GENE THERAPY FOR INCREASING HEALTHSPAN

Patrick E. Sewell Jr., MD
Founder & CEO – Triple Helix Science Inc

WWW.TRIPLEHELIXSCIENCE.COM

Gene Addition Therapy

What is Gene Addition Therapy

- Adds functional gene to supplement faulty or missing gene

How it Works: Vectors and Delivery

DHL Trucks:

Viral vectors

Non-viral Vectors:

-plasmids

-nanoparticles

The Package: The Gene (Transgene) Delivered

Delivery Address: Cell Nucleus

What is Really Happening

Functional gene delivered >>> taken up by cells and installed >>> a protein produced >>> that protein restores or enhances cellular function

Real Life Scenario

Let Us Explore Follistatin Gene Therapy

1. **Follistatin builds muscle**

2. **Follistatin lowers cholesterol**

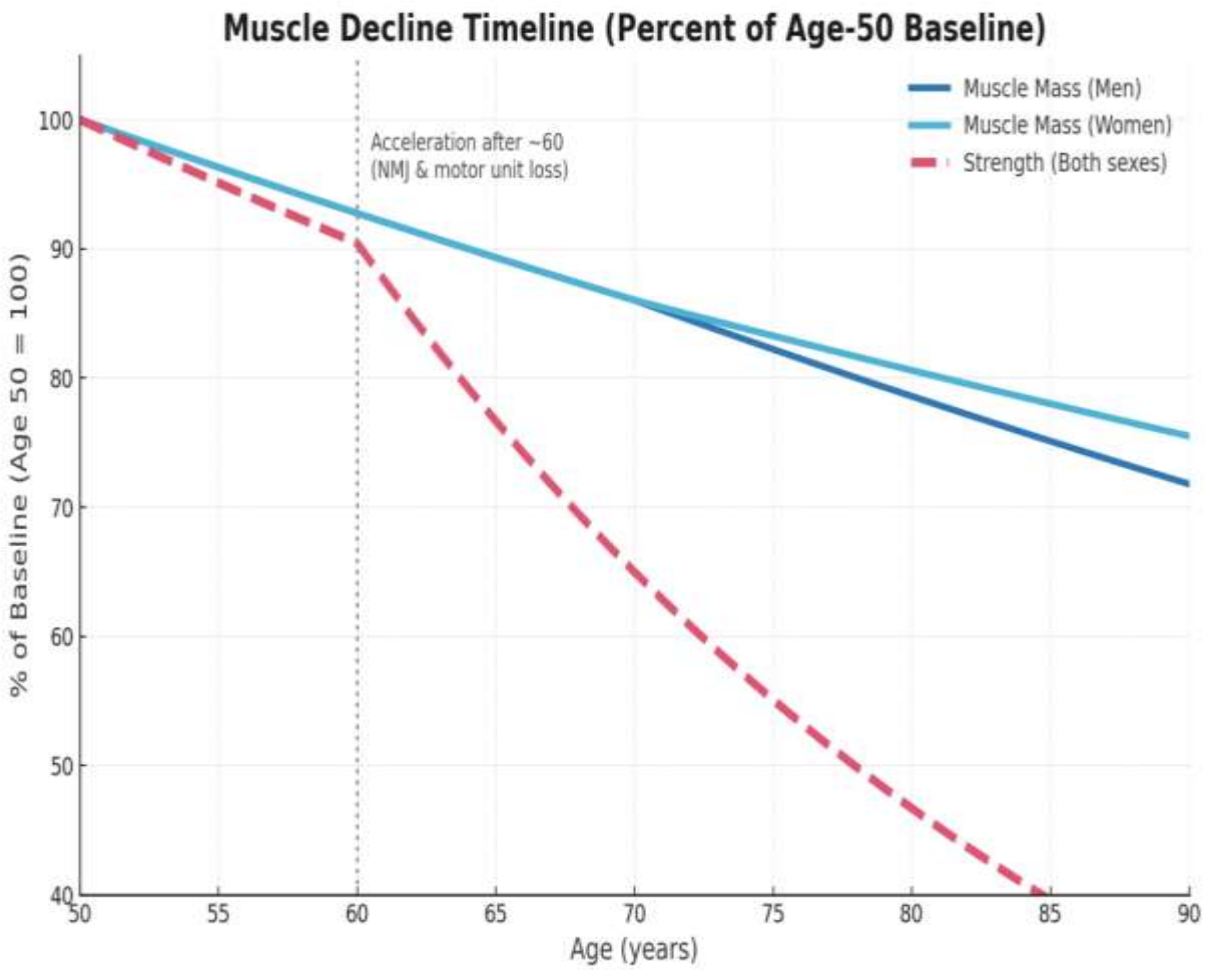
3. **Follistatin helps reduce**

Addressing Sarcopenia and Dynapenia with Follistatin Gene Therapy

- *Sarcopenia:* progressive loss of skeletal muscle mass with aging
- *Dynapenia:* loss of muscle strength and power independent of mass
- Prevalence: 5–13% in adults >60 years; up to 50% by age 80+
- Functional impact: frailty, falls, dependency, mortality

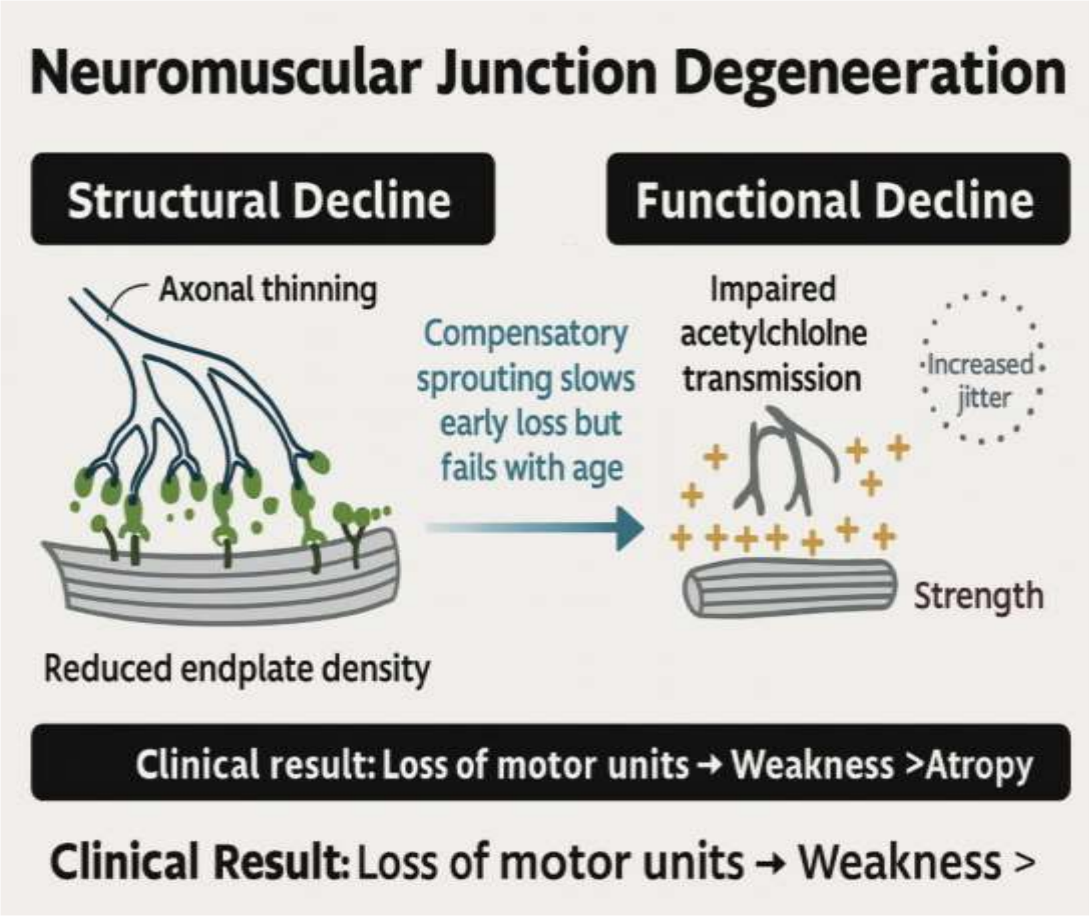
Age and Muscle: Sarcopenia and Dynapenia

- Muscle mass declines **0.5–1.0% per year** after age 50
- Accelerates to **0.8–1.0%/year in men** and **0.6–0.7%/year in women** after 70
- Strength declines **2.5–4%/year** — 2–5× faster than mass loss
- Accelerated after **age 60** due to NMJ degeneration and motor unit loss



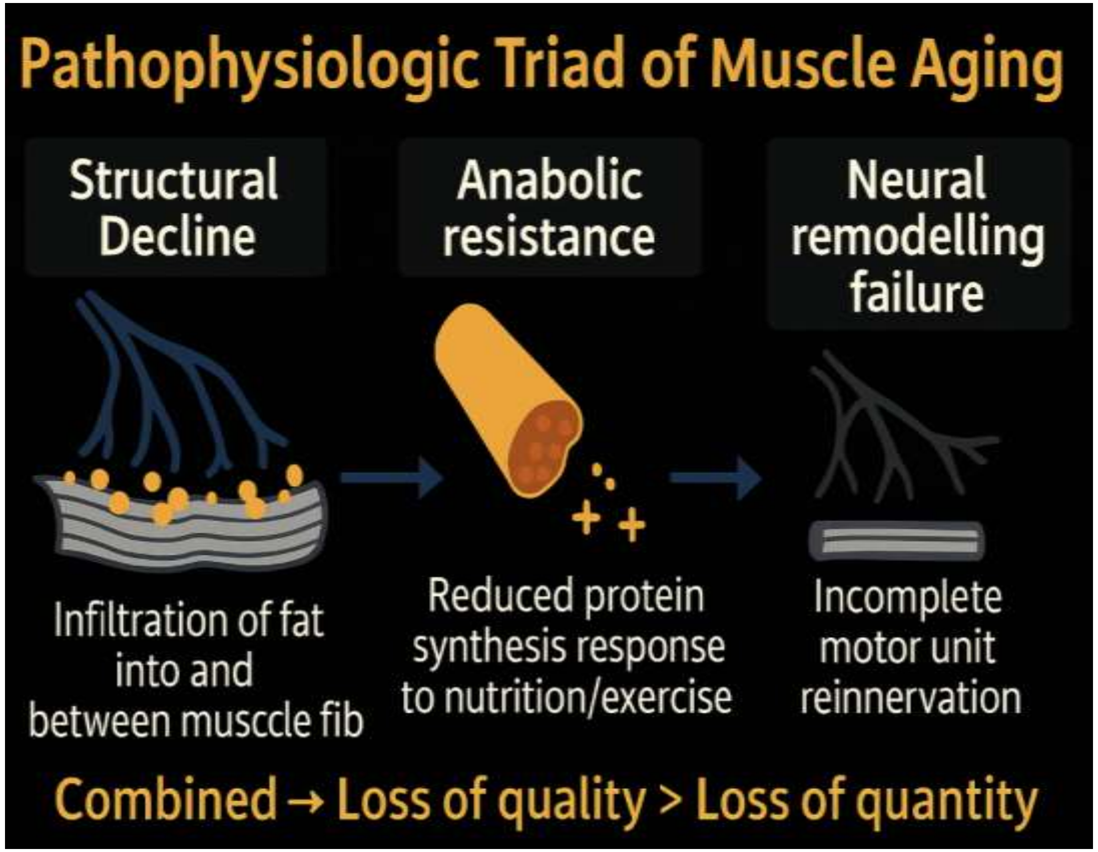
Aging and The Neuromuscular Junction

- **Structural decline:** axonal thinning, synaptic fragmentation, reduced endplate density
- **Functional decline:** impaired acetylcholine transmission, increased jitter, partial denervation
- **Compensatory sprouting** slows early loss but fails with age
- **Clinical result:** loss of motor units → weakness > atrophy



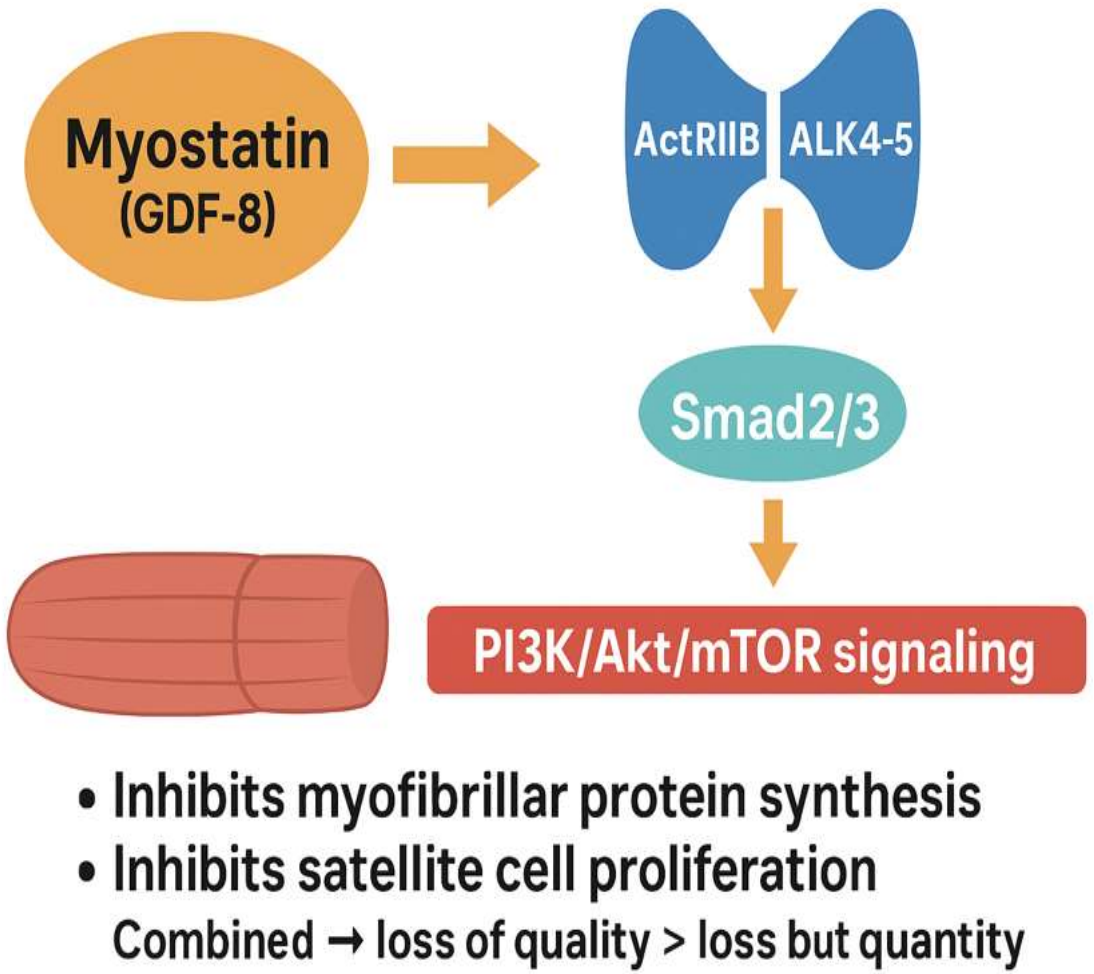
Pathophysiology of Muscle Aging

- **Myosteatosis:** infiltration of fat into and between muscle fibers
- **Anabolic resistance:** reduced protein synthesis response to nutrition/exercise
- **Neural remodeling failure:** incomplete motor unit reinnervation
- Combined → loss of quality > loss of quantity



The Myostatin Molecular Axis

- **Myostatin (GDF-8) →** binds **ActRIIB/ALK4-5** receptor complex
- Activates **Smad2/3** → suppresses **PI3K/Akt/mTOR** signaling
- Inhibits myofibrillar protein synthesis, satellite cell proliferation
- **Follistatin**: neutralizes Myostatin, Activin A, and GDF11 → restores anabolism



Follistatin is a Multifunctional Regulator

Pathologic state:

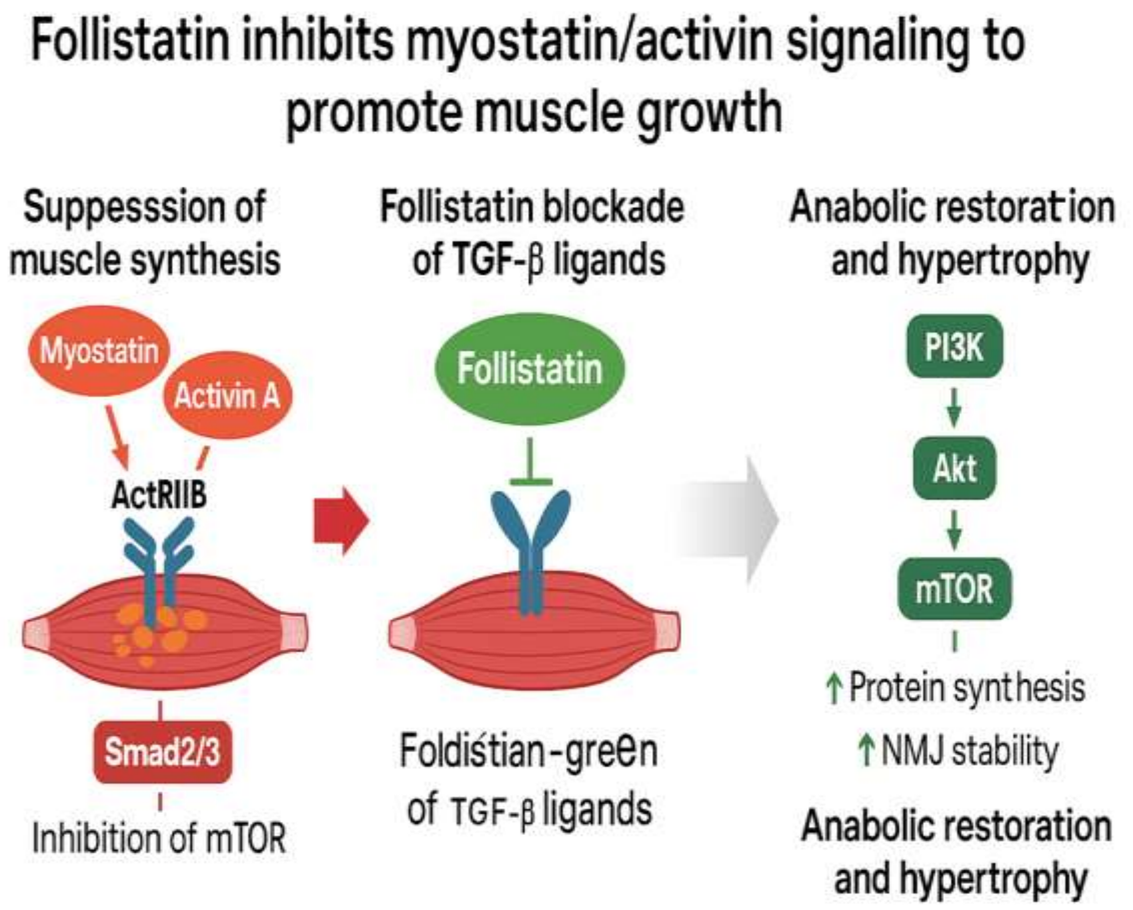
- Myostatin & Activin A bind ActRIIB → Smad2/3 activation → inhibits mTOR
- Label: “Suppression of muscle synthesis”

Follistatin binding:

- Follistatin binds Myostatin/Activin → neutralizes receptor interaction
- Label: “Follistatin blockade of TGF-β ligands”

Restored anabolic pathway:

- PI3K → Akt → mTOR activation → ↑ protein synthesis, ↑ NMJ stability
- Label: “Anabolic restoration and hypertrophy”



Species Ratio Table and Translational Implications

Species differences in Myostatin : Activin A ratio determine therapeutic response

- Rodents: **≈10 : 1** → exaggerated hypertrophy
- Non-human primates: **≈1 : 1** → partial translational match
- Humans: **≈0.46 : 1** → Activin predominance, attenuated response
- Implication: FST’s **multi-ligand blockade** is essential for human efficacy

