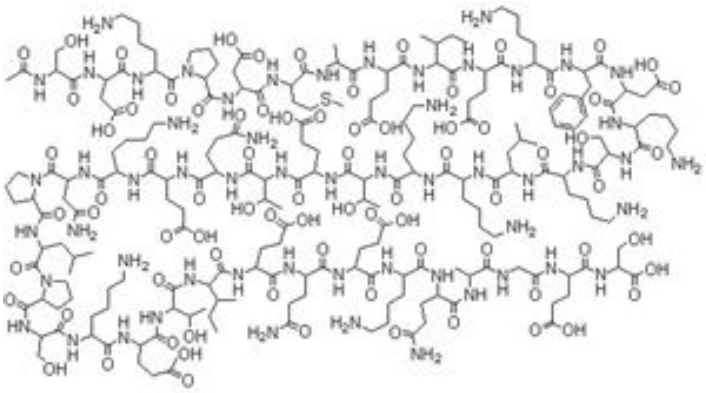
Professional Monograph

Thymosin Beta 4 (Tb4)



INDICATION and USAGE SUMMARY

- Thymosin beta 4 is a synthetic peptide found in most cells and tissues[1]
  - Originally isolated from calf thymus
  - Main intracellular G-actin sequestering peptide
  - **Up-regulates actin**
  - Forms a ternary complex with actin and profilin
  - Increases cells involved in healing
  - Improves cell migration to site of injury
  - Promotes matrix metalloproteinase expression during wound repair
  - Promotes angiogenesis
  - Cytoprotective
  - Helps decrease scar tissue formation
  - Improves T cells
- **Used for clinical conditions where soft tissue recovery or immune support is needed**
  - Sports/athletic injury
  - Soft tissue repair
    - Tendon/ligament/muscle repair
  - Pressure ulcers / venous stasis ulcers
  - Immune support (as monotherapy or in conjunction with Thymosin alpha 1)
  - Brain issues if autoimmunity suspected
  - Multiple sclerosis
  - Ischemic stroke
  - Spinal cord injuries
  - TBI; concussion support (in conjunction with BPC 157)
  - Sepsis
  - Dry eye disorders
  - Ocular tissue injuries including corneal wound healing and repair
    - Chemical burns
    - Diabetes
    - Corneal transplants
  - Cardioprotective
  - NAFLD – non-alcoholic fatty liver disease
  - Lung inflammation / fibrosis
  - May improve hair growth
- General dosage
  - 300 mcg – 1 gram daily, SubQ
  - Depending upon clinical presentation – less for immune, more for repair or most for both issues
  - Do not dose concurrently for more than 3 months
  - Cycle if needed long-term – 3 months on, 6 weeks off or 6 weeks on 6 weeks off
  - Individual dosage requirements may vary based on clinical presentation

Name(s): Thymosin beta 4,, Tb4,TB-500

Sequence:

Ac-Ser-Asp-Lys-Pro-Asp-Met-Ala-Glu-Ile-Glu-Lys-Phe-Asp-Lys-Ser-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-Glu-Lys-Asn-Pro-Leu-Pro-Ser-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Gln-Ala-Gly-Glu-Ser

[1] Sanders MC, Goldstein AL, Wang Y. Thymosin B4 (Fx peptide) is a potent regulator of actin polymerization in living cells. Proc Natl Acad Sci USA. 1992;89:4678-4682.

Molecular formula: C212H350N56O78S

Molar Weight: 4963.4408

Dosage Route: SubQ injection

Dosage:

SubQ General Dosage

- Soft tissue healing support
    - 300 mcg – 1 gm daily
- Dosage depends upon clinical presentation
- Do not dose concurrently for more than 3 months
- Cycle if needed long-term – 3 months on, 6 weeks off or 6 weeks on, 6 weeks off
- Individual dosage requirements may vary based on clinical presentation

Overview

The beta-thymosins (b-thymosins) comprise a family of structurally related, highly conserved amino acid sequences in species ranging from mammals to echinoderms. Of the 16 known family members, thymosin β 4 (Tb4), thymosin β 10 (Tb10), and thymosin β 15 (Tb15) are found in man.[2]

Thymosin beta 4 (Tb4) is a 43 amino acid, 5 kDa polypeptide that is an important mediator of cell proliferation, migration, and differentiation. Tb4 is the most abundant member of the β - thymosin family in mammalian tissue and is regarded as the main G-actin sequestering peptide. It is found in all tissues and cell types except red blood cells. Thymosin beta4 is angiogenic and can promote endothelial cell migration and adhesion, and angiogenesis.[3,4] TB4 also accelerates wound healing and reduces inflammation and scarring when applied in dermal wound-healing assays.[5,6]

Beta thymosins bind and sequester monomeric actin, thus preventing actin polymerization and formation of filamentous actin.[7] Actin is a vital component of cell structure and movement. Actin is involved in many important non-muscle cellular processes including cell locomotion, chemotaxis, phagocytosis, and cytokinesis. Of the thousands of proteins present in cells, actin makes up to 10% of the total proteins in a cell, representing a major role in the genetic makeup of the cell.

