

RESEARCH MONOGRAPH . COMPOUND PROFILE

TB-500 Fragment

A synthetic N-acetylated heptapeptide (Ac-LKKTETQ) derived from the central actin-binding domain of thymosin beta-4, investigated for tissue repair, angiogenesis, anti-inflammatory activity, and wound healing through modulation of actin polymerization dynamics and cellular migration.

DOCUMENT	KDC-MN-1451
ISSUED	2026-05-11
REVISION	1.0
AUTHOR	Kodi <i>ac</i> bio <i>l</i> abs Research
COMPOUND CLASS	Synthetic heptapeptide fragment of thymosin beta-4 encompassing the actin-binding domain (residues 17-23) with N-terminal acetylation

RESEARCH USE ONLY

ABSTRACT

TB-500 Fragment (Ac-LKKTETQ) is a synthetic heptapeptide corresponding to residues 17 through 23 of the endogenous 43-amino acid polypeptide thymosin beta-4, a ubiquitous intracellular G-actin sequestering protein that participates in cytoskeletal organization, cell migration, angiogenesis, and tissue repair. The fragment encompasses the central actin-binding domain of the parent molecule and is N-terminally acetylated to confer resistance to aminopeptidase degradation and to replicate the post-translational modification present on native thymosin beta-4. TB-500 Fragment has been the subject of substantial preclinical investigation since the early 2000s, when Philp et al. (2003) demonstrated that the synthetic LKKTETQ heptapeptide promoted dermal wound repair in db/db diabetic mice and in aged mice at efficacy levels comparable to the full-length thymosin beta-4 molecule, establishing the actin-binding domain as a sufficient pharmacophore for the tissue repair activity of the parent protein. The principal molecular mechanism of TB-500 Fragment is sequestration of G-actin monomers and modulation of actin polymerization dynamics, which promotes cell migration, endothelial tube formation, keratinocyte mobilization, and extracellular matrix remodeling. Downstream signaling involves activation of integrin-linked kinase (ILK), phosphorylation of Akt/protein kinase B, suppression of NF-kappaB-mediated proinflammatory cytokine release, and upregulation of matrix metalloproteinases that facilitate tissue remodeling during wound repair. The pharmacological profile of TB-500 Fragment is therefore characterized by four principal activities: promotion of angiogenesis, acceleration of wound healing, anti-inflammatory

modulation, and facilitation of stem and progenitor cell migration. Preclinical evidence in rodent models has demonstrated accelerated dermal wound closure in diabetic and aged animals, cardioprotection following experimental myocardial infarction (demonstrated with the parent thymosin beta-4 molecule in the landmark Bock-Marquette et al. 2004 Nature study), promotion of hair follicle stem cell activation and hair growth (Philp et al. 2004), and anti-inflammatory activity through suppression of NF-kappaB nuclear translocation and reduction of TNF-alpha and IL-1-beta production. Human clinical data for the heptapeptide fragment itself are limited; the majority of clinical evidence derives from the full-length thymosin beta-4 molecule, which has been evaluated in Phase 2 and Phase 3 clinical trials for dry eye disease (RGN-259, RegeneRx Biopharmaceuticals) and in Phase 2 studies for neurotrophic keratopathy. The Phase 2 dry eye trial demonstrated statistically significant improvements in ocular discomfort (35.1 percent reduction) and corneal fluorescein staining (59.1 percent reduction) relative to vehicle control. The Phase 3 ARISE program in dry eye disease did not meet co-primary endpoints, though statistically significant improvement in ocular grittiness was observed. TB-500 Fragment is not approved by any regulatory authority for human therapeutic use. It is classified as a prohibited substance by the World Anti-Doping Agency under Sections S0 (Non-Approved Substances) and S2 (Peptide Hormones, Growth Factors, Related Substances, and Mimetics) and is prohibited in equine racing competition by multiple national racing authorities. Pharmacokinetic data from preclinical studies indicate a plasma elimination half-life of approximately 1.5 to 3 hours following subcutaneous administration, with subcutaneous bioavailability of approximately 60 to 80 percent relative to intravenous dosing;

tissue-level effects persist substantially longer than plasma residence, consistent with the intracellular mechanism of action. The compound is supplied as a lyophilized powder and is reconstituted in bacteriostatic water for injection; it is stable at refrigerated conditions for up to 30 days after reconstitution. This monograph reviews the chemistry, synthesis, and structural characterization of TB-500 Fragment; the molecular pharmacology of actin sequestration, ILK activation, and NF-kappaB suppression; the pharmacokinetic profile; the preclinical evidence base across wound healing, cardiac repair, hair growth, and anti-inflammatory models; the clinical evidence derived from the parent thymosin beta-4 molecule; sourcing and quality verification; reconstitution and handling; stack interaction considerations; adverse event and safety signal assessment; and a comparative evaluation of five alternative tissue repair and regenerative peptides against TB-500 Fragment on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Common name: TB-500 Fragment.

Systematic peptide name: Ac-LKKTETQ (N-acetyl-L-leucyl-L-lysyl-L-lysyl-L-threonyl-L-glutamyl-L-threonyl-L-glutamine).

Alternative designations: TB-500, TB-500 Fragment (17-23), Thymosin Beta-4 Fragment 17-23, Ac-LKKTETQ, TB4(17-23). The designation "TB-500" originated in the veterinary research literature, where it was used to identify a commercial preparation of the synthetic heptapeptide fragment of thymosin beta-4 for equine tissue repair research [1]. The "Fragment (17-23)" suffix specifies the amino acid positions within the parent 43-residue thymosin beta-4 sequence.

Parent compound: Thymosin beta-4 (Tbeta4, TB4, Timbetasin). The parent molecule is a 43-amino acid polypeptide (molecular weight 4,982 Da) encoded by the TMSB4X gene on the X chromosome in humans [2]. TB-500 Fragment corresponds to the central actin-binding domain of this parent molecule.

Chemical Abstracts Service registry number: 77591-33-4 (Ac-LKKTETQ). The CAS registry number identifies the N-acetylated heptapeptide form that is the standard commercial and research preparation.

Molecular formula: C₃₆H₆₆N₁₀O₁₃ (some sources report C₃₈H₆₈N₁₀O₁₄ depending on the inclusion of bound water or salt counterions in the molecular formula calculation).

Molecular weight: 846.97 g/mol (free peptide, monoisotopic calculation based on C₃₆H₆₆N₁₀O₁₃). Alternative reported values of approximately 889 g/mol in some commercial specifications reflect the acetate salt form or minor calculation differences in the inclusion of counterions.

1.2 Structural class and chemistry

TB-500 Fragment is a linear heptapeptide belonging to the thymosin beta family of actin-binding peptides. The structural class is that of small bioactive peptide fragments derived from larger endogenous regulatory proteins. The peptide contains no disulfide bonds, no cyclic motifs, and no unusual amino acids; all seven residues are proteinogenic L-amino acids in standard peptide bond linkage [3].

Amino acid sequence: Ac-Leu-Lys-Lys-Thr-Glu-Thr-Gln (Ac-LKKTETQ). The N-terminal acetyl group (CH₃CO-) is a deliberate synthetic modification that replicates the N-alpha-acetylation present on the native full-length thymosin beta-4 molecule and confers resistance to aminopeptidase-mediated degradation [4].

Physical properties: White to off-white lyophilized powder. Freely soluble in water and in aqueous buffers at physiological pH. Soluble in dimethyl sulfoxide. The peptide is hygroscopic and should be stored under desiccated conditions prior to reconstitution. The isoelectric point is approximately 9.5, reflecting the two lysine residues (positively charged at physiological pH) and one glutamic acid residue (negatively charged).

Structural features: The LKKTETQ sequence constitutes the minimal actin-binding domain of thymosin beta-4, as defined by deletion mutagenesis and synthetic peptide activity studies [5, 6]. The two lysine residues at positions 2 and 3 (corresponding to positions 18 and 19 of the parent molecule) are critical for electrostatic interaction with the negatively charged surface of G-actin monomers. The threonine and glutamine residues contribute hydrogen bonding contacts that stabilize the peptide-actin complex.

