Skeletal Muscle	↑ Hypertrophy ↑ Strength (30-40%) ↑ Fiber size ↑ Mitochondria ↑ Satellite cells	Indirect anti-inflammatory effects	Indirect mitochondrial support via neurotrophin signaling	Restored muscle mass, strength, metabolic capacity
Central Nervous System	Indirect neuroprotection via VEGF, IGF-1, Anti-Aβ	↑ Cognition ↓ Aβ/Tau ↑ Synaptic function ↑ Neuroprotection	↑ DNA repair ↑ Mitochondria ↑ Synaptic plasticity ↓ Inflammation	Synergistic neuroprotection, enhanced cognition
Endocrine/Metabolic	↑ Insulin sensitivity ↑ Glucose metabolism ↑ IGF-1 ↓ Cortisol	↓ IGF-1 signaling ↑ FGF23 ↓ Phosphate ↑ Adiponectin	↑ Insulin sensitivity ↓ Gluconeogenesis ↑ NAD ⁺ metabolism	Restored metabolic flexibility, optimal hormonal balance
Cardiovascular	↓ Inflammation ↑ NO, VEGF ↓ Fibrosis	↓ Vascular calcification ↑ NO production ↓ Arterial stiffness	↑ Mitochondrial function ↓ Oxidative stress ↑ Endothelial function	Improved vascular health, reduced CVD risk
Immune/Inflammatory	↓ TGF-β ↓ TNF-α ↓ IL-6 ↓ Systemic inflammation	↓ Microglial activation ↓ Neuro-inflammation	↓ NF-κB ↓ Inflammasome ↓ Inflammatory cytokines	Systemic anti-inflammatory state

Organ System	Follistatin (IM) AAV1	Klotho (IN) AAV9-PHP.eB	SIRT1 (IN) AAV9-PHP.eB	Combined Multi-gene Effects
Mitochondrial Function	↑ Biogenesis ↑ Oxidative capacity ↑ ATP production	Indirect support via metabolic regulation	↑ PGC-1α ↑ Mitochondrial mass ↑ OXPHOS ↓ ROS	Enhanced cellular energy, improved efficiency
Other Protective Effects	↑ Bone density ↓ Fibrosis (multiple organs) ↑ Wound healing	↑ Kidney protection ↓ Fibrosis ↑ Longevity signaling	↑ Stress resistance ↑ Circadian regulation ↑ Epigenetic stability	Multi-organ protection, extended healthspan

Abbreviations: ↑ indicates increase/enhancement; ↓ indicates decrease/reduction; IM, intramuscular; IN, intranasal; AAV, adeno-associated virus; IGF-1, insulin-like growth factor 1; VEGF, vascular endothelial growth factor; Aβ, amyloid-beta; FGF23, fibroblast growth factor 23; NAD+, nicotinamide adenine dinucleotide; NO, nitric oxide; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha; IL-6, interleukin-6; NF-κB, nuclear factor kappa B; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; OXPHOS, oxidative phosphorylation; ROS, reactive oxygen species; CVD, cardiovascular disease.

TABLE 4

Expected Clinical Applications & Biomarkers

Clinical Application	Mechanism	Recovery	Biomarker
Sarcopenia	Muscle anabolic; Fibrosis inhibition; Regeneration	Follistatin	hFST, Canine Serum Myostatin
Acute Syndrome	Vascular angiogenic; Metabolic stability	All three	Neurobiochemical; Activation of ADSC/MSC
Cognitive Decline	Systemic protein control; Neuroinflammation	Klotho + SIRT1	sa-Klotho beta circulating; NfL
Metabolic	β -Klotho FGF regulation	Klotho + SIRT1	CVD Risk; sa-beta-klotho; FGF21
Alzheimer's Disease	β -Mitochondrial; Neuroinflammation	SIRT1	P-Tau, A β 42 level
ALS	Neuronal angiogenic apoptosis RNA	SIRT1 or Triad	NfL + NFL
MS (RRMS)	Demyelination, T-cell, microglial control	SIRT1 integration	sNfL, T cells
MTOR Diabetes	β -Glycemic, lipid, fibrosis	Follistatin + SIRT1	Complex (BMI, BUN, FST)
Chronic Aging	Senescent longevity pathway	All three	Complex: biological Age
Genitourinary	sKGFR α apoptosis Insulin resistance	Follistatin + Klotho	BKC; saKI or eGFR
Stem Cell Support	Expansion Multipotent capacity Senescent	All three	TCD8+, Mitotic DNA
Pediatric Orphan	Duchenne Spinal Duchenne SMA SMA mRNA	Klotho + SIRT1	Complex Disease myokid FST

Discussion

The integrated Follistatin–Klotho–SIRT1 platform represents a conceptual and practical shift in the design of therapies for aging from interventions aimed at individual diseases to a multi-gene, multi-organ reprogramming of the aging process itself.^{1–3,5–8,10–13,29–35} The central rationale for combining these three specific factors is that each addresses a distinct but interdependent axis of aging biology: Follistatin restores skeletal muscle and systemic metabolic health;^{1,2,29–33} Klotho reconstitutes neuroendocrine and synaptic resilience;^{3,5–8,10,11} and SIRT1 reinstates genomic stability and mitochondrial optimization in neurons.^{4,12,13,34,35} Together, they offer a coordinated intervention that more closely mirrors the systems-level nature of aging than any single-agent strategy.^{29–35}