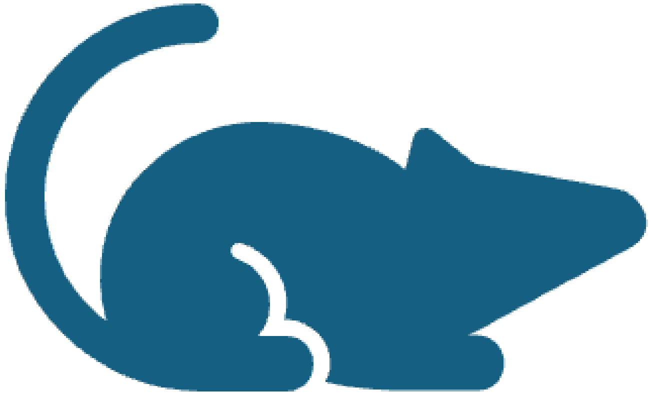


Table 1. Species-Specific Expression of Myostatin Pathway Components

Species	Myostatin (ng/mg)	Activin A (ng/mg)	GDF11 (ng/mg)	Ratio (Myo:Act)	Follistatin Expression	ActRIIB Expression
Mouse	142 ± 28	14 ± 5	10 ± 3	**10.1:1**	Baseline	High
Rat	118 ± 22	19 ± 7	12 ± 4	**6.2:1**	1.1× mouse	Moderate
Dog	72 ± 15	38 ± 12	15 ± 5	**1.9:1**	1.4× mouse	Moderate
Cynomolgus	34 ± 11	52 ± 16	20 ± 6	**0.65:1**	1.6× mouse	High
Human	28 ± 9	61 ± 18	25 ± 7	**0.46:1**	1.8× mouse	High
Pig	45 ± 13	48 ± 15	18 ± 5	**0.94:1**	1.5× mouse	Moderate

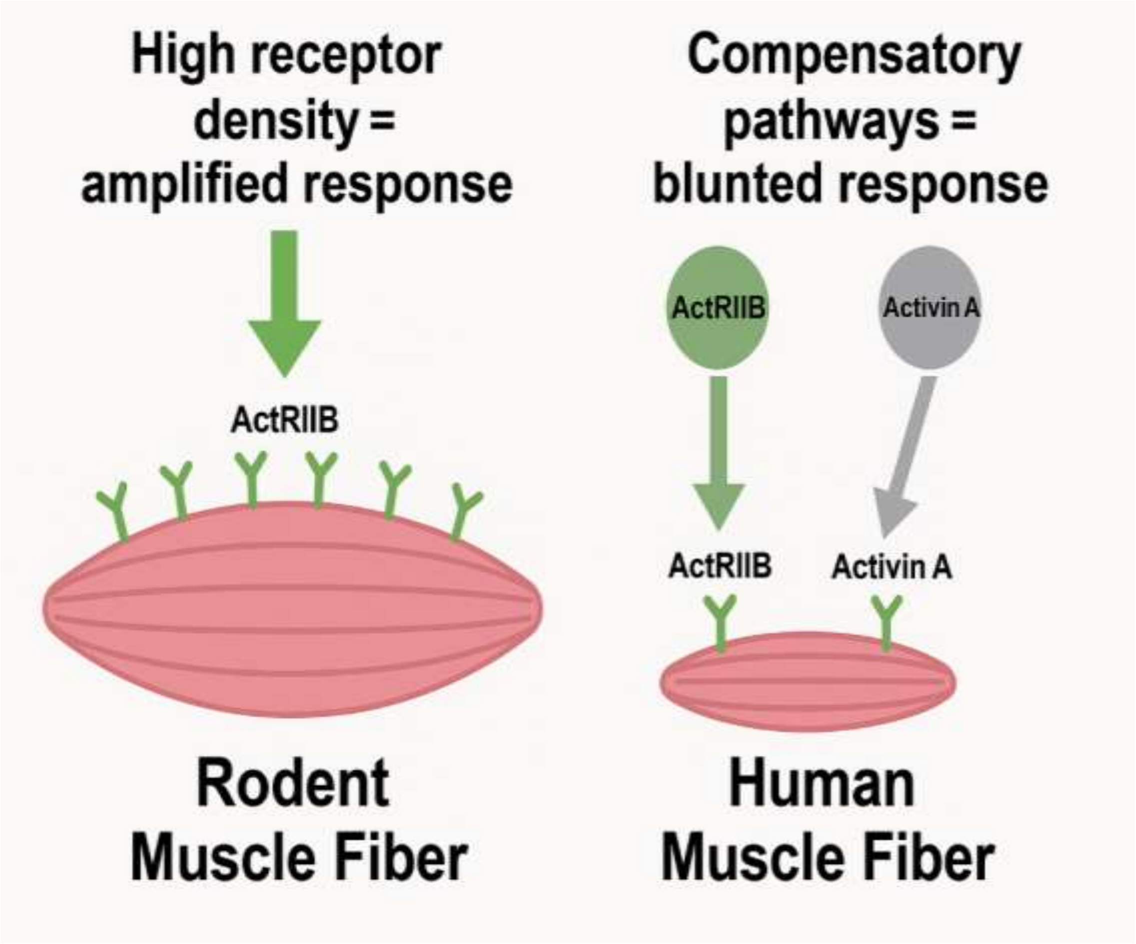
Extrapolating from Mice

Translational Limitations of Rodent Data and Human Receptor Sensitivity

- Rodent models overestimate response due to:
  - High **ActRIIB receptor density**
  - Dominant **myostatin signaling** vs activin A
  - Shorter neuromuscular conduction distances

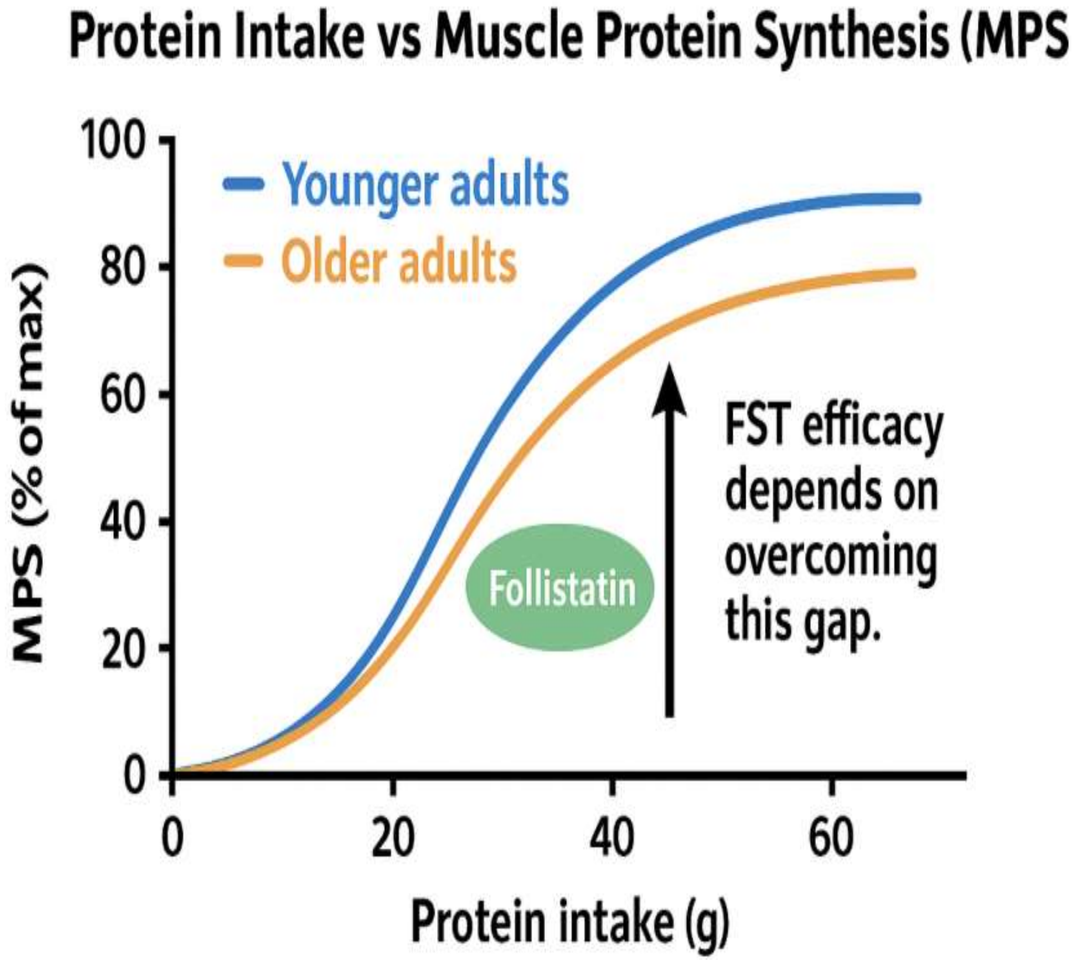
- Humans: lower receptor expression, mixed ligand signaling, slower turnover

- Implication: need for **multi-ligand and NMJ-supportive** therapy for efficacy



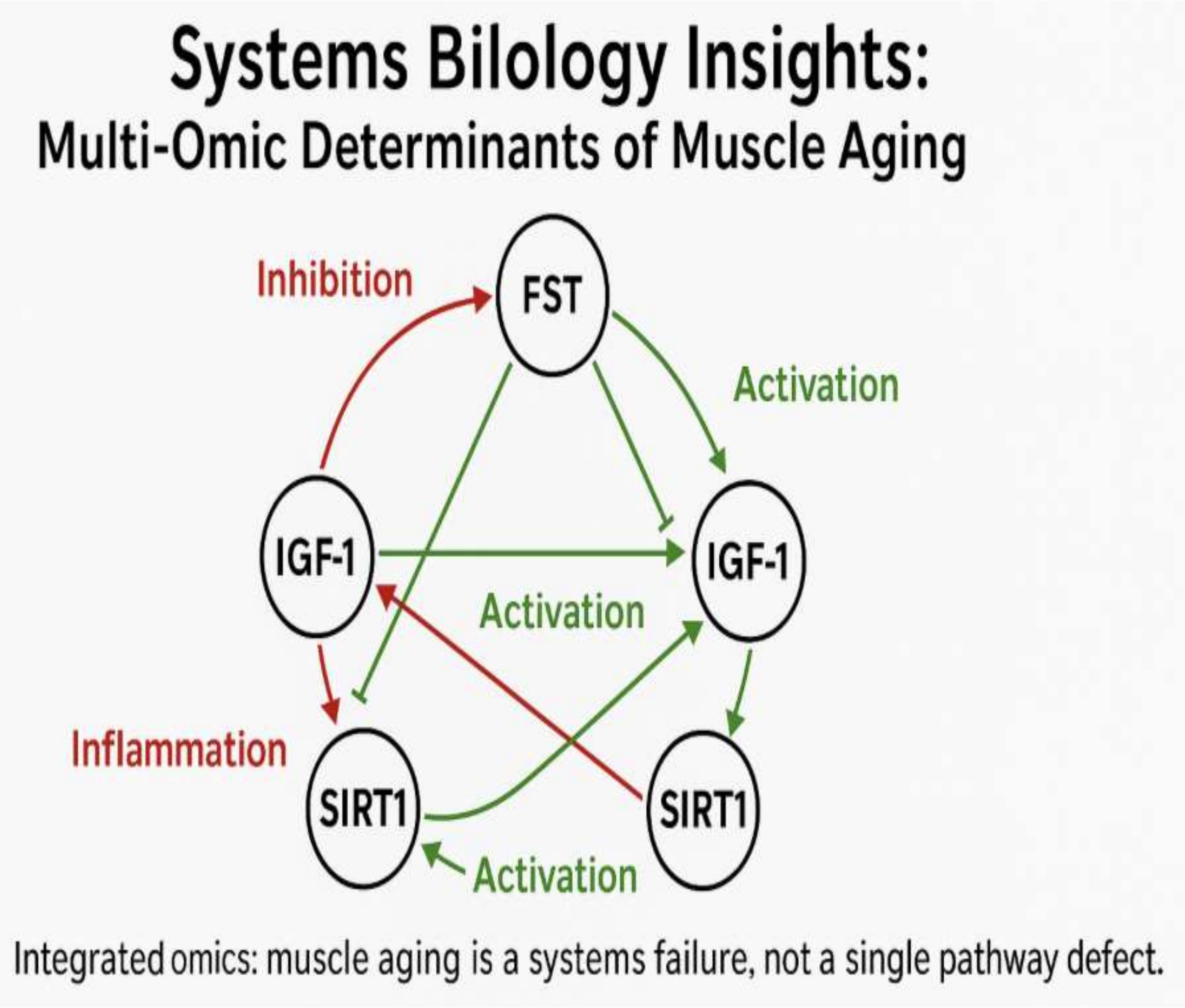
Age and Anabolic Resistance and Nutritional Thresholds

- Aging muscle shows **reduced sensitivity to amino acids** → "anabolic resistance"
- Requires **~1.2–2.0 g protein/kg/day** (vs 0.8 g/kg/day RDA) to stimulate synthesis
- **Leucine** is the key trigger for mTOR activation (threshold ≈ 2.5 g per meal)
- Exercise + protein timing → synergistic rise in MPS (≈ +60 %)
- **Anabolic resistance blunts FST response** unless nutrition is optimized



Multi-Omic Determinants of Muscle Aging

- Integrated **transcriptomic, proteomic, and metabolomic** data reveal complex regulation
- Key disrupted pathways: **mitochondrial energetics**, **inflammation**, **protein turnover**
- **Network analysis (2025):** central hubs = IGF-1, FOXO3, SIRT1, FST, and PGC-1α
- Aging muscle = pro-inflammatory, hypoanabolic, oxidatively stressed state
- **Therapeutic implication:** multi-target modulation (e.g., FST + metabolic support) outperforms single-gene therapy



Systemic vs Targeted Follistatin Delivery

- **Two main delivery systems:**
  - *Minicircle DNA FST*: systemic, non-targeted expression
  - *AAV-FST (AAVX, FS344)*: skeletal muscle–restricted expression
- Systemic exposure → **circulating FST**, broad TGF-β suppression → cardiac risk
- Muscle-targeted AAV-FST → **localized hypertrophy**, preserved cardiac function
- Key safety differentiator: **promoter specificity (MCK, desmin)**

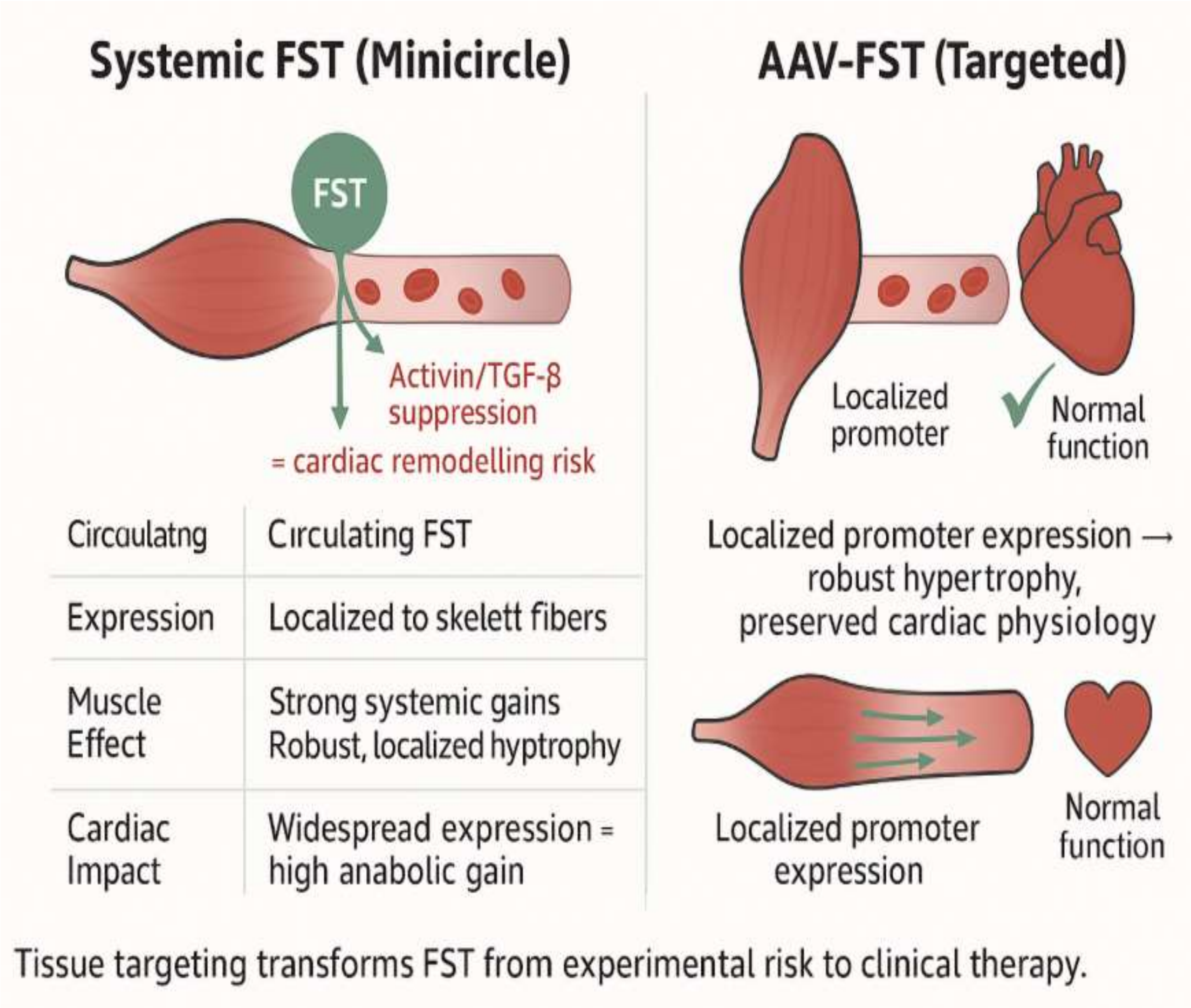
Comparison: Minicircle FST (Systemic) vs AAV-FST (Skeletal-Targ-Targeted)

Feature	Minicircle FST (Systemic)	AAV-FST (Skeletal-Targeted)
Targeting	Non-specific, systemic	Muscle-specific promoters
Expression	Circulating FST	Localized to skeletal fibers
Muscle Effect	Strong systemic gains	Robust, localized hypertrophy
Cardiac Impact	⚠ Hypertrophy, fibrosis, arrhythmia risk	Preserves function, minimal risk
Clinical Outlook	Safety concerns for long-term use	Safer, scalable for chronic therapy

Minicircle delivery produces systemic exposure with cardiac risk, while AAV-FST achieves muscle-targeted expression, ensuring localized hypertrophy and long-term safety.
This distinction defines AAV-FST as the clinically viable therapeutic path forward forward.