1.3 Synthesis

TB-500 Fragment is manufactured by standard solid-phase peptide synthesis (SPPS) using Fmoc (9-fluorenylmethoxycarbonyl) chemistry on a resin support [7]. The synthesis proceeds from the C-terminal glutamine residue through sequential coupling of the seven amino acids with standard coupling reagents (HBTU, HATU, or DIC/Oxyma). N-terminal acetylation is performed on-resin using acetic anhydride or acetyl chloride prior to cleavage from the resin support. Cleavage and side-chain deprotection are achieved with trifluoroacetic acid-based cocktails (typically 95 percent TFA, 2.5 percent triisopropylsilane, 2.5 percent water). The crude peptide is purified by reverse-phase high-performance liquid chromatography (RP-HPLC) on

C18 columns with acetonitrile/water gradient elution. The purified product is lyophilized to a dry powder. Typical synthesis yields for a heptapeptide of this composition are 70 to 90 percent crude and 50 to 70 percent after HPLC purification to greater than 98 percent purity. The synthesis is straightforward relative to longer or more structurally complex peptides, and multiple contract research organizations and commercial peptide suppliers produce TB-500 Fragment at research grade. Industrial-scale synthesis for larger quantities follows the same SPPS methodology with process optimization for coupling efficiency and purification throughput.

2. Abstract

TB-500 Fragment (Ac-LKKTETQ) is a synthetic N-acetylated heptapeptide that reproduces the central actin-binding domain (residues 17 through 23) of the endogenous 43-amino acid polypeptide thymosin beta-4, a ubiquitous intracellular protein that serves as the principal G-actin sequestering factor in mammalian cells and participates in the regulation of actin polymerization, cell migration, angiogenesis, wound healing, and inflammatory signaling [2, 3]. The fragment was identified as a sufficient pharmacophore for the tissue repair activity of the parent molecule through structure-activity studies beginning in the late 1990s, with the definitive demonstration by Philp et al. (2003) that the synthetic LKKTETQ peptide accelerated dermal wound repair in db/db diabetic mice and in aged mice at efficacy comparable to full-length thymosin beta-4 [8].

The molecular pharmacology of TB-500 Fragment centers on its interaction with G-actin monomers. By sequestering monomeric G-actin and preventing premature polymerization into F-actin filaments, the peptide maintains a dynamic pool of actin available for rapid cytoskeletal reorganization during cell migration, a process essential for wound healing, angiogenesis, and inflammatory cell trafficking [5, 6]. Downstream of the actin-binding event, TB-500 Fragment activates integrin-linked kinase (ILK), which in turn phosphorylates Akt/protein kinase B and promotes cell survival signaling [9]. The peptide also suppresses NF-kappaB nuclear translocation and reduces the production of proinflammatory cytokines including TNF-alpha and IL-1-beta, establishing an anti-inflammatory axis that is independent of, though mechanistically linked to, the actin cytoskeletal effects [10, 11].

Preclinical pharmacology encompasses four principal domains. First, wound healing: topical and systemic administration of thymosin beta-4 and the TB-500 Fragment has produced accelerated dermal wound closure, increased collagen deposition, enhanced re-epithelialization, and stimulation of keratinocyte migration in multiple rodent models including full-thickness excisional wounds, diabetic wounds, and aged-animal wounds [8, 12, 13]. Second, cardiac repair: the parent thymosin beta-4 molecule was demonstrated by Bock-Marquette et al. (2004) in *Nature* to activate ILK and promote cardiac cell migration, survival, and functional recovery following experimental myocardial infarction in mice [9]. Third, angiogenesis:

the LKKTETQ actin-binding domain was shown by Grant et al. (2003) to be both necessary and sufficient for the angiogenic activity of thymosin beta-4 in in vitro endothelial tube formation assays and in in vivo Matrigel plug assays [5]. Fourth, hair growth: Philp et al. (2004) demonstrated that thymosin beta-4 increases hair growth by activation of hair follicle stem cells through promotion of stem cell migration, differentiation, and matrix metalloproteinase-2 expression [14].

Human clinical data for the TB-500 Fragment heptapeptide per se are sparse. The clinical evidence base derives principally from the full-length thymosin beta-4 molecule administered as the topical ophthalmic formulation RGN-259 (RegeneRx Biopharmaceuticals). A Phase 2 randomized, double-masked, placebo-controlled trial in severe dry eye disease demonstrated statistically significant improvements in ocular discomfort and corneal staining with RGN-259 relative to vehicle control [15]. A Phase 3 program (ARISE trials) in over 1,600 patients did not meet co-primary endpoints, though significant improvement in ocular grittiness was observed [16]. A Phase 3 trial of RGN-259 in neurotrophic keratopathy demonstrated promotion of epithelial defect healing [17]. No human registration trial has been completed with the isolated TB-500 Fragment heptapeptide.

Pharmacokinetically, TB-500 Fragment exhibits a plasma elimination half-life of approximately 1.5 to 3 hours following subcutaneous administration in preclinical models, with subcutaneous bioavailability of approximately 60 to 80 percent relative to intravenous dosing [18]. The compound distributes widely to tissues with high metabolic activity. Tissue-level pharmacological effects persist for days to weeks after administration, substantially longer than the plasma residence time, consistent with intracellular accumulation and the sustained nature of the actin-binding and ILK-mediated signaling mechanisms.

TB-500 Fragment is not approved by any regulatory authority for human therapeutic use. It is classified as a prohibited substance by the World Anti-Doping Agency and is prohibited in equine racing competition [19, 20]. The compound is supplied as a research-grade lyophilized powder by multiple commercial peptide suppliers. Investigators should obtain analytical confirmation of identity, purity, and peptide content on every lot.

3. Discovery and Development History

3.1 Thymosin discovery and thymosin beta-4 isolation

The discovery of the thymosin peptide family originated in the laboratory of Allan Goldstein at the Albert Einstein College of Medicine in the 1960s. Goldstein and colleagues isolated a lymphocytopoietic factor from calf thymus tissue and designated it thymosin, initially hypothesizing that it was a single polypeptide hormone of the thymus gland involved in T-cell maturation and immune function [21]. Further purification through the 1970s led to the preparation designated thymosin fraction 5, a complex mixture of more than 30 polypeptides with molecular weights ranging from 1,000 to 15,000 Da [22].

Thymosin beta-4 was isolated and characterized as a discrete molecular entity from thymosin fraction 5 by Low, Goldstein, and colleagues in 1981, who determined its complete 43-amino acid sequence and its molecular weight of 4,982 Da [23]. The peptide was initially studied in the context of thymic immunology, as it was shown to induce terminal deoxynucleotidyl transferase activity in thymocyte populations, a marker of T-cell differentiation.

3.2 Identification as an actin-binding protein

The understanding of thymosin beta-4 function was fundamentally transformed in the early 1990s when Safer, Hatch, and colleagues demonstrated that the peptide was the principal G-actin sequestering molecule in mammalian platelets and cells [24]. In 1992, Sanders, Goldstein, and Wang published work demonstrating that thymosin beta-4 was a potent regulator of actin polymerization dynamics in living cells [25]. These findings repositioned the peptide from a thymic hormone to a ubiquitous intracellular cytoskeletal regulatory protein with expression in virtually all nucleated mammalian cell types.

3.3 Wound healing and tissue repair discovery

The tissue repair properties of thymosin beta-4 were discovered by Malinda, Goldstein, and Kleinman and reported in 1999 [12]. Topical or intraperitoneal administration of thymosin beta-4 to rats with full-thickness dermal wounds increased re-epithelialization by approximately 42 percent over controls at 4 days and by 61 percent at 7 days. Treated wounds showed increased collagen deposition and angiogenesis, and thymosin beta-4 stimulated keratinocyte migration at concentrations as low as 10 picograms per milliliter.

3.4 Identification of the actin-binding domain as the active pharmacophore

The critical demonstration that the central actin-binding domain (residues 17 through 23, LKKTETQ) was sufficient for the tissue repair and angiogenic activity of thymosin beta-4 was provided by two studies in 2003. Grant et al. (2003) demonstrated that a synthetic peptide containing the LKKTETQ actin-binding motif promoted angiogenesis in endothelial tube formation assays and in in vivo Matrigel plug models at efficacy comparable to the full-length molecule [5]. Philp et al. (2003) demonstrated that the same synthetic peptide promoted dermal wound repair in db/db diabetic mice and in aged mice with efficacy comparable to full-length thymosin beta-4 [8]. These two studies established the scientific basis for the use of the TB-500 Fragment as a discrete pharmacological agent.

3.5 Cardiac repair and the Nature landmark

In 2004, Bock-Marquette et al. published a landmark study in Nature demonstrating that thymosin beta-4 activated integrin-linked kinase and promoted cardiac cell migration, cardiomyocyte survival, and functional cardiac recovery following experimental myocardial infarction in mice [9]. Treatment with thymosin beta-4 after permanent coronary artery ligation reduced infarct scar volume and preserved cardiac function. This study substantially expanded the research interest in thymosin beta-4 and its derivatives beyond wound healing into cardiovascular repair.

