

Targeted Therapeutic Platform for Limb-Girdle Muscular Dystrophy R4 (LGMD2E)

Precision Translation for Compound Heterozygous SGCB Variants: c.341C>T p.(Ser114Phe) & c.552T>G p.(Tyr184*)

Document Reference:	MED-SGCB-2026-CLIN	Indication:	LGMD R4 (β-Sarcoglycanopathy)
Gene Involved:	SGCB (RefSeq: NM_000232.5)	Clinical Genotype:	Compound Heterozygous (Class 5 Pathogenic)
Allele 1 Variant:	c.341C>T, p.(Ser114Phe) [Exon 3]	Allele 2 Variant:	c.552T>G, p.(Tyr184*) [Exon 4]
Intended Audience:	Attending Neurologist / Geneticist	Manufacturing Scope:	CRO Peptide Synthesis & Analytical QC

SECTION 1: CLINICAL & MOLECULAR PATHOPHYSIOLOGY (FOR MEDICAL TEAM)

1.1 Structural Etiology of β-Sarcoglycan Deficiency

The patient is diagnosed with **Limb-Girdle Muscular Dystrophy Recessive 4 (LGMD R4 / LGMD 2E)**. The *SGCB* gene encodes **β-sarcoglycan** (35 kDa), a single-pass transmembrane glycoprotein that constitutes the indispensable structural anchor of the tetrameric sarcoglycan complex (α-, β-, γ-, and δ-sarcoglycan). In skeletal and cardiac muscle, this complex associates directly with dystrophin and the dystroglycan complex (DGC) to dissipate mechanical shear stress during myofibril contraction.

The "House of Cards" Phenomenon: Assembly of the sarcoglycan subcomplex is hierarchical. β-sarcoglycan is the mandatory nucleating center; absence or missense misfolding of β-sarcoglycan prevents membrane incorporation of α-, γ-, and δ-sarcoglycan subunits, leading to their concurrent endoplasmic reticulum-associated degradation (ERAD). The muscle phenotype therefore exhibits a total loss of the entire sarcoglycan complex, despite normal genetic coding for the other three subunits.

1.2 Precision Molecular Breakdown of the Patient's Variants

- Allele 1: c.341C>T, p.(Ser114Phe) in Exon 3 (Missense Mutation):** Substitution of a polar serine with a bulky hydrophobic phenylalanine disrupts the extracellular/luminal fold of the β-sarcoglycan ectodomain. The mutant protein is synthesized at near-normal rates but is recognized as misfolded by ER quality control chaperones (e.g., BiP/GRP78, calnexin) and redirected to the ubiquitin-proteasome pathway for ERAD. Importantly, functional studies demonstrate that Ser114Phe retains residual intrinsic binding capability if trafficking to the sarcolemma can be pharmacologically enabled.
- Allele 2: c.552T>G, p.(Tyr184*) in Exon 4 (Nonsense Mutation):** Introduction of a premature termination codon (PTC) at residue 184 truncates the critical C-terminal domain required for interaction with δ-sarcoglycan and triggers nonsense-mediated mRNA decay (NMD), resulting in virtually zero functional protein translation from this allele.

1.3 Downstream Pathological Cascade & Cardiac Implications

Loss of the sarcoglycan shock absorber renders the sarcolemma mechanically fragile. Contraction tears create persistent micro-lesions, provoking:

- Uncontrolled Ca²⁺ Influx:** Hyperactivation of cytosolic neutral cysteine proteases (μ- and m-calpains), which digest structural sarcomeric proteins (titin, nebulin, troponin I). *Note: Unlike LGMD R1 (CAPN3 deficiency), calpain activity is elevated and destructive here.*
- Chronic NF-κB Inflammatory Infiltration:** Mechanical shear activates the IKK complex, perpetuating chronic M1 macrophage recruitment and releasing latent TGF-β1.
- Fibro-Adipogenic Replacement:** Persistent activation of fibro-adipogenic progenitors (FAPs), leading to the fibro-fatty destruction of the proximal pelvic and pectoral girdles.
- Cardiomyopathy Risk:** β-sarcoglycan is expressed in vascular smooth muscle and cardiomyocytes. LGMD R4 patients face substantial risk of dilated cardiomyopathy and congestive heart failure, necessitating targeted cardiac cytoprotection.

