

RESEARCH MONOGRAPH . COMPOUND PROFILE

Thymalin

A heterogeneous polypeptide complex isolated from calf thymus, developed at the Military Medical Academy in Leningrad as a thymic bioregulator for immune restoration, distinguished from other thymic peptide preparations by its multicomponent composition containing the immunomodulatory dipeptides L-glutamyl-L-tryptophan and L-lysyl-L-glutamic acid and the tripeptide L-glutamyl-L-aspartyl-L-proline.

DOCUMENT	KDC-MN-1504
ISSUED	2026-05-15
REVISION	1.0
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COMPOUND CLASS	Thymic polypeptide bioregulator complex with immunomodulatory and geroprotective activity

ABSTRACT

Thymalin is a standardized polypeptide complex isolated from the thymus gland of calves by acid hydrolysis and ultrafiltration, containing peptide fractions in the 1,000 to 10,000 dalton molecular weight range. Developed at the Military Medical Academy in Leningrad (now Saint Petersburg) by Vladimir Khavinson and Vyacheslav Morozov in the 1970s, the preparation was registered as an immunomodulatory pharmaceutical in the Soviet Union in 1982 and has remained in clinical use in the Russian Federation for more than four decades. Unlike the structurally defined thymic peptides thymosin alpha-1 (a 28-amino-acid single-sequence peptide), thymulin (a zinc-dependent nonapeptide), and thymopentin (a synthetic pentapeptide fragment of thymopoietin), Thymalin is a multicomponent extract whose principal bioactive constituents have been identified by reversed-phase high-performance liquid chromatography as the dipeptide L-glutamyl-L-tryptophan (Glu-Trp, subsequently developed independently as Thymogen), the dipeptide L-lysyl-L-glutamic acid (Lys-Glu, developed as Vilon), and the tripeptide L-glutamyl-L-aspartyl-L-proline (Glu-Asp-Pro, developed as Crystagen). The molecular mechanism of the immunoprotective activity is attributed to the capacity of these short peptides to bind selectively to double-stranded DNA sequences and to histone proteins, thereby modulating chromatin conformation, gene expression, and the synthesis of immune system proteins including interleukins, interferons, heat-shock proteins, and components of the fibrinolytic system. In experimental systems, Thymalin stimulates the differentiation and functional activity of T-lymphocyte subpopulations (CD4⁺ and CD8⁺), normalizes the

ratio of T-helper to T-suppressor cells, enhances natural killer cell activity and phagocytosis, and modulates the balance between pro-inflammatory and anti-inflammatory cytokines. The geroprotective properties of Thymalin are supported by a prospective clinical observation of 266 elderly subjects over 6 to 8 years conducted at the St. Petersburg Institute of Bioregulation and Gerontology and the Institute of Gerontology of the Ukrainian Academy of Medical Sciences, in which Thymalin-treated subjects exhibited 2.0- to 2.1-fold lower mortality compared to controls receiving standard geriatric care, with further reductions (4.1-fold lower mortality) observed in a subgroup receiving annual combined Thymalin and Epithalamin treatment for 6 years. More recently, a prospective randomized single-blind controlled trial of Thymalin (10 mg intramuscular daily for 10 days) in 80 elderly patients with severe COVID-19 reported a 92 percent increase in blood lymphocytes, 6.5-fold reduction in interleukin-6, halved in-hospital mortality (19.4 percent versus 40.9 percent in controls), and more rapid clinical improvement (80.5 percent versus 59 percent). In vitro studies have demonstrated that Thymalin reduces expression of the stem cell markers CD44 and CD117 by 2- to 3-fold while increasing expression of CD28 (a marker of mature T lymphocytes) by 6.8-fold, consistent with stimulation of hematopoietic stem cell differentiation into functional T cells. The compound is administered by intramuscular or subcutaneous injection in short cyclical courses of 5 to 10 days at doses of 5 to 10 mg daily, with clinical effect reported to persist for weeks to months following each treatment course. The safety record across more than 40 years of clinical use indicates minimal adverse events, principally limited to injection-site reactions. This monograph reviews the composition, extraction, and characterization of Thymalin; the molecular pharmacology of its

constituent peptides at the level of DNA binding, histone interaction, and gene expression regulation; the pharmacokinetic properties; the preclinical evidence base across immune restoration, geroprotection, and oncology models; the clinical evidence in elderly immune decline, respiratory infections, perioperative immune suppression, and COVID-19; sourcing and quality verification considerations; reconstitution and handling protocols; stack interactions with other immunomodulatory agents; the adverse-event and safety profile; and a comparative assessment of five alternative thymic and immunomodulatory peptide preparations against Thymalin on five competency standards (novelty, effect size, promising potential, side-effect profile, and overall validation).

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Nonproprietary name: Thymalin (transliterated from the Russian: Timalin).

Marketed names: Thymalin (Saint Petersburg Institute of Bioregulation and Gerontology; Russian Federation pharmaceutical market). The compound has been marketed under the Thymalin trade name since its original registration in 1982 and is manufactured by several Russian pharmaceutical companies including SAMSON-MED (Saint Petersburg).

Development history identifier: The compound was developed under the research program of the Military Medical Academy in Leningrad (now the S. M. Kirov Military Medical Academy, Saint Petersburg) by Vladimir Khavinson and Vyacheslav Morozov beginning in the early 1970s [1, 2].

Chemical Abstracts Service registry number: No single CAS number is assigned to the Thymalin polypeptide complex, as it is a heterogeneous mixture rather than a single chemical entity. The individual constituent peptides carry separate identifiers: L-glutamyl-L-tryptophan (Thymogen, CAS 13433-09-5); L-lysyl-L-glutamic acid (Vilon); L-glutamyl-L-aspartyl-L-proline (Crystagen).

Pharmacological classification: Immunomodulatory polypeptide bioregulator; thymic extract; cytomedin.

1.2 Structural class and chemistry

Thymalin belongs to the class of compounds designated cytomedins, a term introduced by the Khavinson laboratory to describe tissue-specific peptide bioregulators isolated from animal organs by acid extraction and molecular-weight fractionation [1]. The preparation is not a single peptide with a defined amino acid sequence but rather a standardized mixture of short peptides (2 to 8 amino acid residues) with molecular weights spanning 1,000 to 10,000 daltons.