3.6 Veterinary and anti-doping history

TB-500 entered the veterinary research marketplace in the mid-2000s as a synthetic preparation of the Ac-LKKTETQ fragment for equine musculoskeletal injury research. The compound gained attention in thoroughbred racing as a potential performance-enhancing peptide and was subsequently prohibited by multiple national racing authorities. Toutain et al. (2012) developed liquid chromatography-mass spectrometry methods for the detection of TB-500 and its metabolites in equine urine and plasma, establishing the analytical framework for doping control [20]. The World Anti-Doping Agency added thymosin beta-4 and its derivatives to the Prohibited List, where they remain classified under Sections S0 and S2 [19].

3.7 Clinical development of the parent molecule

RegeneRx Biopharmaceuticals advanced the full-length thymosin beta-4 molecule as the topical ophthalmic formulation RGN-259 through Phase 2 and Phase 3 clinical trials for dry eye disease and neurotrophic keratopathy. The Phase 2 dry eye trial (Dunn et al. 2010) demonstrated statistically significant improvements in ocular discomfort and corneal staining [15]. The Phase 3 ARISE program in over 1,600 dry eye patients did not meet co-primary endpoints, though statistically significant improvement in the secondary endpoint of ocular grittiness was observed [16]. A Phase 3 trial in neurotrophic keratopathy demonstrated promotion of epithelial defect healing and received positive attention as a potential treatment for this orphan indication [17]. As of the most recent monograph revision, RGN-259 has not received regulatory approval in any jurisdiction. No clinical development program using the isolated TB-500 Fragment heptapeptide has been registered.

4. Mechanism of Action

TB-500 Fragment exerts its pharmacological effects through a multi-step molecular mechanism that begins with G-actin sequestration and extends through integrin-linked kinase activation, Akt-mediated survival signaling, NF-kappaB suppression, and modulation of extracellular matrix remodeling. These activities are interdependent and collectively produce the observed tissue repair, angiogenic, anti-inflammatory, and cytoprotective phenotypes.

4.1 G-actin sequestration and cytoskeletal dynamics

The primary molecular target of TB-500 Fragment is monomeric globular actin (G-actin). Thymosin beta-4 is the most abundant G-actin sequestering peptide in mammalian cells, maintaining a large pool of unpolymerized actin monomers available for rapid deployment during cytoskeletal reorganization [24, 25]. The LKKTETQ sequence within TB-500 Fragment constitutes the minimal binding domain for this interaction. The two positively charged lysine residues at positions 18 and 19 of the parent sequence form electrostatic contacts with the negatively charged surface of G-actin, while the threonine and glutamine residues stabilize the complex through hydrogen bonding [5, 6].

By binding and sequestering G-actin, TB-500 Fragment prevents premature polymerization of actin filaments and maintains cytoplasmic actin in a dynamic, readily mobilizable state. When cellular signals trigger migration (chemokines, growth factors, injury cues), the peptide-actin complex can rapidly release monomers for polarized actin polymerization at the leading edge of migrating cells. This mechanism is essential for the directed cell migration that underlies wound healing, angiogenesis, and inflammatory cell trafficking.

4.2 Integrin-linked kinase activation and Akt signaling

Bock-Marquette et al. (2004) demonstrated that thymosin beta-4 activates integrin-linked kinase (ILK) in cardiac and endothelial cells [9]. ILK is a serine/threonine kinase that functions as a scaffold protein at focal adhesion complexes and is a critical node in cell survival and migration signaling. Thymosin beta-4-mediated ILK

activation leads to phosphorylation of Akt/protein kinase B at serine 473, activating the Akt survival pathway. Downstream consequences include inhibition of pro-apoptotic factors (Bad, caspase-9), activation of endothelial nitric oxide synthase (eNOS), and promotion of cell survival under ischemic and inflammatory stress.

The ILK-Akt axis is the principal molecular basis for the cardioprotective activity observed in the myocardial infarction model and for the anti-apoptotic effects observed in endothelial cells, keratinocytes, and corneal epithelial cells exposed to thymosin beta-4 or TB-500 Fragment.

4.3 NF-kappaB suppression and anti-inflammatory activity

Thymosin beta-4 inhibits NF-kappaB nuclear translocation through a mechanism involving its intracellular binding partners PINCH-1 and ILK [10]. Sosne et al. (2007) demonstrated in corneal epithelial cells that thymosin beta-4 treatment significantly decreased nuclear NF-kappaB protein levels, NF-kappaB DNA-binding activity, and p65 subunit phosphorylation following TNF-alpha stimulation [11]. The downstream consequence is reduced transcription of NF-kappaB-dependent proinflammatory genes, including TNF-alpha, IL-1-beta, IL-6, IL-8, and multiple chemokines.

The anti-inflammatory mechanism operates at two levels: direct suppression of NF-kappaB-mediated cytokine production in resident tissue cells (epithelial cells, fibroblasts, cardiomyocytes) and reduction of inflammatory cell recruitment through decreased chemokine expression. This dual anti-inflammatory activity is a significant contributor to the tissue repair phenotype, as excessive or prolonged inflammation impairs wound healing and promotes fibrosis.

4.4 Angiogenic signaling

The angiogenic activity of TB-500 Fragment involves promotion of endothelial cell migration, tube formation, and sprouting through the actin-dependent migration mechanism described above [5]. Additional downstream mechanisms include up-regulation of vascular endothelial growth factor (VEGF) expression, promotion of endothelial progenitor cell mobilization, and facilitation of extracellular matrix remodeling through matrix metalloproteinase induction [26]. Grant et al. (2003)

demonstrated that a seven-amino acid synthetic peptide containing the LKKTETQ actin-binding motif was both necessary and sufficient for the angiogenic activity of thymosin beta-4 in Matrigel-based in vitro and in vivo assays [5].

4.5 Extracellular matrix remodeling

TB-500 Fragment promotes the expression and secretion of matrix metalloproteinase-2 (MMP-2) and other matrix metalloproteinases in keratinocytes, fibroblasts, and endothelial cells [14, 27]. MMP-mediated degradation of the provisional extracellular matrix is a required step in wound healing, angiogenic sprouting, and stem cell migration through tissue. The peptide also promotes the synthesis of laminin-332, an important adhesion and migration factor for epithelial cells, and modulates collagen deposition during the remodeling phase of wound healing [13].

4.6 Composite mechanism summary

The composite pharmacology of TB-500 Fragment at the molecular level is therefore a coordinated program of cytoskeletal mobilization (G-actin sequestration), cell survival enhancement (ILK-Akt axis), inflammatory suppression (NF-kappaB pathway), vascular formation (angiogenic signaling), and tissue remodeling (MMP induction and laminin synthesis). These mechanisms operate in concert to produce the observed acceleration of wound healing, cardioprotection, hair follicle activation, and anti-inflammatory effects. The fragment retains the core actin-binding pharmacophore of the parent thymosin beta-4 molecule and reproduces the principal tissue repair activities, though it lacks the additional functional domains present in the full-length 43-amino acid sequence (including the N-terminal Ac-SDKP tetrapeptide, which has independent anti-fibrotic and anti-proliferative activities mediated through distinct receptor pathways) [28].

5. Pharmacokinetics and Routes of Administration

5.1 Absorption

TB-500 Fragment is administered parenterally in preclinical and research settings, most commonly by subcutaneous injection. The small molecular weight (approximately 847 Da) and high aqueous solubility of the heptapeptide facilitate rapid absorption from the subcutaneous depot. Preclinical pharmacokinetic studies in rodent and equine models indicate time to peak plasma concentration (T_{max}) of approximately 0.5 to 1 hour following subcutaneous administration [18, 20]. Subcutaneous bioavailability relative to intravenous administration is approximately 60 to 80 percent, reflecting modest first-pass degradation by tissue peptidases at the injection site.

Oral bioavailability is low (estimated at 15 to 25 percent), as the peptide is subject to gastric acid hydrolysis and intestinal peptidase degradation. The N-terminal acetylation provides partial protection against aminopeptidase activity but does not confer sufficient stability for reliable oral delivery. Intravenous administration produces immediate peak concentrations and is the reference route for bioavailability calculations.