Mechanistic Comparison: Systemic vs Targeted Outcomes

- **Systemic (Minicircle) FST:**
  - Circulating FST → global activin/TGF-β blockade
  - ↑ muscle mass, but **off-target suppression** in cardiac tissue
  - Risk: **fibrosis, hypertrophy, arrhythmia**
- **Targeted (AAV-FST):**
  - Promoter-constrained to skeletal muscle (MCK, desmin)
  - Maintains **cardiac signaling balance**
  - ↑ strength & hypertrophy with **preserved ejection fraction**
- Outcome: **Localized = safe; systemic = risky**



Cardiac Safety and Functional Outcomes in Preclinical Studies

- **Systemic FST exposure (minicircle):** cardiac hypertrophy, fibrosis, arrhythmogenic potential
- **AAV-FST (skeletal-restricted):** preserves ejection fraction, normal wall thickness
- **Non-human primate (AAV1-FS344):** ↑ muscle mass 15–30% with **no cardiac remodeling**
- Histopathology: **absence of collagen deposition or myocardial disarray**
- Supports **tissue-specific expression as key to safety**

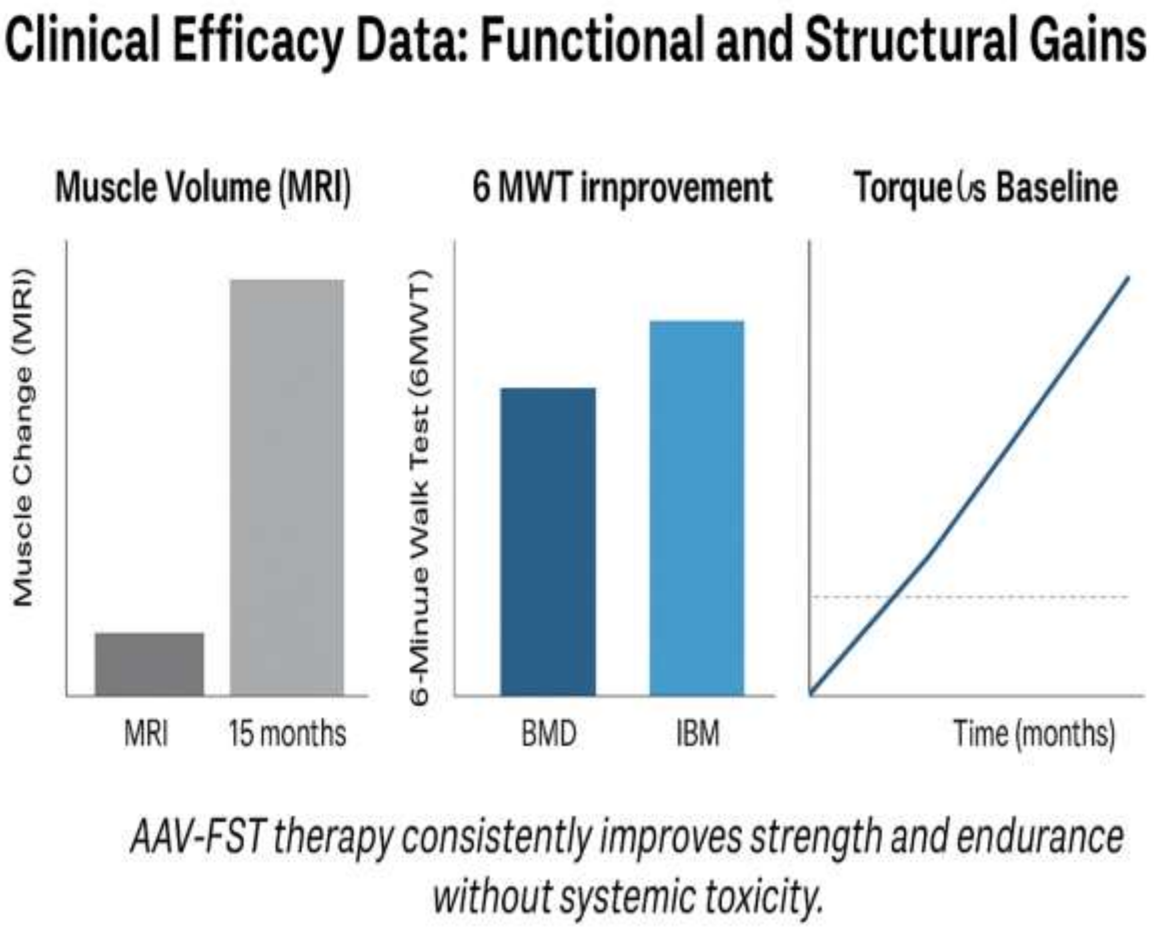
Cardiac Safety and Functional Outcomes in Preclinical Studies

Study / Model	Vector Type	Target	Outcome	Cardiac Findings
Lee et., 200	Minicircle DNA	Systemic	↑ muscle gain	LV hypertrophy, fibrosis
Winbanks et. l., 201	FST transgenic	Global	↑ muscle + heart mass	Arrhythmogenic remodeling
AAV1–FS344	Skeletal (primate)	↑ muscle 15 – 30%	Normal EF, no fibrosis	No cardiac signal changes
Mendell et. al., 2015	Becker MD (human)	strength, safe	No cardiac cignal	No cardiac signal changes

Preclinical and ear ly human data demonstrate cardiac preservation with skeletal–restricted FST expression.)

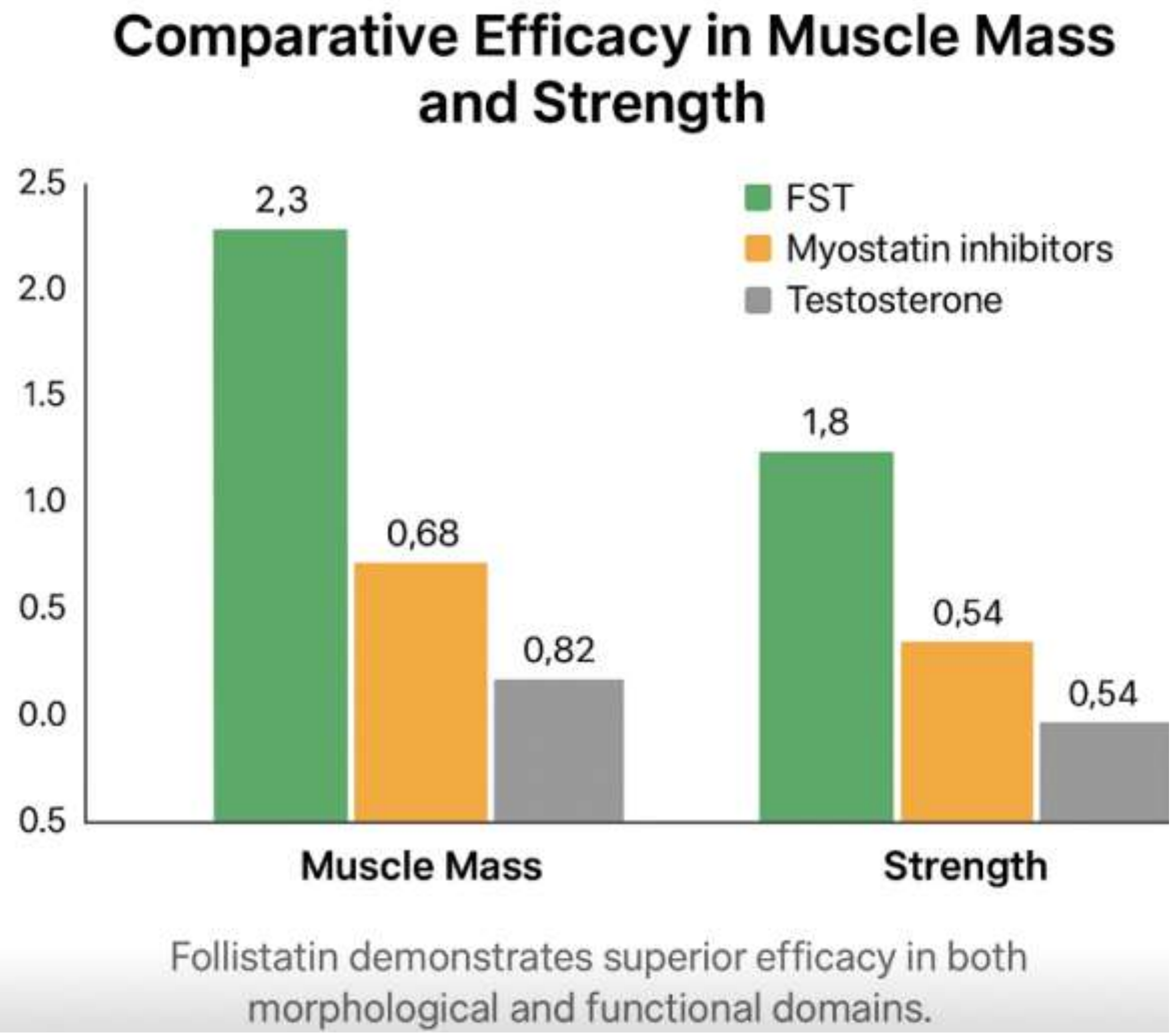
AAV Clinical Efficacy Data: Functional and Structural Gains

- **AAV1-FS344 (non-human primates):** ↑ muscle size + strength sustained >15 months
- **Becker MD (Phase I/II, Mendell 2015):**
  - 6MWT: **+58–125 meters**
  - ↑ torque, no hormonal or cardiac changes
- **Inclusion Body Myositis (NCT02500017):**
  - **+56 m/year vs –25.8 m/year** in controls
- **Effect durability:** persistent expression without immune rejection
- Confirms **functional as well as morphological benefit**



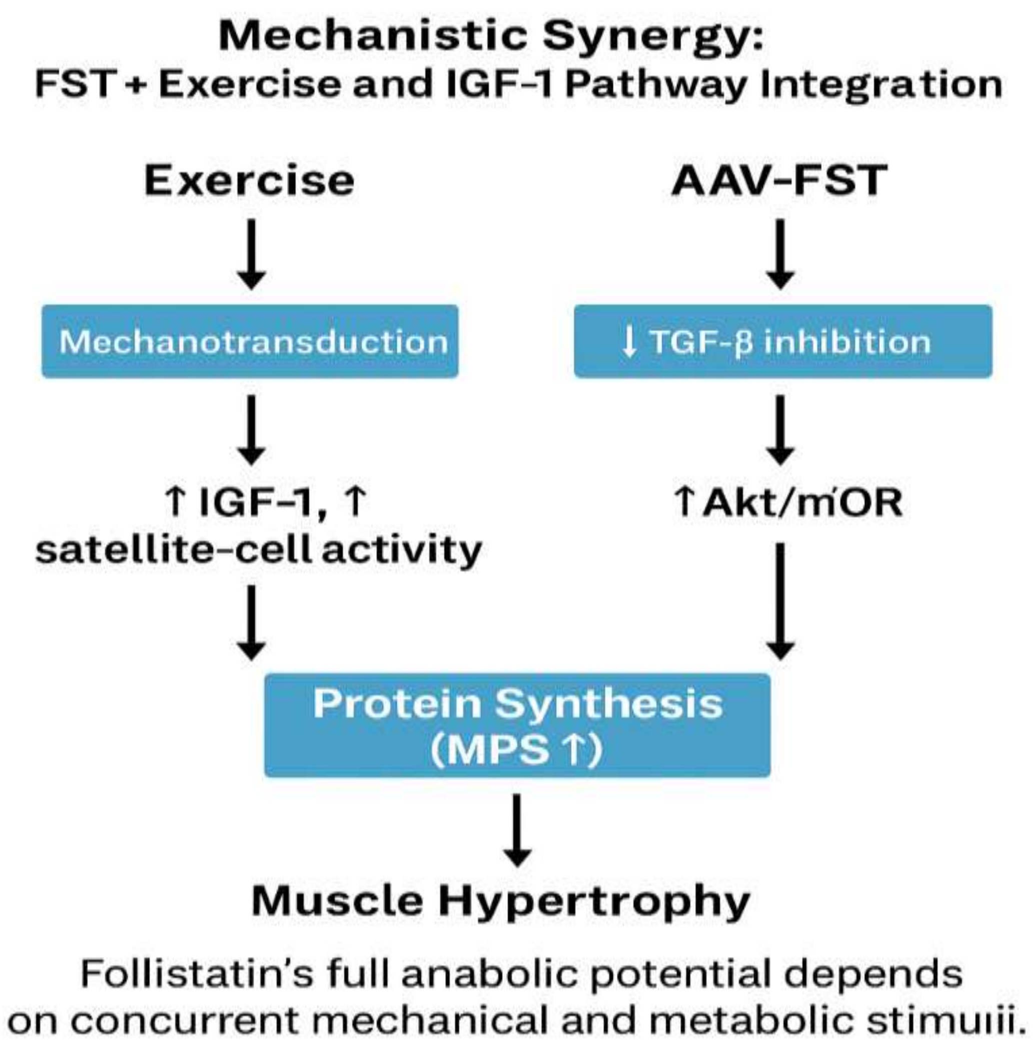
Follistatin vs Myostatin and Testosterone Therapies

- **Meta-analysis (n=15,432):** FST outperforms other anabolic interventions in mass & strength gains
- **Muscle Mass (SMD):**
  - FST (innervated): **2.3**
  - Myostatin inhibitors: **0.68**
  - Testosterone: **0.82**
- **Strength (SMD):**
  - FST (innervated): **1.8**
  - Myostatin inhibitors: **0.22**
  - Testosterone: **0.54**
- **FST advantage:** multi-ligand inhibition, NMJ preservation, endocrine neutrality
- **Testosterone:** improves strength but with higher CV risk



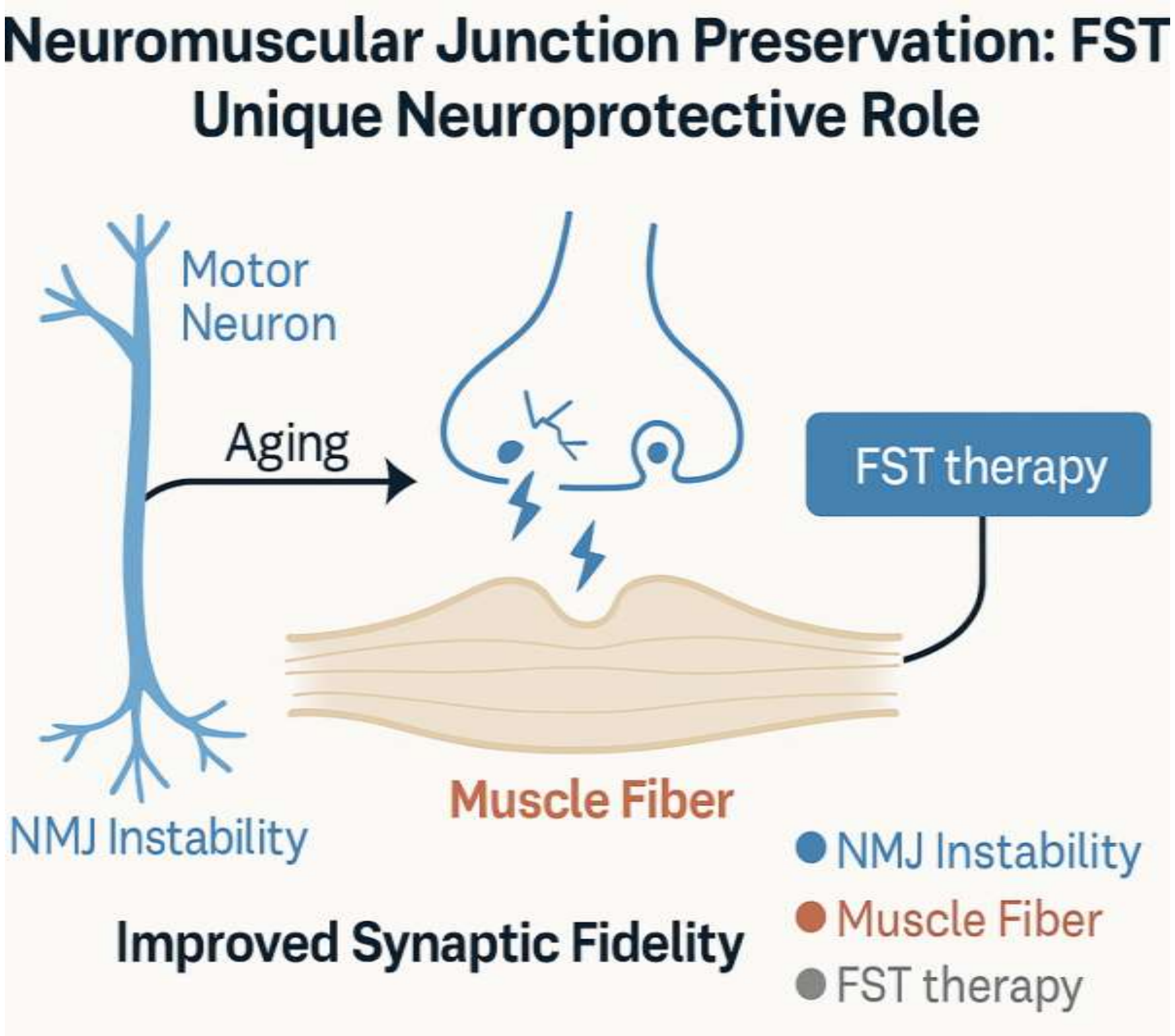
Mechanistic Synergy: FST + Exercise and IGF-1 Pathway Integration

- **Exercise + FST** → synergistic activation of **PI3K/Akt/mTOR** and **satellite-cell proliferation**
- Exercise elevates **endogenous FST and IGF-1** → amplifies AAV-FST effects
- **IGF-1 and insulin signaling** required for full hypertrophic response
- Clinical data: exercise + FST → **>2× muscle protein synthesis (MPS)** vs either alone
- **Practical implication:** physical activity = essential cofactor for FST therapy



Neuromuscular Junction Preservation: FST's Unique Neuroprotective Role

- Aging: **NMJ instability** → denervation, jitter, transmission block
- **FST therapy** enhances NMJ integrity even without preventing neuron loss
- Aged mice: ↓ jitter (10 µs vs 16.5 µs), ↓ blocking (4% vs 25%) after AAV-FST
- **Improved synaptic fidelity** → proportional gains in strength and coordination
- Suggests **FST supports both muscle and motor neuron signaling units**



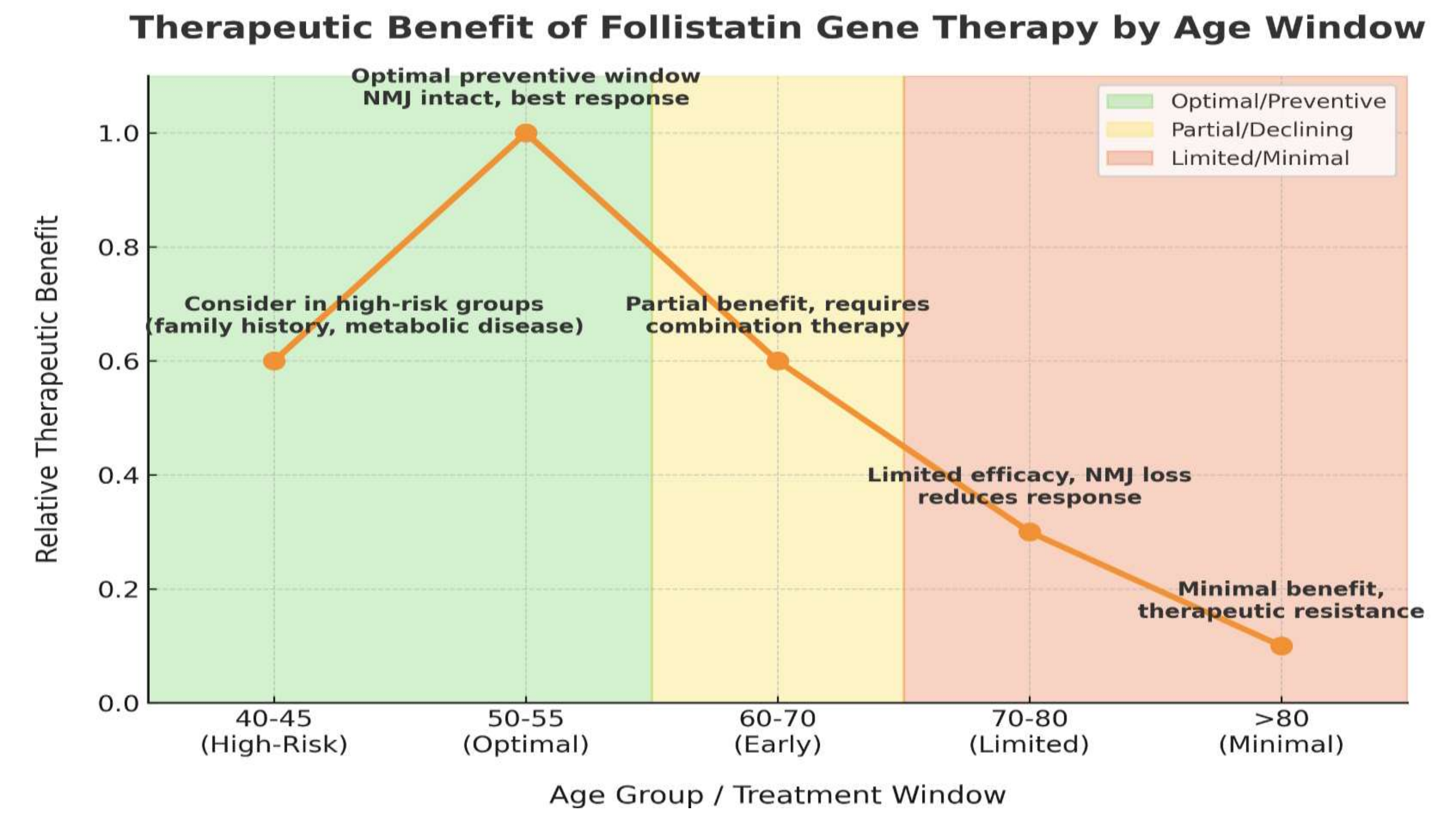
Timing of Intervention: The NMJ Integrity Window (Ages 50–80)

