THYMIC REJUVENATION PROTOCOL

Scientific & Clinical Reference

A pharmacologically detailed framework for thymic immunosenescence reversal targeting cortical and medullary thymic epithelial cell (cTEC/mTEC) regeneration, GH/IGF-1-axis stimulation, mTOR-mediated senescent cell clearance, and iterative 4-week cycling for longevity co-objectives.

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PREFACE

Rationale and Scope

This protocol consolidates the mechanistic rationale for targeting thymic involution as a primary immune-rejuvenation intervention. The thymus undergoes progressive fatty infiltration after puberty, reducing naïve T-cell output by ~90% by the sixth decade. Restoration of functional thymic epithelial cell (TEC) mass and output is the central therapeutic target.

The foundational human clinical evidence is the TRIIM trial (Fahy et al., *Aging Cell*, 2019): a phase II open-label pilot (n=9, males 51–65) demonstrating thymic MRI fat fraction reduction, sjTREC increase, and mean ~2.5-year epigenetic clock reversal using a combination of **recombinant human GH (rhGH, Norditropin 0.015 mg/kg/day)**, DHEA 50 mg/day, and metformin 500 mg BID. This protocol replaces rhGH with **tesamorelin (a GHRH analogue)**, rapamycin for metformin, and imeglimin as an additional metabolic buffer — a set of substitutions grounded in mechanistic pharmacology and aligned with the direction Dr Fahy has stated for future protocol iterations.

Version 4.0 corrections (vs v3.0): (1) Tesamorelin starting dose corrected to 0.5–1 mg/day with IGF-1 z-score titration (the 2 mg/day HIV-lipodystrophy dose is not appropriate for non-HIV individuals with intact GH-axis sensitivity). (2) Erroneous GHRH-receptor desensitisation claim removed — Phase 3 data show no tachyphylaxis at 52 weeks continuous daily dosing. (3) Week-1 IGF-1 monitoring added as the primary titration signal.

SECTION 1

Thymic Biology and Immunosenescence

1.1 Anatomy and Function

The thymus is a bilobed primary lymphoid organ situated in the anterior mediastinum. Its cortical (cTEC) and medullary (mTEC) epithelial cell populations orchestrate T-cell development and central tolerance. cTECs present self-peptide–MHC complexes to drive positive selection; mTECs express AIRE (autoimmune regulator) to drive negative selection. Together they ensure a repertoire of naïve CD4+ and CD8+ T cells that are self-tolerant yet broadly reactive.

1.2 Thymic Involution

Thymic involution begins after puberty under the influence of sex steroids and proceeds at approximately 3% per year through the third decade, accelerating thereafter. By age 50–60, ~70–90% of thymic parenchyma has been replaced by adipose tissue. Naïve T-cell output (measured as sjTREC/mL) falls correspondingly, shifting immune competence toward expanded memory clones with reduced antigenic breadth. This contributes to immunosenescence: impaired vaccine responses, reduced tumour surveillance, and increased autoimmune risk.

1.3 TEC Targets for Rejuvenation

Multiple signals converge on TEC maintenance and regeneration:

Signal	Receptor on TEC	Pharmacological Lever
GHRH (growth hormone-releasing hormone)	GHRH-R (class B GPCR)	Tesamorelin (direct GHRH-R agonist)
IGF-1 (insulin-like growth factor 1)	IGF-1R (RTK)	Secondary to GHRH-R activation
Sex steroids (oestrogen, testosterone)	ERα, AR	DHEA (androgen/oestrogen precursor)
mTOR signalling	mTORC1 (serine/Thr kinase)	Rapamycin (allosteric mTORC1 inhibitor)
Thymulin (thymic hormone)	Thymulin receptor	Zinc as essential cofactor

