Thymosin Beta-4 (TB-4 / TB-500)

A Clinical Learning Guide for Medical Providers

43-Amino Acid Actin-Sequestering Peptide • Tissue Repair & Regeneration • INN: Timbetasin

Evidence base at a glance: A repair/regeneration peptide built around actin dynamics, with the most extensive clinical-trial portfolio of any peptide removed from compounding (6 Phase I/II RCTs, N>400). Strongest human data: topical dry eye and wound healing. NOT FDA-approved, WADA-prohibited (S2), and removed from 503A/503B compounding. Critically, there is NO human subcutaneous PK data — despite SC being how it is used in practice.

1. Peptide Profile

Name: Thymosin Beta-4 (Tβ4); synthetic form marketed as TB-500
Classification: Endogenous actin-sequestering peptide — a tissue-repair/regeneration agent (not primarily an immune peptide, though it has NF-κB-mediated anti-inflammatory effects)
Structure: 43 amino acids (~4,963 Da); gene TMSB4X; WHO INN Timbetasin
Active fragment: Ac-SDKP (N-terminal tetrapeptide) — anti-fibrotic and anti-inflammatory, degraded by ACE
Regulatory status: NOT FDA-approved (orphan designation only, for neurotrophic keratopathy, 2013); investigational in China/Europe; removed from 503A/503B compounding (2023–2024)
Anti-doping: WADA-prohibited at all times (S2 category)
Half-life: ~0.5–2.1 hours IV (dose-proportional); no accumulation after 10–14 days
Immunogenicity: Very low — ~1% anti-drug antibody incidence, transient

A Naming Clarification

"TB-500" is simply the commercial name for synthetic Thymosin Beta-4 — functionally identical to Tβ4. Compounded products sometimes blur this labeling. The separately discussed Ac-SDKP is Tβ4's tetrapeptide metabolite, which is not independently regulated. This guide treats TB-4 and TB-500 as the same molecule.

2. Modes of Action & Mechanisms

Tβ4 sits at the intersection of many signaling cascades, with G-actin dynamics as the central node. The lecturer's analogy: think of a tank tread, where G-actin monomers are continuously laid down so the 'track' (the cell) can keep migrating forward. Ten pathways are validated; nine commonly claimed pathways have no direct evidence.

Primary Mechanism — G-Actin Sequestration

- Forms a 1:1 complex with G-actin, preventing spontaneous polymerization to F-actin; profilin-dependent dissociation then liberates monomers for filament assembly
- Conserved actin-binding motif (LKKTET, residues 17–22 — a WH2 domain); sequesters >50% of unpolymerized actin in PMNs
- This is the foundation for ALL its tissue-repair activity — cell migration, wound closure, angiogenesis (binds ILK in lamellipodia → Akt2 → MMP production → directed migration)

Validated Signaling Pathways

Pathway	Effect
PI3K / Akt / eNOS	Endothelial progenitor cell mobilization; ILK-PINCH-Parvin → cardiomyocyte survival
NF-κB suppression	Blocks IκB phosphorylation / p65 translocation → ↓ TNF-α, IL-1β, IL-6, IL-8, COX-2, iNOS
HIF-1α stabilization	Reciprocal regulation with Tβ4; supports mucosal barrier and collagen formation

Pathway	Effect
ErbB2 / Raf1	Reactivates ErbB → Raf1/ERK → suppresses Bad (cardiomyocyte protection)
TGF-β / SMAD	↓ TGF-β1, TGFβRII, Smad2/3 → attenuates fibrosis
Wnt / β-catenin	Activates Disheveled → β-catenin → hair-follicle morphogenesis
Notch / apoptosis / MAPK	↑ angiogenesis; ↑ Bcl-2, ↓ caspases; antioxidant (↑ SOD, catalase)

Mechanistic honesty (from the source 'Truth Protocol'): Tβ4 does NOT have confirmed direct activity on AMPK, mTOR, NAD/sirtuins, Nrf2, FOXO, insulin/IGF-1, JAK-STAT, cAMP/PKA, Hedgehog, or PPAR. Effects on mTOR/FOXO are theoretical via Akt only. An identified receptor is ATP synthase β-subunit → P2X4 purinergic signaling.

