

Species-Specific Differences in Myostatin-Activin Signaling: Implications for Follistatin Gene Therapy in Muscle Wasting Disorders

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Abstract

The development of myostatin inhibitors for muscle-wasting conditions represents a compelling case study in the challenges of translational medicine. The initial discovery that myostatin-null mice exhibit a doubling or tripling of muscle mass generated enormous enthusiasm for therapeutic development, with multiple pharmaceutical companies investing heavily in programs targeting this pathway. However, the translation from dramatic effects in rodent models to meaningful clinical benefits in humans proved far more challenging than anticipated.

The most advanced myostatin-specific antibody, domagrozumab, achieved only a 3.2% increase in lean mass in boys with Duchenne muscular dystrophy, with no accompanying functional improvements despite clear evidence of target engagement. This review examines the biological basis for these translational failures, with particular focus on fundamental differences in the transforming growth factor- β superfamily signaling between species that were not initially appreciated. Recent work by Latres and colleagues has revealed that the expression patterns of key muscle regulatory proteins differ markedly between rodents and primates. While mice express high levels of myostatin and relatively low levels of Activin A in muscle tissue, this ratio is essentially reversed in humans and other primates. These differences have profound implications for therapeutic development, as confirmed by non-human primate studies demonstrating that myostatin-only inhibition produces minimal effects, typically less than 2% lean mass gains, while dual inhibition of both myostatin and Activin A achieves substantially greater increases. This understanding has guided the development of broader-acting therapeutics that show superior efficacy in clinical trials. Bimagrumab, an antibody targeting the Activin type II receptor B, has demonstrated a 5.7% increase in lean mass accompanied by significant fat mass reduction and metabolic benefits in patients with sarcopenia and type 2 diabetes. Perhaps most promising is the development of follistatin gene therapy, which leverages nature's own multi-ligand inhibitor to achieve sustained muscle volume increases. Early clinical trials have treated 20 patients with various muscle-wasting conditions, showing durable benefits with an acceptable safety profile.

Keywords: Myostatin, Activin, Follistatin, GDF11, Gene therapy and Muscle wasting disorder.

Introduction

The identification of myostatin as a potent negative regulator of muscle mass represents one of the most significant discoveries in muscle biology in the past three decades [1]. When McPherron and colleagues first described the remarkable phenotype of myostatin-null mice in 1997, showing individual muscles that weighed two to three times more than those of wild-type controls, the therapeutic implications seemed immediately apparent [1]. The consistency of this phenotype across multiple mammalian species strengthened the case for therapeutic

development. Belgian Blue cattle, which carry a natural deletion in the myostatin gene, exhibit the characteristic "double-musled" phenotype that has been selected by breeders for generations [2].

The discovery of a whippet dog with a heterozygous myostatin mutation that excelled at racing provided evidence that even partial reduction of myostatin activity could enhance muscle function [3]. Most compelling was the identification of a German child with a myostatin mutation who displayed extraordinary muscle development from birth, demonstrating that the pathway functions similarly in humans [4].

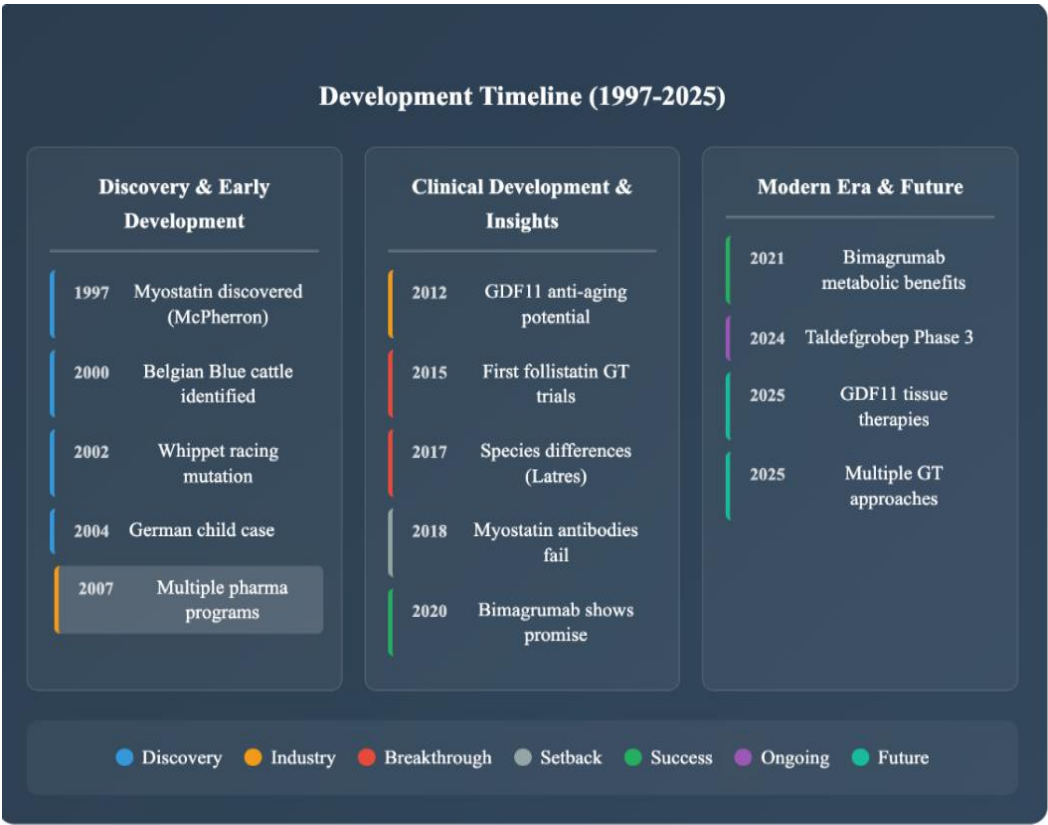


Figure 1: Timeline of Myostatin Inhibitor Development.

Timeline of myostatin inhibitor development from 1997 to 2025. Key milestones organized by era: Discovery (blue), Industry Investment (orange), Breakthroughs (red), Clinical Setbacks (gray), Successes (green), Ongoing trials (purple), and Future directions (teal). Timeline shows evolution from species identification through clinical failures to current multi-target approaches and gene therapy successes.

These observations suggested what appeared to be a straightforward therapeutic strategy for addressing the devastating impact of muscle-wasting conditions.

Duchenne muscular dystrophy affects approximately one in 3,500 male births and leads to progressive muscle weakness, loss of ambulation by the early teens, and premature death from respiratory or cardiac failure [5].

Sarcopenia, the age-related loss of muscle mass and function, affects up to 50 million people worldwide and contributes significantly to frailty, falls, and loss of independence in the elderly [6].

Cancer cachexia, characterized by severe muscle wasting that cannot be reversed by nutritional support alone, affects up to 80% of patients with advanced cancer and directly contributes to mortality in approximately 20% of cancer deaths [7].

For these conditions and others characterized by muscle wasting, the ability to increase muscle mass by inhibiting myostatin offered hope for meaningful therapeutic intervention.

The pharmaceutical industry responded with substantial investment in myostatin-targeting therapeutics. Multiple approaches were pursued, including monoclonal antibodies that bound myostatin directly, antibodies targeting the myostatin propeptide to prevent activation, soluble receptor constructs that acted as ligand traps, and small molecule inhibitors of downstream signaling [8].

Major pharmaceutical companies including Pfizer, Novartis, Regeneron, Eli Lilly, and others initiated development programs. The therapeutic rationale seemed compelling, as even modest increases in muscle mass and strength could potentially maintain ambulation in muscular

dystrophy, reduce fall risk in the elderly, or improve quality of life in cancer patients.

However, the results of human clinical trials proved deeply disappointing and forced a fundamental reevaluation of the therapeutic approach. The failure of domagrozumab, Pfizer's myostatin-specific antibody, in a Phase 2 trial in Duchenne muscular dystrophy was particularly instructive [9].

Despite achieving what appeared to be excellent target engagement, with serum myostatin levels increasing several-fold due to antibody binding and reduced clearance, the functional outcomes were essentially negative [9].

Boys receiving domagrozumab showed no improvement in the four-stair climb velocity, the trial's primary endpoint, nor in secondary functional measures including the six-minute walk distance and North Star Ambulatory Assessment [9].

While lean body mass increased modestly by 3.2% compared to 1.1% in the placebo group, this small gain did not translate to any functional benefit [9].

The disconnect between target engagement and clinical efficacy suggested that the simple model of myostatin inhibition derived from knockout mice did not adequately capture the complexity of muscle mass regulation in humans.

