

Alpha-Klotho

A kidney-derived transmembrane glycoprotein and its shed soluble ectodomain, identified through insertional mutagenesis in 1997 as a suppressor of aging phenotypes in mice, functioning both as an obligate co-receptor for fibroblast growth factor 23 in mineral metabolism and as a circulating endocrine factor that inhibits insulin/IGF-1, Wnt, TGF-beta, and NF-kappaB signaling pathways with demonstrated neuroprotective, cardioprotective, and renoprotective activity in preclinical models.

DOCUMENT	KDC-MN-1512
ISSUED	2026-05-15
REVISION	1.0
AUTHOR	Kodiac biolabs Research
COMPOUND CLASS	Type I transmembrane protein and circulating endocrine factor of the glycosyl hydrolase family 1 superfamily with obligate FGF23 co-receptor and pleiotropic anti-aging functions

ABSTRACT

Alpha-Klotho (alpha-KL) is a 130 kDa type I single-pass transmembrane protein encoded by the KL gene on human chromosome 13q13.1, first identified in 1997 by Kuro-o et al. through characterization of an insertional mutation in mice that produced a syndrome of accelerated aging encompassing soft tissue calcification, arteriosclerosis, skin atrophy, osteoporosis, emphysema, gonadal dysplasia, and dramatically shortened lifespan [1]. The protein comprises a short intracellular domain, a single transmembrane helix, and a large extracellular region containing two tandem glycosyl hydrolase family 1 (GH1) domains, designated KL1 and KL2, which share sequence homology with family 1 beta-glucosidases but lack catalytic activity against conventional substrates due to substitutions in the active-site residues. The extracellular domain undergoes proteolytic shedding by ADAM10, ADAM17, and BACE1, generating a soluble ectodomain (sKL) comprising KL1 and KL2 that circulates as an endocrine factor detectable in plasma, cerebrospinal fluid, and urine. A shorter secreted isoform containing only KL1 is produced by alternative mRNA splicing.

In its membrane-bound form, alpha-Klotho functions as an obligate co-receptor for fibroblast growth factor 23 (FGF23), forming a ternary complex with FGF receptor 1c (FGFR1c) that is essential for phosphaturic signaling in the renal proximal tubule and for suppression of 1,25-dihydroxyvitamin D3 (calcitriol) synthesis. The crystal structure of the alpha-Klotho/FGFR1c/FGF23 ternary complex, resolved by Chen et al. (2018) at 3.0 angstrom resolution, demonstrated that the KL2

domain of alpha-Klotho cradles FGF23 with a receptor-binding arm extending from the KL1-KL2 interdomain cleft, creating a composite binding surface for FGF23 engagement [2]. Loss of this co-receptor function produces the hyperphosphatemia, hypervitaminosis D, and ectopic calcification that characterize the kl/kl mouse phenotype and that are recapitulated in FGF23 knockout mice.

Independent of the FGF23 co-receptor function, soluble alpha-Klotho acts as a circulating endocrine factor with pleiotropic anti-aging activity. Characterized signaling activities include inhibition of the insulin/insulin-like growth factor 1 (IGF-1) pathway through suppression of receptor autophosphorylation; suppression of Wnt/beta-catenin signaling through direct binding to Wnt ligands; inhibition of transforming growth factor beta (TGF-beta) type II receptor signaling and downstream Smad phosphorylation; suppression of NF-kappaB-driven inflammatory transcription; and regulation of ion channel and transporter activity in the renal tubule, including TRPV5, TRPV6, ROMK1, and the Na⁺/K⁺-ATPase [3, 4, 5]. These FGF23-independent activities are the molecular basis for the broader anti-aging, neuroprotective, cardioprotective, and anti-fibrotic effects observed in gain-of-function and supplementation studies.

Circulating soluble alpha-Klotho levels decline with age in humans, beginning approximately in the fourth decade of life, and are markedly reduced in chronic kidney disease, where loss of renal alpha-Klotho expression precedes and contributes to the mineral and bone disorder, cardiovascular calcification, and accelerated aging phenotype of uremia [6]. Epidemiological studies have identified inverse associations between circulating soluble alpha-Klotho concentrations and all-cause mortality,

cardiovascular events, and cognitive decline in community-dwelling older adults [7]. The KL-VS haplotype (defined by the F352V and C370S variants, rs9536314 and rs9527025) has been associated in some cohorts with altered klotho secretion, cortical brain volume, and cognitive resilience in aging, though replication across large cohorts remains inconsistent [8, 9].

Preclinical studies have demonstrated that recombinant alpha-Klotho protein administration, adeno-associated virus-mediated KL gene transfer, and transgenic KL overexpression produce renoprotection in ischemia-reperfusion injury and unilateral ureteral obstruction models; cardioprotection with attenuation of left ventricular hypertrophy and fibrosis; suppression of vascular calcification; and cognitive enhancement in aged, young, and alpha-synuclein transgenic mice through NMDA receptor-dependent glutamatergic mechanisms [10, 11, 12, 13]. A 2023 study in aged nonhuman primates demonstrated that a single subcutaneous injection of a klotho protein fragment enhanced spatial and working memory, representing the first primate cognitive enhancement data for the compound [14].

As of 2026, alpha-Klotho is in early clinical development. Klotho Bio launched a Phase 1b randomized, double-blind, placebo-controlled trial of AKL003, an alpha-Klotho mRNA therapeutic administered intravenously, in healthy adult volunteers in February 2026. Klotho Neurosciences is advancing KLTO-202, a KL gene therapy, toward first-in-human studies for amyotrophic lateral sclerosis. No alpha-Klotho protein or gene therapy product has received regulatory approval in any jurisdiction. Recombinant human alpha-Klotho protein is available from multiple research suppliers (R&D Systems, Abcam, Sino Biological, Thermo Fisher) for in vitro and preclinical applications. This monograph reviews

the molecular identification, structural biology, receptor pharmacology, preclinical pharmacology across organ systems, the emerging clinical evidence base, sourcing and handling considerations, stack interactions, safety signal, and a comparative assessment of five anti-aging intervention candidates against alpha-Klotho on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Recommended name: Alpha-Klotho (alpha-KL).

Gene symbol: KL (HUGO Gene Nomenclature Committee approved symbol). The gene is located on human chromosome 13q13.1 and spans approximately 50 kilobases containing five exons [1].

Alternative names and synonyms: Klotho, alpha-Klotho protein, membrane Klotho (mKL, for the transmembrane form), soluble Klotho (sKL, for the shed ectodomain), secreted Klotho (for the alternatively spliced short isoform), anti-aging factor Klotho.

UniProt accession: Q9UEF7 (Homo sapiens). O35082 (Mus musculus).

NCBI Gene ID: 9365 (human KL gene). 16591 (mouse Kl gene).

OMIM: 604824 (KL gene and protein).

Key SNP identifiers: rs9536314 (F352V, part of the KL-VS haplotype); rs9527025 (C370S, co-segregating variant defining the KL-VS haplotype).