1.4 Biomarkers of Thymic Output

Biomarker	What it Measures	Clinical Access
sjTREC (signal joint T-cell receptor excision circle)	Direct thymic output — circles produced during TCR rearrangement in naïve T cells	qPCR on PBMC DNA; limited specialist labs
Naïve CD4+/CD8+ T cells (flow)	Indirect thymic output; peripheral surrogate	Standard flow cytometry
Thymic MRI fat fraction	Structural thymic parenchyma vs adipose	Research; expensive; used in TRIIM
Epigenetic clock (GrimAge, DunedinPACE)	Biological age acceleration	Commercial (TruDiagnostic, Elysium)

SECTION 2

GH/IGF-1 Axis — Signal Transduction & TRIIM Evidence

2.1 GHRH Receptor Signalling Cascade

Tesamorelin is a synthetic analogue of human GHRH (44 amino acids) modified at the N-terminus (trans-3-hexenoic acid conjugation) to resist dipeptidyl peptidase IV (DPP-4) cleavage, extending its biological half-life from approximately 7 minutes (native GHRH) to 26–38 minutes. Upon GHRH-R engagement, the signalling cascade proceeds:

Step	Molecular Event	Key Mediators
1	GHRH-R activation → Gs protein coupling	Gαs subunit
2	Adenylyl cyclase activation → cAMP elevation	cAMP, PKA
3	PKA phosphorylates CREB → GH gene transcription	CREB, Pit-1
4	GH synthesis and pulsatile exocytosis	GH secretory granules
5	Hepatic GH signalling → IGF-1 synthesis	JAK2–STAT5b, IGF-1
6	Somatostatin negative feedback (intact)	SRIF, SSTR2/5

2.2 Why Tesamorelin Rather Than rhGH

The original TRIIM trial used recombinant human GH (Norditropin, 0.015 mg/kg/day SC). Tesamorelin is a mechanistic substitution — not a replication of TRIIM. The pharmacological advantages of a GHRH analogue over direct rhGH:

Parameter	Tesamorelin	rhGH (TRIIM original)
Mechanism	GHRH analogue → pituitary pulse	Direct GH replacement
Pulsatility	✓ Physiological (somatostatin-gated)	✗ Lost (continuous elevation)
Feedback loop intact	✓ Yes (somatostatin brake)	✗ Bypassed
Direct TEC signalling (GHRH-R)	✓ Yes — TECs express GHRH-R	✗ No
IGF-1 overshoot risk	Low (feedback-limited ceiling)	Real; required dose titration in TRIIM
Diabetogenicity	Mild	Significant (metformin included in TRIIM to counteract)
Thymic endpoint evidence	None (mechanistic extrapolation from TRIIM)	✓ TRIIM demonstrated regeneration
Pituitary required?	✓ Yes (somatotroph reserve needed)	✗ No
Fahy's stated future direction	✓ Aligns (GHRH analogue class)	← Original choice

Key limitation: Clinical thymic regeneration data exist for rhGH (TRIIM). Tesamorelin has never been tested in a thymic endpoint RCT. The assumption that tesamorelin replicates these effects is mechanistically compelling but not yet validated at the thymic-endpoint level.

2.3 TRIIM Trial Summary

Parameter	Detail
Study design	Phase II open-label pilot; n=9 males aged 51–65; 12 months treatment
Interventions	rhGH 0.015 mg/kg/day SC + DHEA 50 mg/day + metformin 500 mg BID
Thymic MRI	Significant fat fraction reduction (p=0.02); thymic parenchyma visible in 7/9
sjTREC	Increased in 7/9 participants (mean +40%)
Epigenetic clock	Mean 2.5-year reversal (Horvath, Hannum); p<0.05
IGF-1	Target: upper quartile of young-adult reference range; monitored q3 months
Key limitation	n=9, no control arm, males only, single site, not blinded

SECTION 3

mTOR Signalling and Immunosenescence

3.1 mTOR Pathway Overview

mTOR is a serine/threonine kinase that integrates nutrient, growth-factor (including IGF-1), and energy signals to regulate cell growth, protein synthesis, and autophagy. It operates in two complexes: mTORC1 (rapamycin-sensitive; anabolism and cell growth) and mTORC2 (largely rapamycin-insensitive; Akt signalling and cytoskeletal organisation). Chronic mTORC1 hyperactivation — driven by persistent nutrient and growth-factor signals during ageing — promotes cellular senescence, suppresses autophagy, and drives terminal T-cell differentiation.