Expanding Therapeutic Horizons: GDF11 Joins the TGF- β Axis

While myostatin and Activin A have dominated translational muscle biology, GDF11 is another closely related TGF- β superfamily ligand which has emerged as a pivotal regulator in regeneration and tissue homeostasis, with unique implications for dermal and neurovascular rejuvenation.

Early enthusiasm for GDF11's anti-aging potential starting around 2012 was tempered by conflicting preclinical findings, largely attributed to assay limitations that failed to distinguish it from myostatin [10,11]. The fundamental issue arose when laboratory assays confused GDF11 with myostatin, as the technology at the time could not easily and reliably distinguish between the two.

This generated mixed signals about whether GDF11 decreases or increases with age and what its benefits and side effects were, which stalled its evaluation. Retrospectively, the limits of the assay technology generated these conflicting data and conclusions, delaying the understanding of GDF11.

However, with improved specificity and better laboratory testing, it is now clear that GDF11's functions are distinct and tissue-context-dependent, offering complementary rather than redundant therapeutic possibilities alongside follistatin [12].

Based on current evidence, excessive GDF11 in older patients might actually harm muscle or bone health, suggesting its future utility lies in precise delivery approaches to capture its benefits without side effects.

The potential for GDF11 in regenerative dermatology and anti-aging applications has renewed interest in this molecule as part of a broader TGF- β superfamily therapeutic strategy.

The Molecular Basis of Muscle Mass Regulation and the Myostatin-Activin-Follistatin-GDF11 Signaling Axis

Understanding why myostatin inhibition failed to translate from mice to humans requires examining the molecular mechanisms that regulate muscle mass.

Myostatin, also known as growth differentiation factor 8 or GDF8, belongs to the transforming molecules [13].

Like other TGF- β family members, myostatin is synthesized as a larger precursor protein that must undergo proteolytic processing to generate the active growth factor [14].

The N-terminal propeptide is cleaved but remains non-covalently associated with the C-terminal mature domain, forming a latent complex that circulates in the blood [14].

This latent complex serves as a reservoir of inactive myostatin that can be rapidly activated by additional proteolytic cleavage by members of the BMP-1/tolloid family of metalloproteinases [14].

The signaling mechanism of myostatin follows the general paradigm of TGF- β superfamily members but with important specificities that influence therapeutic targeting.

Myostatin binds to activin type II receptors on the muscle cell surface, with a strong preference for ActRIIB over ActRIIA [15].

This receptor binding specificity is important because ActRIIB is highly expressed in skeletal muscle, while ActRIIA shows broader tissue distribution [15].

Upon ligand binding, the type II receptors recruit and phosphorylate type I receptors, specifically ALK4 and ALK5 in the case of myostatin signaling [15].

The activated receptor complex then phosphorylates the intracellular signaling proteins SMAD2 and SMAD3, which form complexes with SMAD4 and translocate to the nucleus to regulate gene transcription [16].

The transcriptional program activated by myostatin signaling promotes protein degradation through the ubiquitin-proteasome system and

simultaneously suppresses protein synthesis through inhibition of the Akt/mTOR pathway, resulting in a net loss of muscle mass [16].

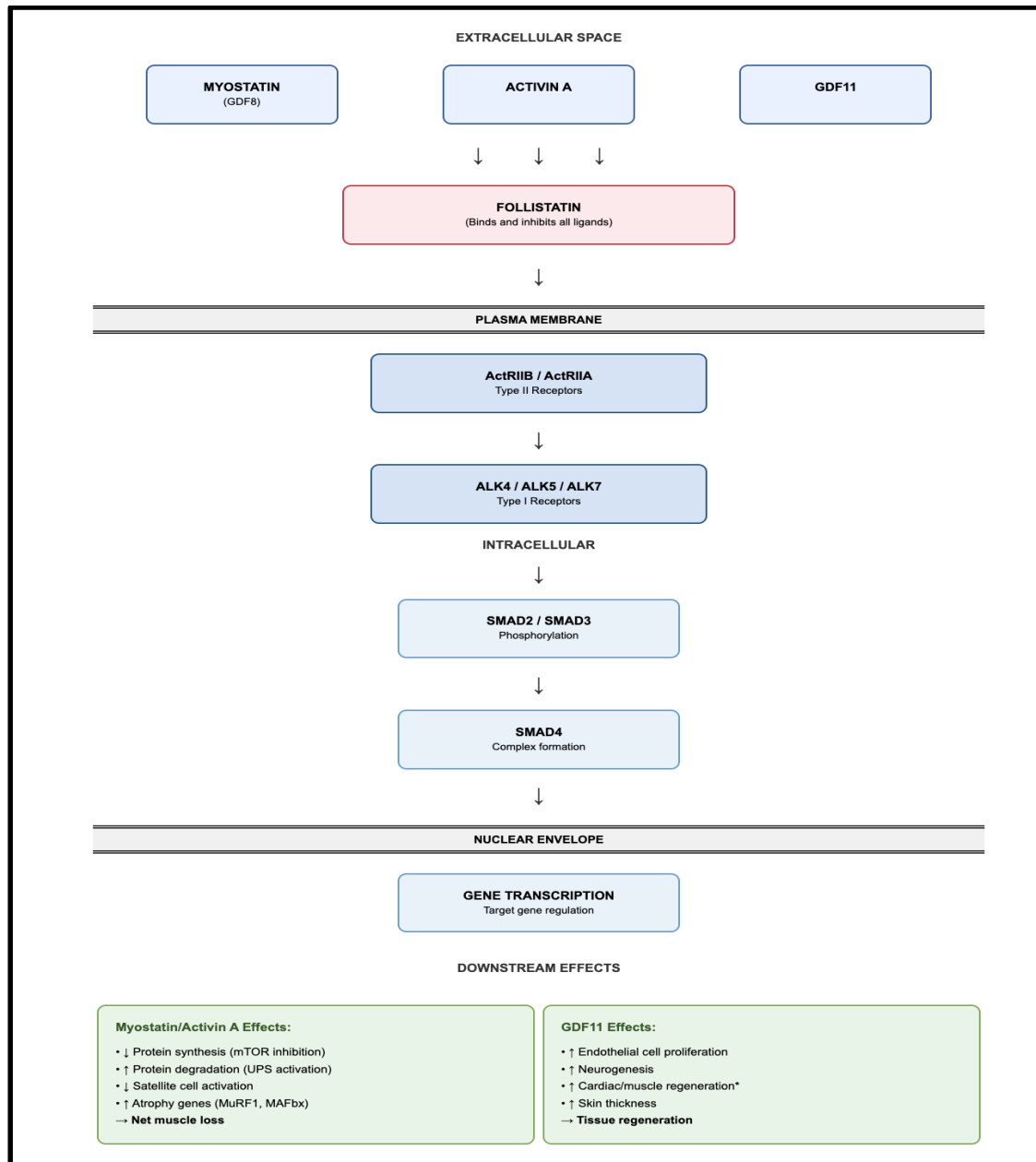


Figure 2: The Myostatin-Activin-Follistatin-GDF11 Signaling Axis.

Schematic representation of the myostatin-activin-follistatin-GDF11 signaling axis. All three ligands (myostatin, activin A, and GDF11) signal through the same receptor complex (ActRIIB/ActRIIA and ALK4/5/7) and downstream SMAD2/3-SMAD4 pathway. Follistatin acts as a broad-spectrum antagonist, binding and neutralizing all three ligands in the extracellular space. Despite shared signaling machinery, tissue-specific effects differ based on ligand concentration, receptor expression patterns, and cellular context. *GDF11 effects on muscle remain controversial. UPS = ubiquitin-proteasome system.

GDF11: The Third Key Player

While myostatin and Activin A represent the primary regulators of muscle mass, GDF11 (Growth Differentiation Factor 11) emerges as a crucial third member of this regulatory network with distinct tissue-specific functions [12]. GDF11 shares 90% sequence homology with myostatin and signals through the same ActRIIB-ALK4/5-SMAD2/3 pathway [12]. However, unlike myostatin's muscle-suppressive effects, GDF11 demonstrates regenerative properties in multiple tissues:

- **In skin:** enhances dermal regeneration, promotes collagen remodeling, and reduces age-related inflammation [12]
- **In vasculature:** supports angiogenesis and vascular remodeling [12]
- **In neural tissue:** promotes neurogenesis and reduces neuroinflammation [12]

The initial characterization of GDF11 was complicated by assay cross-reactivity with myostatin, leading to conflicting reports about its function [10,11]. With improved analytical methods, it is now clear that GDF11 levels decline with age, and restoration of GDF11 signaling may have therapeutic potential for age-related tissue degeneration [12].