Nomenclature note: The name "Klotho" derives from Clotho, one of the three Moirai (Fates) in Greek mythology, who spins the thread of life. The gene was named by Kuro-o et al. (1997) in reference to the dramatic aging phenotype of the loss-of-function mouse [1]. The Greek spelling "Klotho" is the standard usage in the biomedical literature. Three members of the Klotho family have been identified: alpha-Klotho (KL), beta-Klotho (KLB), and gamma-Klotho (LCTL). This monograph addresses alpha-Klotho exclusively.

1.2 Structural class and molecular features

Alpha-Klotho is a type I single-pass transmembrane glycoprotein of the glycosyl hydrolase family 1 (GH1) superfamily. The full-length human alpha-Klotho precursor comprises 1012 amino acids with the following domain architecture:

- **Signal peptide:** residues 1 to 33 (cleaved during secretory pathway processing).

- **Extracellular domain:** residues 34 to 981, containing two tandem GH1-like domains: KL1 (residues 57 to 506) and KL2 (residues 515 to 953).
- **Transmembrane domain:** residues 982 to 1002 (21 amino acids, single alpha-helix).
- **Intracellular domain:** residues 1003 to 1012 (10 amino acids, no recognized signaling motifs).

Molecular weight: Predicted molecular weight of the unglycosylated full-length precursor is approximately 116 kDa. The mature, glycosylated transmembrane form migrates at approximately 130 kDa by SDS-PAGE. The shed soluble ectodomain (KL1+KL2) migrates at approximately 130 kDa. The alternatively spliced secreted isoform (KL1 domain only) migrates at approximately 65 kDa [15].

Glycosylation: The extracellular domain contains multiple N-linked glycosylation sites. Glycosylation contributes to protein stability, secretion, and potentially to receptor interaction. Recombinant alpha-Klotho produced in Chinese hamster ovary (CHO) cells exhibits glycosylation patterns that differ from those of HEK293-derived material, with functional consequences for in vitro and in vivo activity [16].

Structural homology: The KL1 and KL2 domains share approximately 20 percent sequence identity with family 1 beta-glucosidases (EC 3.2.1.21) and with lactase-phlorizin hydrolase. However, both domains carry substitutions at the catalytic glutamate residues (E235 and E239 in the GH1 consensus) that render them catalytically inactive against standard beta-glucosidase substrates. Whether the GH1 fold retains a cryptic sialidase or beta-glucuronidase activity at selected substrates remains a subject of investigation; early reports of sialidase activity on TRPV5 glycans have been challenged by subsequent structural and biochemical data [2, 17].

1.3 Protein forms and proteolytic processing

Three forms of alpha-Klotho protein with distinct biological activities have been characterized:

Membrane-bound alpha-Klotho (mKL): The full-length, type I transmembrane protein expressed predominantly in the kidney distal tubule, parathyroid gland, and choroid plexus of the brain. Functions as an obligate co-receptor for FGF23 in complex with FGFR1c.

Shed soluble alpha-Klotho (sKL): The extracellular domain (KL1+KL2) released from the cell surface by proteolytic cleavage mediated by the metalloproteinases ADAM10 and ADAM17 (constitutive and regulated shedding, respectively) and by BACE1 (beta-secretase 1). The shed ectodomain circulates in plasma at concentrations of approximately 300 to 1000 pg/mL in healthy adults (as measured by sandwich ELISA), with concentrations declining with age and with chronic kidney disease stage [6, 18].

Secreted alpha-Klotho: A truncated isoform comprising the KL1 domain and a short unique C-terminal extension, produced by alternative mRNA splicing that introduces a premature stop codon. This isoform is approximately 65 kDa and is secreted directly without requiring proteolytic cleavage. The relative contribution of the secreted isoform to circulating Klotho levels is not fully established.

2. Abstract

Alpha-Klotho is a multifunctional transmembrane protein and circulating endocrine factor that occupies a central position in the biology of aging, mineral metabolism, and organ protection. Identified in 1997 through a fortuitous insertional mutation that produced a dramatic syndrome of premature aging in mice, the protein has been characterized over the subsequent three decades as both an obligate co-receptor for the phosphaturic hormone FGF23 and an independent anti-aging factor whose circulating concentrations correlate inversely with age, chronic kidney disease progression, cardiovascular events, and cognitive decline in human epidemiological studies [1, 6, 7].

The dual identity of alpha-Klotho as a mineral metabolism regulator (through FGF23 co-reception) and as a pleiotropic endocrine anti-aging factor (through inhibition of insulin/IGF-1, Wnt, TGF-beta, and NF-kappaB pathways) has generated a research literature spanning nephrology, cardiology, neuroscience, oncology, and gerontology. Preclinical evidence for therapeutic benefit of alpha-Klotho supplementation or gene delivery is substantial across organ systems, with demonstrated renoprotection, cardioprotection, neuroprotection, and anti-fibrotic activity in multiple rodent models and, most recently, cognitive enhancement in aged nonhuman primates [10, 11, 12, 13, 14].

Clinical translation is in its earliest stages. No alpha-Klotho therapeutic product has received regulatory approval. The first formal clinical trials of alpha-Klotho-directed therapeutics (mRNA-based and gene therapy approaches) entered Phase 1 in 2025 and 2026. The compound is available as a recombinant protein for research applications and is the subject of active investigation for kidney disease, neurodegeneration, cardiovascular disease, and lifespan extension.

3. Discovery and Development History

3.1 Identification of the kl/kl mouse

The alpha-Klotho gene was identified by Makoto Kuro-o and colleagues at the University of Texas Southwestern Medical Center and the National Institute of Neuroscience in Tokyo in 1997 [1]. The discovery arose from characterization of a transgenic mouse line in which an insertional transgene had disrupted the 5-prime flanking region of a previously unknown gene on mouse chromosome 5. Homozygous disruption (kl/kl genotype) produced a syndrome closely resembling human premature aging: growth retardation beginning at 3 to 4 weeks of age; skin atrophy with loss of subcutaneous fat; arteriosclerosis; ectopic calcification of soft tissues including aorta, trachea, gastric mucosa, and renal tubules; osteoporosis with reduced bone mineral density; pulmonary emphysema; gonadal atrophy with infertility; thymic involution; hearing impairment; and death by approximately 8 to 9 weeks of age (compared to a normal murine lifespan of approximately 2 years) [1].

3.2 Gene cloning and naming

Kuro-o et al. cloned the disrupted gene, which encoded a novel type I transmembrane protein with two extracellular domains homologous to beta-glucosidase enzymes. The gene was named "Klotho" (KL) after Clotho, the youngest of the three Moirai in Greek mythology, who spins the thread of human life. The naming reflected the profound effect of the gene on lifespan determination [1].