Principal bioactive constituents identified by reversed-phase HPLC:

- **EW (Glu-Trp):** The dipeptide L-glutamyl-L-tryptophan, molecular formula C₁₆H₁₉N₃O₅, molecular weight 337.35 g/mol. This dipeptide was isolated from Thymalin by reversed-phase high-performance liquid chromatography and subsequently synthesized as a standalone pharmaceutical registered in the Soviet Union in 1990 under the name Thymogen [3, 4].
- **KE (Lys-Glu):** The dipeptide L-lysyl-L-glutamic acid, molecular formula C₁₁H₂₁N₃O₅, molecular weight 275.30 g/mol. Developed independently as the preparation Vilon, this peptide demonstrates immunomodulatory and gero-protective activity [5].
- **EDP (Glu-Asp-Pro):** The tripeptide L-glutamyl-L-aspartyl-L-proline, molecular formula C₁₄H₂₁N₃O₈, molecular weight 375.33 g/mol. Developed independently as Crystagen, this tripeptide demonstrates immunoprotective activity including heterochromatin remodeling in lymphocytes of elderly subjects [6].

Physical properties: Thymalin is supplied as a white to off-white lyophilized powder, freely soluble in 0.9 percent sodium chloride solution and in sterile water for injection. The preparation is stable as a dry powder at 2 to 8 degrees Celsius with a shelf life of 2 to 3 years.

1.3 Extraction and preparation

The production of Thymalin proceeds through acid hydrolysis of bovine (calf) thymus tissue, followed by sequential ultrafiltration to isolate the peptide fraction below 10 kilodaltons [1, 7]. The resulting polypeptide complex is purified, standardized for biological activity by immunological assay (typically T-lymphocyte rosette-forming capacity or mitogen-stimulated proliferation assay), lyophilized under aseptic conditions, and packaged in sealed glass vials (typically 10 mg per vial) for reconstitution and parenteral administration. The manufacturing process is specified in Russian Pharmacopoeia standards and in the registration dossier maintained by SAMSON-MED and other licensed manufacturers. The extraction methodology is conceptually analogous to the preparation of other tissue-specific cytomedins in the Khavinson system, including Epithalamin (pineal gland extract), Cortexin (cerebral cortex extract), and Retinalamin (retinal extract) [2].

2. Abstract

Thymalin is a polypeptide bioregulator isolated from the thymus of calves by acid hydrolysis and ultrafiltration, containing a standardized mixture of short peptides (principally the dipeptides Glu-Trp and Lys-Glu and the tripeptide Glu-Asp-Pro) in the 1,000 to 10,000 dalton molecular weight range [1]. Developed at the Military Medical Academy in Leningrad by Khavinson and Morozov in the 1970s and registered as an immunomodulatory pharmaceutical in the Soviet Union in 1982, the compound has been in continuous clinical use in the Russian Federation for more than four decades for the treatment of immune deficiency states including post-infectious, post-surgical, post-radiation, and age-related immune decline [2, 7].

The molecular pharmacology of Thymalin is attributed to the capacity of its constituent short peptides to interact with double-stranded DNA at specific nucleotide sequences and with histone proteins (H1, H2b, H3, H4), thereby modulating chromatin conformation from transcriptionally silent heterochromatin to active euchromatin [6, 8]. This epigenetic mechanism regulates the expression of genes encoding immune system proteins (interleukins IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-17A; interferons; tumor necrosis factor alpha), heat-shock proteins (particularly HSPA1A), components of the fibrinolytic and hemostatic systems (plasminogen, tissue plasminogen activator, antithrombin III), and gerontogenes (GDF11, FNDC5, FGF19/21/23, HMGB1) [7, 9].

The principal clinical application of Thymalin is immune restoration in elderly and immunocompromised populations. A prospective clinical observation of 266 elderly subjects over 6 to 8 years demonstrated 2.0- to 2.1-fold lower mortality in Thymalin-treated subjects compared to controls, with a 4.1-fold mortality reduction in a subgroup receiving annual combined Thymalin and Epithalamin treatment [10, 11]. A randomized single-blind controlled trial in 80 elderly patients with severe COVID-19 reported that Thymalin (10 mg intramuscular daily for 10 days) produced a 92 percent increase in blood lymphocytes, 6.5-fold reduction in IL-6, halved in-hospital mortality (19.4 percent versus 40.9 percent), and accelerated clinical improvement [12]. In vitro, Thymalin reduces expression of stem cell markers CD44 and CD117 while increasing expression of the mature T-lymphocyte marker CD28 by 6.8-fold, consistent with stimulation of hematopoietic stem cell differentiation [13].

The compound is administered by intramuscular or subcutaneous injection in cyclical courses of 5 to 10 days at doses of 5 to 10 mg daily. The safety record across more than 40 years of clinical use indicates minimal adverse events. Thymalin is registered as a pharmaceutical in the Russian Federation; it is not approved by the United States Food and Drug Administration, the European Medicines Agency, or other Western regulatory authorities. The evidence base is dominated by Russian-language clinical literature that has not undergone independent replication in double-blind, placebo-controlled, multi-center trials meeting International Conference on Harmonisation standards. Investigators should interpret the available evidence within this methodological context and should obtain analytical confirmation of identity and purity on every research-grade lot.

3. Discovery and Development History

3.1 Origin

Thymalin was developed at the Military Medical Academy in Leningrad (now Saint Petersburg) by Vladimir Khavinson and Vyacheslav Morozov beginning in the early 1970s as part of a systematic program to isolate tissue-specific peptide bioregulators (cytomedins) from animal organs [1, 2]. The program was initiated under the hypothesis that each organ produces low-molecular-weight peptides that regulate its function and that exogenous administration of these peptides can restore function in pathological or age-related decline. The thymus was selected as the source organ for an immunomodulatory preparation on the basis of the known central role of the thymus in T-cell maturation and immune competence and the well-characterized age-related involution of thymic tissue beginning at puberty.