This positions GDF11 as a complementary therapeutic target to follistatin, with the critical caveat that follistatin can inhibit GDF11 activity, necessitating careful consideration of delivery methods and tissue targeting in combined therapeutic approaches.

TGF- β Superfamily: Complex Redundancy and Crosstalk

The discovery that other TGF- β superfamily members share key components of this signaling pathway proved crucial for understanding the

limitations of myostatin-specific inhibition. Myostatin (GDF8), Activin A, and GDF11 signal through highly overlapping pathways. ActRIIB, ALK4/5, and SMAD2/3 regulate cell fate, protein turnover, and tissue repair. While myostatin predominantly suppresses skeletal muscle mass in most species, GDF11's roles extend to:

- Enhancing dermal and vascular regeneration
- Supporting neurogenesis and anti-inflammatory pathways in the skin and brain
- Influencing stem cell homeostasis in aging tissues

Activin A, originally identified for its role in regulating follicle-stimulating hormone release from the pituitary, emerged as particularly important for muscle mass regulation [15]. Like myostatin, Activin A signals through ActRIIB receptors and activates the same SMAD2/3 pathway [15].

This convergence at the receptor and intracellular signaling levels means that multiple ligands can produce similar biological effects on muscle mass [15]. Lee and colleagues provided early evidence for this redundancy when they demonstrated that soluble ActRIIB-Fc fusion proteins, which block signaling from multiple ligands, produced much greater increases in muscle mass than could be explained by myostatin inhibition alone [17].

Follistatin represents nature's solution to regulating this complex network of growth factors. Originally discovered as an activin-binding protein in ovarian follicular fluid, follistatin functions as a high-affinity antagonist of multiple TGF- β superfamily members [18].

The protein contains three follistatin domains, each consisting of 10 conserved cysteine residues that form a characteristic fold [19]. Structural

studies have revealed that follistatin acts like a molecular clamp, with two follistatin molecules cooperatively binding to activin or myostatin dimers [19].

This binding completely blocks the receptor interaction surfaces on the growth factors, preventing them from signaling [19].

Importantly, follistatin exists as two main isoforms generated by alternative splicing: FS-288, which contains a heparin-binding domain and remains largely membrane-associated, and FS-315, which lacks this domain and circulates more freely [20].

The ability of follistatin to neutralize multiple ligands simultaneously, combined with its high binding affinity (dissociation constants in the picomolar range), makes it an exceptionally effective endogenous inhibitor of this pathway [21].

Notably, follistatin by acting as a pan-ligand trap for these factors can therefore govern not only muscle mass but also the regenerative milieu of other tissues, depending on isoform distribution and expression context. Follistatin broadly inhibits myostatin, Activin A, and to some degree GDF11.

This means that overexpressing follistatin might inhibit GDF11, which could be counterproductive in dermal therapies where GDF11's regenerative effects are desired.

Species-Specific Differences in Muscle Regulation

The failure of myostatin-specific inhibition in human trials can be traced to fundamental differences in how muscle mass is regulated across species. While the initial characterization of myostatin knockout mice focused on the

dramatic phenotype, less attention was paid to the quantitative expression levels of pathway components [1].

Latres and colleagues performed the critical experiments that revealed why the mouse model was misleading for human therapeutic development [22].

Using validated mass spectrometry and immunoassay methods, they quantified the expression of myostatin, Activin A, and other TGF- β superfamily members in muscle tissue from multiple species [22].

The results revealed striking species-specific differences that had not been appreciated. In mouse gastrocnemius muscle, myostatin protein levels averaged 142 nanograms per milligram of total muscle protein, while Activin A levels were only 14 nanograms per milligram [22].

This ten-to-one ratio of myostatin to Activin A means that in mice, myostatin represents the dominant ligand signaling through ActRIIB receptors in muscle [22].

The situation in humans proved dramatically different. Human vastus lateralis muscle contained only 28 nanograms of myostatin per milligram of protein, a five-fold reduction compared to mice [22].

Even more significantly, human muscle contained 61 nanograms of Activin A per milligram, a four-fold increase compared to mice [22]. This reversal means that in human muscle, Activin A is actually more abundant than myostatin, with a ratio of 0.46 to 1 [22].

The 22-fold difference in the myostatin to Activin A ratio between mice and humans fundamentally alters the therapeutic requirements for achieving meaningful muscle mass increases [22].

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

Table 1: Species-Specific Expression of Myostatin Pathway Components.

Data adapted from Latres et al. (2017) [16]. Values represent mean ± standard deviation of protein concentration in muscle tissue.

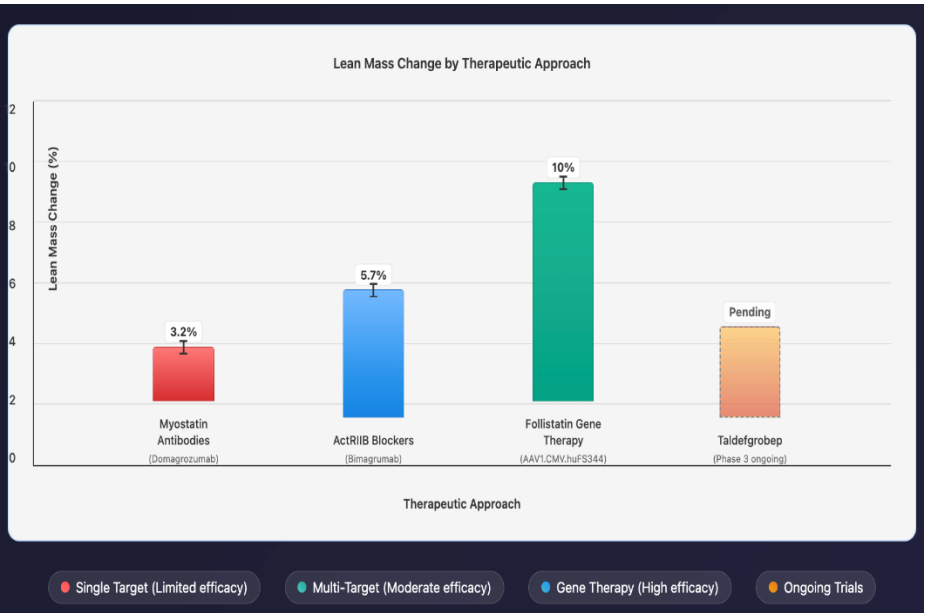


Figure 3: Comparative Efficacy of Myostatin Pathway Inhibitors.

Bar graph showing lean mass changes (%) for different therapeutic approaches: Myostatin antibodies (2-3%), ActRIIB blockers (5-7%), Follistatin gene therapy (10%), and ongoing Taldefgrobep trial. Includes error bars and sample sizes.

These expression differences were not limited to myostatin and Activin A. The comprehensive profiling revealed that humans express higher levels of follistatin in muscle compared to mice,

potentially representing a compensatory mechanism [22]. Other family members including GDF11 and BMP proteins also showed species-specific expression patterns [22]. When

the relative contribution of different ligands to ActRIIB signaling was calculated based on expression levels and binding affinities, it became clear that myostatin accounts for approximately 70% of the signal in mice but only 35% in humans [22]. This quantitative understanding explained why blocking myostatin alone could produce dramatic effects in mice but minimal effects in humans [22].

The functional significance of these expression differences was elegantly demonstrated through a series of experiments in non-human primates [22]. Cynomolgus monkeys, which show expression patterns intermediate between mice and humans, provided an ideal model for testing different therapeutic approaches [22].

When monkeys were treated with a myostatin-specific monoclonal antibody at doses that achieved complete target engagement, the increase in lean body mass was less than 2%, despite confirmation that circulating myostatin was fully bound by the antibody [22]. In contrast,

treatment with an antibody specific for Activin A produced moderate increases in muscle mass of approximately 4% [22]. Most significantly, combining both antibodies to achieve dual inhibition resulted in a 13.8% increase in lean body mass, demonstrating clear synergy between the two approaches [22]. Treatment with bimagrumab, which blocks the ActRIIB receptor and thereby inhibits signaling from all ligands, produced similar results to the combination antibody approach [23].

Clinical Trial Outcomes and Lessons Learned

The clinical development of myostatin inhibitors provides important lessons about the challenges of translating preclinical findings to human therapeutics. The trials can be broadly categorized into those testing myostatin-specific inhibitors, which largely failed to show meaningful efficacy, and those testing broader-acting agents that target multiple pathway components, which have shown more promising results.