5.2 Distribution

TB-500 Fragment distributes widely following systemic absorption. The small molecular weight and absence of disulfide bonds or complex tertiary structure facilitate tissue penetration. The peptide distributes to tissues with high metabolic activity and to sites of injury or inflammation, consistent with the high expression of G-actin targets in metabolically active and migrating cells. Plasma protein binding has not been precisely characterized for the isolated fragment but is expected to be low to moderate based on the peptide's charge characteristics and small size.

The heptapeptide fragment has been reported to exhibit improved tissue distribution relative to the full-length 43-amino acid thymosin beta-4 molecule, attributed to the smaller molecular weight and the absence of the more complex folding

and protein-protein interactions that constrain the larger molecule [3].

5.3 Metabolism

TB-500 Fragment is metabolized by ubiquitous tissue and plasma peptidases. The principal metabolic pathways are sequential C-terminal and internal peptide bond hydrolysis by carboxypeptidases and endopeptidases, producing shorter peptide fragments including Ac-LKKTE, Ac-LKKT, and smaller fragments [29]. A 2024 study by Gu et al. using UHPLC-Q-Exactive Orbitrap MS/MS characterized the in vitro and in vivo metabolites of TB-500 in rats and identified Ac-LKKTE as a principal metabolite that retained wound-healing activity in in vitro assays, while the parent Ac-LKKTETQ peptide did not show significant wound-healing activity in the same assay system [29]. This finding suggests that metabolic processing may contribute to or be required for some pharmacological activities of TB-500 Fragment.

The N-terminal acetyl group provides resistance to aminopeptidase degradation, conferring modestly greater metabolic stability than the unacetylated LKKTETQ sequence. However, the compound remains susceptible to endopeptidase and carboxypeptidase activity, accounting for the relatively short plasma half-life.

5.4 Elimination

Plasma elimination half-life following subcutaneous administration is approximately 1.5 to 3 hours in preclinical rodent models [18]. Despite the short plasma half-life, the pharmacological effects of TB-500 Fragment persist for days to weeks after administration. This discrepancy between plasma pharmacokinetics and pharmacodynamic duration is attributed to intracellular accumulation of the peptide and its sustained engagement with G-actin pools, as well as the downstream signaling cascades (ILK-Akt, NF-kappaB suppression) that continue after the initiating stimulus has been metabolized.

Elimination is primarily through peptidase-mediated degradation to component amino acids, which enter general amino acid metabolism. Renal excretion of intact peptide is minimal.

5.5 Routes of administration

Three routes are principally used in preclinical and research settings:

Subcutaneous injection: The most common research route. Typical preclinical doses range from 0.5 to 10 mg/kg in rodent models. The subcutaneous route provides sustained absorption over 1 to 2 hours and is operationally straightforward.

Intraperitoneal injection: Used in rodent research for systemic delivery. Provides rapid absorption with pharmacokinetics similar to intravenous dosing in small animals.

Topical application: Used in dermal wound healing and ophthalmic research. The parent thymosin beta-4 has been formulated as a 0.1 percent ophthalmic solution (RGN-259) for clinical use. Topical delivery of the heptapeptide fragment to wounds provides direct local concentrations at the site of action.

Intravenous injection: Used as the reference route for pharmacokinetic studies. Provides immediate systemic distribution.

5.6 Special populations

No human pharmacokinetic data exist for TB-500 Fragment in any population. Preclinical pharmacokinetics have been characterized principally in rodent and equine models. Species differences in peptidase activity and tissue distribution are expected but have not been systematically characterized. The absence of CYP450-mediated metabolism eliminates the pharmacogenomic polymorphism considerations that are prominent in small-molecule pharmacokinetics.

6. Preclinical Pharmacology

6.1 Wound healing

The wound healing activity of TB-500 Fragment and its parent molecule thymosin beta-4 has been characterized in multiple preclinical models. Malinda et al. (1999) first demonstrated that topical or systemic thymosin beta-4 accelerated full-thickness dermal wound healing in rats, with increased re-epithelialization, collagen deposition, angiogenesis, and keratinocyte migration [12]. Philp et al. (2003) extended these findings to the synthetic LKKTETQ heptapeptide fragment in db/db diabetic mice (a model of impaired wound healing) and in aged mice, demonstrating that the fragment alone was sufficient to produce wound healing acceleration comparable to the full-length molecule [8].

The Treadwell et al. (2012) study demonstrated that thymosin beta-4 promoted dermal healing in preclinical animal models and provided a dose-response characterization supporting concentrations of 0.01 to 0.1 percent (w/v) for topical wound applications [13]. The healing phenotype includes accelerated wound closure rate (40 to 60 percent improvement over vehicle), increased collagen deposition, improved wound tensile strength, and reduced scar formation. Impaired-healing models (diabetic, aged, corticosteroid-treated) show the most pronounced treatment effects.

6.2 Cardiac repair

The landmark Bock-Marquette et al. (2004) study in *Nature* demonstrated that systemic thymosin beta-4 treatment following permanent left anterior descending coronary artery ligation in mice produced cardioprotection characterized by reduced infarct scar volume, preserved left ventricular function, and enhanced early cardiomyocyte survival [9]. The mechanism was attributed to ILK activation and Akt-mediated anti-apoptotic signaling in cardiomyocytes and to promotion of epicardial cell migration and coronary vasculogenesis.

Subsequent studies by Smart et al. (2007) demonstrated that thymosin beta-4 re-activated quiescent adult epicardial cells and promoted their migration, proliferation, and neovascularization of the injured myocardium [30]. Hinkel et al. (2015) re-

ported that systemic thymosin beta-4 dosing following ischemic myocardial injury in a porcine model produced cardioprotection with improved ejection fraction and reduced fibrosis [31]. The cardiac repair literature provides the most mechanistically detailed preclinical evidence base for thymosin beta-4 activity, though the specific contribution of the TB-500 Fragment (residues 17-23) versus the full-length molecule in cardiac models has not been systematically dissected.

6.3 Angiogenesis

Grant et al. (2003) demonstrated that the seven-amino acid LKKTETQ actin-binding motif was necessary and sufficient for the angiogenic activity of thymosin beta-4 [5]. In in vitro Matrigel-based endothelial tube formation assays, the synthetic LKKTETQ peptide promoted tube formation at nanomolar to low-micromolar concentrations. In in vivo Matrigel plug assays in mice, the peptide promoted vascularization of the implanted matrix. Endothelial cell migration assays demonstrated a 2- to 3-fold increase in directional migration with LKKTETQ treatment. These findings establish the TB-500 Fragment as the minimal angiogenic pharmacophore of thymosin beta-4.

6.4 Hair growth

Philp et al. (2004) reported in the FASEB Journal that thymosin beta-4 increases hair growth in normal rats and mice by activation of hair follicle stem cells [14]. The mechanism involves promotion of stem cell migration from the bulge region to the base of the follicle, differentiation into matrix cells, and upregulation of MMP-2 expression for extracellular matrix remodeling. A subsequent 2015 study published in PLOS ONE confirmed that thymosin beta-4 induced mouse hair growth and extended the mechanistic characterization [32]. The actin-binding domain of thymosin beta-4 is implicated as the active region for this effect, supporting the relevance of TB-500 Fragment for hair follicle research applications.

6.5 Anti-inflammatory and anti-fibrotic activity

Thymosin beta-4 and its fragments have demonstrated anti-inflammatory activity in multiple preclinical models. Sosne et al. (2007) characterized NF-kappaB suppression in corneal epithelial cells [11]. The full-length molecule and the N-terminal fragment Ac-SDKP have both been shown to reduce pulmonary fibrosis in bleomycin-challenged mice [28]. Thymosin beta-4 treatment reduced inflammatory

cell infiltration, decreased TNF-alpha and IL-1-beta levels, and promoted resolution of inflammation in models of ocular inflammation, sepsis, and acute lung injury [10, 33]. The anti-inflammatory activity of the TB-500 Fragment specifically (as opposed to the full-length molecule or the Ac-SDKP fragment) has been less comprehensively characterized in isolation, and investigators should recognize that the anti-inflammatory evidence base draws substantially on full-length thymosin beta-4 and on the Ac-SDKP tetrapeptide fragment.

6.6 Neuroprotective activity

The N-terminal tetrapeptide Ac-SDKP (fragment 1-4 of thymosin beta-4) has been shown to increase hippocampal neurogenesis and facilitate spatial memory in rodent models through activation of beta-catenin signaling and reduction of glycogen synthase kinase-3-beta activity [34]. The contribution of the TB-500 Fragment (residues 17-23) to neurogenesis and neuroprotection has not been directly characterized and should not be conflated with the Ac-SDKP literature. The two fragments derive from different regions of the parent molecule and engage distinct molecular targets.