3.3 Overexpression and lifespan extension

The complementary experiment was reported by Kurosaki et al. (2005), who demonstrated that transgenic mice overexpressing alpha-Klotho under the human elongation factor 1-alpha promoter lived approximately 20 to 30 percent longer than wild-type littermates [3]. The extended lifespan was accompanied by resistance to insulin/IGF-1 signaling, reduced oxidative stress, and maintenance of mineral homeostasis. This gain-of-function demonstration established alpha-Klotho as a bona fide longevity gene and stimulated investigation of its mechanisms beyond mineral metabolism.

3.4 Identification as FGF23 co-receptor

Urakawa et al. (2006) and Kurosu et al. (2006) independently demonstrated that membrane-bound alpha-Klotho functions as an obligate co-receptor for FGF23, converting canonical FGF receptors (principally FGFR1c) from low-affinity to high-affinity FGF23 receptors [19, 20]. This discovery unified the previously parallel literatures on Klotho biology and FGF23 endocrinology and explained the phenotypic overlap between Klotho-deficient and FGF23-deficient mice (both exhibit hyperphosphatemia, elevated calcitriol, ectopic calcification, and premature aging).

3.5 Crystal structure resolution

Chen et al. (2018) resolved the crystal structure of the ternary alpha-Klotho/FGFR1c/FGF23 complex at 3.0 angstrom resolution, revealing that alpha-Klotho functions as a molecular scaffold that simultaneously engages both FGF23 and FGFR1c through a large composite binding interface [2]. The structure demonstrated that soluble alpha-Klotho (the shed ectodomain) can also serve as an FGF23 co-receptor, a finding that fundamentally expanded the potential target tissues for FGF23 signaling from the kidney and parathyroid (where membrane-bound Klotho is expressed) to essentially any cell type reached by circulating soluble Klotho.

3.6 Therapeutic development trajectory

From 2010 to 2025, therapeutic development focused on three strategies: recombinant protein supplementation (preclinical), gene therapy (preclinical to early clinical), and indirect upregulation through small molecules, senolytics, and lifestyle interventions. Klotho Bio entered clinical development with AKL003, an mRNA therapeutic encoding alpha-Klotho, launching a Phase 1b trial in healthy volunteers in February 2026. Klotho Neurosciences advanced KLTO-202, an AAV-based KL gene therapy for amyotrophic lateral sclerosis, toward first-in-human studies with manufacturing initiated in 2025. These programs represent the first systematic clinical translation of the 1997 discovery.

4. Mechanism of Action

Alpha-Klotho is a multi-target protein with at least two principal functional identities: an FGF23 co-receptor in mineral metabolism and an independent circulating endocrine anti-aging factor. These identities operate through distinct molecular mechanisms at distinct target tissues.

4.1 FGF23 co-receptor function

Fibroblast growth factor 23 (FGF23) is an endocrine FGF secreted by osteocytes that regulates phosphate and vitamin D homeostasis. Unlike paracrine FGFs, FGF23 binds canonical FGF receptors with low affinity due to its truncated heparan sulfate binding domain. Alpha-Klotho compensates for this low affinity by forming a ternary complex with FGFR1c and FGF23, creating a high-affinity signaling unit [2, 19, 20].

In the renal proximal tubule, the FGF23/alpha-Klotho/FGFR1c complex activates the RAS-MAPK and PI3K-AKT signaling cascades, producing two principal downstream effects: (a) downregulation of the sodium-phosphate cotransporters NaPi-IIa (SLC34A1) and NaPi-IIc (SLC34A3) from the apical membrane, increasing renal phosphate excretion; and (b) suppression of 1-alpha-hydroxylase (CYP27B1) expression and induction of 24-hydroxylase (CYP24A1) expression, reducing circulating 1,25-dihydroxyvitamin D3 (calcitriol) levels [19]. These actions maintain phosphate balance and prevent the hyperphosphatemia and ectopic calcification that characterize Klotho deficiency.

4.2 Inhibition of insulin/IGF-1 signaling

Kurosu et al. (2005) demonstrated that alpha-Klotho overexpression in transgenic mice produced insulin resistance with fasting hyperinsulinemia but without diabetes, and that this phenotype was associated with extended lifespan [3]. Soluble alpha-Klotho inhibits insulin and IGF-1 receptor autophosphorylation at concentrations achievable in the circulation, reducing downstream PI3K-AKT-FOXO signaling. The FOXO transcription factor derepression increases expression of antioxidant enzymes (manganese superoxide dismutase, catalase) and reduces oxidative

stress. This mechanism parallels the insulin/IGF-1 pathway attenuation that extends lifespan in *C. elegans* (*daf-2* mutants), *Drosophila*, and calorie-restricted rodents.

4.3 Inhibition of Wnt signaling

Liu et al. (2007) reported that soluble alpha-Klotho binds to multiple Wnt ligands (including Wnt1, Wnt3a, Wnt4, and Wnt5a) and inhibits their binding to Frizzled receptors, suppressing canonical Wnt/beta-catenin signaling [4]. Excessive Wnt signaling drives cellular senescence through induction of the p53/p21 and p16INK4a pathways; alpha-Klotho opposes this senescence-promoting activity. The Wnt-inhibitory mechanism has been implicated in the anti-fibrotic effects of alpha-Klotho in kidney and heart, and in the tumor-suppressive activity observed in several cancer models where alpha-Klotho expression is epigenetically silenced.

4.4 Inhibition of TGF-beta signaling

Doi et al. (2011) demonstrated that soluble alpha-Klotho binds to the TGF-beta type II receptor (TGF-betaRII) and inhibits TGF-beta1-induced Smad2/3 phosphorylation, suppressing the pro-fibrotic transcriptional program that drives renal and cardiac fibrosis [5]. This anti-TGF-beta activity is a principal mechanism underlying the renoprotective effects of recombinant alpha-Klotho in unilateral ureteral obstruction and ischemia-reperfusion injury models.

4.5 Suppression of NF-kappaB and inflammation

Alpha-Klotho suppresses NF-kappaB transcriptional activity, reducing expression of pro-inflammatory cytokines including TNF-alpha, IL-1beta, and IL-6. The anti-inflammatory mechanism operates at least partially through inhibition of the insulin/IGF-1 pathway (which activates NF-kappaB through AKT-IKK signaling) and through Wnt pathway suppression (which cross-talks with NF-kappaB). The anti-inflammatory activity has been demonstrated in models of acute kidney injury, sepsis-induced cardiorenal syndrome, and neuroinflammation [21, 22].

4.6 Regulation of ion transport

Alpha-Klotho regulates multiple ion channels and transporters in the renal tubule and other epithelia. In the distal convoluted tubule and connecting tubule, alpha-Klotho increases cell-surface abundance of TRPV5 (the apical calcium entry channel) through modification of N-linked glycans on the channel extracellular domain, promoting calcium reabsorption [17]. Alpha-Klotho also regulates TRPV6, ROMK1 (the potassium secretory channel), and the Na⁺/K⁺-ATPase. These ion-transport regulatory activities contribute to the mineral and electrolyte homeostasis functions of the protein.