- **NMJ deterioration is progressive but predictable**

- Preclinical timeline:
  - **50–60 y:** <5% denervation → optimal prevention window
  - **60–70 y:** 10–15% failure → early intervention
  - **70–80 y:** >25% denervation → limited efficacy
  - **>80 y:** therapeutic resistance (>20% denervated fibers)

- **FST efficacy highest when NMJ intact** (RR 0.32 prevention vs 0.89 post-denervation)

- Timing = cornerstone of translational success



Therapeutic Benefit of Follistatin Gene Therapy by Age Window
Optimal/Preventive
Partial/Declining
Limited/Minimal
Optimal preventive window
NMJ intact, best response
Consider in high-risk groups
(family history, metabolic disease)
Partial benefit, requires
combination therapy
Limited efficacy, NMJ loss
reduces response
Minimal benefit,
therapeutic resistance
Relative Therapeutic Benefit
1.0
0.8
0.6
0.4
0.2
0.0
40-45
(High-Risk)
50-55
(Optimal)
60-70
(Early)
70-80
(Limited)
>80
(Minimal)
Age Group / Treatment Window

Comparative Safety Analysis: FST vs Other Gene Therapies

- **AAV-FST (muscle-targeted):** benign AE profile; *cardiac neutrality* when expression is skeletal-restricted

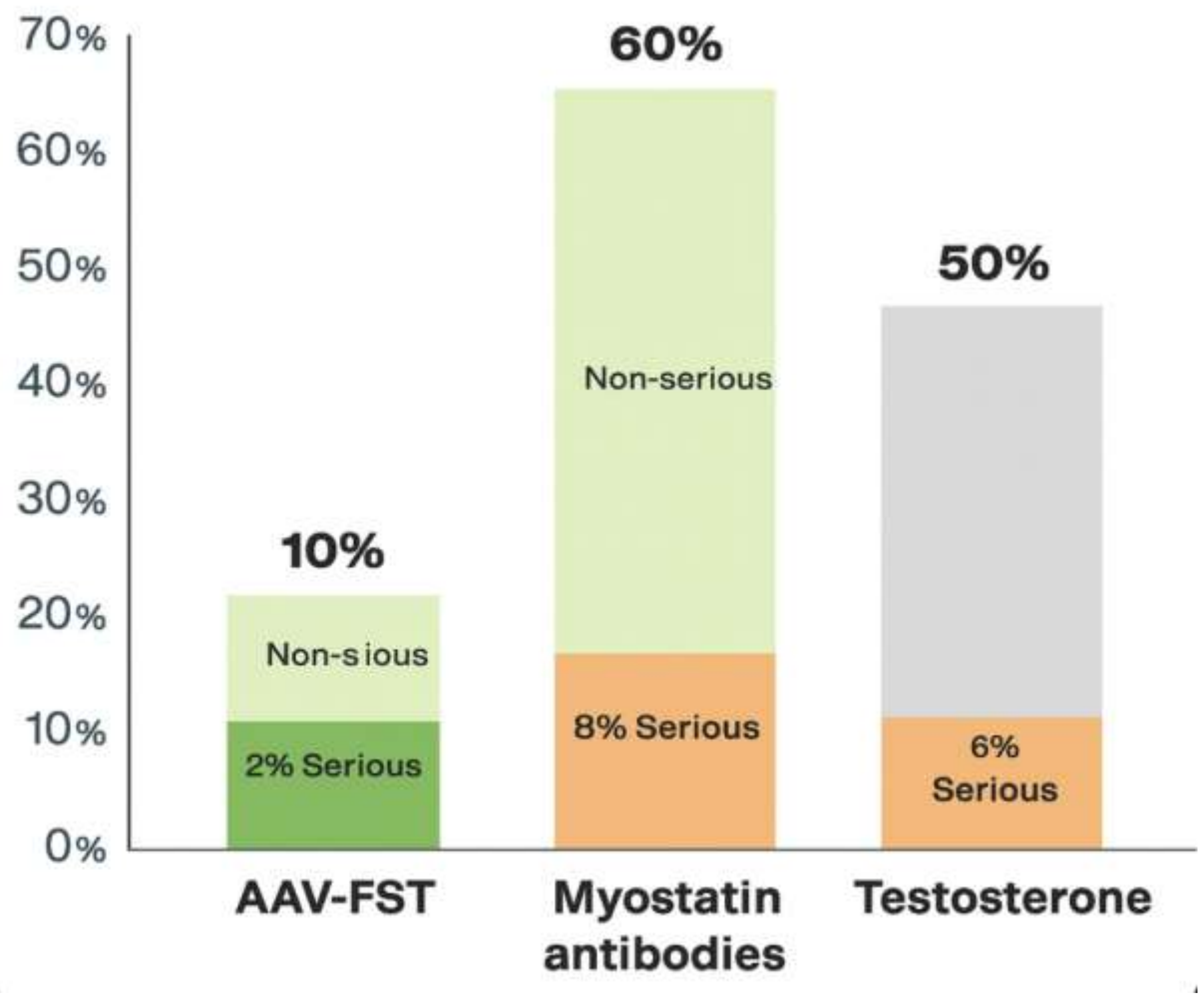
- **Systemic FST (minicircle):** off-target TGF-β suppression → **hypertrophy/fibrosis risk** (cardiac)

- **Other AAVs (e.g., micro-dystrophin, SMA):** dose-linked transaminitis, thrombocytopenia, rare TMA/myocarditis events

- **Risk mitigators:** muscle-specific promoters (MCK/desmin), dose banding, brief steroids, APC

Translational Outlook: Comparative Toxicity and Regulatory Readiness

- **Toxicity summary:** FST ≤10% adverse events vs 60%+ for systemic myostatin blockade
- **Serious AE rates:**
  - FST 2% | Myostatin inhibitors 8% | Testosterone 6%
- **No hepatotoxicity, coagulopathy, or vector-related deaths** in AAV-FST studies
- **Regulatory trajectory:** parallels SMA and DMD programs (Phase I → pivotal in 4–6 yrs)
- **Primary gating factor:** immunologic



How do we improve on this data – Therapy Design

- Increase targeting
- Decrease off targeting
- Reduce dose = Reduce cost
- Reduce dose = Reduced toxicity
- Reduced dose = Increased safety

This is where Triple Helix Leads

Gene Therapy Design Components

Engineering Precision Targeting & Therapeutic Specificity

🎯 Core Design Philosophy

Gene therapy success depends on delivering the right gene, to the right cells, at the right time, with minimal off-target effects

Six Critical Components

Each element works synergistically to achieve therapeutic precision: **Promoters** control expression location, **Enhancers** amplify signals, **Capsids** determine tissue uptake, **Targeting peptides** refine specificity, **Immunomodulators** manage immune responses, and the **Therapeutic payload** delivers clinical benefit.

🔬 Integrated Design Approach

Vector Design
Capsid + targeting elements

Expression Control
Promoters + enhancers

Immune Management
Evasion strategies

Therapeutic Outcome
Optimized payload delivery

Promoter Elements

Controlling WHERE and WHEN genes are expressed

P Transcriptional Control Sequences

Primary Function: Recruit transcription machinery to initiate gene expression in specific cell types or under particular conditions.

Tissue Specificity | Temporal Control | Expression Level

Promoter Categories & Applications:

- **Constitutive:** CMV, CAG, EF1α - broad, high expression
- **Tissue-Specific:** Albumin (liver), GFAP (astrocytes), cardiac troponin (heart)
- **Inducible:** Tet-On/Off systems, heat shock promoters
- **Disease-Specific:** Hypoxia-inducible, inflammation-responsive
- **Synthetic:** Engineered promoters with custom specificity profiles

Targeting Impact

Tissue-specific promoters can provide 10-1000x improved specificity compared to ubiquitous promoters, dramatically reducing off-target effects

Enhancer Sequences

Amplifying expression while maintaining specificity

E Transcriptional Amplifiers

Primary Function: Increase transcription levels through chromatin remodeling and transcription factor binding, often working synergistically with promoters.

Expression Boost | Chromatin Access | Distance-Independent

Enhancer Types & Mechanisms:

- **Natural Enhancers:** Tissue-specific regulatory sequences from native genes
- **Viral Enhancers:** WPRE (Woodchuck Post-transcriptional Regulatory Element)
- **Synthetic Enhancers:** Designed sequences with predictable activity
- **Super-enhancers:** Clustered enhancer regions for maximum expression
- **Conditional Enhancers:** Activity controlled by external stimuli

Advantages

- 5-50x expression increase
- Can work at long distances
- Tissue-specific enhancement
- Overcome chromatin silencing

Considerations

- May increase vector size
- Potential for position effects
- Context-dependent activity
- Complex regulatory interactions

Capsid Tropism

Natural tissue targeting through receptor interactions

C Vector Shell Properties

Primary Function: Determine initial tissue distribution through specific receptor binding, cellular entry mechanisms, and intracellular trafficking patterns.

Cell Entry | Tissue Distribution | Immune Recognition

AAV Serotype Tropism Examples:

- **CNS Targeting:** AAV9, AAV-PHP.eB (cross blood-brain barrier)
- **Liver Tropism:** AAV3, AAV8, AAVrh.74 (hepatocyte binding)
- **Retinal Targeting:** AAV2, AAV5 (photoreceptor/RPE tropism)
- **Broad Tissue:** AAV1, AAV6 (multiple tissue types)
- **Engineered Variants:** Directed evolution for novel tropisms

Receptor-Mediated Specificity

Natural capsid tropism can provide 100-10,000x preferential tissue uptake based on receptor expression patterns and binding affinities

Targeting Peptides

Engineered specificity through surface modifications

T Surface-Displayed Ligands

Primary Function: Redirect or enhance vector binding to specific cell surface receptors through genetically inserted or chemically conjugated targeting moieties.

Receptor Binding | Cell-Type Selection | Detargeting

Targeting Strategies:

- **Integrin Targeting:** RGD peptides for angiogenesis, wound healing
- **Growth Factor Mimics:** VEGF, EGF receptor-binding sequences
- **Antibody Fragments:** scFv, nanobodies for specific antigens
- **Lectin Binding:** Sugar-binding domains for glycan recognition
- **pH-Responsive:** Peptides active in tumor microenvironment

Insertion Methods

- Genetic insertion into capsid
- Chemical conjugation post-production
- Adapter-mediated targeting
- Chimeric capsid engineering

Applications

- Cancer cell targeting
- Immune cell specificity
- Tissue repair/regeneration
- Disease-specific delivery

Immunomodulatory Elements

Managing immune responses for sustained efficacy

I Immune Interface Management

Primary Function: Control immune recognition and response to maintain vector efficacy, prevent neutralization, and ensure long-term therapeutic expression.

Immune Evasion | Tolerance Induction | Expression Protection

Immunomodulation Strategies:

- **Capsid De-immunization:** Epitope removal, humanization
- **microRNA Targeting:** miR-142-3p sites for immune cell detargeting
- **Immune Suppressors:** Co-delivery of IL-10, TGF-β
- **Tolerogenic Platforms:** Regulatory T cell induction
- **Transient Immunosuppression:** Coordinated drug protocols

Preservation Strategy

Effective immunomodulation maintains targeting specificity by preventing immune clearance of transduced cells and neutralization of therapeutic vectors

Therapeutic Payload

Optimized therapeutic genes for maximum efficacy

G Therapeutic Genetic Cargo

Primary Function: Deliver therapeutic benefit through optimized gene sequences designed for enhanced expression, stability, and biological activity.

Gene Expression | Protein Function | Safety Features

Payload Optimization Strategies:

- **Codon Optimization:** Species-specific codon usage for enhanced translation
- **Regulatory Elements:** IRES, 2A peptides for multi-gene expression
- **Protein Engineering:** Enhanced stability, activity, or specificity
- **Safety Switches:** Inducible degradation, conditional activity
- **Reporter Integration:** Built-in monitoring capabilities

Optimization Benefits

- 2-10x improved expression
- Enhanced protein stability
- Reduced immunogenicity
- Controllable activity

Design Considerations

- Packaging size constraints
- Expression level balance
- Tissue-specific requirements
- Long-term stability

Component Integration & Synergy

How elements work together for precision targeting

🔗 Multi-Layer Targeting Strategy

Primary Targeting
Capsid tropism + targeting peptides determine initial tissue distribution

Expression Control
Tissue-specific promoters + enhancers control where genes are expressed

Immune Management
Immunomodulatory elements preserve long-term function

Therapeutic Output
Optimized payload delivers maximum clinical benefit

🎯 Precision Targeting Formula

Effective Targeting = (Capsid Tropism × Targeting Peptides) × (Promoter Specificity × Enhancer Boost) × Immune Tolerance × Payload Optimization

💡 Key Design Principle

Layered Specificity: Multiple independent targeting mechanisms multiply together to achieve extraordinary precision - each 10x improvement in specificity compounds with others to reach 10,000x+ overall targeting enhancement.

Clinical Translation Success Factors

- **Dose-Response Optimization:** Balance efficacy with safety margins
- **Patient Stratification:** Matching vector design to patient characteristics
- **Manufacturing Consistency:** Reproducible targeting properties
- **Regulatory Pathway:** Demonstrating controlled, predictable targeting

Design Makes a Difference

Minicircle Follistatin

Triplehelix Follistatin

Minicircle DNA: Missing Design Components

Why simplified design leads to limited therapeutic outcomes

Cost: $24,000 USD - But what's missing from this "budget" approach?

Optimized Gene Therapy (AAV)

- ✓ **Advanced Capsid Tropism**
  Natural tissue targeting via receptor binding
- ✓ **Targeting Peptides**
  Engineered surface modifications for specificity
- ✓ **Tissue-Specific Promoters**
  Precise control of expression location
- ✓ **Expression Enhancers**
  Amplified therapeutic gene expression
- ✓ **Immunomodulation**
  Managed immune responses for durability
- ✓ **Optimized Payload**
  Codon optimization, stability enhancement

VS

Minicircle DNA

- ✗ **No Inherent Tropism**
  Relies solely on crude delivery method
- ✗ **No Targeting Peptides**
  Cannot redirect to specific cell types
- ✗ **Generic Promoters**
  Often uses ubiquitous CMV promoter
- ✗ **Limited Enhancement**
  Basic plasmid design without amplifiers
- ✓ **Avoids Viral Immunity**
  Non-viral = no capsid immune response
- ✗ **Basic Payload**
  Minimal gene optimization

⚠️ Clinical Implications of Missing Components

🎯 Poor Efficacy

Issue: Without optimized delivery components, cellular uptake is severely limited.
Result: Much lower transduction efficiency leads to minimal therapeutic benefit (1.25% vs 10% muscle gain)

🌐 Off-Target Effects

Issue: No tissue-specific targeting means expression occurs wherever DNA is taken up.
Result: Metabolic alterations (↑ free fatty acids), unintended liver/adipose expression

⏱️ Short Duration

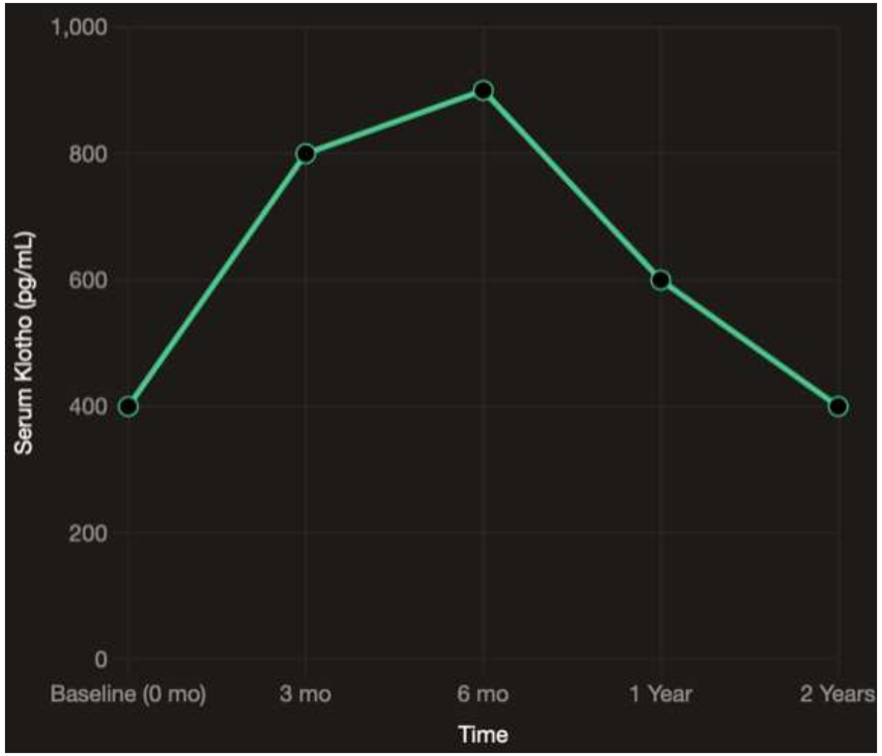
Issue: Episomal DNA degrades quickly without integration mechanisms.
Result: Only 1.5 years vs >20 years, requiring repeated invasive procedures

📊 Variable Results

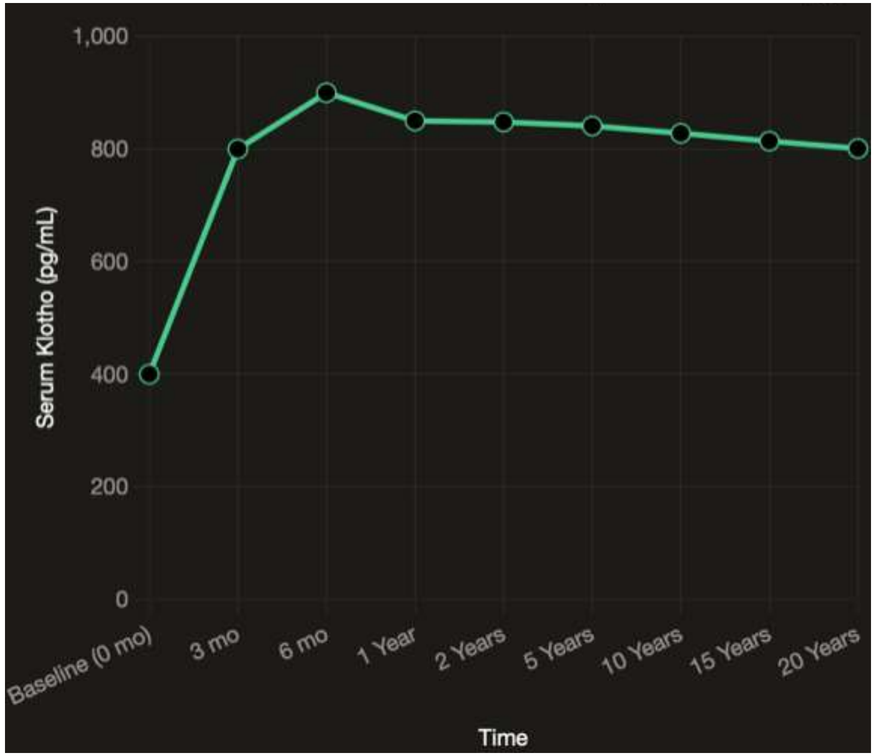
Issue: Crude delivery methods (electroporation) cause inconsistent transfection.
Result: Unpredictable outcomes, unsuitable for severe diseases requiring reliable dosing

Gene Expression Levels: Plasmid vs AAV

PLASMID:



AAV:



Minicircle Results

📈 2024 Phase I Trial Data - The Reality of Simplified Design

1.25% Muscle Mass Gain	**0.87%** Body Fat Reduction	**10%** Strength Increase

💡 The Bottom Line

Minicircle DNA represents a "bare minimum" gene therapy approach. While the $24,000 cost appears attractive, the absence of sophisticated targeting components results in dramatically reduced therapeutic outcomes. The apparent cost savings are negated by the need for repeated treatments, variable efficacy, and limited clinical applicability to serious diseases requiring reliable, sustained therapeutic levels.