Animal studies of disease and repair when using thymosin beta 4 (Tb4), the major actin-sequestering molecule in mammalian cells, have provided a base for the ongoing multicenter clinical trials for wound healing, including dermal, corneal, and cardiac.[8,9] Tb4 has of multiple biological activities, which include down-regulation of inflammatory chemokines and cytokines, and promotion of cell migration, blood vessel formation, cell survival, and stem cell maturation. Thymosin beta 4 inhibits inflammation, microbial growth, scar formation (by reducing the level of myofibroblasts), and apoptosis, and protects cells from cytotoxic damage, including glutamate neuronal toxicity.[10] Thymosin β 4 binds to G-actin, blocks actin

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[2] Stoeva, S., S. Ho ¨rger & W. Voelter. 1997. A novel - thymosin from the sea urchin: extending the phyloge- netic distribution of B-thymosins from mammals to echinoderms. J Pept Sci. 1997;3:282–290.
[3] Philp D, Huff T, Gho YS, et al. The actin binding site on thymosin beta4 promotes angiogenesis. FASEB J. 2003;17(14):2103-5.
[4] Goldstein AL, Hannappei E, Sosne G, et al. Thymosin beta4: a multifunctional regenerative peptide: Basic properties and clinical applications. Expert Opin Biol Ther. 2012;12(1):37-51.
[5] Kleinman HK, Sosne G. Thymosin B4 promotes dermal healing. Vitam Horm. 2016;102:251-75.
[6] Huff T, Müller CS, Otto AM, Netzker R, Hannappel E. β -Thymosins, small acidic peptides with multiple functions. Int J Biochem Cell Biol. 2001;33(3):205–20.
[7] Huff T, Müller CS, Otto AM, Netzker R, Hannappel E. β -Thymosins, small acidic peptides with multiple functions. Int J Biochem Cell Biol. 2001;33(3):205–20.
[8] Philip D, Kleinman HK, Animal studies with thymosin beta, a multifunctional tissue repair and regeneration peptide. Ann NY Acad Sci. 2010;1194:81-6.
[9] Crockford D, Turjman N, Allan C, et al. Thymosin beta4: structure, function , and biological properties current and future clinical applications. Ann NY Acad Sci. 2010;1194:179-89.
[10] Sosne G, Kleinman HK. Primary mechanisms of thymosin b4 repair activity in dry eye disorders and other tissue injuries. Inves Ophth Visual Sci. 2015;56(9):

polymerization, and is coreleased with factor XIIIa by platelets.[11] These activities contribute to the multiple wound healing properties that have been reported in animal and human studies.

Ocular

Tb4 promotes complete and faster corneal healing than saline alone or prescription agents (doxycycline and cyclosporine) in various animal models of eye injury.[12] In human trials, Tb4 eye drops improve both the signs and symptoms of moderate to severe dry eye with effects lasting beyond the treatment period.[13] Thymosin β 4 has also reported efficacy in three phase 2 clinical ocular trials with no evidence of any adverse events.[14] See **table 3** for a summary of corneal wound healing applications.

Table 3: Corneal Wound Healing Applications

Nonmedical application
• Chemical burns
• Patients undergoing photorefractive keratectomy (PRK)
Medical applications
• Stage 2 patients (Mackie classification) with neurotrophic keratitis
• Patients with recurrent corneal erosions
• Patients with map-dot fingerprint and/or Fuch's corneal dystrophies
• Corneal transplants
• Patients undergoing phototherapeutic keratectomy (PTK) for anterior stromal corneal dystrophies

Oral

Tb4 plays a role in suppressing the production of interleukin-8 following stimulation by tumor necrosis factor-alpha, acting as an antimicrobial, anti-inflammatory and antiapoptotic factor on gingival fibroblasts.[15]

Cardiac

Hypoxic heart disease is a predominant cause of disability and death worldwide. Tb4 is the only known molecule to initiate organ wide activation of the embryonic coronary development program in adult mammalian hearts. Tb4 has been reported effective when used to inhibit myocardial cell death, improve angiogenesis, have antifibrotic effects, decrease infarct size and activate endogenous cardiac