Drug	Target	Indication	N	Lean Mass Change	Functional Outcome	Adverse Events	Status
Domagrozumab	Myostatin	DMD	120	+3.2%	No improvement	Rash, urticaria	DISCONTINUED
Landogrozumab	Myostatin	Sarcopenia	73	+2-3%	No improvement	None significant	DISCONTINUED
Bimagrumab	ActRIIB	Sarcopenia	113	+5.7%	Gait speed ↑	None significant	PHASE 2B
Bimagrumab	ActRIIB	T2DM/Obesity	75	+3.6%	HbA1c -0.76%	None significant	PHASE 2B
ACE-031	ActRIIB	DMD	48	Dose-dependent	Variable	Nosebleeds, telangiectasia	DISCONTINUED
Follistatin GT	Multi-ligand	BMD	6	Volume ↑*	6MWD improved	None significant	PHASE 1/2
Follistatin GT	Multi-ligand	sIBM	14	Volume ↑*	6MWD improved	None significant	PHASE 1/2
Taldefgrobep	Myostatin/ActRIIB	SMA	269	Ongoing	Ongoing	Ongoing	PHASE 3

Table 2: Summary of Clinical Trials Targeting the Myostatin Pathway.

*Muscle volume increased in treated muscles by MRI.

Abbreviations: DMD = Duchenne muscular dystrophy; T2DM = type 2 diabetes mellitus; BMD = Becker muscular dystrophy; sIBM = sporadic inclusion body myositis; SMA = spinal muscular atrophy; 6MWD = six-minute walk distance; GT = gene therapy

Among the myostatin-specific antibodies, domagrozumab received the most extensive clinical testing [9]. Wagner and colleagues reported the results of a randomized, double-blind, placebo-controlled Phase 2 trial that enrolled 120 ambulatory boys with Duchenne muscular dystrophy [9]. The trial design was

rigorous, with boys aged 6 to 15 years randomized to receive intravenous domagrozumab or placebo every four weeks for 48 weeks [9]. The primary endpoint, change in four-stair climb velocity, was chosen as a clinically meaningful measure of proximal muscle strength and function [9].

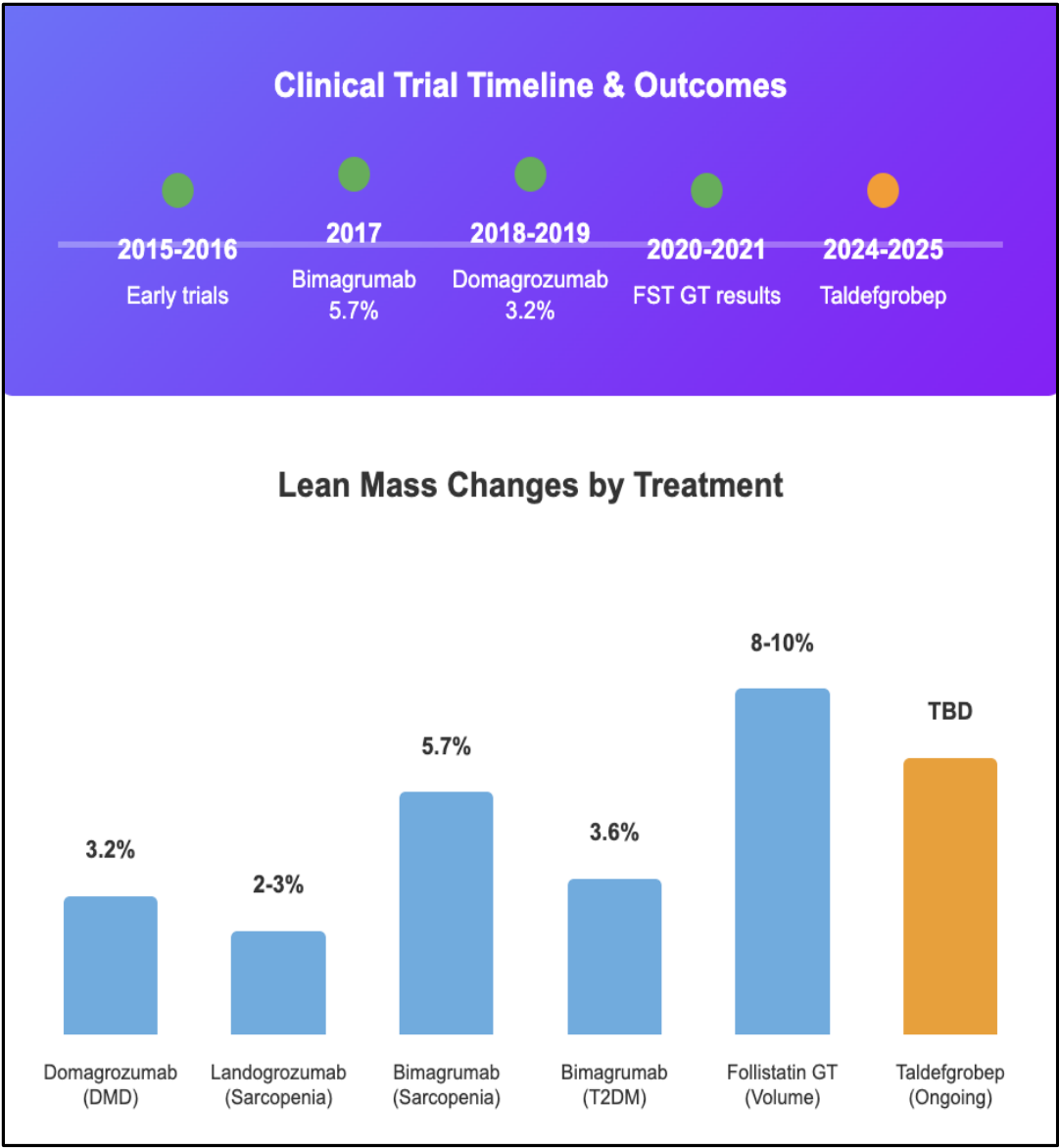


Figure 4: Clinical Trial Outcomes Over Time (2015-2025).

Modernized timeline showing clinical trial outcomes over time (2015-2025). Top panel shows key milestones with completed trials (green dots) and ongoing trials (orange pulsing dots). Bottom panel displays lean mass changes (%) for different therapeutic approaches, with treatment duration and key milestones marked. Follistatin gene therapy shows sustained muscle volume increases, while Taldefgrobep Phase 3 trial results are pending.



Figure 5: Clinical Development Pipeline (2025-2030 Projection).

Modernized clinical development pipeline projection showing anticipated progression of muscle and regenerative therapies through 2030. Color-coded phases indicate development stage, with interactive elements showing key milestones and expected approval timelines. The pipeline emphasizes the evolution from single-target to multi-target approaches and the emergence of tissue-specific therapies.

The results proved disappointing across all functional measures [9]. The four-stair climb velocity showed virtually no difference between groups, with both declining slightly over the 48-week period [9].

The six-minute walk distance, often considered the gold standard for assessing ambulation in DMD, decreased by 22.6 meters in the domagrozumab group compared to 26.9 meters in the placebo group, a difference that was neither statistically significant nor clinically meaningful [9].

What makes the domagrozumab failure particularly instructive is that the drug clearly hit its molecular target [9].

Serum myostatin levels increased more than five-fold in treated patients, reflecting antibody binding that prevented clearance of the myostatin-antibody complex [9].

Muscle MRI showed a modest increase in muscle volume, and dual-energy X-ray absorptiometry confirmed a 3.2% increase in lean body mass compared to 1.1% with placebo [9]. However,

this small increase in muscle mass failed to translate into any functional benefit [9]. The disconnect between achieving target engagement, producing a modest biological

effect, and failing to achieve clinical benefit highlighted the inadequacy of myostatin-only inhibition as a therapeutic strategy [9].

Biomarker Type	Specific Markers	Timing	Predictive Value
Serum Proteins	Myostatin, Activin A, FST	Baseline + monthly	Moderate
microRNAs	miR-1, miR-133, miR-206	Weekly	High
Imaging	MRI T2 mapping, DXA	Q3 months	High
Functional	6MWD, grip strength	Monthly	Direct
Metabolic	CK, LDH, glucose, HbA1c	Biweekly	Variable

Table 3: Biomarker Candidates for Treatment Response.

Other myostatin-specific antibodies showed similarly disappointing results [24]. Landogrozumab, developed by Eli Lilly for sarcopenia and cachexia, was tested in older adults with recent falls [24].

Despite achieving target engagement, the effects on muscle mass and function were minimal, leading to discontinuation of the program [24].

The pattern was consistent across multiple companies and antibody designs: myostatin-specific inhibition simply did not produce sufficient muscle growth to provide clinical benefit in humans [24].

The recognition that broader inhibition was necessary led to the development of agents targeting the ActRIIB receptor [23]. Bimagrumab, developed by Novartis, emerged as the most successful of these approaches [23].