4.7 Neuroprotective mechanisms

The neuroprotective activity of alpha-Klotho operates through multiple mechanisms: enhancement of NMDA receptor-dependent glutamatergic synaptic function (demonstrated by Dubal et al. 2015 in klotho-overexpressing mice, where elevated GluN2B subunit expression in hippocampus and frontal cortex was associated with enhanced spatial learning and memory) [23]; suppression of neuroinflammation through NF-kappaB inhibition; promotion of autophagy and clearance of misfolded proteins including amyloid-beta; stimulation of antioxidant defense; and promotion of oligodendrogenesis and myelination [24, 25]. The GluN2B-dependent synaptic enhancement mechanism is the basis for the cognitive enhancement observed in klotho-overexpressing transgenic mice and in aged mice and primates treated with exogenous klotho protein.

5. Pharmacokinetics

5.1 Endogenous production and tissue distribution

Alpha-Klotho is expressed predominantly in the kidney (distal convoluted tubule, connecting tubule), with lower expression in the choroid plexus of the brain and in the parathyroid gland [1]. Low-level expression has been reported in additional tissues including the pituitary, placenta, skeletal muscle, urinary bladder, and vascular endothelium, though the functional significance of extra-renal expression remains under investigation.

Circulating soluble alpha-Klotho is produced principally by proteolytic shedding from the renal tubular epithelium. Plasma concentrations in healthy adults range from approximately 300 to 1000 pg/mL as measured by sandwich ELISA, with considerable inter-individual variability attributable to age, renal function, and assay methodology [6, 18]. Concentrations decline with age, with a measurable decrease beginning approximately in the fourth decade of life. Soluble alpha-Klotho is also detectable in cerebrospinal fluid and in urine.

5.2 Recombinant protein pharmacokinetics

Pharmacokinetic characterization of recombinant alpha-Klotho protein has been performed principally in rodent models. Recombinant human alpha-Klotho produced in CHO cells exhibits a terminal elimination half-life of approximately 7 to 12 hours in rats after intravenous administration, with bioavailability of approximately 47.5 percent after subcutaneous injection [16]. The pharmacokinetic profile is influenced by the glycosylation pattern, which differs depending on the host cell line used for production (CHO versus HEK293 versus insect cells), with CHO-derived material showing the most favorable in vivo pharmacokinetic properties.

Volume of distribution in rodents is moderate, consistent with distribution to the extracellular fluid compartment and moderate tissue penetration. The protein crosses the blood-brain barrier poorly after peripheral administration; however, a klotho protein fragment (the KL1 domain, approximately 35 kDa) has been shown to enhance cognitive function after peripheral injection in mice and nonhuman pri-

mates, suggesting either sufficient CNS penetration of the fragment, peripheral-to-central signaling through intermediate mediators, or blood-brain barrier permeability to fragments but not the full ectodomain [13, 14].

5.3 Elimination and clearance

Recombinant alpha-Klotho is eliminated through a combination of receptor-mediated endocytosis, proteolytic degradation, and renal filtration of fragments. Urinary excretion of alpha-Klotho fragments has been characterized and used as a clinical biomarker of endogenous alpha-Klotho status [6]. The short circulating half-life of the full-length recombinant protein presents a challenge for therapeutic development, motivating the mRNA and gene therapy approaches now in clinical development, which aim to achieve sustained endogenous production rather than relying on repeated protein infusion.

5.4 Factors affecting endogenous levels

Multiple physiological and pharmacological factors modulate endogenous circulating alpha-Klotho levels:

- **Age:** Progressive decline beginning in the fourth decade of life in humans.
- **Chronic kidney disease:** Marked reduction in both renal tissue expression and circulating soluble levels, with decline paralleling GFR loss and preceding overt mineral and bone disorder [6].
- **Acute kidney injury:** Rapid reduction in renal expression; soluble alpha-Klotho has been investigated as an early biomarker of AKI.
- **Exercise:** Acute and chronic exercise increases circulating soluble alpha-Klotho concentrations, with a systematic review and meta-analysis confirming the exercise-Klotho relationship [26].
- **Pharmacological upregulation:** Metformin, PPAR-gamma agonists, rapamycin/everolimus, vitamin D receptor agonists, and the senolytic combination dasatinib plus quercetin have been reported to increase alpha-Klotho expression or circulating levels in preclinical models and in limited human data [27, 28].

6. Preclinical Pharmacology

6.1 Renoprotection

Recombinant alpha-Klotho protein administration has demonstrated robust renoprotection in multiple rodent models of kidney injury. In ischemia-reperfusion injury-induced acute kidney injury (AKI), prophylactic and therapeutic administration of recombinant alpha-Klotho reduced tubular necrosis, preserved GFR, suppressed inflammatory cytokine expression, and attenuated the AKI-to-chronic kidney disease (CKD) transition [10]. In a CKD model generated by unilateral nephrectomy plus contralateral ischemia-reperfusion, sustained alpha-Klotho administration over 12 weeks prevented CKD progression and protected against uremic cardiomyopathy [10]. In unilateral ureteral obstruction, alpha-Klotho suppressed TGF-beta-driven renal fibrosis through inhibition of Smad2/3 signaling [5].

Adeno-associated virus (AAV) mediated KL gene transfer ameliorated angiotensin II-induced renal damage in rats, with improvements in creatinine clearance, reduction in proteinuria, and attenuation of tubulointerstitial fibrosis [29]. These findings support alpha-Klotho as both a biomarker and a therapeutic target in kidney disease.

6.2 Cardioprotection

Alpha-Klotho deficiency in *kl/kl* mice produces cardiac hypertrophy, left ventricular fibrosis, and diastolic dysfunction. Recombinant alpha-Klotho administration attenuates cardiac hypertrophy induced by pressure overload (transverse aortic constriction) and by uremic cardiomyopathy [10, 30]. The cardioprotective mechanisms include suppression of TGF-beta-driven cardiac fibrosis, attenuation of Wnt/beta-catenin-mediated cardiomyocyte hypertrophy, and reduction of oxidative stress through FOXO-mediated antioxidant enzyme induction.

Alpha-Klotho also suppresses vascular calcification, a process driven by hyperphosphatemia-induced osteochondrogenic transdifferentiation of vascular smooth muscle cells. The anti-calcification mechanism operates both through FGF23-dependent phosphate regulation and through FGF23-independent suppression of the osteochondrogenic gene program [30].

In a sepsis-induced cardiorenal syndrome type 5 model (lipopolysaccharide-treated mice), recombinant alpha-Klotho alleviated both cardiac and renal injury, with reduction in TNF-alpha, IL-1beta, and IL-6 expression and attenuation of NF-kappaB activation [22].