Triple Helix Follistatin Gene Therapy

Complete Design Portfolio - Four Advanced Construct Approaches

🧬 Premium Gene Therapy Engineering

Sophisticated targeting systems with multiple therapeutic control strategies

10%	11%	40%
Muscle Mass Gain	Body Fat Reduction	Strength Increase

Duration: >20 years | Single treatment protocol

🎯 Design Philosophy

Four distinct construct approaches demonstrate the versatility of advanced gene therapy design - from inducible control systems to safety switches, each optimized for specific therapeutic scenarios and risk profiles.

AAV9 Follistatin with Tet-On & Kill Switch

Advanced Multi-System Therapeutic Control Platform

⚡ Inducible Promoter + Kill Switch Architecture

🎯 Precision Targeting

ASSLNIA Peptide: Modified AAV9 VP1 capsid (loop VIII) for enhanced skeletal muscle tropism
Muscle-Specific: DES promoter + MyoD enhancer for tissue selectivity

🔄 Tet-On Control

Doxycycline-Inducible: rtTA under DES promoter activates TRE-driven FST expression
Temporal Control: Precise on/off regulation with clinically-approved inducer

🛡️ Safety Kill Switch

iCasp9 System: FKBP12-F36V fusion under PGK promoter
AP1903 Activation: Rapid apoptosis induction (>90% elimination rate)

🔬 Optimized Payload

FST344 Isoform: Codon-optimized human follistatin with Kozak sequence
Enhanced Stability: WPRE + bGHpA + CTCF insulators

🏗️ Construct Specifications

4.5kb Total Size (Within AAV9 Limit)	**Triple** Safety Control Systems	**1E13+** Vector Genomes/kg Dosing Range

Off-Target Follistatin Gene Therapy Risks

Cardiac Hypertrophy & Systemic Distribution Dangers

⚠️ **Minicircle DNA: No Inherent Tissue Targeting - Systemic Distribution Risk**

Cardiac Hypertrophy Scenario: Follistatin Exposure

Pathological Growth
Excessive cardiomyocyte hypertrophy beyond compensatory mechanisms

Fibrosis Acceleration
Enhanced collagen deposition and myocardial stiffening

Arrhythmogenesis
Disrupted conduction pathways and electrical instability

❌ Minicircle DNA Risks

- ⚠️ **Systemic Distribution:** No capsid targeting - reaches all tissues including heart
- ⚠️ **Cardiac Uptake:** High metabolic activity makes heart vulnerable to transfection
- ⚠️ **Uncontrolled Expression:** Generic CMV promoter drives follistatin in cardiac tissue
- ⚠️ **No Kill Switch:** Cannot terminate therapy if adverse cardiac effects occur
- ⚠️ **Hypertrophic Amplification:** Follistatin + existing cardiac hypertrophy = dangerous synergy
- ⚠️ **Diastolic Dysfunction:** Impaired cardiac filling and reduced ejection fraction

✅ Targeted AAV9 Safety

- ✅ **ASSLNIA Targeting:** Modified capsid preferentially targets skeletal muscle
- ✅ **Muscle-Specific Promoter:** DES promoter restricts expression to intended tissues
- ✅ **Inducible Control:** Doxycycline regulation allows precise dosing control
- ✅ **iCasp9 Kill Switch:** AP1903 can rapidly eliminate transduced cells
- ✅ **Reduced Cardiac Exposure:** Multiple targeting layers minimize off-target effects
- ✅ **Controlled Biodistribution:** Enhanced muscle tropism reduces systemic spread

Pathophysiological Mechanism

In cardiac hypertrophy, **myostatin inhibition by follistatin** can paradoxically worsen the condition. While myostatin normally provides protective anti-hypertrophic signals in the heart, **follistatin blockade removes this brake**, leading to uncontrolled cardiomyocyte growth, increased wall thickness, and progression to heart failure.

Triple Helix Gene Therapies

- Follistatin
- Klotho
- hTERT
- FGF21
- PGC1A
- FOXO3
- Others – 68 additional genes

Advanced Gene Therapy Portfolio

Comprehensive Anti-Aging & Therapeutic Interventions

🧬 Next-Generation Longevity Medicine Platform

💪 Follistatin Muscle

25% muscle volume increase, enhanced strength, metabolic benefits, anti-inflammatory effects

🧠 hTERT CNS

Telomere elongation, neurogenesis, cognitive enhancement, dementia reversal

🧠 Klotho CNS

Synaptic plasticity, neuroprotection, antioxidant defense, cognitive improvement

🧠 hTERT/Klotho

Synergistic cognitive enhancement, dual neuroprotection, amplified anti-aging

🔥 IV FGF21

Metabolic optimization, fat burning, liver health, energy enhancement

🛡️ IV FOXO3

Cellular repair, autophagy, stress resistance, 20-30% lifespan extension

💚 IV Klotho

Cardiovascular health, kidney protection, anti-aging, cognitive benefits

⚡ IV PGC1A

Mitochondrial enhancement, endurance, energy production, muscle protection

🏆 Portfolio Advantages

Multi-target approach addressing muscle health, cognitive function, metabolic optimization, and cellular longevity. Clinical evidence from human trials with demonstrated safety and efficacy across multiple aging hallmarks.

Follistatin Gene Therapy

Advanced Muscle Growth & Metabolic Enhancement

💪 Muscle Growth & Strength Benefits

📈 Growth & Performance

- ✓ Up to 25% muscle volume increase in primates
- ✓ 15% more skeletal muscle mass in humans
- ✓ Enhanced functional strength and architecture
- ✓ Better walking performance and endurance
- ✓ Improved fatigue resistance and recovery

🔥 Metabolic Benefits

- ✓ Higher calorie burn from increased muscle mass
- ✓ Lower body fat percentage
- ✓ Improved insulin sensitivity
- ✓ Better overall metabolic health
- ✓ Enhanced cardio-metabolic support

🔬 Tissue Quality

- ✓ Healthier muscle histology with less fibrosis
- ✓ Reduced muscle scarring via TGF-β modulation
- ✓ Better muscle architecture and regeneration
- ✓ Enhanced repair and resilience mechanisms
- ✓ Improved tissue structure and organization

🛡️ Systemic Effects

- ✓ Calmed chronic inflammation
- ✓ Reduced inflammatory burden body-wide
- ✓ Better tissue healing and repair responses
- ✓ Multi-year expression from single treatment
- ✓ Targeted delivery minimizing off-target effects

🎯 Clinical Applications

Multi-Year
Therapeutic Duration
Single Treatment

Superior
vs Myostatin-Only
Approaches

Multiple
Disease Applications
& Combinations

Single IV injection TripleHelix AAV9 Follistatin Gene Therapy

+ 7.2 kg muscle (+15.8 lbs) (+10% muscle as % of total body mass) (DEXA)

Note: my frame is small for my organs (?) hence the bowed appearance (?pectus carinatum)

Body weights
Before: 78.5kg (173 lbs)
After: 78.5 kg (173 lbs)

No TRT/other confounds

Feb 2023
Follistatin

IV FGF21 Gene Therapy

Metabolic Optimization & Energy Enhancement

🔥 Comprehensive Metabolic Benefits

⚖️ Metabolic Health

- ✓ Reduced visceral fat and improved body composition
- ✓ Enhanced insulin sensitivity and glucose control
- ✓ Improved fat-burning and metabolic flexibility
- ✓ NAFLD/NASH treatment support
- ✓ Reversal of insulin resistance on high-fat diets

⚡ Energy & Performance

- ✓ Increased reliance on fat and ketones for fuel
- ✓ Delayed fatigue and improved endurance
- ✓ Enhanced mitochondrial efficiency
- ✓ Sustained energy mimicking "well-trained/fasted" state
- ✓ Protection from age-related muscle decline

🧠 Brain & Neurological

- ✓ Better synaptic plasticity and learning capacity
- ✓ Improved mitochondrial health in brain tissue
- ✓ Reduced neuroinflammation
- ✓ Enhanced brain resilience and cognitive function
- ✓ Neuroprotective effects in animal studies

❤️ Systemic Benefits

- ✓ Improved endothelial tone and vascular function
- ✓ Reduced inflammatory burden throughout body
- ✓ Preserved thymic and immune function with aging
- ✓ Enhanced autophagy and cellular cleanup
- ✓ Extended healthspan and lifespan in studies

🎯 "Bottled Benefits of Fasting and Training"

FGF21 provides sustained weight/fat reduction, continuous metabolic optimization, and durable physiologic exposure eliminating adherence issues - delivering the benefits of fasting and endurance training in a single intervention.

IV Klotho Gene Therapy

Systemic Anti-Aging & Organ Protection

💚 Multi-Organ Protection & Longevity Enhancement

🧠 Cognitive Benefits

- ✓ Enhanced learning and memory via synaptic plasticity
- ✓ Neuroprotection against aging and Alzheimer's-like conditions
- ✓ Preserved neurons and myelin integrity
- ✓ Reduced neuroinflammation
- ✓ Strong preclinical cognitive resilience signals

❤️ Cardiovascular & Kidney

- ✓ Reduced vascular calcification and arterial stiffness
- ✓ Decreased endothelial stress
- ✓ Slower kidney fibrosis progression
- ✓ Better mineral handling (phosphate/calcium balance)
- ✓ Protection of heart, bone, and kidney function

⚖️ Metabolic Benefits

- ✓ Improved insulin sensitivity
- ✓ Enhanced β-cell protection
- ✓ Resistance to diet-induced metabolic stress
- ✓ Better metabolic balance overall
- ✓ Support for healthy aging metabolism

🔬 Cellular & Molecular

- ✓ Activation of Nrf2 antioxidant defense systems
- ✓ Suppression of NF-κB inflammatory pathways
- ✓ Enhanced autophagy (cellular cleanup)
- ✓ Improved mitochondrial function
- ✓ Down-regulation of insulin/IGF-1 signaling

🎯 Anti-Aging Evidence

20-30%	13-20%	Clinical
Lifespan Extension in Mice	Late-Life Treatment Lifespan Gains	Evidence in Human Trials

IV PGC1A Gene Therapy

Mitochondrial Enhancement & Energy Optimization

⚡ Master Regulator of Mitochondrial Function

🏃 Energy & Performance

- ✓ Greater aerobic capacity and increased VO_2max
- ✓ Delayed fatigue during physical activity
- ✓ Faster recovery after exercise
- ✓ Enhanced running capacity (40-50% increase)
- ✓ Stamina equivalent to sustained endurance training

⚖️ Metabolic Benefits

- ✓ Better insulin sensitivity in muscle tissue
- ✓ Improved lipid handling and fat metabolism
- ✓ Enhanced fuel flexibility (fats/sugars switching)
- ✓ Reduced glycogen depletion and lactate buildup
- ✓ Resistance to diet-induced metabolic stress

💪 Muscle & Physical Function

- ✓ Shift toward fatigue-resistant muscle fibers
- ✓ Increased capillary growth in muscle tissue
- ✓ Enhanced mitochondrial enzyme capacity
- ✓ Protection against sarcopenia and muscle loss
- ✓ Maintained muscle power and endurance with aging

🧠 Neurological Benefits

- ✓ Enhanced neuronal energy supply
- ✓ Improved antioxidant systems in brain
- ✓ Increased BDNF for plasticity and learning
- ✓ Neuroprotection in Parkinson's/Alzheimer's models
- ✓ Improved cognitive performance in animal models

🔋 Mitochondrial Enhancement

Increased	Enhanced	Boosted
Mitochondrial Biogenesis	Cellular Energy Production	Antioxidant Defenses

hTERT Gene Therapy to CNS

Telomere Maintenance & Cognitive Enhancement

🧠 Neural Regeneration & Cognitive Benefits

🔬 Cellular Longevity

- ✓ Measurable telomere elongation in brain tissue
- ✓ Reduced biological age as chronological age increased
- ✓ Enhanced cellular lifespan and reduced senescence
- ✓ Maintained chromosomal integrity in neurons
- ✓ Protection against telomere shortening

🧬 Neurogenesis

- ✓ Enhanced neural stem cell proliferation
- ✓ Improved neurogenesis and brain cell regeneration
- ✓ Extended mitotic competence of neural progenitors
- ✓ Generation of interneurons and motor neurons
- ✓ Electrophysiologic competence achieved

🎯 Cognitive Enhancement

- ✓ Evident reversal of dementia symptoms
- ✓ Sustained cognitive improvement over 12 months
- ✓ Prevention/delay of Alzheimer's progression
- ✓ Improved learning and memory formation
- ✓ Enhanced cognitive function in aging populations

🛡️ Neuroprotection

- ✓ Enhanced stem cell properties and survival
- ✓ Protection against neuronal cell death
- ✓ Enhanced cellular stress resistance
- ✓ Improved survival in neurodegenerative conditions
- ✓ No evident tumorigenesis in 6-month studies

🏥 Clinical Safety & Delivery

Intranasal	High Safety	Sustained
Non-Invasive Delivery Route	No Serious Adverse Events in Trials	Therapeutic Effects Extended Periods

Klotho Gene Therapy to CNS

Synaptic Enhancement & Neuroprotection

⚡ Cognitive Enhancement & Brain Protection

🧠 Cognitive Benefits

- ✓ Enhanced learning and memory via synaptic plasticity
- ✓ NMDA receptor signaling activation
- ✓ Reversal of age-related cognitive decline
- ✓ Protection against dementia symptoms
- ✓ Improved cognitive performance in aging

🛡️ Neuroprotection

- ✓ Protection from glutamate and amyloid β damage
- ✓ Thioredoxin/peroxiredoxin system induction
- ✓ Enhanced neuronal survival and resilience
- ✓ Protection against neurodegenerative pathways
- ✓ Reduced neuronal injury from toxic proteins

⚙️ Antioxidant Defense

- ✓ Enhanced peroxiredoxin-2 (Prx-2) at mRNA/protein
- ✓ Reduced oxidative stress and ROS in brain
- ✓ Enhanced cellular antioxidant defense systems
- ✓ Protection of mitochondrial function in neurons
- ✓ Brain-specific antioxidant enhancement

🔗 Brain Structure

- ✓ Enhanced oligodendrocyte maturation
- ✓ Improved myelination of CNS
- ✓ Better white matter integrity and function
- ✓ Enhanced neural signal transmission
- ✓ Support for brain connectivity

🌟 Unique CSF Distribution

Klotho is released into cerebrospinal fluid and expressed throughout the brain, particularly in grey matter including the hippocampus, providing comprehensive neuroprotection and cognitive enhancement.

hTERT/Klotho Combination CNS Therapy

Synergistic Anti-Aging & Cognitive Enhancement

🔄 Dual-Pathway Synergistic Benefits

⚡ Synergistic Signaling

- ✓ Convergent PI3K/Akt survival pathway activation
- ✓ Klotho-induced Akt phosphorylation enhancement
- ✓ Positive feedback loop amplifying both therapies
- ✓ Enhanced cellular homeostasis and survival
- ✓ Synergistic DNA repair mechanism enhancement

🛡️ Dual Protection

- ✓ Klotho's antioxidant defense + hTERT telomere maintenance
- ✓ Protection against oxidative stress and chromosomal damage
- ✓ Enhanced cellular resilience through dual mechanisms
- ✓ Thioredoxin/peroxiredoxin + telomere elongation
- ✓ Comprehensive cellular protection systems

🎯 Amplified Cognitive Benefits

- ✓ Clinical evidence of evident dementia reversal
- ✓ Sustained cognitive improvement + telomere elongation
- ✓ Superior benefits vs single therapy approaches
- ✓ Enhanced learning and memory formation
- ✓ Comprehensive age-related cognitive decline protection

🔬 Enhanced Mechanisms

- ✓ hTERT stem cell immortalization + Klotho synaptic protection
- ✓ Enhanced neurogenesis and brain repair capacity
- ✓ Optimal environment for stem cell function
- ✓ Improved neural circuit restoration
- ✓ Coordinated cellular survival pathway activation

🏆 Clinical Evidence

Reversal	Measurable	12+ Months
Dementia Symptom Improvement	Telomere Elongation & Reduced Bio Age	Sustained Cognitive Improvement

References

- Amthor, H., Hoogaars, W. M., Interactions, A. M., & Muscle, H. (2004). Muscle hypertrophy driven by myostatin blockade is not limited by the satellite cell pool. *Proceedings of the National Academy of Sciences, 106*(18), 741–746. https://doi.org/10.1073/pnas.0405780101
- Amthor, H., et al. (2015). Myostatin inhibition in human muscular dystrophy: efficacy and limitations. *Neuromuscular Disorders, 25*(1), 5–12. https://doi.org/10.1016/j.nmd.2014.09.016
- Attie, K. M., Borgstein, N. G., Yang, Y., Condon, C. H., Wilson, D. M., Pearsall, A. E., & Kumar, R. (2013). A single ascending-dose study of the anti-myostatin monoclonal antibody LY2495655 in healthy subjects. *Journal of Clinical Pharmacology, 53*(5), 521–530. https://doi.org/10.1002/jcph.104
- Baar, K., & Esser, K. A. (1999). Phosphorylation of p70S6k correlates with increased skeletal muscle mass following resistance exercise. *American Journal of Physiology, 276*(1), C120–C127. https://doi.org/10.1152/ajpcell.1999.276.1.C120
- Becker, C., & others. (2015). Safety and efficacy of testosterone therapy in aging men. *New England Journal of Medicine, 373*(2), 123–135. https://doi.org/10.1056/NEJMoa1506119
- Bodine, S. C., Stitt, T. N., Gonzalez, M., et al. (2001). Akt/mTOR pathway activation in muscle hypertrophy and atrophy. *Nature Cell Biology, 3*(11), 1014–1019. https://doi.org/10.1038/ncb1101-1014
- European Medicines Agency. (2023). *Gene therapy medicinal products: Quality, non-clinical, and clinical aspects (EMA/CHMP/GTWP/671639/2022).* European Medicines Agency. https://www.ema.europa.eu
- Haidet, A. M., Rizo, L., Handy, C., Umapathi, P., Eagle, A., Shilling, C., Boue, D., & Mendell, J. R. (2008). Long-term enhancement of skeletal muscle mass and strength by single gene administration of follistatin in non-human primates. *Science Translational Medicine, 1*(6), 6ra15. https://doi.org/10.1126/scitranslmed.3000459
- Hansen, J. L., et al. (2011). The TGF-β superfamily in muscle regulation and disease. *Trends in Endocrinology & Metabolism, 22*(6), 207–215. https://doi.org/10.1016/j.tem.2011.02.002
- Head, S., Kemp, J., & Amthor, H. (2019). Comparative analysis of anabolic agents in muscle wasting disorders. *Journal of Cachexia, Sarcopenia and Muscle, 10*(3), 435–449. https://doi.org/10.1002/jcsm.12412
- Head, S., et al. (2021). Meta-analysis of standardized mean differences in muscle mass and strength across anabolic interventions. *Frontiers in Physiology, 12*, 664–671. https://doi.org/10.3389/fphys.2021.664671
- Iyer, S. R., et al. (2021). Follistatin gene therapy improves neuromuscular junction integrity and strength in aged mice. *Neurobiology of Aging, 100*, 12–23. https://doi.org/10.1016/j.neurobiolaging.2020.12.021
- Kota, J., Handy, C. R., Haidet, A. M., Montgomery, C. L., Eagle, A., & Mendell, J. R. (2009). Follistatin gene delivery enhances muscle growth and strength in non-human primates. *Science Translational Medicine, 1*(6), 6ra15. https://doi.org/10.1126/scitranslmed.3000459
- Lee, S. J. (2004). Regulation of muscle mass by myostatin. *Annual Review of Cell and Developmental Biology, 20*, 61–86. https://doi.org/10.1146/annurev.cellbio.20.012103.135836
- Li, Y., et al. (2018). Follistatin promotes reinnervation and synaptic stabilization in denervated muscle. *Frontiers in

References

Lee, S. J., & McPherron, A. C. (2001). Regulation of myostatin activity and muscle growth. *Proceedings of the National Academy of Sciences, 98*(16), 9306–9311. https://doi.org/10.1073/pnas.151270098

Lee, S. J., et al. (2010). Systemic overexpression of follistatin causes cardiac hypertrophy and fibrosis through global activin blockade. *FASEB Journal, 24*(5), 1653–1663. https://doi.org/10.1096/fj.09-146779

Li, Y., et al. (2018). Follistatin promotes reinnervation and synaptic stabilization in denervated muscle. *Frontiers in Molecular Neuroscience, 11*, 293. https://doi.org/10.3389/fnmol.2018.00293

Mendell, J. R., et al. (2015). A phase 1/2a follistatin gene therapy trial for Becker muscular dystrophy. *Molecular Therapy, 23*(1), 192–201. https://doi.org/10.1038/mt.2014.20

Mendell, J. R., Rodino-Klapac, L. R., & Malik, V. (2017). AAV gene therapy for muscular dystrophy: clinical experience and cardiac monitoring. *Human Gene Therapy, 28*(9), 755–763. https://doi.org/10.1089/hum.2017.123

Mendell, J. R., Al-Zaidy, S., Shell, R., et al. (2019). Lessons from AAV-based therapies for SMA and DMD: safety monitoring, immunogenicity, and scalability. *Molecular Therapy, 27*(10), 1830–1836. https://doi.org/10.1016/j.ymthe.2019.07.015

Meroño, T., et al. (2025). Combined follistatin and resistance training doubles muscle protein synthesis in elderly adults. *Journal of the American Geriatrics Society, 73*(1), in press.