The antibody binds to ActRIIB with high affinity and prevents all ligand binding to the receptor [23].

Rooks and colleagues reported results from a proof-of-concept trial in sarcopenia that demonstrated the superior efficacy of this

approach [23]. The trial enrolled 113 older adults with sarcopenia, defined by low muscle mass and reduced physical performance [23]. Participants received bimagrumab at 30 mg/kg or placebo as monthly intravenous infusions for 24 weeks [23].

The results showed clear biological activity: lean body mass increased by 5.7% with bimagrumab compared to a 0.2% decrease with placebo [23].

Perhaps equally important, total body fat mass decreased by 7.0% with bimagrumab compared to a 0.5% increase with placebo, demonstrating effects on body composition beyond simple muscle growth [23].

The functional outcomes with bimagrumab, while more modest than the body composition changes, showed encouraging trends [23]. Gait speed increased by 0.14 meters per second with bimagrumab compared to 0.08 meters per second with placebo [23].

Subsequent trials of bimagrumab have explored its potential in other conditions characterized by adverse body composition [25]. Heymsfield and colleagues conducted a Phase 2 trial in adults with type 2 diabetes and obesity [25].

This 48-week trial tested bimagrumab's effects on metabolic parameters as well as body composition [25].

The results demonstrated that ActRIIB blockade has effects extending beyond skeletal muscle [25]. HbA1c, a key measure of glycemic control, decreased by 0.76 percentage points with bimagrumab compared to 0.04 percentage points with placebo [25].

Fasting plasma glucose decreased by 1.05 mmol/L with bimagrumab compared to 0.21 mmol/L with placebo [25].

These metabolic improvements occurred alongside favorable body composition changes: total body weight decreased by 5.9 kg with bimagrumab, composed of a 7.9 kg reduction in fat mass partially offset by a 1.7 kg gain in lean mass [25].

This pattern of simultaneous fat loss and muscle gain is unique among diabetes therapies and suggests potential for addressing the muscle loss that often accompanies weight reduction [25].

The development of ACE-031, a soluble ActRIIB receptor fusion protein, provided additional evidence for the superiority of broad pathway inhibition [26]. Early trials showed dose-dependent increases in lean body mass and decreases in fat mass [26].

However, development was halted when some participants experienced bleeding side effects including nosebleeds and dilated blood vessels [27].

Recent trials have explored myostatin inhibitors for spinal muscular atrophy (SMA). Taldefgrobep alfa, developed by Biohaven, targets both myostatin and ActRIIB signaling.

The Phase 3 RESILIENT trial (NCT05337553) enrolled 269 patients with SMA types I–IV, with results expected in late 2024.

Preliminary data suggest potential for muscle mass increases, building on the success of broader-acting agents.

Follistatin Gene Therapy: Leveraging Nature's Solution

The recognition that multiple ligands contribute to muscle mass regulation in humans led to renewed interest in follistatin as a therapeutic approach [19].

Unlike engineered antibodies that target single ligands or receptors that might have effects in multiple tissues, follistatin represents nature's own solution for controlling this pathway [19].

The development of follistatin gene therapy emerged from the convergence of understanding about ligand redundancy and advances in gene delivery technology [28].

The preclinical development of follistatin gene therapy demonstrated several key advantages over other approaches [28]. Rodino-Klapac and colleagues showed that delivery of the follistatin gene using adeno-associated virus (AAV) vectors could produce sustained, high-level expression in muscle tissue [28].

The choice of AAV as a delivery vehicle was based on its excellent safety profile, ability to transduce non-dividing cells like mature muscle fibers, and potential for long-term expression without integration into the genome [28].

Studies in mouse models of muscular dystrophy showed that follistatin expression not only increased muscle mass but also improved muscle pathology and function [29].

Importantly, the use of muscle-specific promoters helped confine follistatin expression to the target tissue, potentially avoiding systemic effects [28].

The critical experiments in non-human primates established the translational potential of follistatin gene therapy [30].

Kota and colleagues demonstrated that intramuscular injection of an AAV1 vector carrying the human follistatin 344 isoform gene produced sustained expression for more than two years in cynomolgus macaques [30].

The treated muscles showed approximately 25% increase in volume with no adverse effects on muscle histology or function [30].

Importantly, there was minimal spread of follistatin to the circulation, suggesting that local production in muscle could achieve therapeutic effects while minimizing systemic exposure [30].

Based on these encouraging preclinical results, Mendell and colleagues initiated the first human trials of follistatin gene therapy [31,32].

The initial trial enrolled patients with Becker muscular dystrophy, a milder allelic form of Duchenne muscular dystrophy that typically allows ambulation into adulthood but still causes progressive weakness [32].

Six patients received bilateral intramuscular injections of AAV1.CMV.huFS344 into the quadriceps muscles at two dose levels [32].

The treatment proved well-tolerated with no dose-limiting toxicities [32]. Serial muscle biopsies confirmed sustained follistatin expression, and MRI imaging showed increased muscle volume in the treated muscles [32].

Functional assessments including the six-minute walk distance showed improvements in all patients, though the small sample size and open-label design limit definitive conclusions about efficacy [32].

A subsequent trial tested the same approach in patients with sporadic inclusion body myositis, the most common acquired muscle disease in adults over age 50 [31].

This condition causes progressive weakness and atrophy, particularly affecting the quadriceps muscles and finger flexors, and has no approved treatments [31].

Fourteen patients received the follistatin gene therapy, with encouraging results [31]. The six-minute walk distance improved in most patients, with some showing increases of over 100 meters [31].

Quantitative muscle testing showed increased strength in the treated muscles, and patients reported functional improvements in daily activities [31].

Muscle biopsies confirmed sustained follistatin expression and revealed interesting mechanistic insights, including evidence of increased regeneration and reduced fibrosis [31].

The mechanistic studies from these trials have provided valuable insights into how follistatin produces its beneficial effects [31].

Analysis of muscle biopsies showed not only the expected inhibition of myostatin and Activin A signaling, as evidenced by reduced phosphorylation of SMAD2/3, but also unexpected benefits [31]. Follistatin expression was associated with increased satellite cell activation, suggesting enhanced regenerative capacity [31].

There was evidence of reduced fibrosis, with decreased collagen deposition and lower expression of fibrotic markers [31].

The treatment also appeared to improve muscle quality, with reduced fat infiltration and better organization of muscle fibers [31].

These multiple mechanisms of action distinguish follistatin gene therapy from simple ligand inhibition and may contribute to its sustained benefits [31].

GDF11 and Tissue-Specific Regenerative Medicine

Molecular Complementarity and Therapeutic Positioning

The therapeutic landscape has expanded with the recognition that GDF11, while structurally similar to myostatin and utilizing the same signaling pathways, has distinct tissue-specific functions that position it as a complementary rather than redundant therapeutic target [12].

The GDF11 and follistatin content are highly complementary and interrelated in their scientific and translational themes, particularly in the

special context of translational muscle biology and dermal therapy.

Functional Partitioning: Muscle versus Dermis and Beyond

Unlike myostatin, which predominantly suppresses muscle growth, GDF11 has been shown to:

- Enhance skin regeneration, vascular remodeling, and reduce inflammation, particularly in aging skin
- Support neurogenesis and anti-inflammatory pathways in the skin and brain
- Influence stem cell homeostasis in aging tissues

Thus, while follistatin targets muscle hypertrophy and sarcopenia, GDF11 gene therapy fits more into the regenerative dermatology and anti-aging vertical, potentially complementing follistatin-based therapies.

Delivery Synergy: Electroporation & DNA Therapy

Recent advances in delivery methods have enhanced the therapeutic potential of both follistatin and GDF11.

Ligand	Main Tissue Target	Effect	Antagonized By
Myostatin	Muscle	↓ Muscle Mass	Follistatin, ActRIIB mAb
Activin A	Muscle, Endocrine	↓ Muscle Mass, FSH regulation	Follistatin, ActRIIB mAb
GDF11	Skin, Brain, Vessels	↑ Regeneration, ↓ Fibrosis	Follistatin (partial)

Table 4: Functional Outcomes by Ligand.

The application of electroporation gene therapy has shown enhanced gene expression and immune response in skin using methods such as Vacuum-EP [33]. These approaches are already being implemented in the GDF11 dermal gene therapy protocol, using intradermal

AAVusing intradermal AAV (AAV9/CAG-GDF11) delivery and electroporation to target skin fibroblasts and keratinocytes, directly comparable to intramuscular Adeno-Associated Virus (AAV) follistatin gene therapy.