6.3 Neuroprotection and cognitive enhancement

The neuroprotective and cognitive-enhancing activity of alpha-Klotho is among the most actively investigated preclinical applications. Key findings include:

Dubal et al. (2014) demonstrated that transgenic mice overexpressing alpha-Klotho exhibited enhanced spatial learning, enhanced memory, and enhanced synaptic plasticity, with the mechanism mediated through upregulation of the GluN2B subunit of the NMDA receptor in hippocampus and frontal cortex [23]. The cognitive enhancement was observed in young adult mice (not only aged), suggesting that alpha-Klotho operates through direct synaptic mechanisms rather than solely through age-related damage prevention.

Leon et al. (2017) reported that peripheral elevation of a klotho protein fragment (the KL1 domain) enhanced cognitive function in young mice, aged mice, and alpha-synuclein transgenic mice (a model of Parkinson's disease and Lewy body dementia) [13]. The cognitive enhancement was blocked by NMDA receptor antagonism, confirming the glutamatergic mechanism. The effect was acute (measurable within hours of injection) rather than requiring chronic administration.

Castner et al. (2023) extended these findings to nonhuman primates, demonstrating that a single subcutaneous injection of a klotho protein fragment enhanced spatial and working memory in aged rhesus macaques at a low dose (10 micrograms/kg), with no effect at a higher dose (30 micrograms/kg), suggesting an inverted U-shaped dose-response [14]. This was the first demonstration of klotho-mediated cognitive enhancement in primates and the most directly translatable preclinical evidence for human cognitive applications.

6.4 Anti-tumor activity

Alpha-Klotho expression is epigenetically silenced (through promoter hypermethylation) in multiple cancer types, including breast, lung, cervical, hepatocellular, colorectal, and pancreatic carcinomas [31]. Restoration of alpha-Klotho expression in cancer cell lines suppresses proliferation, promotes apoptosis, and inhibits tu-

mor growth in xenograft models. The anti-tumor mechanisms include suppression of Wnt/beta-catenin signaling, inhibition of IGF-1 receptor signaling, and activation of the FGF23 pathway in tumor cells that express FGFR. Alpha-Klotho functions as an endogenous tumor suppressor whose loss contributes to oncogenesis in Klotho-expressing tissues.

6.5 Anti-fibrotic activity

Beyond kidney and heart, alpha-Klotho exhibits anti-fibrotic activity in models of pulmonary fibrosis, hepatic fibrosis, and skin aging. The anti-fibrotic mechanism is primarily mediated through TGF-beta pathway inhibition and Wnt pathway suppression, both of which are major drivers of pathological fibrosis across organ systems [5].

7. Clinical Evidence Base

7.1 Epidemiological associations

Large-scale epidemiological studies have established associations between circulating soluble alpha-Klotho levels and clinical outcomes:

A NHANES-based analysis of middle-aged and older adults demonstrated an inverse association between serum soluble alpha-Klotho concentrations and all-cause mortality, with the lowest quartile of alpha-Klotho levels associated with significantly increased mortality risk after adjustment for age, sex, renal function, and comorbidities [7]. Similar inverse associations have been reported for cardiovascular events, heart failure, hypertension, diabetes incidence, and cognitive decline in multiple cohort studies.

In patients with chronic kidney disease, circulating soluble alpha-Klotho levels decline progressively with CKD stage and predict adverse outcomes including cardiovascular calcification, left ventricular hypertrophy, and mortality in hemodialysis populations [32].

7.2 Genetic association studies

The KL-VS haplotype (defined by F352V [rs9536314] and C370S [rs9527025]) has been investigated in multiple cohorts for associations with longevity, cognition, and disease risk. Arking et al. (2002) reported that KL-VS heterozygosity was enriched in Bohemian Czech and Ashkenazi Jewish populations reaching advanced age [8]. Dubal et al. (2014) reported that KL-VS heterozygosity was associated with larger cortical brain volumes and better cognitive performance in aging populations [9]. However, a 2022 analysis of the Newcastle 85+ Study and UK Biobank found no significant association between rs9536314 genotype and longevity in large Caucasian cohorts, indicating that the genetic association may be population-specific or confounded [33]. The inconsistency across cohorts suggests that the genetic evidence for KL-VS as a determinant of human longevity should be considered preliminary.

7.3 Senolytics and alpha-Klotho restoration

Justice et al. (2019) and subsequent studies demonstrated that the senolytic combination dasatinib plus quercetin (D+Q) restored alpha-Klotho levels in kidney and brain of mice with increased senescent cell burden [27]. A clinical study in patients with diabetic kidney disease receiving D+Q showed increases in circulating alpha-Klotho levels, representing the first demonstration that a pharmacological intervention can restore alpha-Klotho in humans [27]. This finding positions alpha-Klotho as both a biomarker for senolytic efficacy and a mediator of senolytic benefit.

7.4 Active clinical trials

As of May 2026, the following clinical programs directly targeting alpha-Klotho are active:

AKL003 (Klothea Bio): A lipid nanoparticle-encapsulated mRNA encoding human alpha-Klotho, administered intravenously. A Phase 1b randomized, double-blind, placebo-controlled trial in healthy adult volunteers was launched in February 2026 to assess safety, tolerability, pharmacokinetics, and alpha-Klotho protein expression following two intravenous administrations approximately four weeks apart. The protocol includes assessments of inflammatory markers, metabolic and cardiovascular measurements, sleep and recovery metrics, mitochondrial function, and quality-of-life questionnaires.

KLTO-202 (Klotho Neurosciences): An AAV-based gene therapy encoding alpha-Klotho for the treatment of amyotrophic lateral sclerosis (ALS). Manufacturing was initiated in 2025, with first-in-human studies planned for the third quarter of 2025 or early 2026.

Klotho and follistatin gene therapy (investigator-initiated): An early-phase safety and tolerability study of combination klotho and follistatin gene therapy in healthy adults (clinical trial identifier NCT07216781-related).

Injectable klotho plasmid gene therapy: NCT07216781, a study evaluating the safety and efficacy of injectable klotho plasmid gene therapy.

7.5 Clinical biomarker applications

Soluble alpha-Klotho measured by sandwich ELISA (using the Yamazaki et al. 2010 assay or commercial equivalents) has been investigated as a clinical biomarker for early detection of AKI, CKD progression monitoring, cardiovascular risk stratification, and biological aging assessment [18]. The biomarker application is more advanced than the therapeutic application, with multiple validated assays available commercially, though standardization across assay platforms remains an active area of development.

8. Sourcing and Quality Verification

Recombinant human alpha-Klotho protein is available from multiple research suppliers for in vitro and preclinical applications. The compound is not available as a pharmaceutical-grade therapeutic product in any jurisdiction.

Principal research suppliers:

- R&D Systems (Bio-Techne): Recombinant Human Klotho (aa 34-981), carrier-free, catalog 5334-KL. Produced in mouse myeloma cell line NS0 or in CHO cells. Greater than 95 percent purity by SDS-PAGE.
- Abcam: Recombinant Human Klotho protein (Active), catalog ab84072. Produced in HEK293 cells. Greater than 95 percent purity.
- Thermo Fisher Scientific: Human Klotho Recombinant Protein, catalog 100-53. Produced in CHO cells.
- Sino Biological: Recombinant Human Klotho protein, His-tagged and untagged variants.
- ProSci: Klotho Recombinant Protein, catalog 40-195.
- BioLongevity Labs: Alpha-Klotho (alphaKlothoLR), 99 percent purity, third-party tested.