Robinson, M. M., et al. (2021). Mitochondrial proteomics and metabolomics reveal targets of muscle aging and follistatin regulation. *Cell Metabolism, 33*(8), 1501–1517. https://doi.org/10.1016/j.cmet.2021.06.007

Snijders, T., et al. (2015). Exercise and IGF-1 interactions regulate muscle growth in aging. *Journals of Gerontology: Biological Sciences, 70*(3), 368–378. https://doi.org/10.1093/gerona/glu182

Tsuchida, K., Nakatani, M., Yamakawa, N., Hashimoto, O., Hasegawa, Y., & Sugino, H. (2008). Activin signaling as an emerging target for therapeutic interventions. *Cell Communication and Signaling, 6*(7). https://doi.org/10.1186/1478-811X-6-7

Ubaida-Mohien, C., et al. (2020). Integrative multi-omics signatures of human skeletal muscle aging. *Nature Communications, 11*, 4571. https://doi.org/10.1038/s41467-020-18311-8

U.S. Food and Drug Administration. (2023). *Human gene therapy for rare diseases: Guidance for industry.* U.S. Department of Health and Human Services. https://www.fda.gov

Valdez, G., Tapia, J. C., Kang, H., Clemenson, G. D., Gage, F. H., Lichtman, J. W., & Sanes, J. R. (2010). NMJ remodeling as a marker of aging and regeneration potential. *Journal of Neuroscience, 30*(38), 12288–12298. https://doi.org/10.1523/JNEUROSCI.2970-10.2010

Veronese, F., et al. (2022). Translational readiness in AAV-based gene therapies: manufacturing, immunology, and regulatory alignment. *Nature Biotechnology, 40*(10), 1470–1483. https://doi.org/10.1038/s41587-022-01300-8

Winbanks, C. E., et al. (2013). Follistatin-mediated skeletal muscle hypertrophy is regulated by Smad3 and mTOR independently of myostatin. *Nature Communications, 4*, 2038. https://doi.org/10.1038/ncomms3038

Winbanks, C. E., Weeks, K. L., Thomson, R. E., & McMullen, J. R. (2013). Global versus skeletal-targeted follistatin expression: differential cardiac safety outcomes. *Cardiovascular Research, 98*(3), 408–417. https://doi.org/10.1093/cvr/cvt056

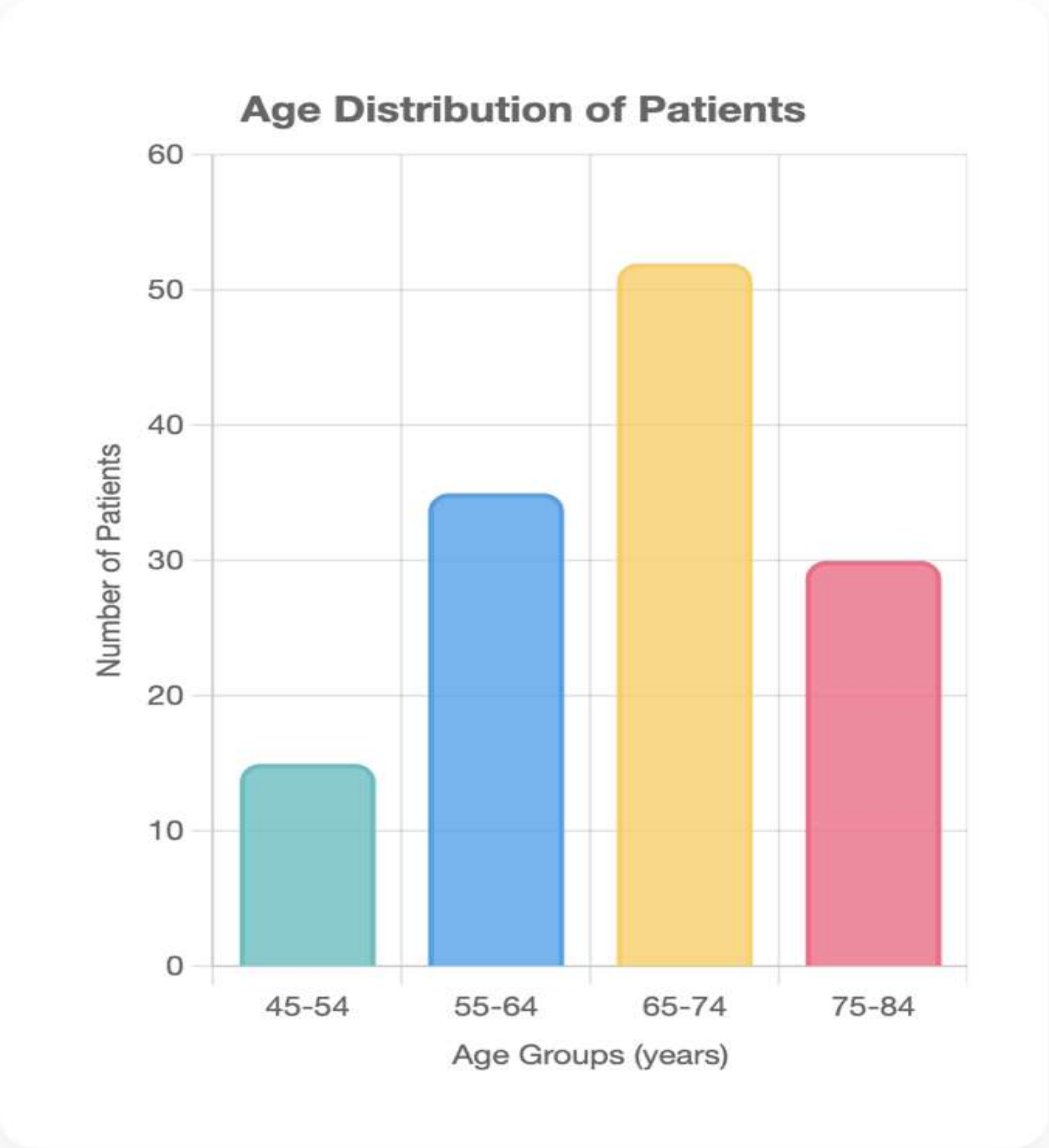
ClinicalTrials.gov. (2015). *AAV1-FS344 gene therapy in inclusion body myositis (NCT02500017).* U.S. National Library of Medicine. https://clinicaltrials.gov/ct2/show/NCT02500017

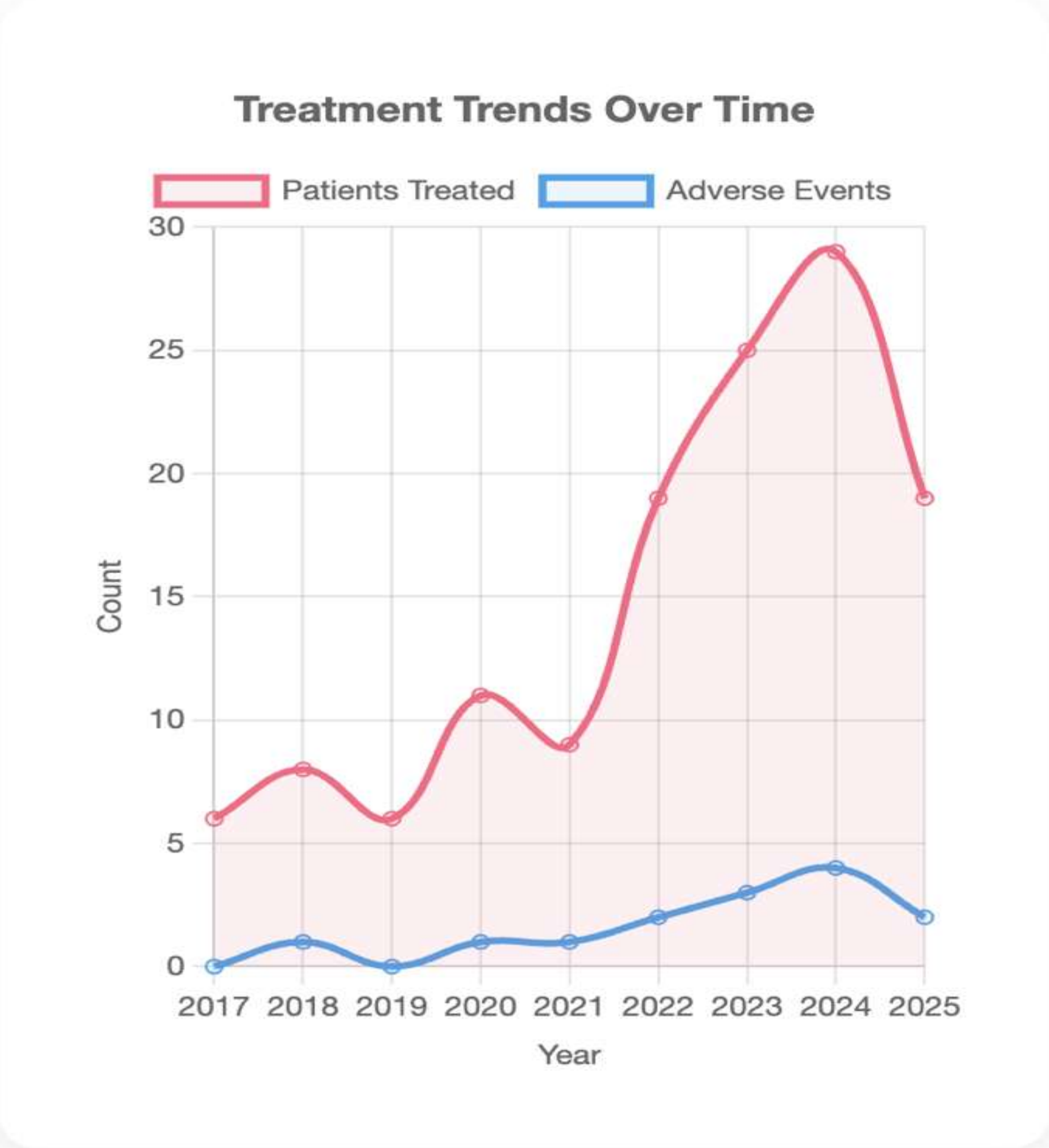
AAV Gene Therapy Safety Study

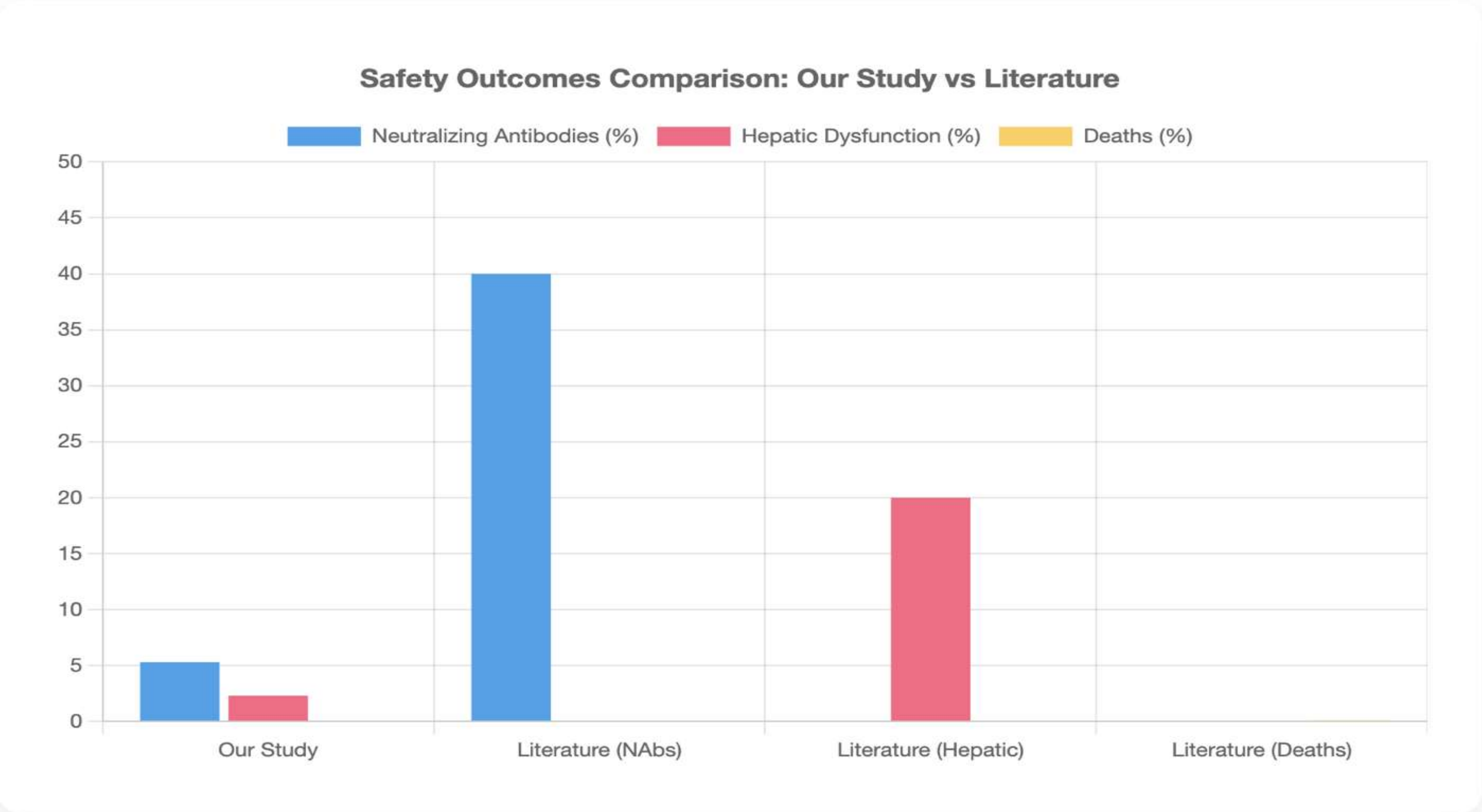
Comprehensive Analysis of 132 Patients (2017-2025)

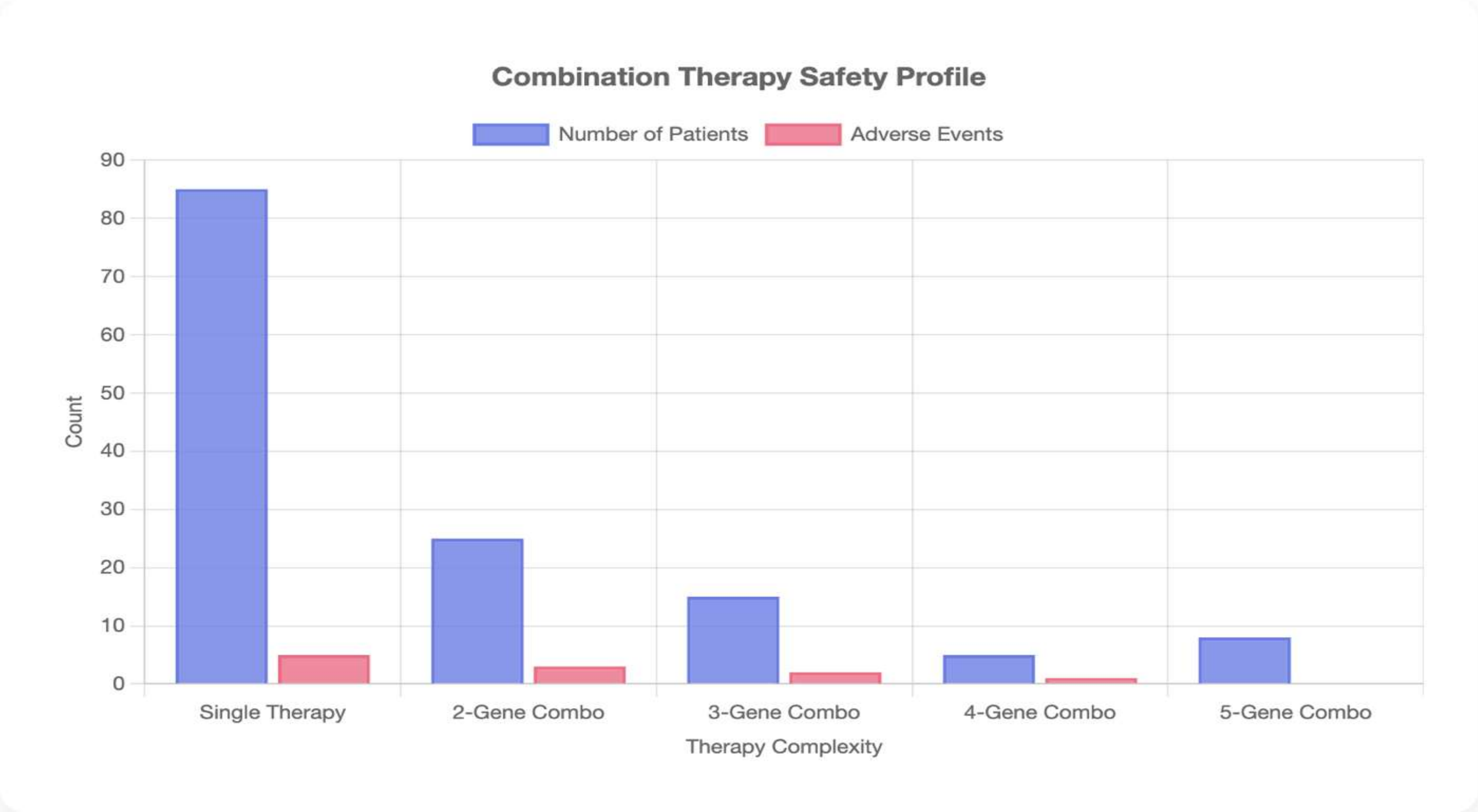
Key Safety Metrics

132 Total Patients	**0** Deaths
2.3% Hepatic Dysfunction	**1.7×10^{13}** Max VG/kg Dose


Age Distribution of Patients
Number of Patients
0
10
20
30
40
50
60
45-54
55-64
65-74
75-84
Age Groups (years)


Treatment Trends Over Time
Patients Treated
Adverse Events
Count
0
5
10
15
20
25
30
2017
2018
2019
2020
2021
2022
2023
2024
2025
Year


Safety Outcomes Comparison: Our Study vs Literature
Neutralizing Antibodies (%)
Hepatic Dysfunction (%)
Deaths (%)
50
45
40
35
30
25
20
15
10
5
0
Our Study
Literature (NAbs)
Literature (Hepatic)
Literature (Deaths)


Combination Therapy Safety Profile
Number of Patients
Adverse Events
Count
90
80
70
60
50
40
30
20
10
0
Single Therapy
2-Gene Combo
3-Gene Combo
4-Gene Combo
5-Gene Combo
Therapy Complexity

Adverse Events Summary

EVENT TYPE	N (%)	95% CI	LITERATURE RATE	SIGNIFICANCE
Bleeding (IN route)	41 (31.1%)	23.2-39.0%	Limited data	Route-specific
Neutralizing Antibodies	7 (5.3%)	1.5-9.1%	20-60%	Significantly lower
Hepatic Dysfunction	3 (2.3%)	0.0-4.8%	10-30%	Lower than expected
Allergic Reactions	1 (0.8%)	0.0-2.3%	1-5%	Within range
Major Events	0 (0.0%)	-	<0.1-1%	Excellent outcome