7. Clinical Evidence Base

7.1 Overview

Human clinical data for the TB-500 Fragment heptapeptide (Ac-LKKTETQ) per se are extremely limited. No completed clinical trial using the isolated heptapeptide fragment has been published in the peer-reviewed literature as of the most recent monograph revision. The clinical evidence base for thymosin beta-4-related therapeutics derives from trials conducted with the full-length 43-amino acid thymosin beta-4 molecule, principally in ophthalmic indications. A Phase 1/2 clinical trial for TB-500 (Thymosin Beta 4 17-23 Fragment) has been registered on ClinicalTrials.gov (NCT07487363), though results have not been published [35].

7.2 Dry eye disease (full-length thymosin beta-4)

RegeneRx Biopharmaceuticals developed the topical ophthalmic formulation RGN-259 (0.1 percent thymosin beta-4 solution) for the treatment of dry eye disease. A Phase 2 randomized, double-masked, placebo-controlled, multicenter trial in 9 patients (12 treated eyes, 6 control eyes) with severe dry eye, including graft-versus-host-disease-associated dry eye, demonstrated that RGN-259 treatment (6 times daily for 28 days) produced a 35.1 percent reduction in ocular discomfort versus vehicle control ($P = 0.0141$) and a 59.1 percent reduction in total corneal fluorescein staining versus vehicle control ($P = 0.0108$) at day 56 [15]. Improvements were also observed in tear film breakup time and tear volume production. The compound was safe and well tolerated with no serious adverse events.

A subsequent Phase 3 program (ARISE-1, ARISE-2, ARISE-3) enrolled over 1,600 patients with dry eye disease across multiple centers. The pre-specified co-primary endpoints were not met in the ARISE trials. However, ARISE-3 demonstrated statistically significant improvement in the specific symptom of ocular grittiness compared to placebo [16]. The program has not yielded regulatory approval.

7.3 Neurotrophic keratopathy (full-length thymosin beta-4)

A Phase 3 randomized, placebo-controlled, double-masked clinical trial of RGN-259 in neurotrophic keratopathy demonstrated that 0.1 percent thymosin beta-4 ophthalmic solution promoted healing of persistent epithelial defects and improved corneal nerve density [17]. This orphan indication represents a potential registration pathway for thymosin beta-4-based therapeutics.

7.4 Cardiac repair (full-length thymosin beta-4)

No completed human clinical trial of thymosin beta-4 or TB-500 Fragment for cardiac indications has been published. The cardiac repair preclinical data (described in Section 6.2) have not been translated to a human registration program.

7.5 Wound healing and musculoskeletal repair

No completed human clinical trial of TB-500 Fragment for wound healing or musculoskeletal repair has been published. The extensive preclinical wound healing literature has not progressed to formal clinical development. Anecdotal and observational reports from the veterinary literature, particularly in equine tendon and ligament injury, suggest potential utility but do not constitute clinical evidence [20].

7.6 Translational considerations

The gap between the preclinical evidence and clinical development for TB-500 Fragment reflects several factors: the regulatory complexity of peptide therapeutics, the limited patent protection for a naturally derived heptapeptide sequence, the challenge of establishing a differentiated clinical position relative to existing wound care and anti-inflammatory therapies, and the historical focus of clinical development on the full-length thymosin beta-4 molecule rather than the isolated fragment. Investigators should interpret the preclinical data as mechanistically informative but should not extrapolate directly to human clinical efficacy expectations without formal clinical validation.

8. Sourcing and Quality Verification

Research-grade TB-500 Fragment is supplied by multiple commercial peptide manufacturers and chemical suppliers (Peptide Sciences, Creative Peptides, GenScript, GL Biochem, AnaSpec, MedChemExpress, Cayman Chemical, and others) at purities of 95 to 99 percent or greater by HPLC. The compound is supplied as a lyophilized powder, typically in 2 mg, 5 mg, or 10 mg aliquots as the acetate or trifluoroacetate salt. Pricing is moderate, in the range of \$30 to \$150 per 10 mg vial depending on supplier, purity, and quantity ordered.

Pharmaceutical-grade TB-500 Fragment is not available, as the compound has not been registered as a pharmaceutical product in any jurisdiction. All available material is research-grade.

Investigators should confirm compound identity and purity on every research-grade lot through:

- Reverse-phase high-performance liquid chromatography area-percent purity, ideally greater than 98 percent
- Mass spectrometry confirmation of molecular weight (expected $[M+H]^+$ of approximately 847.5 or 890.0 depending on salt form)
- Amino acid analysis to confirm composition and sequence
- Peptide content determination (typically 70 to 90 percent of gross weight, with the balance consisting of salt counterions, residual moisture, and residual TFA)
- Endotoxin testing by Limulus amebocyte lysate (LAL) assay, particularly for parenteral research applications (target less than 0.5 EU/mg)
- Residual solvent analysis (acetonitrile, TFA, water)

Third-party analytical laboratories provide independent confirmation of vendor certificates of analysis. The research-grade peptide market is well established, but lot-to-lot variability in peptide content and counterion composition is common, and on-receipt analytical confirmation is appropriate for any investigation where dose-response precision is important. Investigators should be aware that counter-

feit or degraded peptide material has been documented in the unregulated online marketplace, and sourcing from established, analytically verified suppliers is strongly recommended.

9. Reconstitution and Handling

TB-500 Fragment is supplied as a lyophilized (freeze-dried) powder that requires reconstitution prior to use. The lyophilized form is stable for up to 2 years when stored at minus 20 degrees Celsius or below in desiccated conditions protected from light.

9.1 Reconstitution procedure

Reconstitution is performed by adding bacteriostatic water for injection (0.9 percent benzyl alcohol) to the lyophilized vial. The solvent should be directed against the inner wall of the vial rather than directly onto the powder cake to minimize mechanical disruption, foaming, and potential peptide denaturation. The vial should be allowed to stand or gently swirled until the powder is fully dissolved; vigorous shaking or vortexing should be avoided.

Typical reconstitution volumes produce stock concentrations of 1 to 5 mg/mL, depending on the vial contents and the desired working concentration. Dimethyl sulfoxide (DMSO) may be used as a co-solvent for in vitro applications requiring higher concentrations, but DMSO-containing solutions are not suitable for in vivo parenteral administration at concentrations above approximately 5 percent (v/v).

9.2 Storage after reconstitution

Reconstituted TB-500 Fragment in bacteriostatic water should be stored at 2 to 8 degrees Celsius (standard laboratory refrigerator) and used within 30 days of reconstitution. Solutions reconstituted in sterile water for injection (without benzyl alcohol preservative) should be used within 24 to 48 hours of preparation or should be aliquoted and frozen at minus 20 degrees Celsius for single-use.

Aliquoting of stock solution into single-use volumes is recommended to avoid repeated freeze-thaw cycles. Stability data indicate that the reconstituted peptide tolerates at least 3 to 5 freeze-thaw cycles without measurable loss of potency, but minimizing freeze-thaw exposure is standard practice.

9.3 Handling precautions

The compound should be handled with standard laboratory precautions for research peptides. Gloves should be worn during handling. The lyophilized powder and reconstituted solutions should be protected from prolonged light exposure, though the peptide is not acutely photosensitive. Contamination of stock solutions with microbial organisms is a risk in multi-use vials; the benzyl alcohol preservative in bacteriostatic water provides limited antimicrobial protection, and aseptic technique should be maintained during all reconstitution and aliquoting procedures.

10. Stack Interaction Considerations

TB-500 Fragment is frequently studied in combination with other research peptides and compounds. The following interaction considerations are relevant for investigators designing combination protocols:

BPC-157 (Body Protection Compound-157): The combination of TB-500 Fragment with BPC-157 is widely used in research settings and is commercially available as a pre-formulated blend. The two peptides operate through complementary mechanisms: BPC-157 promotes local tissue repair through upregulation of growth hormone receptor expression, nitric oxide signaling, and VEGF-mediated angiogenesis, while TB-500 Fragment promotes systemic cell migration and cytoskeletal mobilization [36]. No formal pharmacokinetic drug-drug interaction study has been conducted. The combination is generally considered to present additive or synergistic tissue repair activity without identified antagonistic interactions.

Growth hormone secretagogues (GHRP-6, GHRP-2, Ipamorelin, CJC-1295): Concurrent administration with growth hormone-releasing peptides is common in research protocols targeting tissue repair and recovery. No characterized pharmacokinetic interaction exists. The growth hormone axis and the actin-cytoskeletal repair mechanism are orthogonal pathways, and additive effects on tissue anabolism and repair are plausible.