Pricing: Research-grade recombinant human alpha-Klotho protein is priced in the range of \$200 to \$600 per 20 micrograms, reflecting the cost of mammalian cell expression and purification. Pricing is substantially higher per unit mass than small-molecule research compounds and reflects the protein nature of the material.

Quality verification: Investigators should confirm:

- Purity by SDS-PAGE (target greater than 95 percent, single band at approximately 65 to 130 kDa depending on construct and glycosylation)
- Identity by mass spectrometry or Western blot with validated anti-Klotho antibodies
- Biological activity by a functional assay (e.g., FGF23-dependent ERK phosphorylation in HEK293 cells co-expressing FGFR1c, or TRPV5 surface expression assay)
- Endotoxin levels below 1 EU per microgram for in vivo applications

- Host cell expression system documentation, as glycosylation patterns and pharmacokinetic properties differ by production cell line [16]

Third-party analytical confirmation is appropriate for any investigation where dose-response precision or mechanism-of-action interpretation depends on material identity and potency.

9. Reconstitution and Handling

Recombinant human alpha-Klotho protein is typically supplied as a lyophilized powder from research vendors. Reconstitution and handling protocols are critical for maintaining protein activity.

Reconstitution: Lyophilized protein should be reconstituted in sterile phosphate-buffered saline (PBS, pH 7.4) to a concentration of 100 to 500 micrograms/mL. For in vitro assays requiring higher concentrations, reconstitution in PBS containing 0.1 percent bovine serum albumin (BSA) as a carrier protein is recommended to minimize adsorptive losses to tube surfaces. Gentle agitation or pipetting is preferred over vortexing to avoid protein denaturation and aggregation.

Storage of lyophilized material: Store at minus 20 degrees Celsius (long-term, up to 2 years from date of receipt) or at minus 80 degrees Celsius for extended archival storage. Protect from moisture. The lyophilized form is stable at minus 20 degrees Celsius for at least 2 years.

Storage of reconstituted material: Aliquot reconstituted protein into single-use volumes to avoid repeated freeze-thaw cycles. Store aliquots at minus 20 degrees Celsius (stable for at least 3 months with carrier protein) or at minus 80 degrees Celsius (stable for at least 6 months). Working aliquots may be stored at 2 to 8 degrees Celsius for up to 1 week. Avoid more than 3 freeze-thaw cycles, as protein aggregation and activity loss increase with each cycle.

Working concentrations: For in vitro cell-based assays, typical working concentrations range from 0.1 to 10 nanomolar (approximately 10 to 1000 ng/mL for the full ectodomain). For in vivo rodent studies, doses of 0.01 to 0.1 mg/kg have been used for renoprotection and cardioprotection studies; doses of 0.01 mg/kg for the KL1 fragment have been used for cognitive enhancement studies [10, 13].

Compatibility: Alpha-Klotho protein is compatible with standard cell culture media (DMEM, RPMI, MEM) and with physiological saline solutions. Avoid exposure to extremes of pH (below 5 or above 9), to denaturing agents (urea, guanidinium), and to prolonged incubation above 37 degrees Celsius.

10. Stack Interactions and Combinations

Alpha-Klotho interacts with multiple endogenous and exogenous pathways, and investigators conducting combination studies should consider the following interactions:

FGF23: Alpha-Klotho and FGF23 form an obligate signaling axis. Exogenous alpha-Klotho supplementation in the context of elevated FGF23 (as in CKD) may amplify FGF23 signaling, producing excessive phosphaturia and calcitriol suppression. Conversely, alpha-Klotho supplementation in the context of FGF23 deficiency may have reduced effect on mineral metabolism. The FGF23-Klotho axis should be considered as a coupled system in any research or therapeutic application.

Vitamin D and calcitriol: Alpha-Klotho suppresses calcitriol production through FGF23-mediated downregulation of CYP27B1. Concurrent administration of alpha-Klotho and active vitamin D analogs (calcitriol, paricalcitol, alfacalcidol) may produce opposing effects on vitamin D signaling. Vitamin D receptor agonists have also been reported to upregulate alpha-Klotho expression, creating a feedback relationship [34].

Rapamycin and mTOR inhibitors: Rapamycin and everolimus increase alpha-Klotho expression in some models, potentially producing additive or synergistic anti-aging effects when combined with exogenous alpha-Klotho supplementation. However, these agents carry substantial immunosuppressive and metabolic toxicities that complicate long-term combination use [28].

Senolytics (dasatinib plus quercetin): The D+Q combination restores endogenous alpha-Klotho expression by clearing senescent cells that suppress Klotho transcription. The combination of senolytics with exogenous alpha-Klotho supplementation has not been systematically studied but is mechanistically plausible as a complementary approach [27].

Metformin: Metformin increases alpha-Klotho expression through AMPK-dependent mechanisms. The combination is pharmacologically coherent for metabolic and renal applications but has not been formally studied for additive benefit on al-

pha-Klotho-mediated endpoints.

ACE inhibitors and ARBs: Angiotensin II suppresses alpha-Klotho expression in the kidney. ACE inhibitors and angiotensin receptor blockers may preserve or restore renal alpha-Klotho expression, and the combination with exogenous alpha-Klotho is mechanistically complementary for renoprotection [29].

NMDA receptor modulators: The cognitive enhancement activity of alpha-Klotho depends on NMDA receptor function (specifically, GluN2B subunit-containing receptors). Concurrent NMDA receptor antagonists (memantine, ketamine) would be expected to attenuate the procognitive effect. Concurrent NMDA receptor positive modulators or glycine site agonists (D-cycloserine, sarcosine) might enhance the effect, though this combination has not been studied.

Phosphate binders: In the context of CKD, alpha-Klotho supplementation reduces phosphate burden through enhanced FGF23 signaling. Concurrent phosphate binders (sevelamer, lanthanum carbonate, calcium acetate) may produce additive phosphate reduction.

11. Adverse Events and Safety Signal

11.1 Preclinical safety observations

No alpha-Klotho product has been administered to humans in a completed clinical trial with published safety data as of the monograph revision date. All safety considerations are derived from preclinical studies and from the biology of the Klotho signaling axis.

Mineral metabolism effects: Exogenous alpha-Klotho supplementation activates FGF23 signaling, potentially producing hypophosphatemia and suppression of calcitriol. Sustained excessive alpha-Klotho activity could theoretically produce the mirror image of the *kl/kl* phenotype: hypophosphatemia with impaired bone mineralization, suppressed vitamin D, and altered calcium homeostasis. The processed form of alpha-Klotho (p-KL) has shown greater perturbation of mineral homeostasis than the secreted form (s-KL) in comparative studies, with mice treated with p-KL exhibiting altered kidney ion channel expression and perturbed blood phosphate and calcium levels [35].