[11] Huff T, Otto AM, Muller CSG, et al. Thymosin beta4 is released from human blood platelets and attached by factor XIIIa (transglutaminase) to fibrin and collagen. FASEB J. 2002;16(7):691-6.
[12] Sosne G, Kleinman HK. Primary mechanism of thymosin b4 repair activity in dry eye disorders and other tissue injuries. Invest Opththalmol Vis Sci. 2015;56(9):5110-7.
[13] Sosne G, Rimmer D, Kleinman HK, et al. Thymosin beta4: a potential novel therapy for neurotrophic keratopathy, dry eye, and ocular surface diseases. Vitamin Horm. 2016;102:277-306.
[14] Crockford D, Turjman N, Allan C, et al. Thymosin beta4: structure, function , and biological properties current and future clinical applications. Ann NY Acad Sci. 2010;1194:179-89.
[15] Reti R, Kwon E, Qui P, et al. Thymosin B4 is cytoprotective in human gingival fibroblasts. Eur J Oral Sci. 2008;116(5):424-30.

progenitors.[16] Treatment with Tb4 is reported to reduce infarct volume and preserves cardiac function in preclinical models of cardiac ischemic injury.[17]

CNS/Brain

As stated earlier, Tb4 is widely distributed in a majority or mammalian tissues and cell types, including those of the CNS (central nervous system). It is expressed in most neural cell types of the developing brain and in a subset of neurons and microglia.[18] Tb4 is locally synthesized in neurons for the regulation of neurite outgrowth.[19] Tb4 is up-regulated in various pathological conditions such as focal ischemia, Alzheimer's disease, Huntington's disease, hippocampal denervation, and kainic acid induced seizure. Its presence in the nervous system likely plays a role in neuroprotection, synaptogenesis, axon growth, cell migration, and plastic changes.[20] If brain changes are suspected due to autoimmunity, Tb4 is a great therapeutic choice.

Sepsis

Sepsis is the dysregulated host response to an infection resulting in life-threatening organ damage. Thymosin Beta 4 is reported to improve mortality when administered intravenously to septic rats.[21] Tb4 decreases inflammatory mediators, lowers reactive oxygen species, up-regulates anti-oxidative enzymes, anti-inflammatory genes, and anti-apoptotic enzymes making it an interesting protein to study in sepsis.

Non Alcoholic Fatty Liver Disease - NAFLD

Studies report that Tb4 is negatively correlated with endotoxemia, and could suppress proinflammatory TLR signaling and reduce inflammatory cytokines.[22] According to the gut-liver axis theory, the effects of Tb4 could play an important role in the treatment of NAFLD. Liang et al detected Tb4 expression in the sera and tissues of patients with chronic hepatitis B combined with NAFLD, and observed that the Tb4 level was negatively correlated with inflammation and fibrosis scores, and Tb4 expression in both serum and liver tissue was negatively correlated with TNF-a expression. Tb4 plays a defensive role in the development of liver disease by inhibiting oxidative stress and proinflammatory factors.[23] When

[16] Shrivastava S, Srivastava D, Olson EN, et al. Thymosin beta4 and cardiac repair. Ann NY Acad Sci. 2010;1194:87-96.
[17] Pipes GT, Yang J. Cardioprotection by thymosin beta4. Vitam Horm. 2016;102:209-26.
[18] Sun W, Lim H. Neurotrophic roles of the beta-thymosins in the development and regenerationof the nervous system. Ann. N.Y. Acad. Sci.. 2007;1112: 210–218.
[19] van Kesteren, RE, Carter C, Dissel HM, et al. Local synthesis of actin-binding protein beta-thymosinregulates neurite outgrowth. J. Neurosci 2006;26:152–157.
[20] Popoli PR, Pepponi A, Martire et al. Neuroprotective Effects of Thymosin B4 in Experimental Models of Excitotoxicity. Ann. N.Y. Acad. Sci.2007;1112: 219–224.
[21] Belsky JB, Rivers EP, Fibin MR, et al. Thymosin beta 4 regulation of actin in sepsis. Expert Opin Biol Ther. 2018:1-5.[Epub ahead of print].
[22] Jiang Y, Han T, Zhang Z, et al. Potential role of thymosin beta 4 in the treatment of nonalcoholic fatty liver disease. Chron Dis Trans Med. 2017;3:165-68.
[23] Liang J, Cai WJ, Han T, Jing L, Ma Z, Gao Y. The expression of thymosin b4 in chronic hepatitis B combined nonalcoholic fatty liver disease. Medicine (Baltimore). 2016;95:e5763.

the concentration of serum Tb4 was compared between patients with NAFLD and healthy controls, serum Tb4 levels in patients with NAFLD were significantly lower. After treatment and subsequent improvement in liver function, the concentration of Tb4 increased.[24] Tian et al observed 83 cases of NAFLD and 80 healthy patients, and reported that Tb4 level can effectively be used as a biomarker of liver function, as increased Tb4 level indicated improved liver function, and decreased Tb4 level indicated severe liver damage.[25] These studies indicate that Tb4 expression is related to liver function in NAFLD patients.

