Follistatin Gene Therapy For Canine Degenerative Disease

A Proof-of-Concept Study with Delayed-Start Control Design

Executive Summary

Building on a human Phase 1 clinical trial[17] that demonstrated safety and preliminary efficacy of follistatin gene therapy, this proof-of-concept study translates this approach to companion canines suffering from degenerative conditions. Using a delayed-start control design, 30-50 dogs will be randomized to receive either follistatin plasmid gene therapy or delayed treatment after a 3-month observation period.

This exploratory study will focus on elderly dogs, or those already suffering from osteoarthritis or another degenerative condition. This approach leverages the established safety profile from human trials to address unmet needs in veterinary medicine for disease-modifying treatments. The delayed-start design ensures all participants eventually receive the treatment while providing robust control data.

By targeting both muscle preservation and fat loss (via myostatin inhibition) and inflammation reduction (via activin neutralization), follistatin gene therapy offers a novel multi-mechanism approach to degenerative disease management.

The study will generate proof-of-concept data to inform larger controlled trials, while potentially providing immediate therapeutic benefit to companion animals with limited treatment options. The results will establish the foundation for expanding follistatin gene therapy applications across both veterinary and human medicine.

Study Objectives

- **Translate human safety data**: Confirm the safety profile of follistatin gene therapy established in human Phase 1 trials when applied to the canine model
- **Proof-of-concept efficacy**: Generate preliminary evidence of the therapeutic benefit in canine degenerative diseases to justify larger controlled studies
- **Validate delayed-start design**: Demonstrate the feasibility and scientific rigor of delayed-treatment control methodology in veterinary gene therapy research
- **Dose-response characterization**: Evaluate therapeutic effects across multiple dosing tiers to optimize future treatment protocols
- **Disease-specific responses**: Assess differential therapeutic responses across degenerative conditions (osteoarthritis, degenerative myelopathy, intervertebral disc disease)

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1. Introduction and Background

1.1 Degenerative Disease Burden in Canines

Degenerative diseases represent a significant health burden in the aging canine population. Osteoarthritis (OA) affects approximately 20% of dogs over one year of age, with prevalence increasing to over 80% in geriatric dogs. This painful, progressive joint disease is characterized by cartilage degradation, synovial inflammation, and bone remodeling, leading to decreased mobility and quality of life. Rheumatoid arthritis (RA), while less common, presents as a more aggressive autoimmune-mediated polyarthritis with significant inflammatory components.

Other degenerative conditions of interest include Degenerative Myelopathy (DM), a progressive spinal cord disorder similar to amyotrophic lateral sclerosis in humans; Intervertebral Disc Disease (IVDD), characterized by degeneration and potential herniation of intervertebral discs; and Degenerative Lumbosacral Stenosis (DLSS), which causes compression of cauda equina nerves and subsequent pain and neurological deficits.

1.2 Current Treatment Landscape

Current treatment options for canine degenerative diseases are primarily focused on symptom management rather than disease modification. A breakdown of the classes of drugs used to treat the most common canine degenerative disease, osteoarthritis:

- **Disease-Modifying Osteoarthritis Drugs**: Adequan® (polysulfated glycosaminoglycan) is the only approved treatment for osteoarthritis dogs, and its mechanism of action is not understood
- **Intra-articular Therapies**: Steroids, hyaluronic acid, platelet-rich plasma, stem cells, Tin-117m
- **Pain Management**: Anti-NGF monoclonal antibodies, amantadine, gabapentin, cannabinoids, Librela
- **Alternative Therapies**: Acupuncture, massage, shockwave therapy
- **Supplements**: Egg shell membrane, *perna canaliculus*, undenatured type II collagen

Despite these options, many dogs continue to experience disease progression and decreased quality of life, highlighting the need for novel therapeutic approaches that address the underlying pathophysiology of these conditions.

1.3 Follistatin and Gene Therapy

Follistatin (FST) is a naturally occurring glycoprotein that binds and inhibits members of the TGF-β family, particularly myostatin and activin. Follistatin levels are naturally elevated by exercise and are usually highest during puberty. By inhibiting myostatin (a negative regulator of muscle growth) and activin (a pro-inflammatory mediator), follistatin promotes muscle formation while simultaneously reducing inflammation—two mechanisms highly relevant to degenerative disease management.