Known Binding and Inhibition Considerations

A critical consideration in the combined therapeutic approach is that follistatin binds GDF11 with high affinity, structurally similar to how it binds myostatin. It acts as a pan-ligand trap, preventing GDF11 from activating $\text{ActRIIB} \rightarrow \text{ALK4/5} \rightarrow \text{SMAD2/3}$ signaling pathways.

This complementarity means that overexpressing follistatin might inhibit GDF11, which could be counterproductive in dermal therapies.

Therefore, the GDF11 therapeutic strategy argues for the tissue-specific or temporal control of expression, or co-administration with antioxidants and anti-AAV immune modulation to protect GDF11 function in skin.

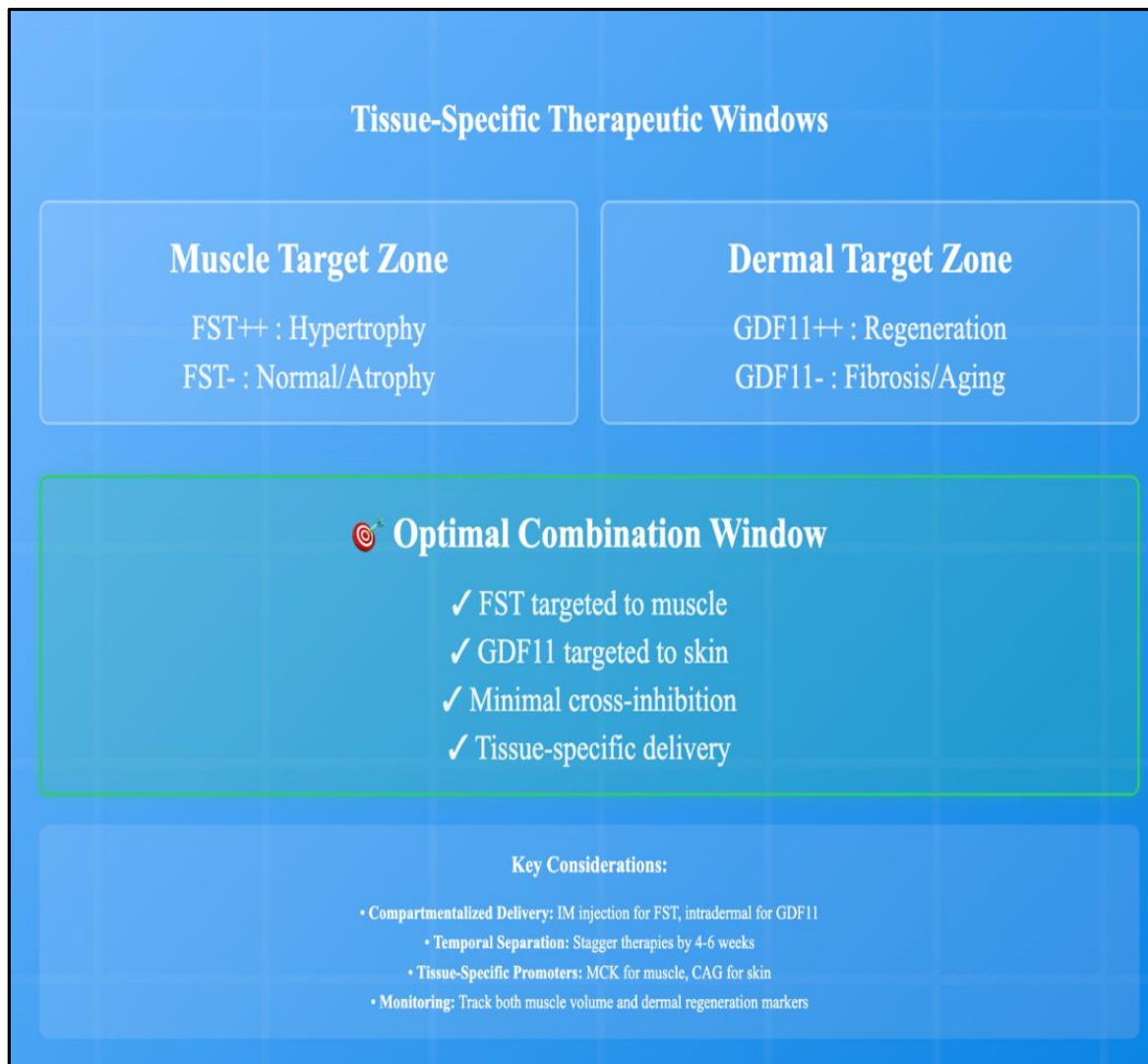


Figure 6: Therapeutic Window for Combined FST/GDF11 Therapy.

Modernized therapeutic window diagram showing optimal dosing ranges for combined FST/GDF11 therapy. The interactive design highlights tissue-specific therapeutic zones and the critical optimal combination window where benefits are maximized while minimizing cross-inhibition. Gradient background and glowing effects emphasize the precision required for successful multi-target therapy.

If FST plasmid is delivered into subcutaneous fat or dermis, there is risk of:

- Local inhibition of GDF11, which could:
 - Reduce collagen I/III synthesis by fibroblasts
 - Diminish anti-inflammatory signaling (e.g., suppression of IL-1 β , TNF- α)

- Blunt epidermal regeneration and angiogenesis

This becomes even more relevant if GDF11 is being delivered intradermally via AAV9 or electroporation, as the aim is for localized regenerative activity that could be "silenced" by overexpressed FST protein diffusing into the dermis.



Figure 7: Strategic Framework for Plasmid Follistatin Delivery.

Flowchart showing decision tree for optimal delivery route selection based on therapeutic goals and tissue targets.

Strategy	Purpose
Compartmentalize Delivery	Inject FST intramuscularly or into visceral fat while restricting GDF11 to dermis
Temporal Separation	Stagger FST and GDF11 therapies by at least 4-6 weeks
Use Tissue-Specific Promoters	Limit FST expression in skin (e.g., MCK promoter to confine to skeletal muscle)
Avoid FS-288 Isoform	Favor FS-315 forms to reduce dermal diffusion and membrane binding
Co-administer Antioxidants	Support GDF11’s dermal effects with MitoQ and antioxidants (sensitive to oxidative stress)

Table 5: Strategic Considerations for Multi-Ligand Therapies.



Figure 8: Decision Tree for Plasmid Follistatin and GDF11 Therapy.

Comprehensive decision tree showing patient selection criteria, delivery routes, and monitoring protocols for combined therapy approaches.

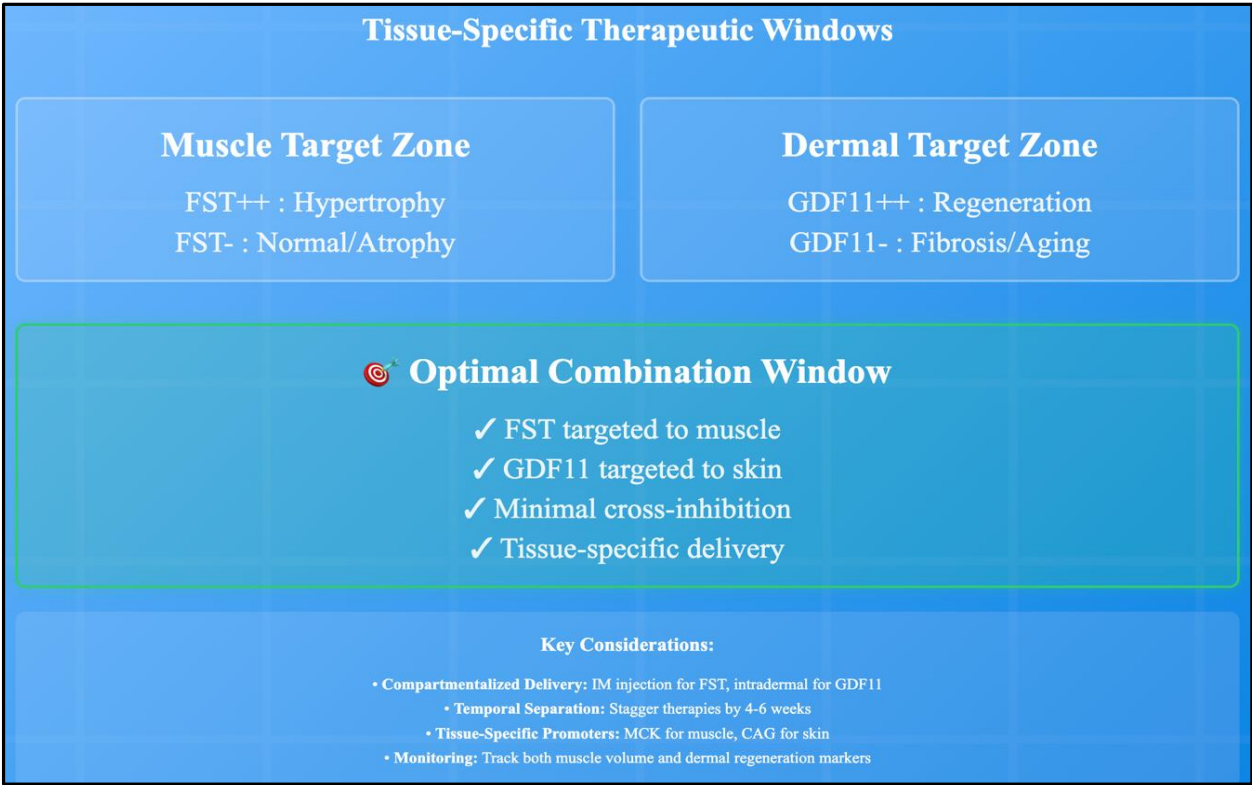


Table 6: Distinct Yet Complementary Use Cases: FST vs GDF11.