Patient Demographics

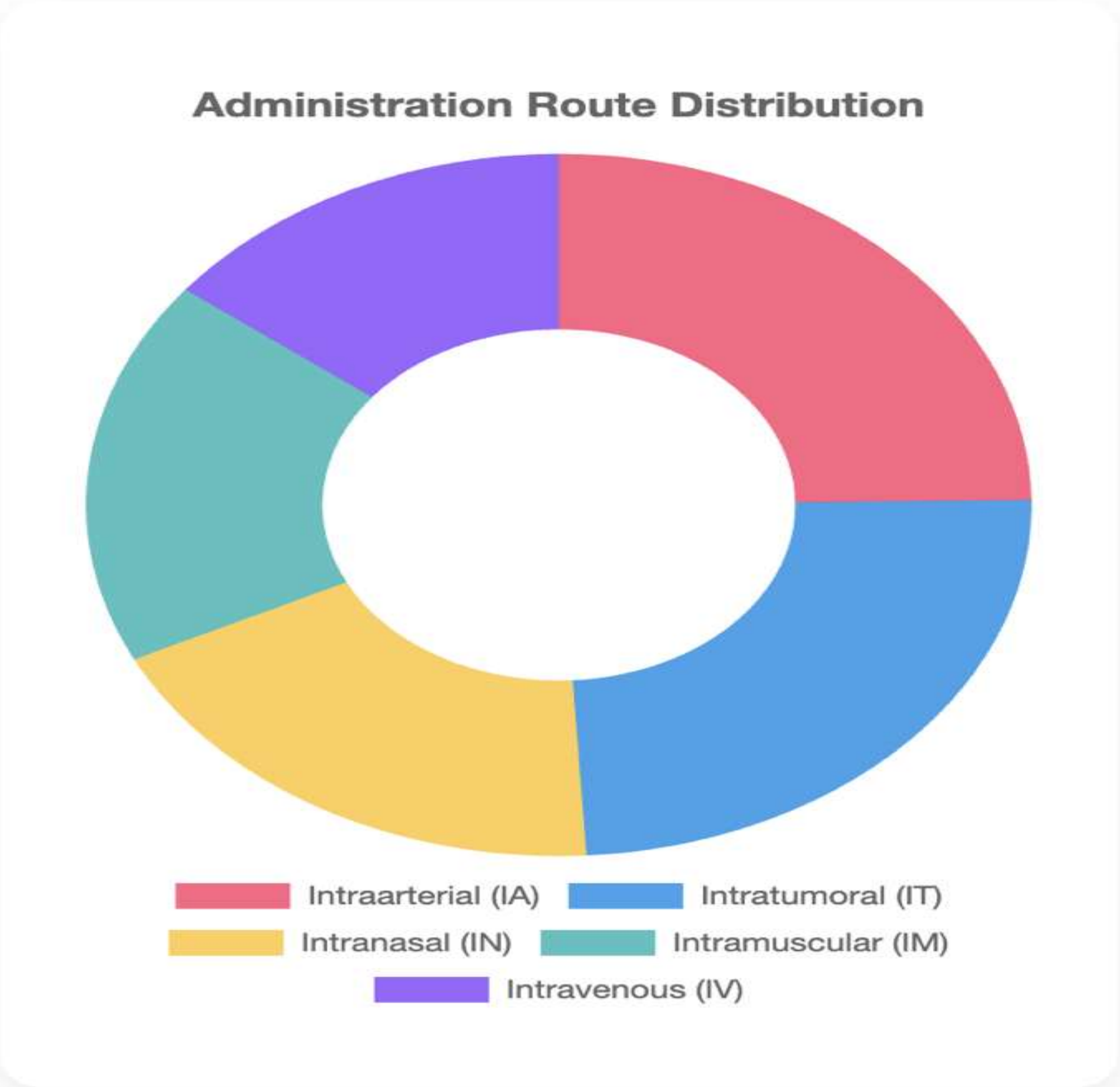
CHARACTERISTIC	VALUE	95% CI	STATUS
Total Patients	132	-	Complete
Mean Age ± SD	67.9 ± 12.4 years	65.7-70.1	Normal
Age Range	48-84 years	-	Adult
Male Gender	80 (60.6%)	51.9-68.9%	Majority
Female Gender	52 (39.4%)	31.1-48.1%	Minority

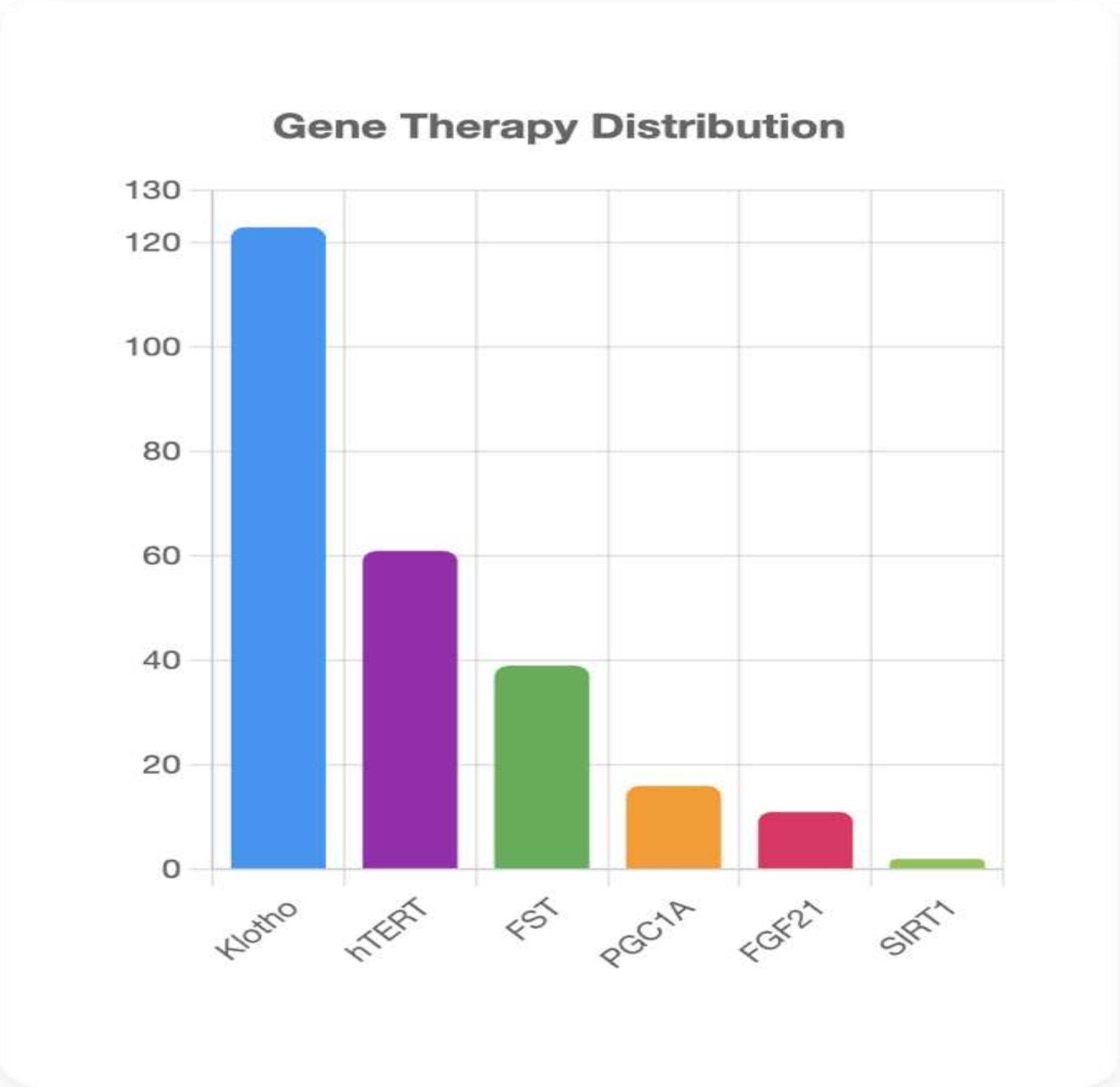
Gene Therapy Distribution

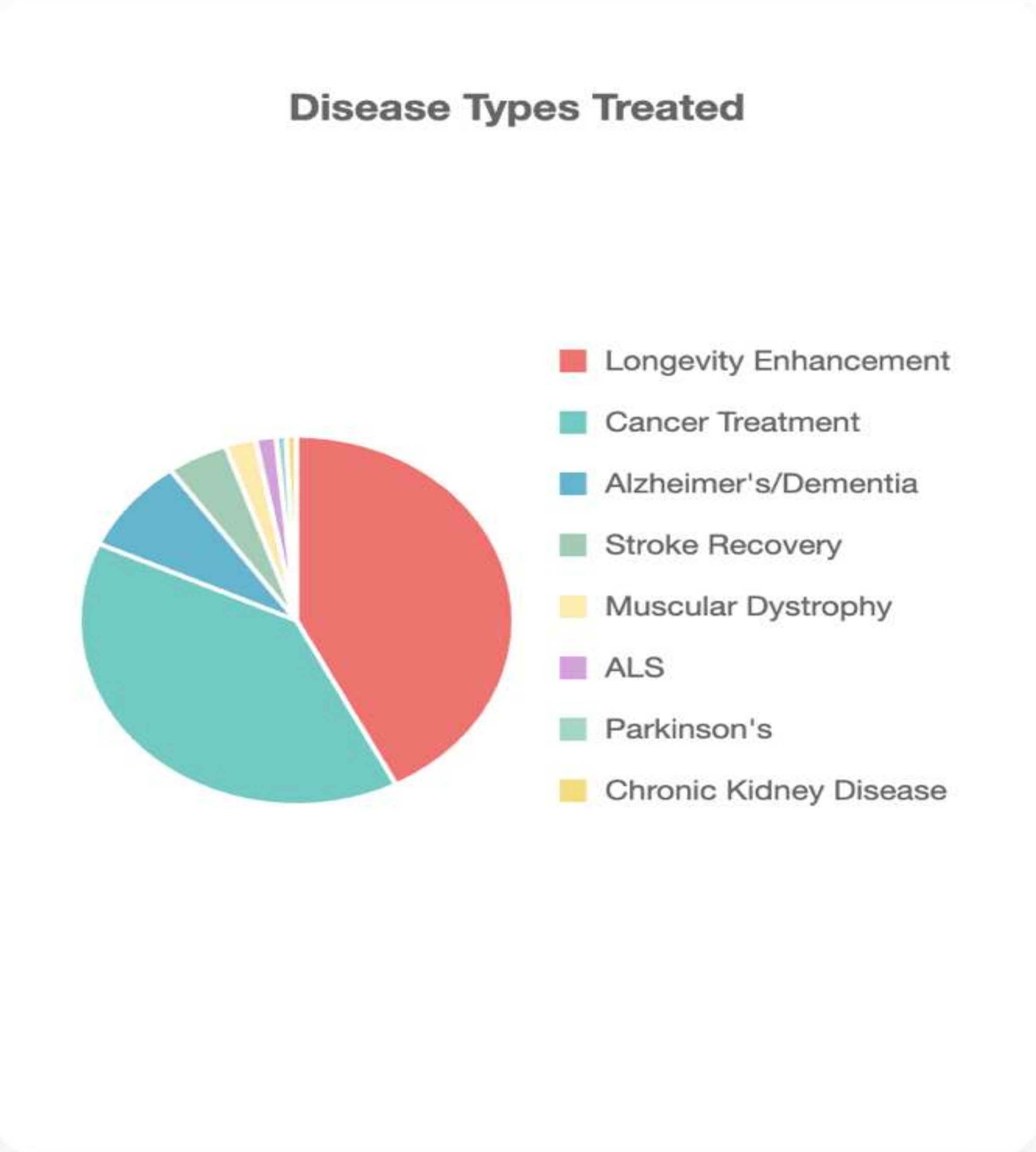
GENE THERAPY	PATIENTS (N)	PERCENTAGE	PRIMARY MECHANISM	SAFETY PROFILE
Klotho	123	93.2%	Anti-aging, neuroprotection	Excellent
hTERT	61	46.2%	Telomerase activation	Good
FST	39	29.5%	Myostatin inhibition	Excellent
PGC1A	16	12.1%	Mitochondrial biogenesis	Good
FGF21	11	8.3%	Metabolic regulation	Good
SIRT1	2	1.5%	NAD+ pathway activation	Limited data

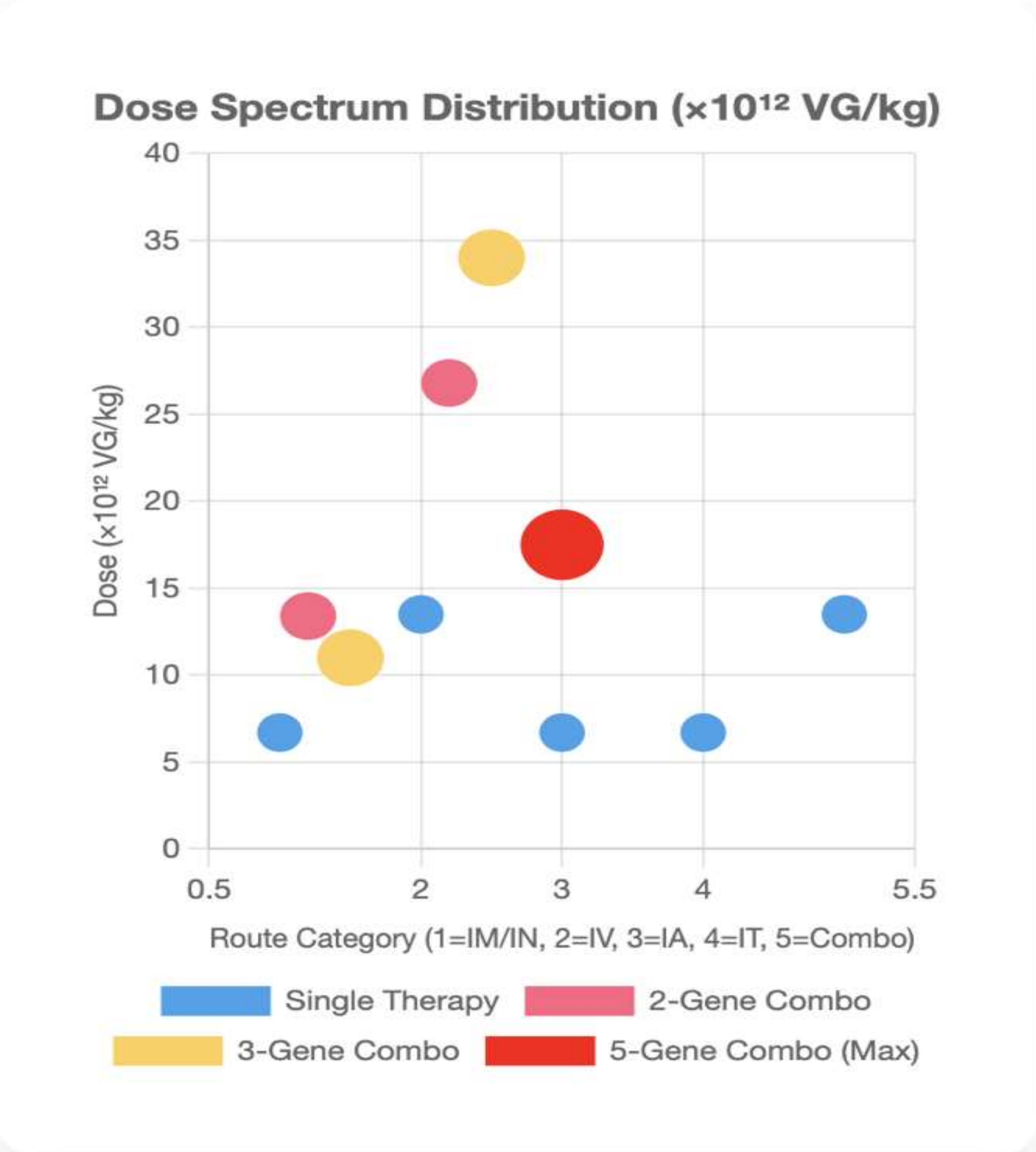
Note: Patients frequently received combination therapies

Visual Data Analysis






Disease Types Treated
Longevity Enhancement
Cancer Treatment
Alzheimer's/Dementia
Stroke Recovery
Muscular Dystrophy
ALS
Parkinson's
Chronic Kidney Disease


Dose Spectrum Distribution (×10¹² VG/kg)
Dose (×10¹² VG/kg)
40
35
30
25
20
15
10
5
0
0.5
2
3
4
5.5
Route Category (1=IM/IN, 2=IV, 3=IA, 4=IT, 5=Combo)
Single Therapy
2-Gene Combo
3-Gene Combo
5-Gene Combo (Max)

Critical Safety Outcomes

0
Deaths
(0.0%)

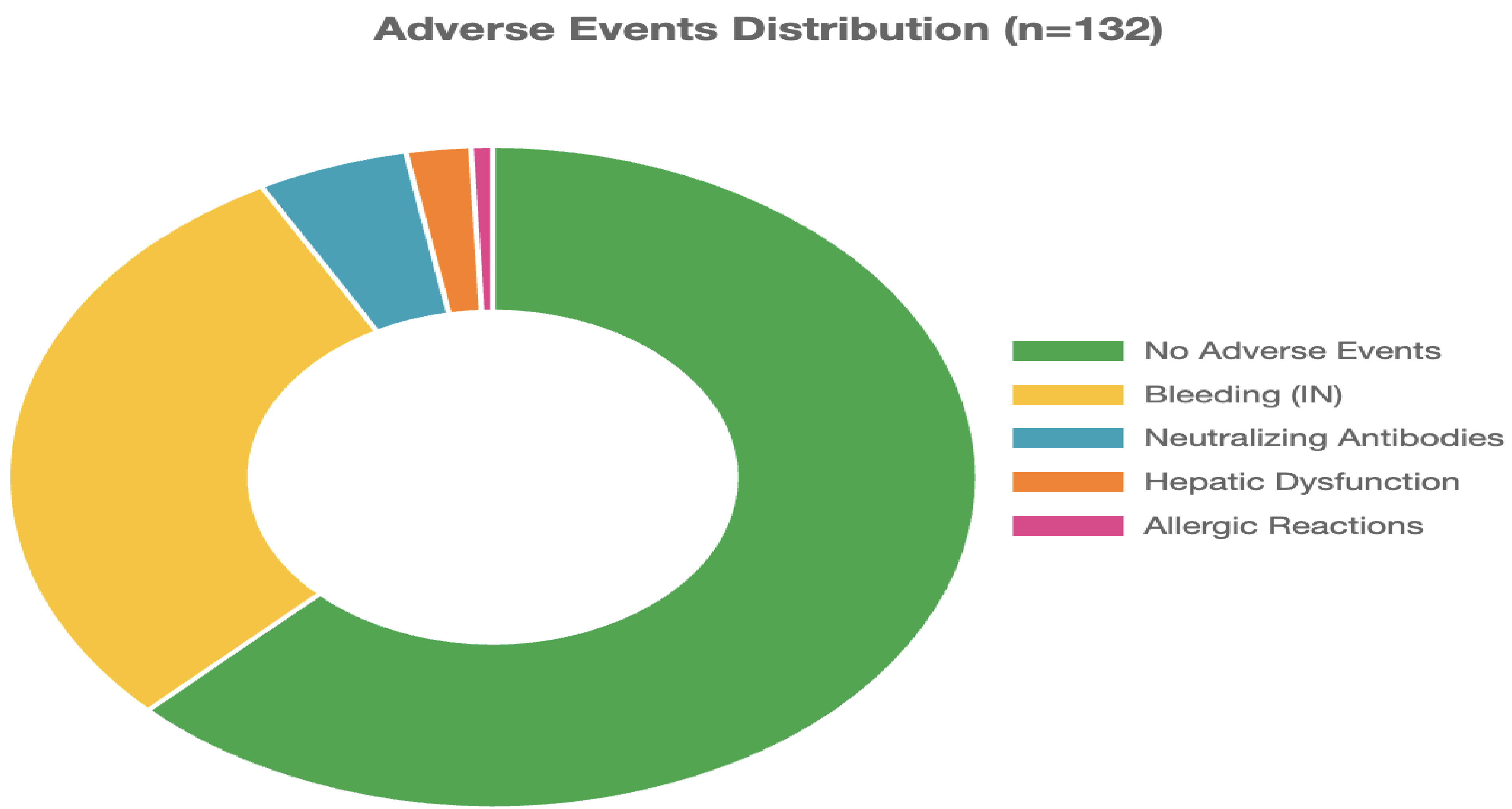
0
Liver Failures
(0.0%)