3.2 Preclinical development

The initial preclinical characterization of Thymalin was conducted in the mid-1970s in animal models of immune suppression, including cyclophosphamide-induced immunodeficiency, thymectomy-induced T-cell depletion, and age-related immune decline in rodents [1, 7]. These studies demonstrated that intramuscular administration of the thymic polypeptide complex restored T-lymphocyte counts, normalized T-helper to T-suppressor ratios, enhanced phagocytic activity, and improved resistance to experimental infections. The preparation was also shown to stimulate regeneration and hematopoiesis in models of bone marrow suppression.

3.3 Pharmaceutical registration

Following completion of preclinical and clinical evaluation according to Soviet pharmaceutical standards, Thymalin received registration as an immunomodulatory drug in the Soviet Union in 1982. It was approved for the treatment of immune deficiency states associated with acute and chronic infections, surgical procedures, radiation exposure, chemotherapy, and age-related immune decline [2, 7]. The compound has maintained its registration in the Russian Federation through successive re-registration cycles and remains available as a prescription pharmaceutical.

3.4 Identification of active constituent peptides

A critical advance in the understanding of Thymalin came from the isolation and identification of its individual bioactive peptide constituents by reversed-phase HPLC [3]. The dipeptide Glu-Trp was isolated from the Thymalin complex and demonstrated to reproduce the principal immunomodulatory effects of the parent preparation. This dipeptide was subsequently synthesized and registered as an independent pharmaceutical, Thymogen, in the Soviet Union in 1990 [4]. The dipeptide Lys-Glu (Vilon) and the tripeptide Glu-Asp-Pro (Crystagen) were similarly identified as bioactive constituents and developed as independent preparations [5, 6].

3.5 Expansion of the research program

From the 1990s through the present, the Khavinson laboratory at the St. Petersburg Institute of Bioregulation and Gerontology has extended the Thymalin research program to encompass geroprotective applications, molecular mechanisms of peptide-DNA and peptide-histone interaction, and applications in COVID-19 [7, 10, 12, 13]. The laboratory has also developed a broader class of tissue-specific peptide bioregulators following the same extraction and characterization methodology, including Epithalamin (pineal gland), Cortexin (cerebral cortex), and Retinalamin (retina), creating the conceptual framework of "peptide bioregulation" as a therapeutic paradigm [2].

3.6 Current regulatory and research status

Thymalin remains registered and clinically available in the Russian Federation. It is not approved by the United States Food and Drug Administration, the European Medicines Agency, or other Western regulatory authorities. The compound has not undergone registration-quality clinical trials meeting International Conference on Harmonisation standards. The evidence base is substantial by volume but is concentrated in Russian-language journals, with limited independent replication by Western research groups. Recent publications in English-language journals (Biology Bulletin Reviews, Advances in Gerontology, Bulletin of Experimental Biology and Medicine, International Journal of Molecular Sciences) have increased international accessibility of the primary data [7, 9, 12, 13].

4. Mechanism of Action

Thymalin exerts its immunomodulatory and geroprotective effects through a multi-level mechanism encompassing direct peptide-DNA interaction, peptide-histone binding, epigenetic modulation of gene expression, and downstream effects on immune cell differentiation, cytokine production, and hemostatic balance. The mechanism is immunomodulatory rather than purely immunostimulatory: the compound tends to normalize aberrant immune parameters toward physiological range rather than uniformly amplifying immune responses [7, 9].

4.1 Peptide-DNA interaction

The constituent short peptides of Thymalin (KE, EW, EDP) demonstrate sequence-specific binding to double-stranded DNA. The KE dipeptide shows pronounced selective binding to the TCGA DNA sequence, as demonstrated by molecular modeling and biochemical binding assays [8]. This interaction is proposed to modulate chromatin conformation at specific genomic loci, converting transcriptionally silent heterochromatin to active euchromatin and thereby increasing the transcriptional availability of genes encoding immune system proteins and regulatory factors.

4.2 Peptide-histone interaction

The constituent peptides bind preferentially to histone proteins H1/1, H1/3, H1/6, H2b, H3, and H4 at sites that normally interact with DNA [8]. This histone binding is proposed to alter nucleosome positioning and chromatin accessibility, complementing the direct DNA-binding mechanism. The combined peptide-DNA and peptide-histone interactions represent an epigenetic regulatory mechanism that operates at the level of chromatin remodeling rather than at the receptor-ligand level characteristic of conventional pharmacology.

4.3 Gene expression regulation

Through the chromatin-remodeling mechanism, Thymalin and its constituent peptides regulate the expression of multiple gene families [7, 9]:

Immune system genes: Interleukins IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-17A; interferons (IFN-gamma); tumor necrosis factor alpha (TNF-alpha). The regulatory effect is bidirectional: in states of excessive pro-inflammatory cytokine production (such as the cytokine storm in severe COVID-19), Thymalin reduces expression of IL-6 and TNF-alpha; in states of immune suppression, the compound enhances expression of IL-2 and IFN-gamma [7, 12].

Heat-shock proteins: Thymalin upregulates expression of HSPA1A and other heat-shock proteins involved in cellular stress responses and protein quality control [7].

Hemostatic and fibrinolytic proteins: The compound regulates expression of plasminogen, tissue plasminogen activator, urokinase plasminogen activator, and antithrombin III, contributing to normalization of blood coagulation and fibrinolytic activity [7, 9].

Gerontogenes and aging-related factors: Thymalin modulates expression of GDF11, FNDC5, FGF19/21/23, HMGB1, and MIC1/GDF15, factors implicated in the regulation of aging processes [7].

4.4 Immune cell differentiation and activation

At the cellular level, Thymalin produces the following characterized effects:

T-lymphocyte differentiation: The compound stimulates the differentiation and functional activity of T-lymphocyte subpopulations, particularly CD4⁺ T-helper cells and CD8⁺ cytotoxic T cells, and normalizes the CD4/CD8 ratio [7, 12].

Hematopoietic stem cell differentiation: In vitro studies demonstrate that Thymalin reduces expression of the stem cell markers CD44 and CD117 by 2- to 3-fold while increasing expression of CD28 (a costimulatory molecule expressed on mature T lymphocytes) by 6.8-fold, consistent with stimulation of hematopoietic stem cell differentiation along the T-cell lineage [13].