[LGMD R4 PRECISION PARADIGM] | [GENE / PROTEIN LEVEL RESCUE] [DOWNSTREAM SARCOLEMMA SHIELD] | | ↳ Allele 1: Pharmacological Chaperones ↳ Layer 1: Sarcolemmal Sealant (P188 Mimetic) | (Trafficking rescue of Ser114Phe out of ERAD) | • Plugs contraction-induced micro-tears | | • Halts catastrophic Ca^{2+} influx ↳ Allele 2: Translational Read-Through | | (Ribosomal bypass of Tyr184* stop codon) ↳ Layer 2: Cyclic Calpastatin 14-mer | | • Shields titin/troponin from hyperactive calpain ↳ Clinical Horizon: AAVrh74-SGCB Gene Replacement | (Investigational Gene Therapy Vector) ↳ Layer 3: Hydrocarbon-Stapled NBD Peptide | • Blocks IKK/NF- κ B; suppresses FAP fibrosis | ↳ Layer 4: SS-31 Cardiolipin Stabilizer • Rescues mitochondrial ATP; shields heart

Clinical Contraindication: Myostatin / ActRIIB Inhibitors

Stimulating muscle hypertrophy via ActRIIB blockade (e.g., bimagrumab, follistatin mimetics) in β -sarcoglycan-deficient muscle accelerates mechanical contraction force against unanchored, fragile sarcolemma, worsening micro-tearing and accelerating necrosis. ActRIIB antagonists are strictly contraindicated until sarcolemmal stabilization is validated.

SECTION 2: CUSTOM PEPTIDE SYNTHESIS SPECIFICATIONS (FOR MANUFACTURER / CRO)

The following four peptide entities represent the cytoprotective shield designed for research and translation. All syntheses must adhere to strict solid-phase peptide synthesis (SPPS) parameters with documented analytical RP-HPLC ($\geq 98.0\%$ purity) and High-Resolution Mass Spectrometry (HRMS).

ENTITY 1 Sarcolemmal Sealant-C20 (Biomimetic Membrane Patch) PURITY $\geq 98\%$

Primary Function: Amphipathic α -helical membrane-partitioning peptide engineered to plug micro-tears in fragile sarcoglycan-null bilayers, functionalized with a fatty diacid for human serum albumin hitchhiking.

Chemical Sequence & Structural Architecture:

Ac-F-K-A-L-K-E-L-L-K-A-A-L-E-L-L-K-K([2-(2-(2-aminoethoxy)ethoxy)acetyl]2-(γ -Glu)-icosanedioic acid)-A-A-L-K-A-L-A-NH₂

Synthesis Parameters:

- **N-Terminus:** Acetylated (Ac-); **C-Terminus:** Carboxamide (-NH₂).
- **Side-Chain Functionalization (Lys17):** Orthogonally protected Lys(Mmt/Alloc) selectively deprotected on-resin, coupled with dual AEEA linkers (8-amino-3,6-dioxaoctanoic acid), γ -glutamic acid, and mono-tBu-protected icosanedioic acid (C20 diacid).
- **Purity & QC:** Analytical RP-HPLC purity $\geq 98.0\%$ (area% at 214/280 nm); ESI-MS deconvoluted monoisotopic mass confirmation; residual TFA $< 1.0\%$ (acetate exchange preferred).

ENTITY 2 Hydrocarbon-Stapled NBD-C18 Peptide (Selective IKK/NF- κ B Antagonist) PURITY $\geq 98\%$

Primary Function: Uncouples NEMO (IKK γ) from the IKK core exclusively under mechanical inflammatory stress, arresting fibro-adipogenic macrophage scarring.

Chemical Sequence & Structural Architecture:

Ac-T-A-L-D-X1-S-W-L-X2-T-E-K([2-(2-(2-aminoethoxy)ethoxy)acetyl]2-(γ -Glu)-octadecanedioic acid)-NH₂

Synthesis Parameters:

- **Macrocyclization:** Residues **X1 (pos 5)** and **X2 (pos 9)** are (S)-2-(4'-pentenyl)alanine (S5). Macrocyclic ring-closing metathesis (RCM) performed on-resin using 1st/2nd generation Grubbs catalyst to form an all-hydrocarbon (i, i+4) olefin staple.
- **Linker & Diacid:** Conjugated at C-terminal Lys12 with dual AEEA, γ -Glu, and octadecanedioic acid (C18 diacid).
- **Purity & QC:** Analytical RP-HPLC $\geq 98.0\%$; confirmation of complete olefin metathesis (loss of 28 Da for ethylene byproduct); ESI-MS confirmation.

ENTITY 3

Cyclic Calpastatin Subdomain 14-mer (Active-Site Calpain Inhibitor)

PURITY ≥98%

Primary Function: Conformationally locked pseudo-substrate inhibitor targeting Ca²⁺-activated μ-calpain; shields sarcomeric titin and troponin without cross-reacting with the 20S/26S proteasome.