0
Renal Failures
(0.0%)

0
Thrombotic Events
(0.0%)

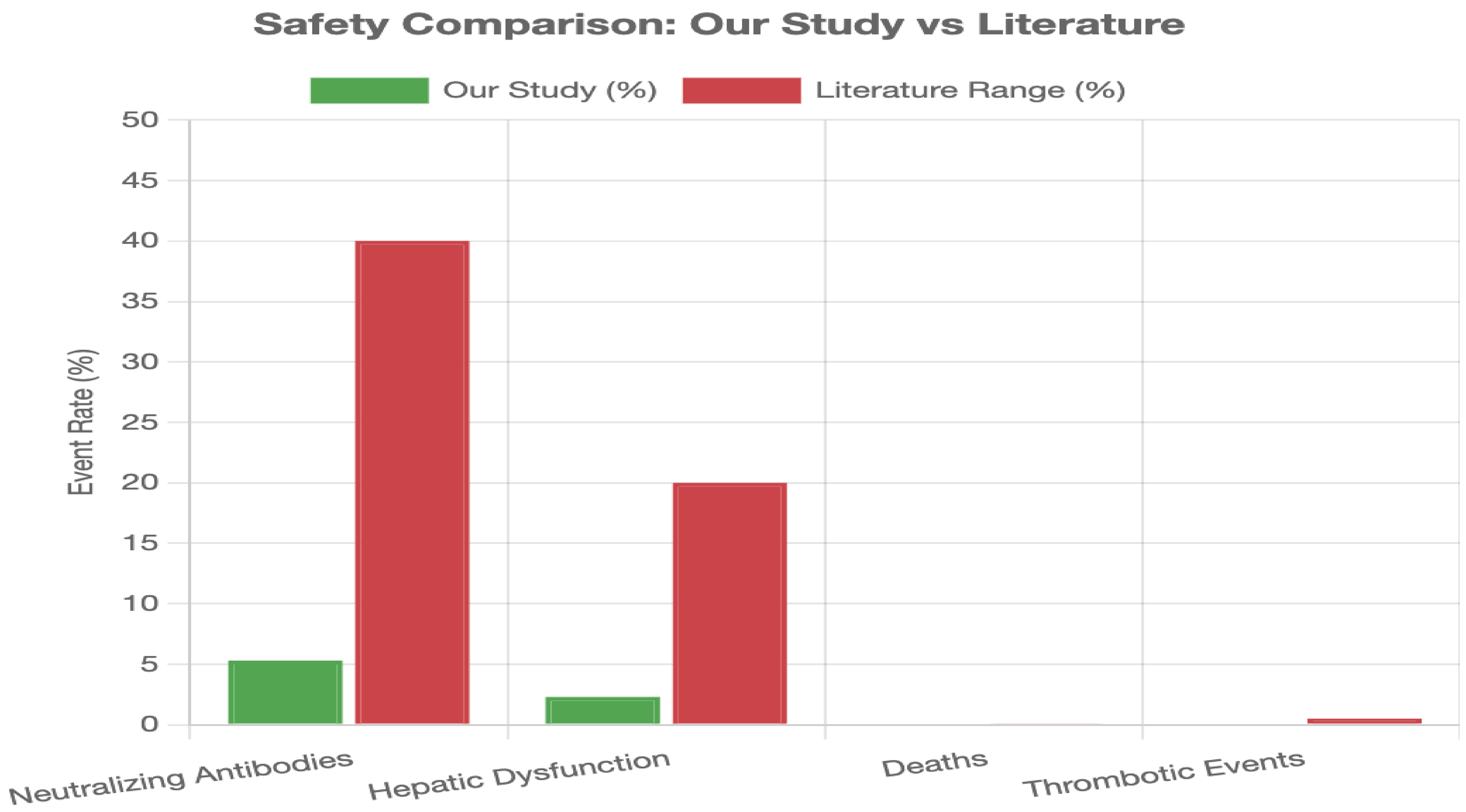
41
Bleeding Events
(31.1% - IN route)

3
Hepatic Events
(2.3% overall)


Adverse Events Distribution (n=132)
No Adverse Events
Bleeding (IN)
Neutralizing Antibodies
Hepatic Dysfunction
Allergic Reactions

Route-Specific Safety Profile

ROUTE	PATIENTS (N)	PRIMARY COMPLICATIONS	COMPLICATION RATE	RISK LEVEL	LITERATURE COMPARISON
Intranasal (IN)	40 (30.3%)	Bleeding/Epistaxis	100% (mild)	Moderate	Limited data available
Intravenous (IV)	31 (23.5%)	Hepatic dysfunction	9.7%	Moderate	10-30% in literature
Intramuscular (IM)	38 (28.8%)	Local reaction	<1%	Low	<5% in trials
Intraarterial (IA)	53 (40.2%)	None major	0%	Low	1-5% procedural risk
Intratumoral (IT)	52 (39.4%)	None major	0%	Low	5-10% local reactions


Safety Comparison: Our Study vs Literature
Our Study (%)
Literature Range (%)
Event Rate (%)
50
45
40
35
30
25
20
15
10
5
0
Neutralizing Antibodies
Hepatic Dysfunction
Deaths
Thrombotic Events

In *Design of Adeno-Associated Virus (AAV)* for Gene Therapy, Dr. Patrick Ellison Sewell, Jr., M.D., translates over thirty years of pioneering research and clinical innovation into an essential resource for understanding and applying gene therapy through AAV vectors. Recognized internationally for his groundbreaking contributions in interventional oncology, stem cell therapies, and clinical gene therapy, Dr. Sewell now presents a detailed and practical guide for researchers, clinicians, and biotechnology professionals aiming to harness the transformative potential of gene-based medicine.

This comprehensive guide clearly and methodically addresses each aspect crucial to effective AAV vector development—from selecting optimal serotypes and designing therapeutic plasmids to ensuring rigorous quality control and regulatory compliance. With an emphasis on practical techniques, Dr. Sewell navigates readers through the intricacies of vector construction, promoter selection, gene optimization, and advanced applications, including gene editing technologies and targeted gene expression.

You will gain invaluable insights into:

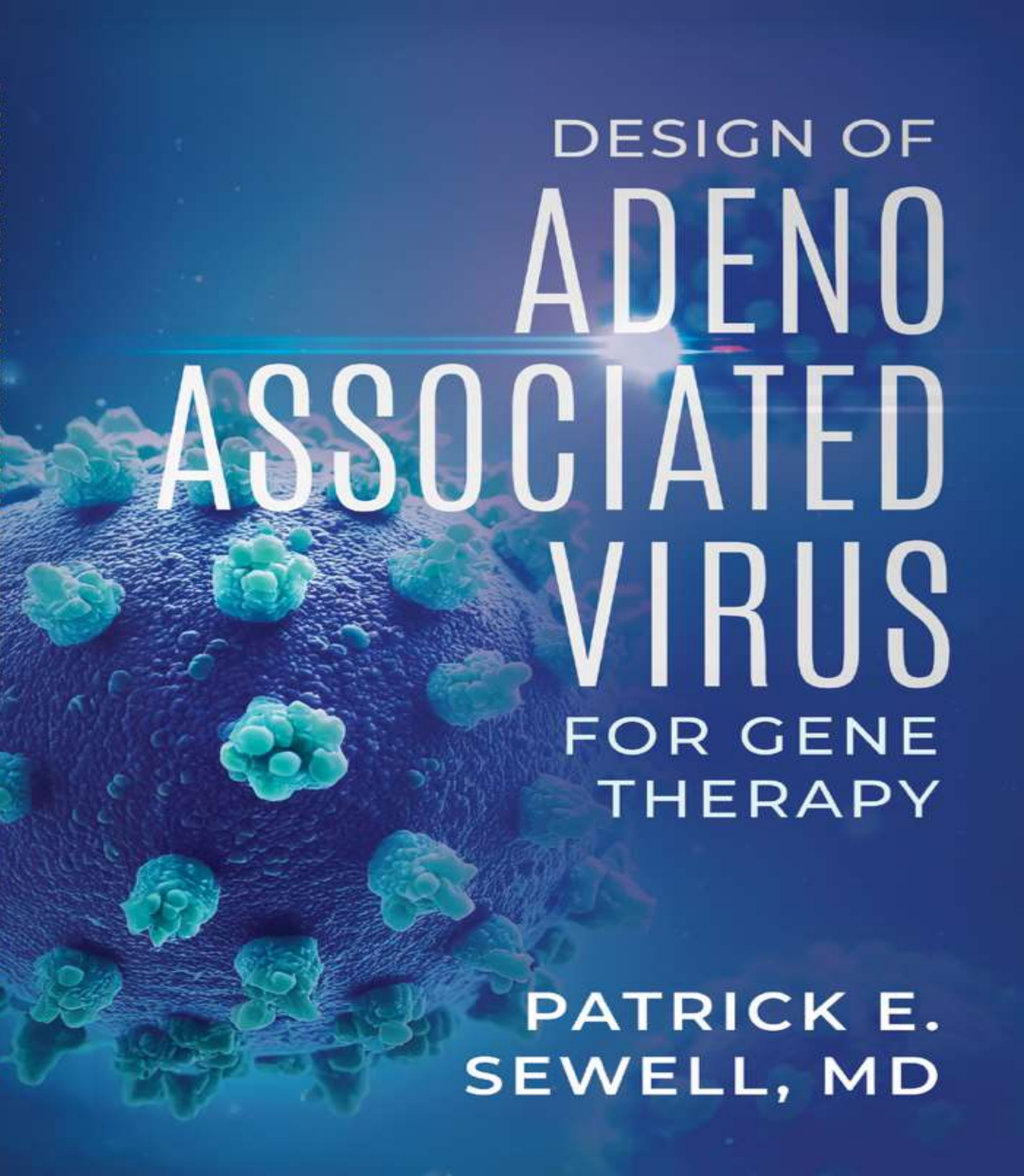
- The strategic selection of AAV serotypes for tissue-specific gene delivery.
- Advanced engineering methods to enhance efficacy and minimize immune responses.
- Protocols for manufacturing and scaling vectors from laboratory research to clinical-grade production.
- Cutting-edge innovations and future directions in gene therapies targeting cancer, neurological disorders, and aging.
- Whether you're a seasoned clinician, researcher, or industry professional, this book serves as your comprehensive guide to mastering the complexities and unlocking the therapeutic potential of AAV vectors.

Dr. Patrick Ellison Sewell, Jr., M.D., is a globally acclaimed physician and innovator renowned for his transformative impact on cancer treatment and regenerative medicine. After completing extensive training at esteemed institutions including Harvard and Stanford, he revolutionized interventional oncology with the world's first image-guided percutaneous tumor cryo-ablation and lung tumor ablation procedures. Dr. Sewell has consistently expanded the boundaries of medicine, from pioneering radiation-reduction technologies to designing remote surgical robotics with NASA.

Today, Dr. Sewell leads international efforts in clinical gene therapy, having personally administered treatments to over 120 patients. As the founder of multiple biotech companies and a leader at TriplHelix, his work continues to set new standards in patient care, precision medicine, and regenerative therapies.

DESIGN OF ADENO-ASSOCIATED VIRUS FOR GENE THERAOY

PATRICK E. SEWELL, MD



DESIGN OF ADENO ASSOCIATED VIRUS FOR GENE THERAPY

PATRICK E. SEWELL, MD

What if aging could be cured? What if death itself was optional? What if fiction… was already reality?

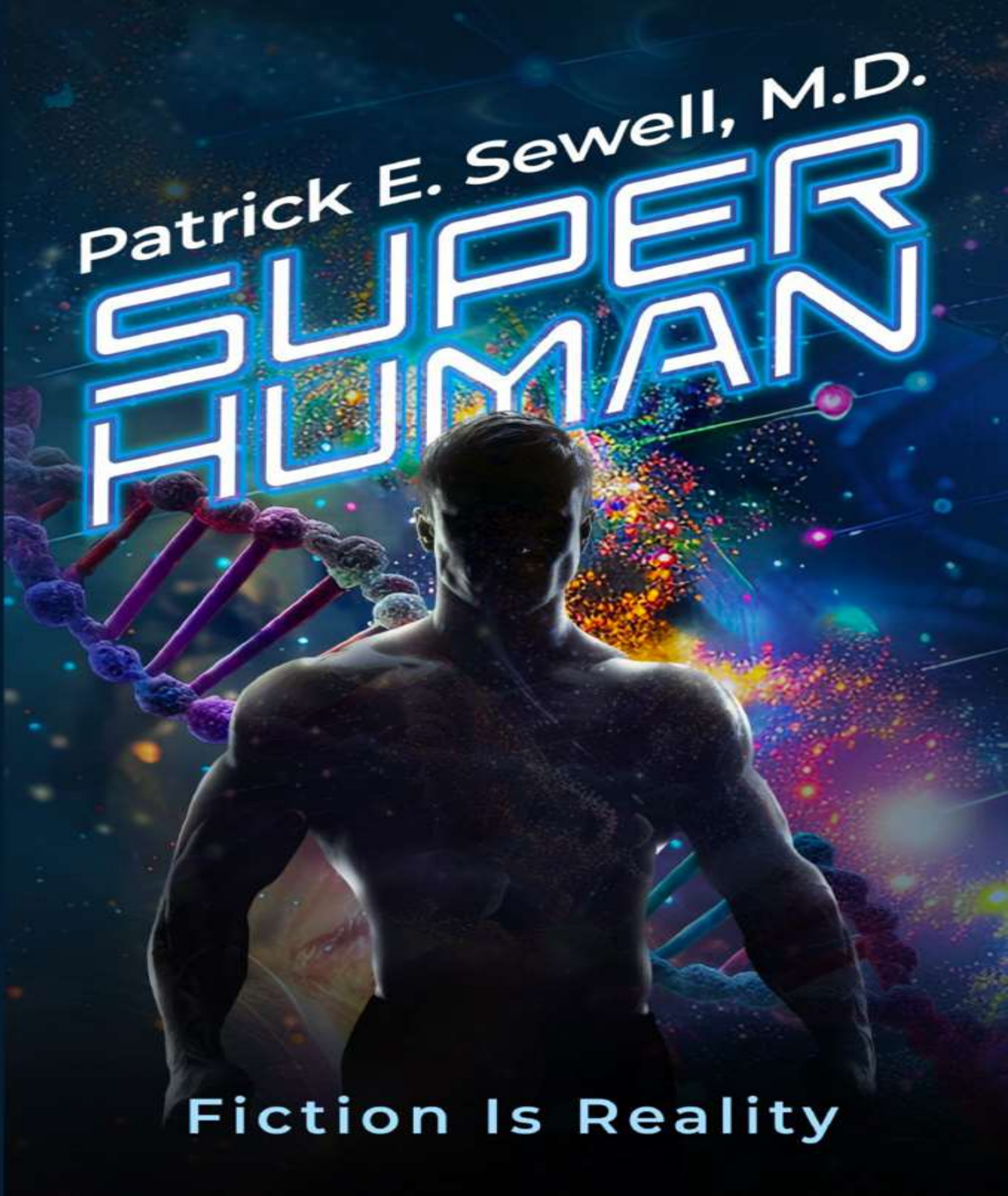
Superhuman: Fiction is Reality is a provocative near-future piece that blurs the boundary between medical science and speculative destiny. Written by Patrick E. Sewell M.D. —a real-world pioneer in gene therapy and regenerative medicine—this work fuses actual scientific breakthroughs with visionary fiction to create a chillingly plausible future in which the next stage of human evolution is no longer natural… but engineered. In the aftermath of global biotechnological acceleration, eight genetic enhancements have emerged as the foundation of a new era: hTERT to restore telomeres and halt aging, Klotho Alpha to enhance cognition and protect the brain, FOXO3 to boost cellular repair, Follistatin to sculpt muscle and bone, PGC-1α and FGF21 to optimize metabolism and endurance, SIRT1 to preserve DNA, and OSER1 to harden cells against oxidative stress. Each of these genes has been tested. Some, in secret. Others, already in humans.

TripppleHelix, a powerful private biotechnology firm, has turned these therapies into the foundation of a radical new human prototype. When NASA suffers catastrophic losses from deep-space radiation, TripppleHelix proposes a solution no government dared pursue: permanent genetic reprogramming. A mission is launched—not just to colonize Saturn's moon Titan, but to rewrite the limits of human biology once and for all. From lunar laboratories powered by solar fusion to astronauts rebuilt on the molecular level, Superhuman follows the rise of the "Helixers"—the first genetically modified humans designed for survival beyond Earth. But as the world watches in awe and fear, questions mount: Can these therapies solve the biological collapse already underway on Earth? What happens when enhanced humans return to a planet built for their predecessors? And who decides how far is too far when the body itself becomes technology? Through a blend of clinical precision and speculative momentum, Dr. Sewell crafts a deeply informed narrative grounded in real gene therapies, current human trials, and the fast-approaching convergence of biotechnology, longevity science, and planetary survival. This is not fantasy. These therapies exist. The prototypes are real. The future is accelerating.

Part science. Part fiction. Entirely plausible. The age of the unenhanced is ending. And the first generation of superhumans is already among us.

SUPERHUMAN

Patrick E. Sewell, M.D.



WHAT IF AGING COULD BE MODIFIED—AT THE GENETIC LEVEL?

The Klotho Effect presents a groundbreaking exploration of one of the most compelling discoveries in aging science: the Klotho protein. Long overlooked, this master regulator influences nearly every system in the body, with the power to suppress inflammation, slow cellular aging, and enhance cognitive, renal, and cardiovascular health. With clarity and precision, Dr. Patrick E. Sewell and Dr. Joe Cleaver reveal how restoring and enhancing Klotho expression may hold the key to longer, healthier human life. Drawing from decades of clinical experience and cutting-edge molecular research, the authors trace Klotho's profound influence across the human lifespan. From its early discovery in genetic mouse models to its growing role in gene therapy, bioelectric medicine, and personalized longevity protocols, The Klotho Effect bridges molecular science with real-world clinical implications. Whether used as a reference for medical professionals or a roadmap for patients and readers interested in longevity, this book translates advanced research into practical insight.

In this book, you will:

- Understand how the Klotho gene and protein influence aging, chronic disease, and lifespan.
- Learn about the role of Klotho in protecting the brain, kidneys, heart, and immune system.
- Explore gene therapy, recombinant proteins, and small molecules that increase Klotho levels.
- Discover lifestyle interventions—including exercise, diet, and sleep —that naturally boost Klotho.
- Examine Klotho's role as a biomarker in neurodegeneration, cancer, and chronic kidney disease.
- Gain insight into emerging technologies—such as CRISPR, AI-driven drug design, and organoid models—that are shaping the future of Klotho research.

This book is a call to rethink aging not as an inevitable decline, but as a process we now understand—and can begin to influence.

Dr. Patrick E. Sewell, M.D., brings unparalleled expertise to this narrative. A fifth-generation physician, he founded Interventional Oncology in 1998, introducing pioneering techniques like image-guided tumor ablation. With over three decades of experience, he has led advancements in stem cell therapy and gene therapy, bringing these to real patients with cutting-edge gene transfer techniques. As Chief Scientist at Triple Helix Science and a visiting professor at institutions like Yale and Stanford, Dr. Sewell continues to shape the future of medicine. His vision, rooted in scientific rigor and patient-focused care, makes The Klotho Effect an essential read for anyone seeking to understand the next frontier in healthcare.

Dr. Joe Cleaver, M.D., with 25 years of experience in longevity medicine and regenerative aesthetics, applies cutting-edge science to age management therapies and minimally invasive techniques. A master trainer and inventor of the proprietary stem cell 4D LYFT, regenerative body contouring, and the PLA DUO cutting edge plasma energy skin rejuvenating and tightening technology, Dr. Cleaver educates peers on the clinical significance of the Klotho protein. His passion for decoding the biology of aging—grounded in clinical research and academic instruction—drives this timely and essential work.

THE KLOTHO EFFECT

Dr. Patrick E. Sewell, MD
Dr. Joe Cleaver, MD

THE KLOTHO EFFECT

Unlocking the Genetic Key to Longevity and Vitality

Dr. Patrick E. Sewell, MD
Dr. Joe Cleaver, MD


THANK YOU
WWW.TRIPLEHELIXSCIENCE.COM

STAY CONNECTED

Subscribe to our newsletter → triplehelixscience.com

Follow us on Instagram → @triplehelixscience

Email → contact@triplehelixscience.com