Natural killer cell activation: Thymalin enhances natural killer cell cytotoxic activity and increases circulating NK cell counts in immunocompromised subjects [12].

Phagocytosis enhancement: The compound increases the phagocytic activity of neutrophils and macrophages and enhances the oxidative burst capacity of phagocytic cells [7].

Cyclic nucleotide modulation: The EW dipeptide modulates the intracellular balance between cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) in lymphocytes, a mechanism shared with other thymic hormones and proposed as the proximate signal for T-cell activation [4].

4.5 Anti-inflammatory and cytokine-modulatory activity

Thymalin modulates the balance between pro-inflammatory and anti-inflammatory cytokines. In elderly subjects, the compound has been shown to reduce excessive TNF-alpha and IL-6 production (associated with the chronic low-grade inflammation of aging, termed "inflammaging") while maintaining adequate IFN-gamma and IL-2 levels needed for effective immune surveillance [7, 12]. This immunomodulatory (rather than immunostimulatory) profile distinguishes Thymalin from conventional immune stimulants and is the basis for its application in conditions characterized by immune dysregulation rather than simple immune deficiency.

4.6 Oncogene regulation

The KE dipeptide component suppresses HER-2/neu oncogene expression by approximately twofold in transgenic mice, reduces matrix metalloproteinase-9 (MMP-9) expression, and increases Ki-67 and CD98hc expression [7]. The EDP tripeptide inhibits proliferation of K-562 tumor cells while enhancing normal lymphocyte activity, suggesting selective antiproliferative effects on transformed cells [6].

5. Pharmacokinetics and Routes of Administration

5.1 Absorption

Thymalin is administered parenterally (intramuscular or subcutaneous injection). After subcutaneous administration, the peptide components are deposited in the hypodermis and diffuse into the systemic circulation over approximately 6 to 8 hours, producing relatively stable serum concentrations without the rapid peak-and-trough pattern of intravenous administration [14]. After intramuscular injection, absorption is more rapid, with peak plasma concentrations of the constituent peptides achieved within approximately 2 to 3 hours. Oral bioavailability has not been formally characterized but is expected to be negligible for the larger peptide components due to gastrointestinal proteolysis, although the very short dipeptides (Glu-Trp, Lys-Glu) may retain partial oral absorption through intestinal peptide transporters. All registered formulations are parenteral.

5.2 Distribution

Formal volume-of-distribution studies for the Thymalin polypeptide complex have not been published in the English-language literature. Given the low molecular weight of the constituent peptides (275 to 375 g/mol for the principal components), rapid tissue distribution following parenteral administration is expected. The constituent dipeptides and tripeptides are expected to distribute freely to the extravascular compartment. Central nervous system penetration data are not available, although the Khavinson laboratory has reported behavioral and neuroendocrine effects (including modulation of melatonin secretion through the pineal-thymic axis) that suggest at least indirect central effects [10].

5.3 Metabolism

The constituent peptides of Thymalin are metabolized through standard peptide degradation pathways: aminopeptidases, carboxypeptidases, and endopeptidases present in plasma and tissues cleave the dipeptides and tripeptides to their con-

stituent amino acids. The metabolic half-life of short peptides (2 to 3 amino acid residues) in plasma is typically minutes to tens of minutes. The clinical effect of Thymalin persists for weeks to months following a 5- to 10-day treatment course, a duration that cannot be explained by conventional pharmacokinetic persistence and is attributed to the epigenetic mechanism (chromatin remodeling and gene expression changes that persist after the peptide is cleared) rather than to sustained plasma concentrations [7, 8].

5.4 Elimination

The amino acid components produced by peptide degradation are incorporated into normal amino acid metabolism and protein synthesis pathways. Renal clearance of intact peptide is expected to contribute minimally to total elimination given the rapid enzymatic degradation. No dose adjustment for renal or hepatic impairment has been formally characterized, although the absence of hepatic CYP-mediated metabolism and the small molecular size of the constituents suggest that significant drug accumulation in organ impairment is unlikely.

5.5 Routes of administration

Intramuscular injection: The principal route used in the Russian clinical literature and the route specified in the registration dossier. Standard protocol: Thymalin 10 mg reconstituted in 1 to 2 mL of 0.9 percent sodium chloride, injected into the deltoid or gluteal muscle once daily for 5 to 10 consecutive days [7, 12].

Subcutaneous injection: Widely used in research and clinical practice with reported comparable efficacy to intramuscular administration. Standard subcutaneous injection sites include the abdomen, outer thigh, and upper arm [14].

Intravenous administration: Not a standard route. The compound has not been characterized for intravenous use in published clinical studies.

5.6 Dosing regimens

The registered dose in the Russian Federation is 5 to 10 mg daily by intramuscular injection for courses of 3 to 10 days, with courses repeated at intervals of 1 to 6 months as clinically indicated. The COVID-19 clinical trial used 10 mg daily for 10 days [12]. The geroprotective studies used annual or biannual courses [10, 11].

6. Preclinical Pharmacology

6.1 Immune restoration models

Thymalin has been characterized in multiple rodent models of immune suppression. In cyclophosphamide-induced immunodeficiency in mice and rats, intramuscular Thymalin administration restored T-lymphocyte counts, normalized the CD4/CD8 ratio, enhanced mitogen-stimulated lymphocyte proliferation, and improved resistance to subsequent infectious challenge [1, 7]. In thymectomized animals, Thymalin partially restored T-cell numbers and function, although the effect was less complete than in pharmacological immunosuppression models, consistent with the requirement for residual thymic stromal tissue to support the full differentiation pathway.

6.2 Geroprotective effects in animal models

In aging rodent models, chronic cyclical Thymalin administration (repeated short courses over the lifespan) produced measurable preservation of thymic cortical architecture, maintained the cortex-to-medulla ratio (the loss of which is a hallmark of thymic involution), increased macrophage and mast cell populations in aged tissues, and reduced the incidence of spontaneous tumors [7, 15]. The synthetic dipeptide L-Glu-L-Trp (the principal Thymalin constituent) administered chronically to rats slowed aging as assessed by survival curves and inhibited spontaneous carcinogenesis, with treated animals showing significantly lower tumor incidence and longer median lifespan [15].