3.2 mTOR in the Thymic Microenvironment

In aged mice, rapamycin administered late in life increased thymic output measurably and partially reversed thymic involution. mTORC1 hyperactivation in thymic stromal cells promotes premature TEC senescence, reduces AIRE expression in mTECs, and impairs the cTEC/mTEC ratio. The pharmacological distinction critical for this protocol: continuous high-dose rapamycin produces immunosuppression; intermittent low-dose produces immune reconditioning.

3.3 The mTOR/IGF-1 Tension and Its Resolution

IGF-1, elevated by tesamorelin, activates PI3K–Akt–mTOR, creating a pharmacological tension: tesamorelin drives thymopoiesis via GHRH-R and IGF-1R on TECs, while rapamycin counters mTOR-driven TEC senescence. Resolution: rapamycin is dosed once weekly (Sunday), allowing IGF-1-driven thymopoietic signalling on the remaining 6 days while periodic mTORC1 suppression limits senescence accumulation. Tesamorelin’s feedback-limited IGF-1 ceiling makes this balance more controllable than uncapped rhGH.

SECTION 4

Component Pharmacology — Primary Agents

4.1 Tesamorelin (GHRH Analogue)

Full-length (44 AA) synthetic GHRH analogue with N-terminal trans-3-hexenoic acid modification conferring DPP-4 resistance (~26–38 min half-life vs ~7 min for native GHRH). FDA-approved as Egrifta (2010) and Egrifta SV (2025) for HIV-associated lipodystrophy. **For thymic rejuvenation use, tesamorelin acts both via the pituitary (GHRH-R → GH pulse → IGF-1) and directly on thymic epithelial cells (TECs express GHRH-R independently).**

Dosing: Individualised, IGF-1 Z-Score-Guided

> **Starting dose: 0.5 mg/day SC (non-HIV individuals).** The 2 mg/day dose validated in HIV-associated lipodystrophy trials is appropriate for a state of GH-axis blunting and peripheral GH resistance caused by HIV/antiretroviral therapy. Non-HIV individuals with intact GH-axis sensitivity will respond at substantially lower doses. Dose should be titrated based on IGF-1 z-score, not on a fixed-dose protocol. Target: IGF-1 z-score 0 to +1.5 (hard ceiling +2.0). Maximum dose if needed: 1.0–1.5 mg/day.

PK/PD Parameter	Value
Half-life (plasma)	26–38 minutes (DPP-4 resistant; native GHRH ~7 min)
Route	Subcutaneous injection, abdomen, daily (preferably evening)
Starting dose (non-HIV)	0.5 mg/day; assess IGF-1 z-score at 7 days
Titration ceiling	1.0–1.5 mg/day; never exceed IGF-1 z-score +2.0
HIV lipodystrophy dose	2 mg/day (NOT applicable to this protocol)
Time to IGF-1 steady state	5–7 days of consistent daily dosing
GHRH-R tachyphylaxis?	NO — Phase 3 data: 52-week continuous daily use shows no attenuation of efficacy or IGF-1 response
Somatostatin feedback	Intact — natural ceiling on IGF-1 elevation
Storage	Lyophilised vial; refrigerate; reconstitute with diluent; use within 24h

Phase 3 Evidence (HIV Lipodystrophy)

Falutz et al. (JCEM 2010) pooled LIPO-010 and CTR-1011: 2 mg/day for 52 consecutive weeks produced 15–19% VAT reduction at week 26, sustained/slightly increased at week 52. IGF-1 elevation maintained throughout. No tachyphylaxis or GHRH-R desensitisation observed at any timepoint. Upon discontinuation, VAT reaccumulated, confirming continued drug effect at week 52.