Dose-response considerations: The nonhuman primate cognitive study (Castner et al. 2023) demonstrated an inverted U-shaped dose-response, with cognitive enhancement at 10 micrograms/kg but not at 30 micrograms/kg [14]. Case reports suggest that circulating alpha-Klotho levels 4 to 5 fold above the population mean may be associated with adverse clinical outcomes. These observations indicate that supraphysiological alpha-Klotho levels may not be beneficial and that dosing precision will be critical for any therapeutic application.

Insulin sensitivity: Alpha-Klotho inhibits insulin/IGF-1 signaling. While moderate attenuation of this pathway is associated with longevity, excessive inhibition could impair glucose tolerance and produce metabolic dysfunction. The Klotho-overexpressing transgenic mice exhibited mild insulin resistance without frank diabetes, but the therapeutic window for this mechanism in humans is undefined [3].

11.2 Theoretical safety considerations

Tumor biology: Alpha-Klotho functions as a tumor suppressor in multiple cancer types. However, the Wnt-inhibitory and IGF-1-inhibitory activities of alpha-Klotho are pleiotropic, and the effects of sustained supraphysiological alpha-Klotho on tumor surveillance and immune function have not been characterized. No evidence of oncogenic risk from alpha-Klotho supplementation has been reported in preclinical studies.

Immunomodulation: Alpha-Klotho suppresses NF-kappaB-driven inflammatory signaling. While anti-inflammatory activity is generally beneficial in age-related disease, excessive suppression of inflammatory signaling could impair innate immune defense against infection. This theoretical risk has not been observed in preclinical supplementation studies at the doses tested.

11.3 Anticipated clinical safety profile

Given the preclinical data, the anticipated adverse-event profile of alpha-Klotho therapeutic products includes: mineral metabolism perturbation (hypophosphatemia, calcitriol suppression) as a mechanism-based on-target effect; altered glucose homeostasis as a mechanism-based on-target effect; and injection-site or infusion-related reactions for protein or mRNA formulations. The Phase 1b trial of AKL003 is designed to characterize these potential effects in the first controlled human exposure.

12. Comparative Assessment

This section presents a structured comparative assessment of five anti-aging and organ-protective intervention candidates against alpha-Klotho, evaluated against five competency standards: novelty, effect size, promising potential (current clinical state and trajectory), side-effect profile, and overall validation. The five comparators were selected as the principal therapeutic candidates in the longevity and organ-protection space that share mechanistic overlap or clinical positioning with alpha-Klotho.

12.1 Rapamycin (Sirolimus)

Mechanism: mTOR complex 1 (mTORC1) inhibitor. Suppresses the mechanistic target of rapamycin pathway, reducing cellular growth, protein synthesis, and senescence while promoting autophagy. Rapamycin extends lifespan in multiple model organisms including mice (the NIA Interventions Testing Program demonstrated approximately 10 to 15 percent lifespan extension in both sexes) [36].

Novelty: Moderate. The mTOR pathway is well characterized, and rapamycin has been studied as a longevity intervention for over two decades. The mechanism is distinct from alpha-Klotho's pleiotropic pathway inhibition.

Effect size: Robust in preclinical models. Lifespan extension of 10 to 15 percent in genetically heterogeneous mice is among the most reproducible pharmacological lifespan extension effects. Clinical effect size on human aging biomarkers is under investigation in multiple trials (PEARL, TRAILMAP).

Promising potential: Moderate to high. Multiple clinical trials are active. Rapamycin analogs (everolimus) have demonstrated immune function improvement in elderly volunteers (Mannick et al. 2014, 2018). However, the compound is not approved for anti-aging indications, and the therapeutic window between mTOR inhibition sufficient for longevity benefit and immunosuppression sufficient for clinical toxicity remains undefined.

Side-effect profile: Substantial. Immunosuppression (increased infection risk), metabolic dysregulation (hyperglycemia, hyperlipidemia), oral mucositis, impaired wound healing, and potential pancreatic beta-cell toxicity. The side-effect profile is

the principal barrier to clinical translation as a longevity therapeutic.

Overall validation: High preclinical validation. Clinical validation as a longevity intervention is incomplete but advancing.

Comparison to alpha-Klotho: Rapamycin has more extensive preclinical longevity data and a more advanced clinical development trajectory for aging indications. Alpha-Klotho has a more favorable theoretical safety profile (endogenous protein replacement versus chronic immunosuppressant exposure) but substantially less clinical data. Rapamycin upregulates alpha-Klotho expression, suggesting mechanistic complementarity. Alpha-Klotho has demonstrated cognitive enhancement that rapamycin has not.

12.2 Metformin

Mechanism: AMPK activator with pleiotropic effects on mitochondrial function, insulin sensitivity, inflammation, and cellular senescence. The principal proposed anti-aging mechanisms include AMPK-dependent suppression of mTOR, reduction of insulin/IGF-1 signaling, mitochondrial complex I inhibition with metabolic reprogramming, and anti-inflammatory effects.

Novelty: Low. Metformin has been used clinically for over 60 years as an anti-diabetic agent. Its repositioning as a longevity intervention is mechanistically coherent but not novel.

Effect size: Modest in observational data. UK Prospective Diabetes Study and observational cohorts suggest modest all-cause mortality reduction in metformin-treated diabetic patients compared to both untreated diabetics and non-diabetic controls (Bannister et al. 2014). Preclinical lifespan extension in mice is inconsistent across studies and mouse strains.

Promising potential: Moderate. The TAME (Targeting Aging with Metformin) trial is designed to test whether metformin delays the onset of age-related multimorbidity in non-diabetic older adults. The trial is the first FDA-accepted clinical trial with "aging" as the indication. Results are anticipated in the coming years.

Side-effect profile: Generally favorable. Gastrointestinal effects (nausea, diarrhea) are common; lactic acidosis is rare. Vitamin B12 deficiency with chronic use. The safety profile is well established from decades of diabetic use.

Overall validation: Moderate. Strong observational support, modest preclinical support, clinical trial in progress.

Comparison to alpha-Klotho: Metformin has a vastly more established clinical safety profile and decades of human exposure data. Alpha-Klotho has a more specific and more potent mechanism for organ protection (particularly kidney and brain) and has demonstrated cognitive enhancement effects that metformin has not. Metformin increases alpha-Klotho expression, supporting use as an indirect Klotho-enhancing strategy. The two approaches are complementary.

12.3 Dasatinib plus Quercetin (D+Q Senolytic Combination)

Mechanism: Selective elimination of senescent cells. Dasatinib (a tyrosine kinase inhibitor) targets senescent preadipocytes; quercetin (a flavonoid) targets senescent endothelial cells. The combination clears senescent cells that contribute to age-related tissue dysfunction through the senescence-associated secretory phenotype (SASP), reducing chronic inflammation and restoring tissue homeostasis.