Chemical Sequence & Structural Architecture:

cyclo(Asp-Pro-Met-Ser-Thr-Ile-Tyr-Ile-Glu-Glu-Leu-Gly-Lys-Lys) [Head-to-tail amide backbone cyclization]

Synthesis Parameters:

- **Cyclization:** Synthesized on 2-chlorotrityl chloride resin; cleaved with intact side-chain protecting groups using 1% TFA/DCM. Macrocyclization achieved in dilute solution (1 mM) via PyBOP/HOAt/DIEA, followed by global TFA deprotection.
- **Purity & QC:** Analytical RP-HPLC ≥98.0%; ESI-MS verification of cyclic monomer (absence of linear or uncyclized dimer species).

ENTITY 4

SS-31 / Elamipretide Analogue (Mitochondrial & Cardiac Cytoprotection)

PURITY ≥98%

Primary Function: Partitions into inner mitochondrial membrane (IMM) cardiolipin, preventing electron leakage, ROS generation, and cardiac cell apoptosis.

Chemical Sequence & Structural Architecture:

D-Arg-2',6'-Dmt-Lys-Phe-NH₂ [Dmt = 2',6'-dimethyltyrosine; C-terminal carboxamide]

Synthesis Parameters:

- **Chiral Building Blocks:** Incorporates D-Arginine at position 1 and unnatural 2',6'-dimethyl-L-tyrosine (Dmt) at position 2.
- **Purity & QC:** Analytical RP-HPLC ≥98.0%; ESI-MS confirmation; optical rotation verification for stereochemical purity.

SECTION 3: PHARMACOKINETICS & SYNCHRONIZED DOSING PROTOCOL

ENTITY	ROUTE	FREQUENCY	ELIMINATION T _{1/2}	TARGET BIOLOGICAL MECHANISM
Sealant-C20	Subcutaneous	Twice-Weekly (q3.5d)	5–7 days (Albumin bound)	Maintains steady-state sarcolemmal hydrophobic barrier against mechanical shear.
Stapled NBD-C18	Subcutaneous	Twice-Weekly (q3.5d)	3–4 days (Albumin bound)	Continuous inhibition of NF-κB p65 nuclear translocation and FAP transdifferentiation.
Cyclic Calpastatin	Subcutaneous	Twice-Weekly (q3.5d)	16–24 hours	Suppresses active μ-calpain proteolysis during daily ambulatory mechanical loading.
SS-31 Tetrapeptide	Subcutaneous	Once Daily (Morning)	Plasma: 2–4 h; Tissue: 16 h	Direct mitochondrial IMM polarization prior to daily diurnal muscular exertion.

Co-Formulation & Injection Guidelines

- **Shield Depot Injection (Twice Weekly - Mon AM / Thu PM):** Entity 1 (Sealant-C20), Entity 2 (Stapled NBD-C18), and Entity 3 (Cyclic Calpastatin) may be co-administered subcutaneously into abdominal or thigh adipose tissue in buffered isotonic sterile saline (pH 7.2–7.4). Albumin binding creates a continuous slow-release systemic reservoir.
- **Mitochondrial Maintenance (Daily Morning):** Entity 4 (SS-31) must be administered daily as a rapid-uptake morning subcutaneous injection. Avoid lipidation to preserve rapid mitochondrial partition.

SECTION 4: OBJECTIVE CLINICAL MONITORING & SAFETY BENCHMARKS

CLINICAL PARAMETER	TARGET BIOMARKER / MODALITY	ASSESSMENT SCHEDULE	EFFICACY / SAFETY THRESHOLD
Sarcolemmal Integrity	Serum Creatine Kinase (CK), Myoglobin	Baseline, Wk 2, Wk 4, Wk 12, Wk 24	Target ≥40% reduction from baseline; indicates reduced membrane leakage.
Myocardial Function	Cardiac MRI (T1/ECV, LVEF), hs-cTnI	Baseline, Week 12, Week 24	Stabilization of myocardial strain; absence of late gadolinium enhancement (LGE).
Fibrotic Modulation	Serum TGF-β1, MMP-9, Fibronectin	Baseline, Week 4, Week 12	Suppression of circulating fibrogenic cytokines driven by NBD peptide.
Renal Clearance Safety	Urinary β2-microglobulin, Cystatin-C	Monthly	Verification of proximal renal tubule health (monitoring megalin/cubilin flux).
Functional Ambulation	NSAA, Timed 10M Walk, 4-Stair Climb	Baseline, Week 12, Week 24	Functional stability / arrest of pelvic girdle deterioration.

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