4.2 Thymosin α1 (Tα1)

A 28-amino-acid thymic peptide (synthetic: Zadaxin) with TLR2/TLR9 agonist activity and direct effects on thymic epithelial cell maturation and AIRE expression. Approved in >35 countries for hepatitis B/C, sepsis, and adjunctive oncology therapy. In vitro and murine data support TEC maturation; human thymic endpoint data are lacking.

Parameter	Value
Dose	1.6 mg SC twice weekly (Monday/Thursday)

Parameter	Value
Half-life	2 hours (plasma); tissue effects more prolonged
Mechanism	TLR2/TLR9 agonism; DC maturation; NK activation; AIRE upregulation in mTECs
Evidence tier	Extrapolation from infection/oncology trials; no thymic RCT
Safety	Excellent; no serious adverse events in large HBV/HCV trials

4.3 Rapamycin (Sirolimus)

Allosteric mTORC1 inhibitor via FKBP12–mTOR complex formation. FDA-approved as an immunosuppressant (organ transplant); repurposed here at intermittent low dose for senolytic and immuno-reconditioning effects. The Mannick et al. (2014, *Sci Transl Med*) everolimus study demonstrated enhanced influenza vaccine response and reduced PD-1+ exhausted T cells in healthy older adults.

Parameter	Value
Dose in this protocol	5 mg once weekly (Sunday)
Rationale for weekly dose	Avoids continuous immunosuppression; allows mTORC2 recovery; senescence clearance effect
Half-life	~62 hours (blood); reaches steady-state in ~6–12 weeks
Key risk	Wound healing impairment; metabolic dysregulation (LDL, triglycerides); myelosuppression at higher doses
Contraindications	Active serious infection; concurrent strong CYP3A4 inhibitors (azoles, macrolides)

4.4 Imeglimin (Twymeeg)

First-in-class glimin agent approved in Japan (2021) for type 2 diabetes. Acts via mitochondrial complex I modulation to enhance glucose-stimulated insulin secretion (GSIS) and reduce hepatic gluconeogenesis. Functions as the metabolic safety buffer for GH-axis insulin resistance (replacing metformin from the original TRIIM protocol).

Parameter	Value
Dose	1000 mg PO twice daily (with meals)
Mechanism	ETC complex I/III inhibition; mitochondrial ROS reduction; GSIS enhancement
GI tolerability	Favourable vs metformin; lower GI adverse-event rate in trials
Renal dosing	Reduce to 500 mg BID if eGFR 30–45; avoid <30
Role in protocol	Counteract GH-axis insulin resistance; replace metformin role from TRIIM

SECTION 5

Component Pharmacology — Daily Base Stack

Six components taken every day regardless of cycle phase, providing foundational TEC support, anti-inflammaging activity, and metabolic buffering throughout the protocol.

Component	Dose / Timing	Primary Rationale
DHEA	25–50 mg po, morning	Androgen receptor signalling on TECs; insulin sensitisation; original TRIIM component
Imeglimin (Twymeeg)	1000 mg po with breakfast + dinner	Mitochondrial GSIS enhancement; GH-axis insulin resistance buffer
Zinc + Copper	Zn 20 mg + Cu 2 mg, morning	Thymulin zinc-dependent cofactor; antioxidant
Vitamin D3 + K2	3000–5000 IU D3 + 150 µg K2, morning	VDR signalling on TECs; calcium homeostasis; immune regulation
Omega-3 FA	2–3 g EPA+DHA with meals	NF-κB suppression; membrane fluidity in aged immune cells
Melatonin	0.5–1 mg, 30 min before sleep	Thymus-protective antioxidant; GH pulse amplifier (nocturnal)