3. Points of Clinical Relevance

1. **It is fundamentally a repair peptide — actin is the single best node**
   Unlike the other immunomodulators in this series, Tβ4's defining action is regulating actin dynamics to drive cell migration and tissue rebuilding. Its anti-inflammatory effects (via NF-κB) are real but secondary. Frame it for patients as a recovery/repair enhancer, with immune-calming as a bonus rather than the primary purpose.
2. **The regulatory and anti-doping status is a hard practical reality**
   Tβ4/TB-500 is NOT FDA-approved, is WADA-prohibited at all times (S2), and was removed from 503A/503B compounding in 2023–2024. Any athlete subject to testing must avoid it entirely, and clinicians should be explicit that sourcing now sits outside the regulated compounding pathway.
3. **There is NO subcutaneous PK data — yet SC is how it is actually used**
   Every Phase I/II trial used IV or topical routes. Subcutaneous bioavailability, dosing, and kinetics have never been established in humans, so the common SC regimens are anecdotal extrapolations from IV data. This is the single most important gap to disclose.
4. **Best human evidence is topical (dry eye, wound healing), not systemic**
   The strongest, cleanest data are ophthalmic: RGN-259 reduced corneal staining and dry-eye symptoms with zero adverse events, and is in/near Phase III for neurotrophic keratopathy. Topical gel accelerated chronic wound healing by ~1 month in responders. Systemic regenerative claims are far less substantiated.
5. **Cardiac regeneration is promising preclinically but unproven in humans**
   Epicardial progenitor mobilization is robust in mice, and a 2025 Phase IIb STEMI trial showed infarct reduction in a subgroup (within 8h post-PCI) — but the intention-to-treat result was not significant. Cardiac regeneration in humans cannot yet be confirmed; larger, time-sensitive trials are the next step.
6. **The Ac-SDKP metabolite links Tβ4 to ACE inhibitors**
   Tβ4's active fragment Ac-SDKP is degraded by ACE, so ACE inhibitors elevate endogenous Ac-SDKP — a plausible synergistic anti-fibrotic interaction worth noting in patients already on an ACE-I. Conversely, as a pro-angiogenic agent, Tβ4 may counteract anti-VEGF/anti-angiogenic therapies.
7. **Angiogenic potential makes active malignancy a theoretical contraindication**
   TMSB4X is overexpressed in some tumors, and Tβ4 is pro-angiogenic — so active malignancy is a theoretical contraindication. Importantly, causation vs. correlation is unestablished, and angiogenic potential alone does not make it cancer-promoting; this requires individualized caution and cancer-screening context, not blanket alarm.

4. General Dosing & Delivery Options

Trial-derived dosing exists only for IV and topical routes. ALL subcutaneous dosing is anecdotal, with no human PK to support it. Oral use is expected to fail due to GI degradation. Presented for educational context only.

Published Trial Protocols

Setting	Dose	Route	Schedule
Phase I (synthetic)	42–1,260 mg	IV	Single, then daily × 14d
Phase I (rh-Tβ4)	0.05–25 μg/kg	IV	Single, then daily × 10d
Phase IIb STEMI	0.5–1.0 μg/kg	IV	Daily × 7d post-PCI
Dry eye (RGN-259)	0.1%	Topical	6× daily × 28d
Wound healing	0.01–0.1% gel	Topical	Daily × 84d
SC (anecdotal only)	~2.5 mg 2×/wk	SC	Loading → weekly maintenance

Evolving Off-Label (Anecdotal) Practice

In off-label use for wound healing and injury recovery, practice typically employs the subcutaneous route at under ~1 mg/day — commonly 200–300 μg two to three times daily, given either at the injury/wound site or systemically. Dosing cycles run roughly 6–12 weeks and may be cycled depending on the injury. Again, none of this is supported by human SC pharmacokinetic data.

Pharmacokinetics (IV) & Metabolism

- t½ ~0.5–2.1 h (dose-dependent); dose-proportional Cmax/AUC; no accumulation after 10–14 days
- Metabolism: N-terminal Ac-SDKP cleaved by prolyl oligopeptidase, then degraded by ACE
- Anti-drug antibody incidence ~1%, transient; brain peak ~40 min post-IP (mouse)

5. Evidence Profile

Human RCTs

Study	Design / N	Key Outcome
Ruff 2010	Phase I RCT, IV, N=40	No DLTs/SAEs; t½ 0.95–2.1 h
Wang 2021	Phase I RCT, IV, N=54	Dose-proportional PK; ADA ~1%
Sosne 2015 (a)	Phase II RCT, topical, N=72	Corneal staining ↓ 27% (P=0.008)
Sosne 2015 (b)	Phase II RCT, topical, N=15	Dry-eye symptom score ↓ 35% (P=0.014)
Zhang 2025	Phase IIb RCT, IV, N=96	Subgroup infarct ↓ (P=0.016); ITT not significant