6.3 Hematopoietic stem cell differentiation

Linkova et al. (2020) demonstrated in vitro that Thymalin stimulates differentiation of human hematopoietic stem cells (HSCs) by reducing expression of CD44 (a glycoprotein involved in cell adhesion and stem cell maintenance) and CD117 (c-Kit receptor, a marker of intermediate-stage HSC differentiation) by 2- to 3-fold while increasing expression of CD28 (a costimulatory molecule on mature T cells) by 6.8-fold [13]. This differentiation pattern indicates that Thymalin drives HSCs through the intermediate progenitor stage toward the mature T-lymphocyte phenotype.

6.4 Anti-tumor activity

The EDP tripeptide (Crystagen) component inhibits proliferation of K-562 chronic myelogenous leukemia cells in vitro while simultaneously enhancing the proliferative activity of normal peripheral blood lymphocytes [6]. The KE dipeptide (Vilon) suppresses HER-2/neu oncogene expression by approximately twofold in transgenic mice bearing HER-2/neu-positive tumors [7]. The spleen explant growth zone is increased by 20 to 50 percent by Thymalin treatment, while apoptosis of immune cells is reduced by 29 to 42 percent compared to control [7].

6.5 Hemostatic effects

In preclinical pneumonia models, Thymalin normalized blood coagulation parameters and fibrinolytic activity, reducing markers of disseminated intravascular coagulation and systemic inflammatory response syndrome [7, 9]. These effects are attributed to regulation of gene expression for plasminogen, tissue and urokinase plasminogen activators, and antithrombin III.

6.6 Telomere length regulation

The KE dipeptide normalizes telomere length in phytohemagglutinin-stimulated lymphocytes, a finding consistent with the geroprotective mechanism and potentially contributing to the long-term preservation of immune cell replicative capacity observed in the clinical geroprotective studies [7].

7. Clinical Evidence Base

7.1 Immune restoration in elderly populations

The principal geroprotective clinical dataset is the Khavinson and Morozov prospective study of 266 elderly subjects (aged 60 years and older) observed over 6 to 8 years at the St. Petersburg Institute of Bioregulation and Gerontology and the Institute of Gerontology of the Ukrainian Academy of Medical Sciences [10, 11]. Subjects were allocated to four groups: Thymalin treatment, Epithalamin (pineal peptide bioregulator) treatment, combined Thymalin and Epithalamin treatment, and a control group receiving standard geriatric care. Bioregulator courses were administered during the first 2 to 3 years of the observation period.

Mortality results: The Thymalin-treated group exhibited 2.0- to 2.1-fold lower mortality compared to controls. The Epithalamin-treated group exhibited 1.6- to 1.8-fold lower mortality. The combined treatment group exhibited 2.5-fold lower mortality. A separate subgroup that received annual combined Thymalin and Epithalamin courses for 6 continuous years exhibited 4.1-fold lower mortality compared to controls [10, 11].

Physiological improvements: Treated subjects demonstrated normalization of cardiovascular indices (blood pressure, electrocardiographic parameters), endocrine parameters (cortisol, insulin, thyroid hormones), immune parameters (T-lymphocyte counts, CD4/CD8 ratio, natural killer cell activity), and neurological function. Acute respiratory disease incidence was reduced 2.0- to 2.4-fold in treated groups compared to controls [10].

Reduced disease incidence: The bioregulator-treated groups showed reduced incidence of clinical manifestations of ischemic heart disease, hypertension, deforming osteoarthritis, and osteoporosis compared to controls [10, 11].

Methodological limitations: The study was not double-blind, was not placebo-controlled (the control group received standard care rather than matched placebo injections), and allocation methodology is not described to the standard of modern randomized controlled trial reporting. The results have not been independently

replicated by groups outside the Khavinson research network. These limitations are significant and should inform the evidential weight assigned to the mortality and morbidity outcomes.

7.2 COVID-19

Kuznik et al. (2021) conducted a prospective, randomized, single-blind, controlled trial of Thymalin in 80 elderly patients (aged 60 years and older) hospitalized with severe COVID-19 at the Chita State Medical Academy Hospital [12]. The investigation group (n=36) received standard COVID-19 therapy plus Thymalin 10 mg in 2 mL of 0.9 percent NaCl by intramuscular injection daily for 10 days. The control group (n=44) received standard therapy plus placebo.

Immune parameters: Thymalin increased blood lymphocytes by 92 percent compared to minimal change in controls. T-lymphocytes, B-lymphocytes, and NK cells increased 2.2-, 2.0-, and 2.4-fold, respectively. CD4+ and CD8+ T cells each increased 2.2-fold [12].

Inflammatory markers: IL-6 decreased 6.5-fold in the Thymalin group versus no significant change in controls. C-reactive protein decreased 3.3-fold (Thymalin) versus 2.2-fold (control). D-dimer decreased 1.5-fold (Thymalin) versus no change (control) [12].

Clinical outcomes: In-hospital mortality was 19.4 percent in the Thymalin group versus 40.9 percent in controls. Clinical improvement was observed in 80.5 percent of Thymalin-treated patients versus 59 percent of controls (p=0.039). Time to lymphocyte recovery was 7.36 days (Thymalin) versus 10.78 days (control, p=0.002). Recovery from lymphopenia was observed in 66.6 percent (Thymalin) versus 43.18 percent (control, p=0.037) [12].

Statistical analysis: Wilcoxon signed-rank test with Hommel correction was used for multiple pairwise comparisons; p value less than 0.05 was considered statistically significant [12].

7.3 Post-surgical and post-radiation immune restoration

Thymalin has been used in the Russian Federation for perioperative immune support in patients undergoing major surgery, and for immune restoration following radiation therapy and chemotherapy. The clinical literature in these indications is

primarily Russian-language and is summarized in review articles [1, 7]. Reported outcomes include normalization of T-lymphocyte counts, improved wound healing, reduced post-operative infection rates, and faster recovery of white blood cell counts following myelosuppressive chemotherapy.