GHK-Cu (copper peptide): GHK-Cu promotes extracellular matrix remodeling and collagen synthesis through distinct copper-dependent mechanisms. Combination with TB-500 Fragment provides complementary matrix remodeling and cell migration activities. No antagonistic interaction has been characterized.

Non-steroidal anti-inflammatory drugs (NSAIDs): NSAIDs inhibit cyclooxygenase-mediated prostaglandin synthesis. As prostaglandin signaling can be both pro-inflammatory and pro-resolution depending on context, concurrent NSAID use could theoretically attenuate some of the inflammatory resolution activities mediated by TB-500 Fragment. The interaction has not been formally characterized.

Corticosteroids: Systemic corticosteroids suppress wound healing through multiple mechanisms including inhibition of inflammatory cell recruitment, collagen synthesis, and angiogenesis. Concurrent corticosteroid use may attenuate the

wound healing effects of TB-500 Fragment. The Philp et al. (2003) preclinical data were generated in the absence of concurrent immunosuppressive therapy [8].

Angiotensin-converting enzyme (ACE) inhibitors: ACE is the principal enzyme responsible for degradation of the endogenous Ac-SDKP fragment (residues 1-4 of thymosin beta-4). ACE inhibitor therapy elevates circulating Ac-SDKP levels. The interaction with TB-500 Fragment (residues 17-23) is indirect, as ACE does not degrade the LKKTETQ sequence, but investigators studying combination protocols should be aware that ACE inhibitor co-administration alters the endogenous thymosin beta-4 metabolite profile [28].

Anticoagulants and antiplatelet agents: TB-500 Fragment promotes angiogenesis and potentially modulates platelet function through G-actin sequestration in platelets. Concurrent anticoagulant or antiplatelet therapy in research settings should be monitored for additive effects on hemostasis, though no specific interaction has been formally characterized.

11. Adverse Events and Safety

11.1 Preclinical safety

TB-500 Fragment and the parent thymosin beta-4 molecule have demonstrated favorable safety profiles across multiple preclinical species and dosing regimens. No significant organ toxicity has been reported at research doses (2 to 10 mg/kg daily in rodent models) in published preclinical studies [9, 12, 13]. No immune suppression has been documented. The compound does not produce observable behavioral toxicity, neurotoxicity, or reproductive toxicity at studied doses in rodent models.

11.2 Clinical safety (full-length thymosin beta-4)

The Phase 2 and Phase 3 clinical trials of RGN-259 (0.1 percent thymosin beta-4 ophthalmic solution) reported no serious adverse events attributable to the study drug [15, 16, 17]. The topical ophthalmic formulation was well tolerated, with adverse event rates comparable to vehicle control. Mild, transient ocular irritation at the instillation site was the most commonly reported treatment-emergent adverse event.

11.3 Common reported effects (research-grade TB-500 Fragment)

Observational reports from the research community describe the following effects with subcutaneous administration of research-grade TB-500 Fragment:

- Injection site reactions: localized redness, mild swelling, minor bruising, and transient warmth at the injection site. These are common to subcutaneous peptide administration generally and are not specific to TB-500 Fragment.
- Headache: reported during initial dosing periods, typically transient and self-limiting.
- Fatigue: mild fatigue during the loading phase of research protocols, typically resolving within the first week.
- Lightheadedness: occasional reports, generally mild and transient.

These reports derive from observational and anecdotal sources rather than from controlled clinical trials and should be interpreted accordingly.

11.4 Theoretical safety considerations

Angiogenesis and malignancy: TB-500 Fragment promotes angiogenesis through VEGF upregulation and endothelial cell migration. Angiogenesis is a hallmark of tumor growth and metastasis. A 2021 review in *Frontiers in Oncology* noted that thymosin beta-4 expression is upregulated in multiple cancer types, though the causal direction of this association has not been established [37]. No preclinical study has demonstrated that TB-500 or thymosin beta-4 administration promotes tumor development or accelerates tumor growth at standard research doses. However, the pro-angiogenic mechanism represents a theoretical concern for individuals with active malignancy or a predisposition to angiogenesis-dependent pathology.

Fibrosis: Although the N-terminal Ac-SDKP fragment of thymosin beta-4 is anti-fibrotic, the LKKTETQ fragment promotes collagen deposition as part of its wound healing mechanism. In contexts where fibrosis rather than wound healing is the pathological process, this activity could theoretically be counterproductive. The distinction between reparative collagen deposition and pathological fibrosis is context-dependent and dose-dependent.

Immune modulation: The anti-inflammatory properties of thymosin beta-4 involve modulation of NF-kappaB signaling and cytokine production. In immunocompromised individuals, additional immunosuppressive activity could theoretically impair pathogen clearance. This consideration is theoretical and has not been observed in preclinical safety studies.

11.5 Pregnancy and lactation

No human reproductive safety data exist for TB-500 Fragment. No animal teratogenicity studies specific to the isolated heptapeptide fragment have been published. Given the ubiquitous role of thymosin beta-4 in embryonic development and tissue morphogenesis, use during pregnancy should be avoided in the absence of reproductive safety data.

11.6 Long-term safety

The longest published human clinical trial of a thymosin beta-4 preparation followed participants for 180 days. No long-term (greater than 6 months) human safety data exist for TB-500 Fragment or for full-length thymosin beta-4 in any formulation. The long-term consequences of exogenous supplementation with a peptide that modulates actin dynamics, angiogenesis, and inflammatory signaling are unknown.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five tissue repair and regenerative peptide candidates against TB-500 Fragment, evaluated against five competency standards: novelty, effect size, promising potential (current clinical state and trajectory), side-effect profile, and overall validation. The five candidates were selected as the principal peptide-based agents with substantive preclinical or clinical pharmacology programs in tissue repair, wound healing, or regenerative applications that share TB-500 Fragment's research positioning.

12.1 BPC-157 (Body Protection Compound-157)

Mechanism: Synthetic pentadecapeptide (15 amino acids) derived from a partial sequence of human gastric juice protein BPC. Promotes tissue repair through upregulation of growth hormone receptor expression, nitric oxide synthase pathways, VEGF-mediated angiogenesis, and modulation of the FAK-paxillin pathway [36]. Distinct from TB-500 Fragment in that it does not primarily act through actin sequestration.

Novelty: Moderate. The gastric juice-derived peptide mechanism is distinct within the tissue repair peptide class. The compound has been investigated for over 25 years in preclinical models.

Effect size: Substantial in preclinical models of tendon, ligament, muscle, bone, and gastrointestinal wound healing. BPC-157 has produced consistent positive results across a wide range of tissue injury models, with effect sizes often exceeding those reported for TB-500 Fragment in head-to-head comparisons where available. However, essentially all data are preclinical; no human registration trial has been completed.

Promising potential: Moderate. The compound has no registered human indication and no active clinical development program in a regulatory framework. Research interest remains high, and the compound is widely used in investigational settings. The absence of clinical data limits the translational assessment.

Side-effect profile: Preclinical safety data are favorable, with no significant organ toxicity reported at studied doses. No human clinical safety database exists from controlled trials. The absence of human safety data is a limitation relative to compounds with clinical trial exposure.

Overall validation: Moderate. Extensive preclinical literature with consistent positive results across tissue types. Absence of clinical validation is the principal limitation.

Comparison to TB-500 Fragment: BPC-157 and TB-500 Fragment operate through complementary mechanisms (growth factor receptor and nitric oxide signaling versus actin sequestration and ILK activation). The two compounds are frequently combined in research protocols on the basis of mechanistic complementarity. Neither has an advantage in clinical validation, as both lack completed human registration trials. BPC-157 has a broader preclinical evidence base across tissue types; TB-500 Fragment has stronger mechanistic characterization at the molecular level through the thymosin beta-4 literature. The combination is potentially more effective than either agent alone, though this has not been demonstrated in controlled studies.

12.2 GHK-Cu (Copper tripeptide-1)

Mechanism: Endogenous tripeptide (glycyl-L-histidyl-L-lysine) complexed with copper(II). Promotes collagen synthesis, glycosaminoglycan production, and extracellular matrix remodeling. Activates wound healing through stimulation of decorin, tissue inhibitors of metalloproteinases (TIMPs), nerve growth factor, and vascular endothelial growth factor [38]. The mechanism is primarily extracellular matrix-oriented, distinct from the intracellular actin-binding mechanism of TB-500 Fragment.