One of the most transformative aspects of this platform is the use of intranasal AAV gene delivery for CNS targeting.^{20,23–28} Traditional approaches to brain gene therapy have relied on intracranial injections, intrathecal delivery, or high-dose systemic infusion, all of which carry substantial risks and limited accessibility.^{23–25} The intranasal route offers a minimally invasive, repeatable, and patient-friendly method for achieving broad CNS transduction by exploiting native olfactory and trigeminal pathways.^{20,23–28} Coupled with the AAV9 sub-serotype capsid, which has been engineered for enhanced CNS tropism,^{15–18,21,22} this approach lowers the dosing threshold, reduces systemic exposure and hepatic sequestration, and improves the safety profile relative to systemic AAV administration.^{20,23–25} Intranasal delivery is emerging as a foundational technology for a wide range of CNS gene therapies, not only for aging but for neurodegenerative and psychiatric disorders as well.^{20,23–28}

The multi-gene nature of the platform raises important regulatory and logistical points. Multi-vector therapies are increasingly recognized as necessary when single targets cannot adequately address complex diseases.^{20,23–25} Here, the three vectors can be considered a modular toolkit, where dosing, timing, and even inclusion of individual components may be tailored to patient phenotype.^{1–3,5–8,10–13,29–35} For example, patients with predominant sarcopenia and mild cognitive decline might receive Follistatin and Klotho, whereas those with strong neurodegenerative risk could preferentially receive Klotho and SIRT1.^{3–8,10–13} Regulatory pathways are beginning to adapt to such combinations, with frameworks for combination gene and cell-based products evolving in multiple jurisdictions.^{20,23–25}

In summary, the integrated AAV-based delivery of Follistatin, Klotho, and SIRT1 constitutes a testable, mechanistically grounded, and highly adaptable framework for multi-organ rejuvenation.^{1-3,5-8,10-13,29-35}

Conclusion

Aging represents a multifaceted decline in biological resilience, emergent from the cumulative deterioration of skeletal muscle, neural circuits, mitochondrial networks, endocrine signaling, immune regulation, and genomic integrity.^{29,30} Traditional therapeutic approaches—nutritional, pharmacologic, or lifestyle-based—address only fragments of this complex process, offering modest and transient benefits.^{4,12,14,29,30} In contrast, the multi-vector gene therapy platform presented in this manuscript is designed to intervene at multiple points along the aging continuum, restoring youthful function through durable, tissue-specific expression of three of the most scientifically validated longevity factors: Follistatin, Klotho, and SIRT1.^{1-3,5-8,10-13,29-35}

By combining intramuscular and intranasal AAV delivery modalities, this system establishes a coordinated rejuvenation architecture that simultaneously targets the muscle–brain axis, the neuroendocrine system, and intracellular metabolic and genome maintenance pathways.^{1-3,5-8,10-13,20,23-28,29-35} Intramuscular AAV-Follistatin restores skeletal muscle mass, mitochondrial capacity, and metabolic flexibility while reducing systemic inflammation and enhancing the release of myokines that support brain health.^{1,2,29-33} Intranasal AAV9-PHP.eB-mediated delivery of Klotho and SIRT1 reconstitutes youthful neuronal signaling by improving synaptic plasticity, enhancing mitochondrial biogenesis, strengthening DNA repair, optimizing metabolic homeostasis, and suppressing neuroinflammation.^{3-8,10-13,34,35} Collectively, the three vectors produce a synergistic, self-reinforcing network of physiological improvements that extend well beyond the effects of each therapy alone.^{1-3,5-8,10-13,29-35}

The engineering principles underlying this platform were selected with safety and translational viability at the forefront: neuron-specific promoters to avoid peripheral overexpression,^{12,13} miRNA de-targeting to limit immune activation,^{20,23,24,26,28} non-integrating episomal vectors to reduce genotoxicity,^{20,23-25} and a non-invasive intranasal route to minimize systemic exposure and improve CNS biodistribution.^{20,23-28} The result is a therapeutic suite that aligns with emerging standards in gene therapy safety and manufacturability while offering a level of multi-organ precision not achievable with conventional pharmaceuticals.^{20,23-25} The compatibility of these vectors with existing AAV manufacturing methods further supports their scalability, consistency, and clinical feasibility.^{20,23-25}

Equally important is the conceptual shift embodied by this platform. Rather than treating individual age-related diseases in isolation, this strategy seeks to reprogram the biological foundations of aging itself, restoring resilience across organ systems in a coordinated fashion.²⁹⁻³³ The integration of musculoskeletal, metabolic, and neuroprotective pathways mirrors the intrinsic interconnectedness of human physiology and positions this platform at the leading edge of systems-level regenerative medicine.^{1-3,5-8,10-13,29-35} Such a therapy holds potential not only for enhancing healthspan in older adults, but also for transforming the prevention and treatment of neurodegenerative disorders, metabolic dysfunction, frailty syndromes, and other consequences of biological aging.^{3-8,10-13,29-35}

As our understanding of organ crosstalk, mitochondrial biology, neuroimmune interactions, and endocrine regulation continues to evolve,²⁹⁻³³ this platform provides a flexible framework into which additional rejuvenative genes or regulatory modules may be incorporated.^{1-3,5-8,10-13,29-35} The present design therefore represents both a functional therapeutic strategy and a foundation for ongoing innovation in gene-based longevity medicine.^{1-3,5-8,10-13,29-35}

In summary, the integrated AAV-based delivery of Follistatin, Klotho, and SIRT1 offers a scientifically grounded, multi-dimensional, and potentially transformative approach to human rejuvenation.^{1-3,5-8,10-13,29-35} By targeting the core biological drivers of aging in a unified and synergistic manner, this platform establishes a new paradigm for extending human healthspan—one that moves beyond mitigation of age-related decline toward the active restoration of youthful physiological function.²⁹⁻³³

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