7.4 Acute and chronic infections

The registered indication in the Russian Federation includes adjunctive therapy for acute and chronic viral and bacterial infections, including pneumonia, sepsis, and infectious purulent processes. Thymalin treatment in pneumonia patients has been reported to normalize blood coagulation and fibrinolytic activity, reduce systemic inflammatory response markers, and accelerate clinical recovery [7, 9].

7.5 Immunogram normalization in elderly subjects

The EDP tripeptide (Crystagen) component, studied as an independent preparation, achieved immunogram normalization (defined as normalization of T- and B-lymphocyte counts, CD4/CD8 ratio, and immunoglobulin levels) in 82 percent of elderly patients compared to 56 percent of controls receiving standard care [6].

7.6 Hematopoietic stem cell activation in COVID-19 context

Khavinson and Linkova (2021) published data on hematopoietic stem cell differentiation as a proposed mechanism for Thymalin efficacy in COVID-19, noting that the compound stimulates CD28+ T-lymphocyte production from hematopoietic precursors, potentially compensating for the lymphopenia characteristic of severe SARS-CoV-2 infection [13, 16].

7.7 Limitations of the clinical evidence base

The clinical evidence for Thymalin is substantial in volume but limited in methodological rigor by contemporary Western standards. The following limitations should be acknowledged in any assessment of the evidence:

(1) The majority of clinical studies were conducted in the Russian Federation and published in Russian-language journals with limited accessibility to the international research community. (2) Most studies are open-label, non-blinded, or single-

blind rather than double-blind. (3) Placebo controls are frequently absent or consist of standard-care comparators rather than matched injectable placebos. (4) Sample sizes are modest by contemporary registration-trial standards. (5) Independent replication by research groups outside the Khavinson research network is essentially absent. (6) The compound has not undergone regulatory evaluation by the FDA, EMA, or other Western regulatory authorities that apply ICH-GCP standards.

8. Sourcing and Quality Verification

Pharmaceutical-grade Thymalin is manufactured by licensed Russian pharmaceutical companies (SAMSON-MED, others) and is available through Russian pharmacy distribution channels. The pharmaceutical-grade material is the highest-quality source for research applications where regulatory equivalence to the clinical trial material is operationally important. Supply outside the Russian Federation is through import from Russian distributors or through research peptide suppliers.

Research-grade Thymalin is available from multiple peptide research suppliers as a lyophilized polypeptide complex, typically in 10 mg or 20 mg vials. The individual constituent peptides (Glu-Trp, Lys-Glu, Glu-Asp-Pro) are also available as synthetic preparations from peptide research suppliers at greater than 95 percent purity by HPLC.

Quality verification recommendations for research-grade lots:

- High-performance liquid chromatography with UV detection to confirm the peptide profile and assess relative abundance of the principal constituent peptides
- Mass spectrometry (MALDI-TOF or ESI-MS) to confirm molecular weight distribution within the expected 1,000 to 10,000 dalton range and to identify the principal peptide components
- Amino acid analysis to confirm the expected amino acid composition (enrichment for glutamic acid, tryptophan, lysine, aspartic acid, and proline relative to a random protein hydrolysate)
- Endotoxin testing (Limulus amebocyte lysate assay) for preparations intended for parenteral research use, given the bovine tissue origin
- Sterility testing for reconstituted preparations intended for injection
- Certificate of analysis from the manufacturer or supplier, with independent third-party confirmation recommended for any investigation where dose-response precision is important

The heterogeneous nature of the Thymalin complex introduces lot-to-lot variability that is inherently greater than that of single-sequence synthetic peptides. Investigators requiring maximal reproducibility may prefer to work with the indi-

vidual synthetic constituent peptides (Glu-Trp, Lys-Glu, Glu-Asp-Pro) rather than the native extract, at the cost of potentially missing synergistic interactions between the components.

Third-party analytical laboratories (Janoshik Analytical, Anabolic Lab, others) provide independent confirmation of vendor certificates of analysis. Given the biological-extract nature of Thymalin and the potential for bovine-derived contaminants, on-receipt analytical confirmation is strongly recommended for any research application.

9. Reconstitution and Handling

Thymalin is supplied as a lyophilized powder in sealed glass vials, typically containing 10 mg per vial.

Reconstitution protocol: Add 1 to 2 mL of sterile 0.9 percent sodium chloride solution or bacteriostatic water for injection to the vial. Gently swirl to dissolve; do not shake vigorously, as vigorous agitation may denature the peptide components. The standard reconstitution volume is 1 mL per 5 mg or 2 mL per 10 mg, producing a solution concentration of 5 mg/mL suitable for volumetric dosing with standard insulin syringes marked in 0.1 mL increments [14].

Appearance of reconstituted solution: The solution should be clear and colorless to faintly yellow, free of visible particulate matter. Any solution that is cloudy, discolored, or contains visible particles should be discarded.

Storage of lyophilized powder: 2 to 8 degrees Celsius (standard refrigeration), protected from light. Do not freeze the lyophilized powder. Shelf life is typically 2 to 3 years when stored under proper conditions.

Storage of reconstituted solution: When reconstituted with bacteriostatic water for injection, the solution should be refrigerated at 2 to 8 degrees Celsius and used within 28 days. When reconstituted with sterile water for injection (without bacteriostatic preservative), the solution should be used within 24 hours. Do not freeze reconstituted Thymalin; ice crystal formation during freezing physically disrupts peptide bonds and may reduce or eliminate biological activity [14].

Aliquoting: For research applications requiring multiple injections from a single vial, aliquoting into single-use volumes in sterile microcentrifuge tubes at the time of reconstitution is recommended to minimize the number of needle punctures through the vial septum and to reduce the risk of microbial contamination.

Injection technique: For intramuscular administration, standard aseptic injection technique with a 25- to 27-gauge needle of appropriate length for the injection site. For subcutaneous administration, a 29- to 31-gauge insulin syringe is appropriate. Common subcutaneous injection sites include the abdominal subcutaneous tissue (avoiding the periumbilical area), the outer thigh, and the posterior upper arm.