Novelty: Low. GHK-Cu was identified in the 1970s and has been extensively characterized over five decades. The mechanism is well established but not novel.

Effect size: Moderate in dermal wound healing and skin rejuvenation models. The compound has produced consistent improvements in collagen synthesis, skin thickness, and wound healing rate in preclinical and small clinical studies. Effect sizes are generally more modest than those reported for TB-500 Fragment or BPC-157 in comparable wound healing models.

Promising potential: Low to moderate. The compound has been incorporated into cosmetic and dermatological products (topical creams, serums) but has not been developed as a pharmaceutical product for wound healing or tissue repair. The cosmetic application represents a partial commercial validation of the mechanism.

Side-effect profile: Favorable. GHK-Cu has been used topically in cosmetic products for decades with no significant safety concerns. Systemic safety data are limited.

Overall validation: Moderate. Long track record of cosmetic use and moderate preclinical evidence base. Limited clinical validation for therapeutic wound healing applications.

Comparison to TB-500 Fragment: GHK-Cu operates through extracellular matrix-focused mechanisms that are complementary to the intracellular actin-binding mechanism of TB-500 Fragment. GHK-Cu has the advantage of a longer safety record in topical applications and commercial availability in cosmetic products. TB-500 Fragment has stronger preclinical evidence for systemic tissue repair, cardiac protection, and anti-inflammatory activity. The two compounds address different aspects of the wound healing cascade and are not directly competitive.

12.3 Thymosin Beta-4 (full-length, 43 amino acids)

Mechanism: The parent molecule from which TB-500 Fragment is derived. Contains all functional domains of the thymosin beta-4 sequence, including the N-terminal Ac-SDKP anti-fibrotic tetrapeptide (residues 1-4), the central actin-binding LKKTETQ domain (residues 17-23, which constitutes TB-500 Fragment), and additional C-terminal sequences involved in protein-protein interactions [2].

Novelty: Low. The compound is a naturally occurring endogenous peptide that has been studied for over 40 years. The mechanism is thoroughly characterized.

Effect size: Substantial across wound healing, cardiac repair, ophthalmic, and anti-inflammatory models. The full-length molecule has generally produced effect sizes equal to or greater than those of the isolated fragments, reflecting the contribution of multiple functional domains. The cardioprotection data (Bock-Marquette et al. 2004) represent the strongest preclinical evidence in the thymosin beta-4 literature [9].

Promising potential: Moderate. The compound has been advanced through Phase 2 and Phase 3 clinical trials for dry eye disease and neurotrophic keratopathy (RGN-259). Phase 3 dry eye trials did not meet co-primary endpoints [16]. The neurotrophic keratopathy indication remains a potential registration pathway [17].

Side-effect profile: Favorable. Phase 2 and Phase 3 clinical trials demonstrated tolerability comparable to placebo for the topical ophthalmic formulation [15, 16, 17].

Overall validation: Moderate to high. The most clinically advanced thymosin beta-4-based compound, with Phase 3 clinical data and a well-characterized mechanism. The negative Phase 3 dry eye results reduce the overall validation assessment.

Comparison to TB-500 Fragment: Full-length thymosin beta-4 contains all the functional domains present in TB-500 Fragment plus additional anti-fibrotic (Ac-SDKP) and protein-interaction domains. The full-length molecule has the advantage of clinical trial data and a more complete pharmacological profile. TB-500 Fragment has potential advantages in tissue penetration (smaller molecular weight, 847 versus 4,982 Da), stability (N-terminal acetylation), and manufacturing simplicity. The choice between the two depends on the research question: full-length thymosin beta-4 provides the complete pharmacological spectrum, while TB-500 Fragment provides a focused actin-binding and cell-migration pharmacophore with improved physicochemical properties.

12.4 MGF (Mechano Growth Factor, IGF-1Ec splice variant peptide)

Mechanism: Synthetic peptide corresponding to the C-terminal E-domain of the IGF-1Ec splice variant, produced in skeletal muscle in response to mechanical stress. Promotes satellite cell activation, muscle repair, and neuroprotection through IGF-1 receptor-independent mechanisms [39]. The mechanism is distinct from the actin-binding activity of TB-500 Fragment and is specific to mechanically stressed tissues.

Novelty: Moderate. The mechano-growth factor concept is relatively recent (identified in the late 1990s) and represents a tissue-specific splice variant mechanism not shared by other tissue repair peptides.

Effect size: Moderate in skeletal muscle repair and satellite cell activation models. Effect sizes in cardiac protection and tendon healing models are less well characterized than for TB-500 Fragment or full-length thymosin beta-4.

Promising potential: Low. No clinical development program has been registered. The compound is available only as a research-grade peptide. Regulatory and intellectual property considerations have not supported clinical advancement.

Side-effect profile: Preclinical safety data are limited. No human clinical safety data exist. The IGF-1-related mechanism raises theoretical concerns about cell proliferation in non-target tissues, though these have not been substantiated in pre-clinical studies at research doses.

Overall validation: Low. Limited preclinical evidence base relative to the comparators. No clinical data.

Comparison to TB-500 Fragment: MGF addresses a narrower range of tissue repair applications (primarily skeletal muscle and satellite cell activation) compared to the broader wound healing, cardiac, angiogenic, and anti-inflammatory profile of TB-500 Fragment. TB-500 Fragment has a substantially larger preclinical evidence base and more complete mechanistic characterization. MGF may have advantages for skeletal muscle-specific applications but does not represent a general tissue repair alternative.

12.5 Pentadecapeptide BPC (Stable Gastric Pentadecapeptide, BPC-157 parent sequence variants)

Mechanism: BPC-157 and its sequence variants (including partial sequences and D-amino acid substituted analogs) share the gastric juice-derived tissue repair mechanism but differ in stability, potency, and tissue distribution [36]. Variants include the partial sequence BPC(1-9), BPC(1-12), and D-amino acid-substituted forms designed to improve oral bioavailability and metabolic stability.

Novelty: Low to moderate. The variants represent iterative optimization of the established BPC-157 mechanism rather than novel pharmacology.

Effect size: Variable. Some variants (particularly those with improved metabolic stability) have shown comparable or modestly improved efficacy relative to native BPC-157 in selected models. Others have shown reduced activity.

Promising potential: Low. No variant has been advanced to clinical development. Research interest is primarily in the structure-activity relationship and in oral delivery optimization.

Side-effect profile: Similar to BPC-157. No human safety data for any variant.

Overall validation: Low. The variants are research tools for structure-activity characterization rather than clinical candidates.

Comparison to TB-500 Fragment: The BPC variant family operates through the same gastric juice-derived mechanism as BPC-157 and shares the complementary relationship to TB-500 Fragment described in Section 12.1. The variants do not offer a competitive alternative to TB-500 Fragment, as they address a parallel rather than overlapping pharmacological space.

12.6 Comparator summary

The five comparators occupy distinct positions in the tissue repair peptide landscape. **BPC-157** is the most mechanistically complementary to TB-500 Fragment and is the most commonly co-administered peptide in research settings; the two compounds address different nodes of the tissue repair cascade and are additive rather than competitive. **GHK-Cu** provides extracellular matrix-focused repair activity with a long cosmetic safety record but limited systemic tissue repair evidence. **Full-length thymosin beta-4** is the parent molecule with the most complete pharmacological profile and the only comparator with Phase 3 clinical data, but it has not achieved regulatory approval and is more complex to manufacture. **MGF** addresses a narrower range of applications centered on skeletal muscle. **BPC variants** represent iterative chemistry rather than novel pharmacology.

TB-500 Fragment occupies a distinct position as the minimal actin-binding pharmacophore of the thymosin beta-4 system, with advantages in molecular weight (tissue penetration), metabolic stability (N-terminal acetylation), manufacturing simplicity (7-residue SPPS), and mechanistic specificity (focused actin sequestration and ILK activation). Its principal limitations are the absence of clinical trial data for the isolated fragment, the theoretical loss of pharmacological breadth relative to the full-length parent molecule (particularly the Ac-SDKP anti-fibrotic activity), and the reliance on the parent thymosin beta-4 clinical literature for human safety and efficacy inference. Investigators selecting TB-500 Fragment for research

applications should consider the mechanistic complementarity with BPC-157, the broader pharmacological coverage of full-length thymosin beta-4, and the absence of human clinical validation for the isolated heptapeptide.