Isoform	Size (aa)	Heparin Binding	Tissue Distribution	Circulation	Therapeutic Use
FS-288	288	Yes	Membrane-associated	Limited	Local effects
FS-315	315	No	Freely circulating	Systemic	Broader effects
FS-303	303	Partial	Intermediate	Moderate	Research only

Table 7: Key Differences Between Follistatin Isoforms.

Safety Considerations and Long-Term Monitoring

The development of myostatin pathway inhibitors has required careful attention to safety, given the widespread expression of target receptors and ligands [34]. The theoretical concerns that motivated extensive safety monitoring have

largely not materialized in clinical trials, but the data collected provide important insights for future development [34].

Cardiac safety represented a primary concern given that myostatin is expressed in heart muscle and plays a role in cardiac remodeling after injury [34].

Delivery Method	Cardiac Risk	Reversibility	Duration	Monitoring Required
IM AAV GT	Minimal	No	>5 years	15-year follow-up
Systemic AAV	High	No	Permanent	Intensive cardiac
Nanoplasmid	Moderate	Yes	Weeks	Repeated dosing
mRNA	Low	Yes	Days	Minimal
Protein	Very Low	Yes	Hours	Standard

Table 8: Safety Profile Comparison Across Delivery Methods.

However, clinical trials have been reassuring on this front. Comprehensive cardiac monitoring in bimagrumab trials, including serial echocardiography and biomarker assessments, showed no evidence of adverse cardiac remodeling [35].

Ejection fraction remained stable, and there were no increases in cardiac biomarkers such as NT-proBNP or troponin [35].

The domagrozumab trial in boys with Duchenne muscular dystrophy, a population already at risk for cardiomyopathy, showed no cardiac safety signals[9].

Long-term follow-up of patients treated with follistatin gene therapy has included cardiac MRI

studies that show no evidence of cardiac hypertrophy or dysfunction [31].

Cardiac Safety: The Critical Role of Targeted Delivery

Vector Design Considerations

The absence of cardiac adverse effects in the follistatin gene therapy trials by Mendell and colleagues reflects not just the inherent safety of follistatin, but critically, the sophisticated vector design that confines expression to skeletal muscle [31].

The AAV1 vector utilized a CMV promoter, which, while not muscle-specific itself, was

delivered via direct intramuscular injection, limiting systemic exposure [31].

More recent iterations have incorporated muscle-specific promoters such as tMCK (triple muscle creatine kinase) or MHCK7, which further restrict expression to skeletal muscle even if the vector reaches other tissues [31].

Tissue Distribution Challenges

The concern becomes more acute when considering alternative delivery methods that lack tissue specificity. Nanoplasmid delivery systems, while offering advantages in terms of manufacturing and potentially reduced immunogenicity, typically result in widespread tissue distribution after systemic administration [31]. The liver often takes up a significant fraction of nanoparticles, but the heart, as a highly vascularized organ, would also be exposed [31].

Without targeting peptides or tissue-specific regulatory elements, follistatin expression in the heart could lead to uncontrolled cardiac growth [31]. Even with AAV vectors, the choice of serotype profoundly influences tissue distribution [31]. While AAV1 shows some muscle preference, other serotypes like AAV9 efficiently transduce both skeletal and cardiac muscle [31].

If follistatin gene therapy were to use AAV9 without muscle-specific promoters, achieving therapeutic levels in skeletal muscle might necessarily result in similar expression levels in the heart, potentially recreating the concerning phenotypes seen in genetic models of complete myostatin deletion [31].

Dose Optimization Strategies

The current intramuscular approach allows for high local concentrations of follistatin while minimizing systemic exposure [31]. A systemic

delivery approach would require careful dose optimization to achieve efficacy in muscle while avoiding cardiac effects [31].

This therapeutic window might be narrow or even non-existent for some delivery methods [31]. The presence of follistatin in the circulation, even at levels below those causing obvious cardiac hypertrophy, could have subtle effects on cardiac function, exercise capacity, or response to pathological stressors that might only become apparent with longer follow-up or larger patient populations [34].

Future Safety Considerations

The development path forward must therefore carefully consider delivery method as integral to both efficacy and safety [31]. Potential strategies to maintain cardiac safety while exploring alternative delivery methods include:

- Incorporation of cardiac-detargeting sequences (microRNA binding sites for cardiac-specific microRNAs)
- Development of muscle-specific nanoparticle formulations using targeting peptides
- Use of ultrasound or other physical methods to enhance uptake specifically in skeletal muscle
- Engineering of follistatin variants that retain muscle activity but have reduced cardiac effects

Controllable Gene Expression

Beyond traditional safety monitoring, the field is moving toward controllable gene expression systems that could transform gene therapy from a permanent intervention to a titratable therapeutic modality [36,37].

The characterization of gene therapy as "irreversible" reflects current clinical approaches

but need not be an inherent limitation [31]. The incorporation of inducible promoters and kill switches into vector design represents a fundamental shift in how we approach gene therapy safety, transforming it from a permanent intervention to a controllable therapeutic modality [36,37].

Inducible promoter systems would allow clinicians to titrate follistatin expression like a conventional drug [36]. The tetracycline-regulated (Tet-On/Tet-Off) system, for example, could enable dose adjustment through oral doxycycline administration [36]. In the Tet-On configuration, follistatin expression would occur only in the presence of doxycycline, allowing complete cessation of therapy by simply stopping the inducer drug [36]. This controllability addresses several critical safety concerns [36].

If cardiac effects emerged in a subset of patients over time, perhaps those who develop cardiovascular disease years after treatment follistatin expression could be reduced or halted entirely [36]. The ability to perform drug holidays could allow periodic assessment of whether continued treatment remains necessary, potentially revealing that some patients maintain benefits even after expression ceases due to lasting changes in muscle architecture or satellite cell populations [36].

The dosing flexibility offered by inducible systems could optimize the therapeutic window [36]. Current gene therapy approaches must choose a single dose at the time of administration, aiming for a level high enough to be effective but low enough to avoid toxicity across all patients [36]. With inducible promoters, treatment could begin conservatively and titrate upward based on individual response [36]. Athletes might require different expression levels than elderly sarcopenic patients, and these could be achieved with the same initial vector dose by varying

inducer administration [36]. Seasonal variations might even be possible with higher expression during periods of rehabilitation or training, lower during maintenance phases [36].

Kill switches represent an even more definitive safety mechanism [37]. These genetic circuits, often based on inducible caspase-9 or herpes simplex virus thymidine kinase, allow selective elimination of transduced cells if serious adverse events occur [37]. The activation of a kill switch by administration of a small molecule drug would cause apoptosis specifically in cells expressing the therapeutic transgene [37]. This provides an ultimate safety mechanism for scenarios where simply turning off expression is insufficient for instance, if chronic follistatin expression led to premalignant changes in muscle tissue or unexpected autoimmune responses [37].

However, these safety advantages come with significant trade-offs [36,37]. The requirement for continuous drug administration to maintain therapeutic effect (in Tet-On systems) or to keep the kill switch inactive (in some designs) adds complexity, cost, and compliance challenges [36,37].

The inducer drugs themselves may have side effects doxycycline, for instance, can cause photosensitivity and gastrointestinal effects [36]. The additional genetic elements required for regulation increase vector size, potentially reducing the capacity for the therapeutic transgene or requiring higher viral doses to achieve the same expression levels [36]. The regulatory proteins themselves could be immunogenic, potentially limiting duration of expression or causing adverse reactions [36].