While injection of follistatin itself is possible, its short half life means that frequent injections are necessary to achieve therapeutic effects. Plasmid gene therapy offers an alternative approach to increasing follistatin expression *in vivo*. Minicircle plasmids, which have had their bacterial components removed, allow for semi-permanent (~1 year) gene expression, potentially providing sustained therapeutic effects from a single administration. This approach has been validated in a recent human Phase 1 trial conducted by Minicircle Inc., demonstrating safety and preliminary efficacy in terms of improved body composition and reduced inflammatory markers[17].

1.4 Follistatin and Arthritis

Follistatin's therapeutic potential in arthritic conditions stems from its dual mechanism targeting both inflammatory pathways and muscle preservation. As an extracellular decoy receptor, follistatin binds and neutralizes activins—proinflammatory cytokines of the TGF-β superfamily that drive synovial inflammation and cartilage degradation in arthritic joints.

Preclinical evidence demonstrates follistatin's protective effects in joint disease[15]. In carrageenan-induced arthritis models, follistatin pretreatment significantly reduced synovitis, decreased macrophage infiltration into synovial membranes, and inhibited proteoglycan erosion in articular cartilage. These findings indicate that activin-follistatin signaling directly regulates joint inflammation and cartilage homeostasis.

Beyond anti-inflammatory effects, follistatin addresses the muscle wasting commonly associated with degenerative joint disease. By inhibiting myostatin, follistatin promotes muscle formation and maintenance, potentially improving biomechanical joint support and reducing compensatory stress on affected joints. This dual action is particularly relevant in obesity-associated osteoarthritis, where follistatin gene therapy has demonstrated systemic reduction of inflammatory adipokines and cytokines while protecting against post-traumatic cartilage degeneration[20].

The sustained expression achievable through plasmid gene therapy (approximately 12 months) offers significant advantages over traditional anti-inflammatory treatments, potentially providing consistent disease modification rather than intermittent symptom relief.

1.5 Follistatin and Metabolism: Pathways and Adaptive Capacity

Follistatin functions as a powerful metabolic regulator extending far beyond its role as a TGF-β superfamily inhibitor. The protein significantly impacts muscle metabolism by enhancing skeletal muscle hypertrophy through mTOR pathway activation, operating via the Smad3/Akt/mTOR/S6K/S6RP signaling cascade[1,2]. In muscle tissue, follistatin activates PI3K/Akt/mTOR pathways while inhibiting muscle-wasting factors like FoxO1 and MuRF1, promoting protein synthesis and cellular energy production essential for muscle growth and maintenance[3].

Follistatin plays a crucial role in adipose tissue metabolism and thermogenesis by promoting brown adipocyte differentiation and upregulating thermogenic genes including UCP1, PGC1α, and PRDM16[4,5]. The protein enhances cellular respiration in adipocytes and increases energy expenditure through AMPK pathway activation, promoting irisin secretion that induces white adipose tissue browning[6]. In hepatic metabolism, follistatin helps maintain glucose homeostasis through liver-mediated mechanisms, with secretion regulated by the glucagon-to-insulin ratio, and can alleviate hepatic steatosis by reducing lipogenesis and improving insulin sensitivity[7,8].

The follistatin system demonstrates remarkable adaptive capacity through multiple feedback mechanisms, including a well-characterized activin-follistatin negative feedback loop that creates self-regulation[9]. Different follistatin isoforms show tissue-specific distribution—FST315 serves as the major circulating form while FST288 acts locally—allowing both systemic and localized metabolic control[10]. The system responds to environmental cues including exercise, cold exposure, and nutritional status, adapting metabolic processes to changing physiological demands[11].

While muscle anabolic and brown adipose tissue activation pathways are well-characterized, important knowledge gaps remain. Follistatin exhibits paradoxical effects, improving muscle insulin sensitivity while potentially causing insulin resistance in adipose tissue and decreased glucose tolerance[12,13]. The therapeutic window remains unclear, as excessive activation may lead to unwanted metabolic consequences, highlighting the need for further research into the system's adaptive limits and long-term effects of sustained elevation[14].