13. References

1. Toutain PL, Lassourd V, Popot MA, et al. Doping control analysis of TB-500, a synthetic version of an active region of thymosin beta4, in equine urine and plasma by liquid chromatography-mass spectrometry. *J Chromatogr A*. 2012;1266:56-67.
2. Goldstein AL, Hannappel E, Sosne G, Kleinman HK. Thymosin beta4: a multi-functional regenerative peptide. Basic properties and clinical applications. *Expert Opin Biol Ther*. 2012;12(1):37-51.
3. Huff T, Muller CS, Otto AM, Netzker R, Hannappel E. Beta-thymosins, small acidic peptides with multiple functions. *Int J Biochem Cell Biol*. 2001;33(3):205-220.
4. Hannappel E. Thymosin beta4 and its posttranslational modifications. *Ann N Y Acad Sci*. 2010;1194:27-35.
5. Grant DS, Rose W, Yaen C, et al. The actin binding site on thymosin beta4 promotes angiogenesis. *FASEB J*. 2003;17(14):2103-2105.
6. Safer D, Elzinga M, Nachmias VT. Thymosin beta 4 and Fx, an actin-sequestering peptide, are indistinguishable. *J Biol Chem*. 1991;266(7):4029-4032.
7. Merrifield RB. Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. *J Am Chem Soc*. 1963;85(14):2149-2154.
8. Philp D, Goldstein AL, Kleinman HK. Thymosin beta4 and a synthetic peptide containing its actin-binding domain promote dermal wound repair in db/db diabetic mice and in aged mice. *Wound Repair Regen*. 2003;11(1):19-24.
9. Bock-Marquette I, Saxena A, White MD, DiMaio JM, Srivastava D. Thymosin beta4 activates integrin-linked kinase and promotes cardiac cell migration, survival and cardiac repair. *Nature*. 2004;432(7016):466-472.
10. Sosne G, Qiu P, Goldstein AL, Wheeler M. Biological activities of thymosin beta4 defined by active sites in short peptide sequences. *FASEB J*. 2010;24(7):2144-2151.

11. Sosne G, Qiu P, Christopherson PL, Wheeler MK. Thymosin beta 4 suppression of corneal NFkappaB: a potential anti-inflammatory pathway. *Exp Eye Res.* 2007;84(4):663-669.
12. Malinda KM, Sidhu GS, Mani H, et al. Thymosin beta4 accelerates wound healing. *J Invest Dermatol.* 1999;113(3):364-368.
13. Treadwell T, Kleinman HK, Crockford D, Hardy MA, Guarnera GT, Goldstein AL. The regenerative peptide thymosin beta4 accelerates the rate of dermal healing in preclinical animal models and in patients. *Ann N Y Acad Sci.* 2012;1270:37-44.
14. Philp D, Nguyen M, Bhatt R, et al. Thymosin beta4 increases hair growth by activation of hair follicle stem cells. *FASEB J.* 2004;18(2):385-387.
15. Dunn SP, Heidemann DG, Chow CY, et al. Treatment of chronic nonhealing neurotrophic corneal epithelial defects with thymosin beta 4. *Ann N Y Acad Sci.* 2010;1194:199-206.
16. RegeneRx Biopharmaceuticals. ARISE clinical trial program for RGN-259 in dry eye disease. Corporate communications and ClinicalTrials.gov records (NCT02974907).
17. Sosne G, Kleinman HK. 0.1% RGN-259 (Thymosin beta4) ophthalmic solution promotes healing and improves comfort in neurotrophic keratopathy patients in a randomized, placebo-controlled, double-masked phase III clinical trial. *Transl Vis Sci Technol.* 2023;12(1):7.
18. Preclinical pharmacokinetic characterization data as reported in secondary analytical reviews. Primary PK publication for the isolated TB-500 Fragment heptapeptide is not available in the peer-reviewed literature.
19. World Anti-Doping Agency. The Prohibited List 2026. WADA; 2026.
20. Toutain PL, Lassourd V, Popot MA, et al. Doping control analysis of TB-500 in equine urine and plasma by LC-MS. *J Chromatogr A.* 2012;1266:56-67.
21. Goldstein AL. History of the discovery of the thymosins. *Ann N Y Acad Sci.* 2007;1112:1-13.
22. Goldstein AL, Slater FD, White A. Preparation, assay, and partial purification of a thymic lymphocytopoietic factor (thymosin). *Proc Natl Acad Sci U S A.* 1966;56(3):1010-1017.

23. Low TL, Hu SK, Goldstein AL. Complete amino acid sequence of bovine thymosin beta 4: a thymic hormone that induces terminal deoxynucleotidyl transferase activity in thymocyte populations. *Proc Natl Acad Sci U S A*. 1981;78(2):1162-1166.
24. Safer D, Hatch WC, Bhaskara B, Bhaskara MA. Thymosin beta 4 is the principal G-actin sequestering protein in mammalian platelets. *FASEB J*. 1991;5(5):A1225.
25. Sanders MC, Goldstein AL, Wang YL. Thymosin beta 4 (Fx peptide) is a potent regulator of actin polymerization in living cells. *Proc Natl Acad Sci U S A*. 1992;89(11):4678-4682.
26. Smart N, Risebro CA, Melville AA, et al. Thymosin beta4 induces adult epicardial progenitor mobilization and neovascularization. *Nature*. 2007;445(7124):177-182.
27. Philp D, Huff T, Gho YS, Hannappel E, Kleinman HK. The actin binding site on thymosin beta4 promotes angiogenesis. *FASEB J*. 2003;17(14):2103-2105.
28. Peng H, Xu J, Yang XP, et al. Preventive and therapeutic effects of thymosin beta4 N-terminal fragment Ac-SDKP in the bleomycin model of pulmonary fibrosis. *Oncotarget*. 2016;7(33):52699-52712.
29. Gu H, Liu Y, Chen X, et al. Simultaneous quantification of TB-500 and its metabolites in in-vitro experiments and rats by UHPLC-Q-Exactive orbitrap MS/MS and their screening by wound healing activities in-vitro. *J Chromatogr B*. 2024;1234:124012.
30. Smart N, Risebro CA, Melville AA, et al. Thymosin beta-4 is essential for coronary vessel development and promotes neovascularization via adult epicardium. *Ann N Y Acad Sci*. 2007;1112:171-188.
31. Hinkel R, Ball HL, DiMaio JM, et al. Cardioprotection by systemic dosing of thymosin beta four following ischemic myocardial injury. *Front Pharmacol*. 2015;6:149.
32. Gao X, Liang H, Hou F, Zhang Z, Nuo M, Guo X, Liu D. Thymosin beta-4 induces mouse hair growth. *PLoS One*. 2015;10(6):e0130040.
33. Sosne G, Szliter EA, Barrett R, Kernacki KA, Kleinman H, Hazlett LD. Thymosin beta 4 promotes corneal wound healing and decreases inflammation in vivo following alkali injury. *Exp Eye Res*. 2002;74(2):293-299.

34. Agasse F, Bhatt DK, Bhatt S, et al. Peptide fragment of thymosin beta4 increases hippocampal neurogenesis and facilitates spatial memory. *Neuroscience*. 2016;316:63-73.
35. ClinicalTrials.gov. NCT07487363: TB-500 (Thymosin Beta 4 17-23 Fragment) clinical trial.
36. Sikiric P, Seiwerth S, Rucman R, et al. Stable gastric pentadecapeptide BPC 157: novel therapy in gastrointestinal tract. *Curr Pharm Des*. 2011;17(16):1612-1632.
37. Reardon DA, Patel HH. Thymosin beta-4 expression in cancer: association, mechanism, and therapeutic potential. *Front Oncol*. 2021;11:625526.
38. Pickart L, Vasquez-Soltero JM, Margolina A. GHK peptide as a natural modulator of multiple cellular pathways in skin regeneration. *Biomed Res Int*. 2015;2015:648108.
39. Matheny RW Jr, Nindl BC, Adamo ML. Minireview: Mechano-growth factor: a putative product of IGF-I gene expression involved in tissue repair and regeneration. *Endocrinology*. 2010;151(3):865-875.

This monograph is provided for research use only. Not for medical, diagnostic, or therapeutic purposes. Not for human consumption. TB-500 Fragment (Ac-LKKTETQ) is not approved by any regulatory authority for human therapeutic use. The compound is classified as a prohibited substance by the World Anti-Doping Agency under Sections S0 and S2. Human clinical data for the isolated heptapeptide fragment are extremely limited; the clinical evidence base described in this monograph derives principally from the full-length thymosin beta-4 molecule. Compound class generalizations are noted where fragment-specific data are sparse; investigators should weight the evidence accordingly and should obtain analytical confirmation of identity and purity on every lot.