SECTION 6

Protocol Design — Cycle Structure and Interactions

6.1 4-Week Cycle Structure

Week	Tesamorelin	Thymosin α1	Rapamycin	Notes
1	0.5–1 mg/day (titrating)	1.6 mg Mon + Thu	5 mg Sunday	IGF-1 z-score drawn Day 7 — primary titration signal
2	Titrated dose	1.6 mg Mon + Thu	5 mg Sunday	Continue at confirmed dose
3	Titrated dose	1.6 mg Mon + Thu	5 mg Sunday	Continue; confirm no adverse effects
4 (clearance)	Off (rapamycin clearance)	—	—	Allows mTOR/GH tension reset; metabolic labs

The week-4 tesamorelin break is not driven by GHRH-receptor desensitisation concerns (Phase 3 data confirm no tachyphylaxis at 52 weeks continuous use). The break is a pharmacokinetic and safety decision: rapamycin (t½ ~62h) needs clearance before the next cycle, and the off-week provides a structured window for metabolic and safety monitoring.

6.2 Key Pharmacological Interactions

Combination	Interaction	Verdict
Tesamorelin + Imeglimin	Tesamorelin raises glucose (GH-IR); imeglimin GSIS directly compensatory	Synergistic
Tesamorelin + Rapamycin	IGF-1 drives mTOR; rapamycin limits downstream senescence — temporal separation resolves tension	Managed by timing
Thymosin α1 + Rapamycin	Tα1 promotes DC/NK activation; rapamycin at low weekly dose does not suppress DC function	Compatible
DHEA + Rapamycin	DHEA may modestly raise IGF-1; rapamycin modulates mTOR downstream — net effect small	No interaction
Rapamycin + CYP3A4 inhibitors	Azole antifungals, macrolides substantially raise rapamycin levels — avoid co-administration	CONTRAINDICATION

SECTION 7

Clinical Monitoring Framework

Monitoring serves three purposes: (1) dose titration, particularly tesamorelin based on IGF-1 z-score; (2) early detection of adverse effects; and (3) longitudinal tracking of protocol efficacy.

7.1 Pre-Treatment Baseline Assessment

Test	Rationale
IGF-1 (fasting) with age/sex z-score	Establish baseline GH-axis status; primary tesamorelin titration reference
Fasting glucose, fasting insulin, HbA1c	Baseline metabolic status; screen for pre-existing insulin resistance before GH-axis stimulation
CMP (comprehensive metabolic panel)	Renal function (eGFR for imeglimin dosing); hepatic function; electrolytes
Lipid panel (fasting)	Baseline prior to rapamycin, which may modestly raise LDL/TG
CBC with differential	Baseline immune cell counts; flag cytopenias before rapamycin
Testosterone + SHBG (males)	Sex hormone status; DHEA supplementation decision
DEXA body composition	Visceral fat and lean mass baseline; surrogate efficacy endpoint for tesamorelin
sjTREC level (if available)	Baseline thymic output measure; most direct efficacy biomarker
MRI thymus (optional)	Structural baseline; high cost; used in TRIIM

7.2 Week 1 — IGF-1 Response and Dose Titration

The week-1 IGF-1 draw is the most important early monitoring test in this protocol. Tesamorelin reaches IGF-1 steady state within 5–7 days of consistent daily dosing. A fasting IGF-1 measurement at day 7, interpreted as an age/sex-adjusted z-score, provides the primary titration signal before proceeding to week 2. Always request the age/sex-adjusted reference range or z-score from the laboratory (Quest Diagnostics and LabCorp both provide this). An absolute ng/mL value alone is insufficient.