RegeneRx

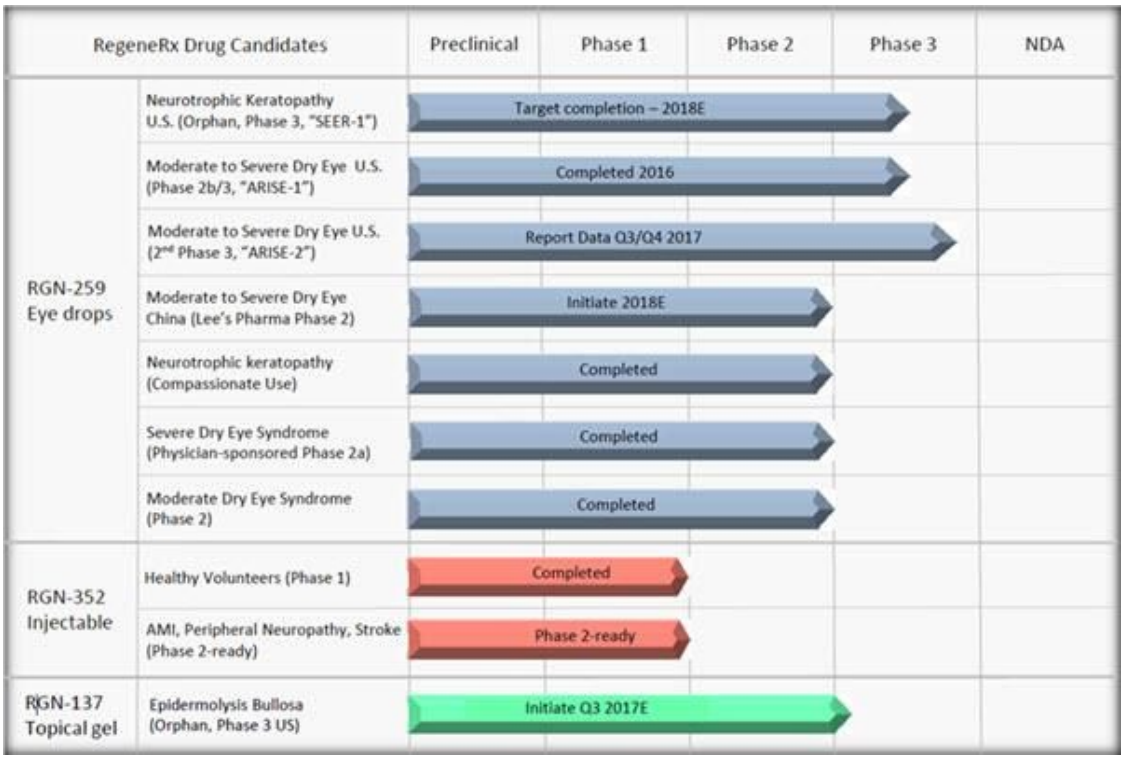
RegeneRx Pharmaceuticals, Inc. is a publicly traded, clinical-stage, biopharmaceutical company focused on tissue protection, repair and regeneration. RegeneRx acquired the rights to Tb4 from the NIH in 1999. RegeneRx has concentrated on the development of Tb4 for tissue and organ protection, repair and regeneration. RegeneRx currently has three drug candidates in clinical development for ophthalmic, cardiac and dermal indications, three active strategic licensing agreements in China, Pan Asia (Korea, Japan, and Australia, among others) and in the U.S. and Canada. RGN-259, the Company's T β 4-based ophthalmic drug candidate is being developed for dry eye syndrome and for the treatment of neurotrophic keratopathy (NK), both of which are being developed in the U.S and Canada through its joint venture, ReGenTree. ReGenTree has reported results of its recently completed Phase 2/3 U.S. trial in patients with dry eye syndrome and is conducting a Phase 3 clinical trial for the treatment of patients with NK, for which it has been granted orphan status by the U.S. FDA. RGN-352, the Company's T β 4-based injectable drug candidate is a Phase 2-ready drug candidate designed to be administered systemically to prevent and restore tissue damage associated with acute events such as heart attacks, strokes, and other similar injuries. RGN-137, the Company's T β 4-based dermal gel, is in phase 2 clinical development. See table 2 for more information on RegeneRx Tb4 Drug Candidates.