Light sensitivity: The tryptophan-containing EW dipeptide is susceptible to photodegradation. While light protection is not strictly required for short-term storage, wrapping vials in aluminum foil or storing in opaque containers is reasonable practice for extended storage of reconstituted solutions.

10. Stack Interactions and Combinations

Thymalin interacts with other agents through its immunomodulatory mechanism rather than through cytochrome P450-mediated pharmacokinetic interactions. The absence of hepatic CYP-dependent metabolism eliminates the class of drug-drug interactions that dominate conventional small-molecule pharmacology. Investigators conducting research with Thymalin in combination with other compounds should consider the following interactions:

Immunosuppressive agents: Concurrent use of Thymalin with calcineurin inhibitors (cyclosporine, tacrolimus), mTOR inhibitors (sirolimus, everolimus), or anti-proliferative agents (mycophenolate mofetil, azathioprine) may produce pharmacodynamic antagonism, as Thymalin stimulates the immune cell populations that these agents suppress. The combination is contraindicated in organ transplant recipients where maintenance of immunosuppression is critical [17].

Other thymic peptide preparations: Concurrent use with thymosin alpha-1 (thymalfasin), thymulin, thymopentin, or Thymogen (the synthetic EW dipeptide) produces additive immunomodulatory effects. Whether the combination is synergistic, additive, or redundant has not been formally characterized in controlled studies. The combination of Thymalin with its own synthetic constituent Thymogen is pharmacologically redundant.

Epithalamin and Epitalon (pineal peptide bioregulators): The combination of Thymalin with Epithalamin (the native pineal polypeptide extract) or Epitalon (the synthetic tetrapeptide Ala-Glu-Asp-Gly derived from Epithalamin) has been studied in the geroprotective clinical trials and produced the largest mortality reductions observed in the Khavinson dataset (4.1-fold mortality reduction with annual combined treatment) [10, 11]. The proposed mechanism involves functional synergy between the thymic and pineal peptide bioregulatory systems (the "pineal-thymic axis"), with Thymalin restoring immune competence and Epithalamin restoring circadian melatonin secretion and neuroendocrine regulation.

Corticosteroids: Concurrent use with glucocorticoids (dexamethasone, prednisone, methylprednisolone) may partially attenuate the immunostimulatory effects of Thymalin through glucocorticoid-mediated lymphocyte suppression. In the COVID-19 clinical trial, Thymalin was administered concurrently with standard COVID-19 therapy that included dexamethasone, and the immunomodulatory effect was preserved [12].

Vaccines: Due to the immunomodulatory action of Thymalin, concurrent administration with live attenuated vaccines should be approached with caution. A waiting period of at least 2 weeks between Thymalin course completion and live vaccine administration is recommended in the Russian clinical literature [17].

Interferons and interleukin-based therapies: Concurrent use with exogenous interferon-alpha, interferon-beta, or interleukin-2 therapy may produce additive or unpredictable immunomodulatory effects, as Thymalin independently regulates the expression of these cytokines at the gene level.

Conventional small-molecule pharmaceuticals: The absence of CYP-mediated metabolism means that Thymalin is not expected to produce pharmacokinetic interactions with drugs metabolized by CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, or other major hepatic CYP isoforms. No CYP-based drug-drug interactions have been reported in the clinical literature.

Other peptide bioregulators (Cortexin, Retinalamin): The Khavinson system of tissue-specific peptide bioregulators includes preparations targeting multiple organ systems. Concurrent use of Thymalin with Cortexin (cerebral cortex peptides) or Retinalamin (retinal peptides) has been used clinically in the Russian Federation for multi-system geriatric support. The interactions are pharmacodynamic (additive tissue-specific bioregulatory effects) rather than pharmacokinetic [2].

11. Adverse Events and Safety

11.1 Common adverse events

The safety record of Thymalin across more than four decades of clinical use in the Russian Federation indicates a favorable tolerability profile. The most commonly reported adverse events are [7, 17]:

- Injection-site reactions: local pain, erythema, or induration at the intramuscular or subcutaneous injection site (incidence not precisely quantified in published studies but described as infrequent)
- Transient low-grade fever (less than 38 degrees Celsius) during the initial days of treatment, attributed to immune activation
- Mild fatigue or malaise during the treatment course

These events are generally self-limiting and do not require dose adjustment or treatment discontinuation.

11.2 Rare adverse events

Rare adverse events reported in the clinical literature or in post-marketing surveillance include [17]:

- Allergic reactions including urticaria, rash, and pruritus (rare, attributed to bovine protein sensitivity in the native extract)
- Restlessness (akathisia) and irritability or mood instability (rare, mechanism unclear)
- Transient muscle atrophy at the injection site (rare, associated with repeated intramuscular injections at the same site)
- Polyarthralgia combined with hand edema (rare, reported in isolated cases)

Anaphylaxis has not been reported in the published clinical literature but remains a theoretical risk for any protein-derived injectable preparation of animal origin.

11.3 Contraindications

Thymalin is contraindicated in the following situations [17]:

- Known hypersensitivity to thymic peptides or to any component of the preparation
- Organ transplant recipients receiving immunosuppressive therapy (due to the risk of graft rejection from immune stimulation)
- Active autoimmune disease in flare (due to the potential for exacerbation of autoimmune responses; use in stable autoimmune disease requires careful risk-benefit assessment)
- Pregnancy and lactation (insufficient safety data; animal reproductive toxicity studies have not demonstrated teratogenicity, but human data are absent)
- Patients with known severe allergies to animal-derived peptide products

11.4 Safety in special populations

Elderly subjects: The geroprotective clinical studies specifically enrolled elderly subjects (aged 60 years and older) and reported favorable tolerability with no increase in adverse events compared to younger adult populations. The COVID-19 trial enrolled subjects with a mean age in the seventh decade and reported no serious adverse events attributable to Thymalin [12].

Pediatric subjects: Limited data are available on pediatric use. Thymalin has been used in the Russian Federation in children with recurrent infections and immune deficiency states; the published pediatric literature reports tolerability comparable to adult use at weight-adjusted doses [7].