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3. Study Design

3.1 Study Population

- 30-50 canines
- Inclusion Criteria
    - Eight years of age AND/OR
    - Diagnosed by a vet as having Osteoarthritis, Rheumatoid Arthritis, Degenerative Myelopathy, Intervertebral Disc Disease,, or Degenerative Lumbosacral Stenosis
- Canines experiencing osteoarthritis are required to forgo treatments of Adequan or Librela for two months prior and after the treatment’s administration
- Other treatments are permitted so long as the data and dosage of their administration is recorded
- Exclusion Criteria
    - Life expectancy of less than six months
    - Inability to stand or walk (necessary for radiography and gait analysis)
    - Possibility of becoming pregnant[16]

3.2 Treatment Protocol

- Single administration of follistatin plasmid gene therapy via intramuscular injection
- Three-tiered dose escalation:
  - Tier 1: x1 dose - 10 ug plasmid/30ug PEI MAX MW40000, per 1kg animal weight
  - Tier 2: x3 dose (following safety confirmation in Tier 1)
  - Tier 3: x5 dose (following safety confirmation of Tier 2)
- Safety confirmation will be considered three days without a serious adverse event following treatment administration, witnessed in at least two participants.
- Dosage will be scaled appropriately based on canine body weight.

3.3 Study Timeline

- Screening/Baseline Assessment (Week 0)
- Treatment Administration (Week 2)
- Follow-up Assessments:
  - Week 2: Initial safety assessment
  - Month 1: First efficacy evaluation
  - Month 3: Comprehensive evaluation
  - Month 6: Mid-point assessment
  - Month 12: Final evaluation
- Optional extended monitoring beyond 12 months

3.4 Outcome Measures

Primary Outcome Measures:

- **Safety**: Adverse events, clinical pathology (CBC, chemistry, urinalysis)
- **Mobility**: Gait analysis via StanceAnalyzer[19], range of motion assessments, activity levels via biometric collars
- **Pain**: Veterinary pain assessment, owner-reported pain scores
- **Disease Progression**: Radiographic evaluation, joint fluid analysis (for arthritis cases)

Secondary Outcome Measures:

- **Functional Assessments**: Liverpool Osteoarthritis in Dogs (LOAD) questionnaire, veterinary joint range of movement assessment
- **Body Composition**: Weight, muscle mass evaluation
- **Inflammatory Markers**: C-reactive protein, inflammatory cytokines
- **Metabolic Parameters**: Glucose, insulin, lipid profiles
- **Biomarkers of Aging**: Epigenetic age (DNA methylation patterns)[21]

3.5 Comparative Analysis

- Comparison of outcomes against historical data from:
  - Published canine OA/RA intervention studies
  - Dog Aging Project database [22]
  - Natural history of each degenerative condition

4. Methodology

4.1 Recruitment

- Participant recruitment through:
  - Collaborating veterinary practices
  - Shelter networks and rescue organizations
  - Referrals from veterinary specialists

4.2 Screening and Enrollment

- Comprehensive veterinary examination
- Radiographic confirmation of degenerative condition (or family/genetic predisposition towards)
- Baseline blood work and urinalysis
- Owner education and informed consent

4.3 Assessment Procedures

Veterinary Reported Outcomes

- Orthopedic and range of motion examinations
- Neurological evaluations (for DM, IVDD, DLSS)
- Imaging: Radiography, potentially MRI or CT for selected cases
- Joint fluid analysis (for arthritis cases)
- Body Condition Score & weight
- Pain evaluation using validated veterinary scales

Owner-Reported Outcomes

- LOAD questionnaire
- Quality of life assessments
- Video records of mobility

Objective Measurements

- Activity monitoring via biometric collars
- Gait analysis using pressure-sensitive platform (StanceAnalyzer)
- Range of motion evaluation by vet
- Body composition assessments

Laboratory Testing

- Inflammatory markers
- Metabolic parameters
- Serum follistatin levels
- Epigenetic age determination

4.4 Safety Monitoring

- Continuous adverse event reporting system
- Scheduled veterinary examinations

5. Ethical Considerations

5.1 Animal Welfare

- Study design informed by prior safety testing:
    - Rodent model evaluation
    - Human Phase 1 trial data
    - Preliminary testing in study team members
- All procedures approved by an Institutional Animal Care and Use Committee (IACUC) or ad-hoc 6 person committee
- Pain management protocols in place
- Clear criteria for study withdrawal if adverse events occur

5.2 Owner Involvement

- Comprehensive informed consent process
- Regular communication with owners
- Transparency regarding experimental nature of treatment

5.2 Withdrawal Guidelines

5.2.1 Mandatory Withdrawal Criteria

Participants must be immediately withdrawn from the study if any of the following occur:

Immediate Safety Concerns:

- Anaphylaxis or severe allergic reaction to treatment
- Development of systemic illness directly attributable to gene therapy
- Significant elevation in liver enzymes (>3x upper limit of normal)
- Severe injection site reactions with necrosis or non-healing wounds
- Development of autoimmune conditions post-treatment

Progressive Deterioration:

- Rapid decline in mobility requiring emergency intervention
- Development of neurological symptoms inconsistent with baseline condition
- Inability to ambulate or stand (unless pre-existing)
- Significant weight loss (>15% baseline) not attributable to other causes

5.2.2 Discretionary Withdrawal Criteria

The following warrant case-by-case evaluation for potential withdrawal:

- Moderate adverse events persisting beyond 30 days
- Owner non-compliance with assessment schedules (>2 missed appointments)

- Development of concurrent serious illness unrelated to study treatment
- Behavioral changes indicating persistent discomfort or distress

5.2.3 Owner-Initiated Withdrawal

Owners may withdraw their animal at any time without penalty.

5.2.4 Withdrawal Process

1. Immediate Assessment: Complete veterinary examination within 72 hours of withdrawal decision
2. Documentation: Record reason for withdrawal, current health status, and treatment received
3. Final Data Collection: Attempt to collect all planned assessments up to withdrawal point
4. Follow-up: Contact owner at 30 and 90 days post-withdrawal to assess animal's condition
5. Safety Reporting: Report all safety-related withdrawals to IACUC within 48 hours

Animals withdrawn for safety reasons will receive appropriate veterinary care at study expense until condition stabilizes..

6. Data Analysis Plan

6.1 Primary Analyses

- Safety: Frequency and severity of adverse events across dosage tiers
- Efficacy: Pre-post changes in mobility, pain, and disease markers
- Subgroup analysis by condition type, age, breed, and size

6.2 Secondary Analyses

- Dose-response relationships
- Comparative efficacy across different degenerative conditions
- Correlation between objective measurements and owner-reported outcomes
- Effects on biomarkers of aging and metabolism

7. Potential Impact and Future Directions

7.1 Significance

This study represents a novel approach to addressing degenerative diseases in companion animals. By targeting both muscle formation and inflammation through a single gene therapy, we may achieve more comprehensive management of conditions that significantly impact canine quality of life. The extended duration of effect (potentially up to one year) could dramatically improve treatment compliance and consistency compared to daily medication regimens.

7.2 Future Research

Results from this pilot study could inform:

- The design of larger controlled trials with placebo groups
- Refinement of dosing protocols for different breeds and sizes
- Potential applications in other species, including feline degenerative conditions
- Development of combination therapies for synergistic effects

7.3 Translational Value

Insights gained may also have translational relevance to human degenerative diseases, particularly given the similar pathophysiology between canine and human OA and the promising results already observed in the human Phase 1 trial.

While a number of projects aim to bring a follistatin gene therapy to market for humans, the question of which disease indication to pursue looms over any Phase 2 study. While animal evidence suggests that lifespan can be extended, it is unclear if regulators will approve a gene therapy targeting ‘frailty’ or ‘sarcopenia’, as they are not considered acute conditions. By demonstrating that follistatin can treat arthritis, a pathway for regulatory approval opens for gene therapy.

8. Timeline

	Day -14	Day 1	Day 90	Day 180	Day 270	Day 360
Treatment Administration		A	B			
Placebo Administration		B				
Physical Examination		✔	✔	✔		
Radiography		x		x		x
Blood Panel		✔	A	B		✔
Urinalysis		✔	A	B		✔
LOAD Questionnaire	✔		✔	✔	✔	✔
Dog Aging Project Questionnaire	✔			✔		✔
Dog Aging Project Movement Analysis	O			O		O
Gait Analysis		✔	✔	✔		✔
Biometric Collar	✔	✔	✔	✔	✔	✔
Gene Sequence	O					
Synovial Fluid Analysys		O		O		
Blood draw For follistatin serum levels		O	O	O		O
Epigenetic Age	O					O
Video of Movement	X			X		X

Key: ✔ = All Participants
X = Participants with Degenerative Disease
A = Immediate start group
B = Placebo / delayed start group
O = Optional

9. Team and Facilities

9.1 Facilities

- Partner veterinary clinics for recruitment and assessments (TBD)
- Laboratory facilities for sample processing (TBD)
- CryptPad for E2E encrypted data management system for secure storage