Recently, RegeneRx announced publication of a pilot clinical trial demonstrating that Tb4 was effective in the treatment of patients after an acute ST segment elevation myocardial infarction (STEMI).[26] The trial was designed to study whether endothelial progenitor cells (EPCs or immature cells) treated with T β 4 and transplanted into STEMI patients would improve cardiac and clinical function compared to a control group. Ten patients with STEMI were included; they were randomized to 2 groups: a T β 4-pre-treated EPC transplantation group (experimental group; n = 5) and an EPC transplantation group (control group; n = 5). EPCs were pre-treated with T β 4 24 hours before transplantation in

[24] Dong QY, Han T, Wang LF, Dong YP, Liu Y, Wang B. The signif- icance of serum thymosin b4 levels in patients with non-alcoholic fatty liver disease. Tianjin Med J. 2013;41:97e100 [in Chinese].
[25] Tian Y, Wang YB, Tan LL, Wang YY, Cheng Y. Appraisal value of Tb4 on liver function of non-alcoholic liver disease. Chin J Gastroenterol Hepatol. 2014;23:432e434 [in Chinese].
[26] Zhu J, Song J, Yu L, et al. Safety and efficacy of autologous thymosin B4 pre-treated endothelial progenitor cell transplantation in patients with acute ST segment elevation myocardial infarction: A pilot study. Cytotherapy. 2016;18(8):1037-42.

experimental group. Cardiac function was evaluated using echocardiography and emission computed tomography, as well as the 6-min walking test before and 6 months after the intervention. After 6 months, the left ventricular ejection fraction based on two different measurements improved by more than 50% ($p<0.05$), and the stroke volume, amount of blood ejected by the left ventricle, improved by approximately 50% (($p<0.05$) in the T β 4-pre-treated group. After 6 months of follow-up, the average 6-min walking distance was improved by 14% ($p<0.01$). There were no severe complications related to the procedure in either group during the follow-up.

Table 2: RegeneRx Tb4 Drug Candidates



Summary of beneficial effects of thymosin beta 4 (Tb4) include:[27,28]

- Differentiation of endothelial cells (blood and lymphatic vessels)
- Growth of new blood vessels - angiogenesis
- Keratinocyte migration
- Improves collagen deposition
- Decreases scar tissue formation
- Overall improved tissue repair
- Faster healing of wounds
- Repair of tendons and ligaments
- Improved flexibility of joints
- Prevents the formations of adhesions and fibrous bands in muscles, tendons and ligaments
- Decreases inflammation in various tissue types
- Increased muscle growth
- Increased in endurance and strength
- Relaxed muscle spasm and improved muscle tone
- Repair damaged heart tissue following a heart attack
- Healing of ulcers and lesions (including pressure, venous, stomach and intestinal ulcers)
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- Promotes hair growth
- Protects and restores neurons after brain injury
- Protects brain neurons from autoimmune inflammation

Potential Side Effects and/or Contraindications

- Thymosin beta 4 peptide administered subcutaneously is reported safe and efficacious in recommended dosages.
- As with all injections, redness and pain at the site of injection may be present.
- Do not use for more than 3 months without cycling (3 months on, 6 weeks off or 6 weeks on, 6 weeks off).
- Based on FDA recommendations and various guidance documents developed by the International Conference of Harmonization (ICH), 23 nonclincal studies have been performed to date that demonstrate the safety of Tb4 for its current and planned uses in man.[29]

[27] Yarmola EG, Kilmenko ES, Fujita G, et al. Thymosin beta4: actin regulation and more. Ann NY Acad Sci. 2007;1112:76-85.
[28] Treadwell T, Kleinman HK, Crockford D, et al. The regenerative peptide thymosin B4 accelerates the rate of dermal healing in preclinical and clinical animal models and in patients. Ann NY Acad Sci. 2012;1270:37-44.
[29] Crockford D, Turjman N, Allan C, et al. Thymosin beta4: structure, function , and biological properties current and future clinical applications. Ann NY Acad Sci. 2010;1194:179-89.

DISCLAIMER: Statements made are for educational purposes and have not been evaluated by the US Food and Drug Administration (FDA). They are not intended to diagnose, treat, cure, or prevent any disease. Peptides should only be administered by licensed and qualified health care professionals.