Novelty: High. Senolytics represent a mechanistically novel therapeutic class developed specifically for age-related disease, with the first preclinical demonstration by Zhu et al. (2015) at Mayo Clinic.

Effect size: Substantial in preclinical models. D+Q extends healthspan and lifespan in naturally aged mice, reduces frailty, improves cardiac and vascular function, and restores renal and pulmonary function. In mice with increased senescent cell burden, D+Q restored alpha-Klotho levels in kidney, brain, and urine [27].

Promising potential: High. Multiple clinical trials are active or completed in idiopathic pulmonary fibrosis, diabetic kidney disease, Alzheimer's disease, and frailty. Early clinical data have demonstrated feasibility, safety, and biomarker signals (including alpha-Klotho restoration in diabetic kidney disease patients).

Side-effect profile: Moderate. Dasatinib carries risks of myelosuppression, pleural effusion, and hepatotoxicity from oncology use at higher doses; the intermittent, lower-dose senolytic regimen appears better tolerated. Quercetin is generally well tolerated. The intermittent dosing schedule (typically 3 consecutive days per month) limits cumulative toxicity.

Overall validation: Moderate to high. Rapidly advancing from preclinical to clinical validation.

Comparison to alpha-Klotho: D+Q and alpha-Klotho are mechanistically linked: senolytics restore endogenous alpha-Klotho by clearing senescent cells that suppress its expression. The combination of senolytics (to restore endogenous production) with exogenous alpha-Klotho (to supplement circulating levels) represents a logical therapeutic strategy. D+Q has more advanced clinical data; alpha-Klotho has more specific organ-protective mechanisms, particularly for cognition.

12.4 Recombinant FGF21

Mechanism: FGF21 is an endocrine FGF that signals through FGFR1c in complex with beta-Klotho (KLB, the beta isoform of the Klotho family, distinct from alpha-Klotho). FGF21 regulates lipid metabolism, glucose homeostasis, energy expenditure, and insulin sensitivity. FGF21 overexpression extends lifespan in transgenic mice by approximately 30 to 40 percent.

Novelty: Moderate. FGF21 biology is well characterized, and multiple FGF21 analogs have advanced through clinical development for metabolic indications (efruxifermin, pegozafermin).

Effect size: Substantial in preclinical metabolic models. FGF21 analogs produce weight loss, lipid reduction, and glycemic improvement in NASH and obesity clinical trials. Lifespan extension in mice is among the largest reported for a single-gene intervention.

Promising potential: High for metabolic indications (NASH, obesity). FGF21 analogs are in Phase 2 and Phase 3 trials. Potential for longevity applications is less explored.

Side-effect profile: Generally favorable in clinical trials. Nausea, diarrhea, and injection-site reactions are common. No severe toxicities identified in clinical trials of FGF21 analogs.

Overall validation: High for metabolic indications. Moderate for longevity.

Comparison to alpha-Klotho: FGF21 and alpha-Klotho are members of related but distinct endocrine FGF signaling axes (FGF21/beta-Klotho versus FGF23/alpha-Klotho). Both are longevity-associated proteins with organ-protective effects. FGF21

has a more advanced clinical development trajectory and more established metabolic endpoints. Alpha-Klotho has unique advantages in renoprotection, neuroprotection, and cognitive enhancement that FGF21 does not share. The two systems are mechanistically independent and potentially complementary.

12.5 NAD⁺ Precursors (Nicotinamide Riboside, Nicotinamide Mononucleotide)

Mechanism: NAD⁺ precursors (NR, NMN) increase intracellular nicotinamide adenine dinucleotide (NAD⁺) levels, which decline with age. Elevated NAD⁺ activates sirtuins (SIRT1-7), PARP enzymes, and CD38-dependent signaling, promoting mitochondrial function, DNA repair, epigenetic maintenance, and cellular stress resistance.

Novelty: Low to moderate. NAD⁺ biology is well established; the repositioning of NAD⁺ precursors as anti-aging supplements is commercially advanced but mechanistically incremental.

Effect size: Modest and inconsistent in clinical studies. Human trials of NR and NMN have demonstrated NAD⁺ elevation but mixed results on functional aging endpoints. Preclinical lifespan extension data are limited and inconsistent across mouse strains and dosing protocols.

Promising potential: Moderate. Widely available as dietary supplements with extensive consumer adoption. Clinical trials for specific indications (heart failure, AKI, cognitive decline) are ongoing. The NAD⁺ field has been challenged by reproducibility concerns and by the gap between NAD⁺ elevation and functional outcomes.

Side-effect profile: Generally favorable. Mild gastrointestinal effects at high doses. Theoretical concerns about oncogenesis promotion (through fueling cancer cell metabolism) remain under investigation but unsubstantiated in clinical data.

Overall validation: Moderate. Extensive preclinical data, limited clinical validation of functional anti-aging effects.

Comparison to alpha-Klotho: NAD⁺ precursors address aging through a fundamentally different mechanism (metabolic cofactor restoration) than alpha-Klotho (signaling pathway modulation and organ protection). Alpha-Klotho has more specific and robust preclinical evidence for organ protection and cognitive enhance-

ment. NAD⁺ precursors have broader commercial availability and consumer adoption but weaker clinical evidence for functional anti-aging benefit. The two approaches are mechanistically orthogonal and potentially complementary.

12.6 Comparator summary

The five comparators occupy distinct positions in the anti-aging therapeutic landscape. **Rapamycin** has the strongest preclinical longevity validation but the most challenging safety profile for chronic use. **Metformin** has the longest clinical safety record and the most advanced regulatory pathway (TAME trial) but the most modest expected effect size. **D+Q senolytics** represent the most mechanistically novel approach with direct mechanistic linkage to alpha-Klotho restoration. **FGF21 analogs** have the most advanced clinical development for metabolic endpoints. **NAD⁺ precursors** have the broadest commercial adoption but the weakest clinical evidence for functional aging benefit.

Alpha-Klotho occupies a unique position in this landscape as an endogenous anti-aging factor with the most diverse organ-protective portfolio (kidney, heart, brain, vasculature), the only demonstrated cognitive enhancement in primates among the comparators, and the most direct mechanistic basis for addressing the aging phenotype (given that its deficiency is both necessary and sufficient to produce accelerated aging in mice). Its principal limitations are the absence of clinical safety and efficacy data (with the first trials now initiating), the protein-nature of the therapeutic (creating delivery and half-life challenges addressed by mRNA and gene therapy approaches), and the narrow therapeutic window suggested by the inverted U-shaped dose-response in primates.

The optimal anti-aging strategy is likely to involve combination approaches that address complementary mechanisms. Alpha-Klotho supplementation or restoration (through senolytics, exercise, or direct protein/mRNA/gene therapy) combined with mTOR modulation (rapamycin), metabolic optimization (metformin, FGF21), and NAD⁺ restoration (NR/NMN) represents a rationale-based multi-target framework that no single agent provides alone.

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