IGF-1 Z-Score at Week 1	Interpretation	Action
< 0 (below age-adjusted midpoint)	Sub-therapeutic; somatotroph reserve may be lower than expected	Increase to 1.0 mg/day (if at 0.5 mg) or 1.5 mg/day (if at 1.0 mg); recheck at week 2
0 to +1.5 (target range)	Optimal therapeutic response within normal range	Maintain dose; next IGF-1 at end of cycle (week 4)
+1.5 to +2.0 (approaching ceiling)	Elevated but within reference range; approaching hard limit	Maintain dose; do not increase; recheck at week 4; consider 0.25 mg reduction
> +2.0 (above reference range)	Above normal range for age — reduce immediately	Reduce by 0.25–0.5 mg/day; recheck IGF-1 at day 14; do not proceed at current dose

7.3 Ongoing Monitoring Schedule

Timepoint	Tests	Purpose
Week 1 (day 7)	Fasting IGF-1 (z-score), fasting glucose	Tesamorelin dose titration; early metabolic safety
Week 4 (cycle 1 end)	IGF-1 (z-score), glucose, CBC, CMP, fasting lipids	Confirm dose stability; rapamycin metabolic signal
Each cycle end (monthly)	IGF-1 (z-score), glucose, HbA1c, CBC, CMP	Ongoing safety and efficacy surveillance
3 months (cycle 3)	Full baseline repeat + DEXA	Body composition change; visceral fat response
6 months (cycle 6)	Full panel + sjTRECs + optional epigenetic clock	First meaningful thymic output assessment
12 months	Full panel + sjTRECs + epigenetic clock + optional MRI thymus	Comprehensive efficacy assessment

7.4 Target Values

Biomarker	Target Range	Notes
IGF-1 z-score	0 to +1.5; ceiling +2.0	Always z-score adjusted for age/sex; never interpret absolute ng/mL alone
Fasting glucose	< 100 mg/dL (< 5.6 mmol/L)	GH mildly diabetogenic; monitor closely in first cycle
HbA1c	< 5.7%	Flag rising trend across cycles
LDL cholesterol	Maintain baseline or < 130 mg/dL	Rapamycin may modestly raise LDL
sjTREC level	Upward trend from baseline	Absolute values vary by lab; trend over time is the endpoint
Epigenetic age	Stable or decreasing vs chronological age	TRIIM demonstrated ~2.5-year mean reduction at 12 months
Visceral fat (DEXA)	Reduction from baseline	Expected within 3–6 months of tesamorelin; corroborates GH-axis response

SECTION 8

Evidence Quality and Limitations

8.1 Evidence Quality by Component

Component	Strongest Evidence	Evidence Tier
rhGH → thymic regeneration	TRIIM RCT (n=9); sjTREC + MRI endpoints	Phase II pilot (limited n)
Tesamorelin → GH pulse + IGF-1	Phase 3 RCTs (n=800+) in HIV lipodystrophy	High for GH/VAT; zero for thymic endpoint
Rapamycin → immune rejuvenation	Mannick 2014 (everolimus; n=218)	Phase IIb; surrogate endpoints
Thymosin α1 → TEC maturation	Murine + infection/oncology trials	Extrapolation; no thymic RCT
DHEA → thymic effects	Murine + small human studies	Low-moderate; TRIIM component
Imeglimin → GH-IR buffer	Phase 3 T2DM trials (Japan)	Mechanistic extrapolation to this role

8.2 Principal Limitations

Non-negotiable clinical caveat: This protocol is a mechanistic extrapolation from the TRIIM trial. No published RCT has tested this specific combination. The tesamorelin substitution for rhGH is scientifically reasoned but **unvalidated at the thymic-endpoint level.** Physician supervision with endocrinology and immunology input is required before initiating any component.

- TRIIM used rhGH (Norditropin) — tesamorelin is a substitution; thymic data do not transfer directly
- n=9 in TRIIM; results not validated in larger or female cohorts
- No direct comparator between tesamorelin dose levels and thymic output
- Rapamycin long-term safety profile at longevity doses in healthy individuals is not established
- Imeglimin not approved in the US or EU; access may require compassionate use or off-label import
- Thymosin α1 thymic endpoint evidence is preclinical only
- Tesamorelin response is pituitary-dependent: individuals with low somatotroph reserve may have attenuated response

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