9.2 Research Team

- Principal Investigator - Tristan Roberts
- Canine Research Specialist - Emmanuel Bijaoui
- Veterinary Specialist - Brandy Nunez
- Gene Therapy Expert - Andreas Stuermer
- Data Analyst - TBD

10. Preliminary Data

The foundation for this study is built upon two key sources of preliminary data:

1. **Human Phase 1 Trial (Minicircle Inc.)**: This study of 43 adults (ages 23-88) demonstrated:
   - Safety: No serious adverse events
   - Efficacy: Improvements in body composition (average 1.96 lbs lean mass gain, 0.87% body fat reduction)
   - Biological aging: 7.1-year reduction in extrinsic epigenetic age
   - Anecdotal reports of reduced arthritis symptoms in the subset of participants with pre-existing joint issues
   - Preprint for this study is available here
   - Clinical Trials.gov Identifier: NCT06411366
2. **Case Study - Canine Application**: A single large, aged dog (Skye) with severe arthritis received the follistatin vector and experienced dramatic reduction in pain behaviors that persisted for over six months, suggesting potential efficacy in the canine model.

Additionally, published research on follistatin gene therapy in a mouse model of osteoarthritis supports the therapeutic potential of this approach, with evidence of reduced inflammation and improved joint function.

11. Conclusion

This proposed study of follistatin gene therapy in canines with degenerative diseases addresses an important unmet need in veterinary medicine. By leveraging a gene vector to deliver follistatin, we aim to provide a longer-lasting, more comprehensive approach to improving mobility, reducing pain, and potentially modifying disease progression in affected dogs.

The results will provide critical insights into the safety, optimal dosing, and relative efficacy across different degenerative conditions, establishing the foundation for future larger-scale trials and potentially transforming the management of these challenging conditions in companion animals.

Appendix A: Diagnostic Criteria for Inclusion

Osteoarthritis (OA)

Clinical Signs:

- Joint stiffness, particularly after rest
- Decreased range of motion
- Lameness or altered gait
- Joint swelling or effusion
- Pain on joint manipulation

Diagnostic Requirements:

- Radiographic evidence of joint space narrowing, osteophyte formation, or subchondral sclerosis
- OR clinical diagnosis by licensed veterinarian based on physical examination and history
  OR LOAD score >11
- Exclusion of infectious or immune-mediated arthritis

Rheumatoid Arthritis (RA)

Clinical Signs:

- Symmetric polyarthritis affecting multiple joints
- Morning stiffness lasting >30 minutes
- Joint swelling with warmth
- Systemic signs (fever, lethargy, inappetence)

Diagnostic Requirements:

- Positive rheumatoid factor OR anti-cyclic citrullinated peptide antibodies
- Radiographic evidence of erosive joint disease
- Synovial fluid analysis showing inflammatory changes (>3,000 nucleated cells/μL)

Degenerative Myelopathy (DM)

Clinical Signs:

- Progressive hindlimb weakness and ataxia
- Loss of proprioception in hindlimbs

- Upper motor neuron signs progressing to lower motor neuron signs
- No evidence of spinal pain

Diagnostic Requirements:

- Genetic testing positive for SOD1 mutation (at-risk breeds)
- MRI or myelography ruling out compressive lesions
- Progressive clinical course over 6+ months

Intervertebral Disc Disease (IVDD)

Clinical Signs:

- Acute or chronic back pain
- Neurological deficits ranging from mild ataxia to paralysis
- Spinal hyperesthesia
- Bladder/bowel dysfunction in severe cases

Diagnostic Requirements:

- Radiographic evidence of disc space narrowing or calcification
- Advanced imaging (MRI/CT) confirming disc herniation or protrusion
- Neurological examination consistent with spinal cord compression

Degenerative Lumbosacral Stenosis (DLSS)

Clinical Signs:

- Hindlimb lameness or weakness exacerbated by exercise
- Pain on lumbosacral manipulation
- Difficulty rising or reluctance to jump
- Possible urinary/fecal incontinence

Diagnostic Requirements:

- Radiographic evidence of lumbosacral spondylosis or transitional vertebrae
- Advanced imaging showing cauda equina compression
- Clinical signs consistent with L7-S1 dysfunction
- Exclusion of hip dysplasia as primary cause of lameness

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