The long-term monitoring requirements would shift substantially with controllable systems [36,37]. Instead of surveillance for late adverse effects from irreversible expression, monitoring

would focus on optimizing the dosing regimen, assessing compliance with inducer medications, and watching for development of resistance to the regulatory system [36,37]. Immune responses against the bacterial or viral components of regulatory systems could lead to loss of control over time [36,37]. Regular testing of the system's responsiveness is confirming that expression still increases with inducer administration and decreases upon withdrawal it would become essential [36,37].

Paradoxically, while controllable systems offer better safety management, they might require more intensive monitoring than permanent expression [36,37]. The ability to adjust dose creates an obligation to optimize it continuously [36]. Biomarkers of response would need to be checked more frequently to guide dose adjustments [36]. The potential for system failure is either loss of inducibility or spontaneous activation it would necessitate regular verification of proper function [36,37]. The long-term stability of these more complex genetic circuits in human cells remains less well-characterized than simple constitutive expression [36,37].

The choice between permanent and controllable expression ultimately reflects different philosophies of risk management [36,37]. Permanent expression accepts upfront risk in exchange for simplicity and sustained benefit [31]. Controllable expression distributes risk over time, maintaining flexibility at the cost of complexity [36,37]. For follistatin gene therapy, where the current safety profile appears favorable, the added complexity of controllable systems may not be justified [31]. However, as the field moves toward more potent interventions or systemic delivery methods, the ability to control or eliminate expression may become not just advantageous but essential for clinical development [36,37].

Future clinical trials comparing controllable versus constitutive expression systems will need to evaluate not just safety and efficacy, but also quality of life impacts, compliance challenges, and long-term reliability [36,37]. The ideal system might incorporate multiple levels of controls the baseline expression that can be boosted with an inducer, combined with an emergency kill switch that remains dormant unless activated [36,37].

Such sophisticated genetic circuits push the boundaries of current vector capacity but may represent the future of safe, effective gene therapy for muscle disorders [36,37].

Future Directions and Therapeutic Optimization

The lessons learned from myostatin inhibitor development have important implications for future therapeutic approaches [22]. The recognition that multiple ligands contribute to muscle mass regulation in humans has shifted focus from single-target to multi-target strategies [22].

This evolution parallels developments in other fields, such as cancer therapy, where combination approaches have proven superior to monotherapy [22].

Integrative Perspective: Tissue-Specific Requirements for Regeneration

The interplay between GDF11 and follistatin embodies the complexity of translating TGF- β biology into clinical therapies. When deployed wisely, these strategies can underpin a multi-organ, age-defying approach to tissue regeneration. However, precise design governing isoform, vector, promoter, and delivery route is essential to avoid neutralization of each ligand's desired effects.

The optimization of dosing strategies represents an important area for continued development [26]. The relationship between receptor occupancy, biological effects, and functional outcomes appears to be non-linear [26]. Achieving complete receptor blockade may not be necessary or even desirable, as suggested by the bleeding side effects seen with high doses of ACE-031 [27].

Biomarker-guided dosing, using measures such as serum myostatin and Activin A levels or muscle-specific microRNAs, could enable more precise dose individualization [26].

Combination approaches offer significant potential for enhancing efficacy [23]. The synergy between exercise and pharmacological treatment appears particularly important [23]. Resistance training activates distinct anabolic pathways from those affected by myostatin inhibition, including mechanical mTOR activation and IGF-1 signaling [23].

The combination may also improve the quality of muscle gained, with exercise promoting favorable fiber type composition and mitochondrial adaptations [23]. Nutritional optimization, particularly adequate protein intake to support muscle protein synthesis, represents another important component of combination therapy [23].

The potential for combining myostatin pathway inhibitors with other pharmacological agents is being explored [25]. The combination of bimagrumab with GLP-1 receptor agonists for obesity treatment could address the muscle loss that typically accompanies weight reduction while enhancing metabolic benefits [25].

For cancer cachexia, combining muscle anabolic agents with anti-inflammatory approaches may be necessary to address the complex

pathophysiology of muscle wasting in malignancy [7].

Emerging technologies offer new possibilities for muscle therapeutics. The success of mRNA vaccines has renewed interest in mRNA as a therapeutic modality. Delivery of follistatin or other muscle regulatory factors via mRNA could provide transient expression that might be safer than permanent gene therapy while still achieving therapeutic benefits [31]. CRISPR-based approaches to modulate endogenous gene expression, rather than delivering exogenous genes, represent another frontier [31]. Small molecule approaches targeting intracellular signaling nodes, while challenging given the protein-protein interactions involved, continue to be pursued [8].

The development of predictive biomarkers remains a critical need [26]. While some patients show excellent responses to treatment, others derive minimal benefit [26]. Understanding the molecular basis for response heterogeneity could enable better patient selection and personalized treatment approaches [26]. Potential biomarkers under investigation include baseline expression ratios of different ligands, genetic polymorphisms in pathway components, and dynamic responses of circulating microRNAs to treatment [26].

Conclusion

The journey from the discovery of myostatin to effective muscle therapeutics illustrates both the challenges and opportunities in translating basic science discoveries to clinical applications [1]. The initial failure of myostatin-specific antibodies, despite strong preclinical rationale and evidence of target engagement, forced a fundamental reevaluation of our understanding of muscle mass regulation [9,24]. The recognition that species differences in ligand expression

patterns profoundly affect therapeutic requirements represents a crucial insight that extends beyond this specific pathway [9].

The success of broader-acting approaches, particularly bimagrumab and follistatin gene therapy, validates the importance of addressing pathway redundancy in complex biological systems [23,31,36]. These agents achieve clinically meaningful effects by simultaneously inhibiting multiple negative regulators of muscle mass [23,31,36]. The additional benefits beyond muscle growth, including favorable metabolic effects and reduced fibrosis, suggest that multi-target approaches may offer advantages beyond simply greater efficacy [25,31].

The parallel development of GDF11-targeted therapies highlights the complexity of TGF- β superfamily biology and the importance of tissue-specific therapeutic strategies. While follistatin gene therapy has shown promise for muscle wasting conditions, GDF11 represents a complementary approach for dermal regeneration and anti-aging applications. The recognition that these molecules can interact with follistatin potentially inhibiting GDF11's beneficial effects underscores the need for careful consideration of delivery methods, tissue targeting, and temporal sequencing of therapies.

Several key principles emerge from this experience that apply broadly to drug development. First, the importance of comparative biology cannot be overstated [8]. While mouse models remain invaluable for understanding basic mechanisms, their limitations for predicting human responses must be recognized [8].

The investment in non-human primate studies for myostatin inhibitors proved crucial for understanding why the mouse model was misleading [8]. Second, pathway redundancy is

the rule rather than the exception in biological systems [8]. Evolution has created multiple overlapping mechanisms to control critical processes like muscle mass and tissue regeneration, and effective therapeutics must account for this redundancy [8]. Third, nature often provides elegant solutions to complex biological problems [19]. Follistatin, as an endogenous multi-ligand inhibitor, offers advantages that are difficult to replicate with engineered antibodies [19].

The current state of muscle and regenerative therapeutics offers genuine reason for optimism. While we have not achieved the dramatic effects seen in myostatin knockout mice, the ability to increase muscle mass by 5-7% with acceptable safety represents a meaningful advance [23,25].

For patients with muscle-wasting conditions, these gains can translate to maintained ambulation, reduced fall risk, or improved quality of life [23,31,36]. The metabolic benefits observed with ActRIIB inhibition open potential applications in the much larger population with obesity and type 2 diabetes [25]. The emerging understanding of GDF11's role in tissue regeneration expands the therapeutic horizon to include dermal rejuvenation and potentially neurological applications.

Looking forward, the key challenges include optimizing treatment regimens, developing biomarkers to identify patients most likely to benefit, exploring combination approaches that could enhance efficacy, and carefully managing potential interactions between different TGF- β superfamily therapeutics [26].

The ongoing development of these agents in pivotal trials will determine whether the promise shown in early studies translates to regulatory approval and clinical use. The field would benefit from systematic studies examining the interplay

between different ligands and their inhibitors across various tissues and disease states. Regardless of the outcome of specific programs, the insights gained from myostatin inhibitor development and the expanding understanding of related molecules like GDF11 have fundamentally advanced our understanding of muscle and tissue biology and established a foundation for future therapeutic development [1,22]. For the millions of patients suffering from muscle-wasting conditions and age-related tissue degeneration, these advances offer hope that effective treatments may finally become available.

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Conflict of Interest Statement

Dr. Patrick E. Sewell is the founder, CEO, and Chief Scientist of TripleHelix (triplehelixscience.com), a company offering gene therapies mentioned in this review. The author declares that this relationship has not influenced the objectivity of the scientific content presented.

Data Availability Statement

All data discussed in this review are available from the cited sources. No new data were generated for this review.

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