What Can Be Confirmed

- **Safety:** IV Tβ4 well tolerated up to 1,260 mg × 14d (N≈94 Phase I); no dose-limiting toxicities or SAEs
- **Pharmacokinetics:** IV t½ 0.5–2.1 h, dose-proportional, no accumulation
- **Dry eye:** topical RGN-259 reduces corneal staining and discomfort vs placebo, with zero AEs
- **Wound healing:** topical gel accelerated chronic healing by ~1 month in responders
- **STEMI subgroup:** rh-Tβ4 within 8h post-PCI reduced infarct at 90d (subgroup); ITT not significant

Preclinical Promise (not yet human-confirmed)

- Cardiac regeneration via epicardial progenitor mobilization (mice)
- Neuroprotection/neurorestoration in TBI/stroke (rodent only)
- Anti-fibrosis; chronic granulomatous disease repair via HIF-1α (preclinical)

Critical gaps (verbatim from the source 'Truth Protocol' framing): cannot confirm SC bioavailability or SC dosing; cannot confirm long-term safety beyond 14 days IV; cannot confirm oral bioavailability; no formal drug-interaction studies; cannot confirm direct activation of AMPK/mTOR/NAD/sirtuins/etc.; cannot confirm cancer promotion (overexpression seen in tumors — causality unestablished); cannot confirm human cardiac regeneration or neuroprotection.

6. Clinical Considerations

Contraindications (Theoretical)

- **Active malignancy:** pro-angiogenic potential + TMSB4X tumor overexpression — avoid pending individualized assessment (not clinically validated as a contraindication)
- **Pregnancy & lactation:** excluded from all trials; no safety data — avoid
- **WADA-tested athletes:** prohibited at all times (S2) — absolute for competitive athletes

Drug Interactions (no formal studies)

- **ACE inhibitors:** elevate the Ac-SDKP metabolite — potential synergistic anti-fibrotic effect
- **Anti-VEGF / anti-angiogenic agents:** Tβ4 is pro-angiogenic and may counteract them — avoid the combination
- **Anticoagulants:** Tβ4 is present in platelets; no clinical interaction data

Monitoring Parameters

- CBC with differential; comprehensive metabolic panel
- SCC antigen (transient elevation noted in a Phase I recombinant study)
- ECG (transient changes noted in Phase I); cancer-screening context given angiogenic properties
- Downstream inflammatory markers (IL-1β, IL-6, TNF-α, CRP, ESR) where an anti-inflammatory effect is the goal — no universal validated biomarker exists

Safety Profile

- Across N>400 combined Phase I/II subjects: no dose-limiting toxicities, no serious adverse events; AEs mild/moderate
- Zero adverse events in the ophthalmic (dry eye) trials; ~1% transient anti-drug antibodies; long-term human data absent

Sourcing & Regulatory Status

Tβ4/TB-500 is not FDA-approved (orphan designation only for neurotrophic keratopathy), is WADA-prohibited, and has been removed from 503A/503B compounding. With manufacturing only GMP-standardized for trials, product quality and sourcing are genuine clinical variables that should be discussed with patients under explicit informed consent.

7. Final Note

Thymosin Beta-4 is the repair specialist of this series — a peptide whose entire biology radiates from a single elegant node, the sequestration of G-actin, which underwrites cell migration, wound closure, angiogenesis, and tissue rebuilding, with NF-κB-mediated anti-inflammation as a meaningful secondary effect. Among regenerative peptides removed from compounding, it has the most developed clinical-trial portfolio, and its safety signal across N>400 subjects is reassuring.

The honest framing, which the source lecture itself models carefully, is a sharp split between mechanism and proof. The confirmed wins are narrow and largely topical — dry eye and surface wound healing — while the headline systemic promises (cardiac regeneration, neuroprotection, anti-fibrosis) remain preclinical or subgroup-only. Two practical realities dominate any clinical conversation: there is no human subcutaneous pharmacokinetic data despite SC being the de facto route of use, and the agent is WADA-banned and outside the regulated compounding pathway. Tβ4 is best understood as a

mechanistically compelling repair peptide with strong topical evidence, promising-but-unproven systemic potential, and significant regulatory and data gaps that demand candor and careful informed consent.

Bottom line: A repair/regeneration peptide anchored in actin dynamics, with the strongest trial portfolio among compounding-removed peptides — but confirmed benefit is essentially topical (dry eye, wound healing), systemic claims are unproven, there is NO human SC pharmacokinetic data, and it is WADA-banned and removed from compounding. Investigational/off-label only, with explicit informed consent.

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