Renal and hepatic impairment: No formal pharmacokinetic studies in organ impairment. The absence of CYP-mediated metabolism and the small molecular weight of the constituents suggest that dose adjustment is unlikely to be necessary, but formal data are lacking.

11.5 Long-term safety

The 6- to 8-year geroprotective study provides the longest follow-up data and reported no long-term safety signals attributable to Thymalin. The treated groups had lower mortality and lower incidence of cardiovascular disease, respiratory infections, and musculoskeletal disease compared to controls [10, 11]. These data are

consistent with a favorable long-term safety profile, although the limitations of the study design (non-blinded, non-placebo-controlled) should be considered in evaluating the safety signal.

11.6 Bovine-origin safety considerations

As a bovine-derived biological product, Thymalin carries theoretical risks associated with animal-origin preparations, including potential contamination with prions (bovine spongiform encephalopathy agent) and other adventitious agents. Manufacturing processes include acid hydrolysis and ultrafiltration steps that reduce but may not entirely eliminate these risks. Investigators should confirm that source material originates from BSE-free herds and that the manufacturing process includes validated viral and prion inactivation or removal steps.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five alternative thymic and immunomodulatory peptide preparations against Thymalin, evaluated on five competency standards: novelty, effect size, promising potential (current clinical state and trajectory), side-effect profile, and overall validation. The comparators were selected as the principal thymic peptide-based immunomodulatory agents with substantive clinical or preclinical evidence.

12.1 Thymosin Alpha-1 (Thymalfasin, Zadaxin)

Mechanism: Thymosin alpha-1 is a 28-amino-acid peptide originally isolated from thymosin fraction 5 (a partially purified bovine thymus extract) by Allan Goldstein at the George Washington University [18]. It stimulates T-cell maturation via differentiation of CD4+ and CD8+ progenitors, activates dendritic cells and natural killer cells, and stimulates cytokine production (IL-2, IFN-gamma). The mechanism is predominantly at the cell-surface receptor and intracellular signaling level, in contrast to the epigenetic (DNA/histone-binding) mechanism proposed for Thymalin.

Development trajectory: Thymalfasin (Zadaxin, SciClone Pharmaceuticals) was approved as an orphan drug by the FDA for treatment of malignant melanoma, chronic active hepatitis B, DiGeorge syndrome, and hepatocellular carcinoma. The compound is registered in more than 35 countries. Clinical trials in chronic hepatitis B demonstrated 40.6 percent complete virological response with 1.6 mg subcutaneous injection twice weekly [19]. In hepatitis C, combination therapy with interferon-alpha produced 22.4 percent sustained biochemical response versus 9.3 percent with interferon alone [20]. The safety profile across more than 600,000 treated patients is excellent.

Novelty: Low to moderate. Thymosin alpha-1 is the longest-studied and most extensively characterized single-sequence thymic peptide, but the immunostimulatory mechanism is well established and does not introduce novel pharmacological concepts.

Effect size: Moderate. Hepatitis B virological response rates of 40 percent and hepatitis C combination-therapy response improvements are clinically meaningful. The immune-reconstitution effects in immunocompromised populations are reproducible across multiple studies and research groups [18, 19].

Promising potential: High. Active pharmaceutical registration in multiple jurisdictions, ongoing clinical trials in cancer immunotherapy and infectious disease, and a robust manufacturing and supply chain.

Side-effect profile: Excellent. Across more than 600,000 treated patients and decades of use, thymosin alpha-1 has maintained an outstanding safety profile with minimal adverse events [18].

Overall validation: High. The most extensively validated thymic peptide preparation globally, with regulatory approval, large-scale clinical trial data, and independent replication by multiple international research groups.

Comparison to Thymalin: Thymosin alpha-1 has substantially stronger regulatory validation, larger clinical datasets, and independent international replication. Thymalin has the theoretical advantage of multicomponent activity (multiple peptides with potentially synergistic epigenetic mechanisms) and the geroprotective evidence base. The two compounds target overlapping but distinct mechanisms: thymosin alpha-1 operates through receptor-mediated cell signaling, Thymalin through epigenetic chromatin remodeling.

12.2 Thymulin (Facteur Thymique Serique, FTS)

Mechanism: Thymulin is a nonapeptide (nine amino acids: Glu-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn) produced exclusively by thymic epithelial cells [21]. It is biologically active only when bound to zinc (the zinc-thymulin complex is the active form). Thymulin promotes T-cell differentiation, modulates cytokine production, and has analgesic and anti-inflammatory properties. The zinc dependence is a unique feature not shared by other thymic peptides.

Development trajectory: Thymulin has been characterized primarily as a research tool for understanding thymic endocrine function. A synthetic zinc-bound form has been studied in preclinical models of immune deficiency, pain, and inflammation. Clinical development has been limited; no pharmaceutical registration has been achieved in any jurisdiction [21].

Novelty: Moderate. The zinc-dependent activation mechanism is unique among thymic peptides and introduces an interesting mineral-cofactor dimension to thymic immunology.

Effect size: Preclinical effects are consistent across studies. Clinical effect size data are absent due to the lack of clinical trials.

Promising potential: Low to moderate. The compound remains a research tool without a clinical development pathway.

Side-effect profile: Preclinical only. No human safety database exists.

Overall validation: Low. Extensive preclinical characterization but no clinical validation.

Comparison to Thymalin: Thymulin is a structurally defined single peptide with a unique zinc-dependent mechanism, in contrast to the heterogeneous multicomponent Thymalin extract. Thymalin has the advantage of four decades of clinical use and a substantial (if methodologically limited) clinical evidence base. Thymulin has the advantage of structural precision but lacks any clinical validation.

12.3 Thymopentin (TP-5, Timunox)

Mechanism: Thymopentin is a synthetic pentapeptide (Arg-Lys-Asp-Val-Tyr) corresponding to residues 32 through 36 of the 49-amino-acid thymic hormone thymopoietin [22]. It stimulates early T-cell differentiation, modulates helper and suppressor T-cell function, and enhances production of interleukin-2 and colony-stimulating factors. The mechanism operates through cell-surface binding and intracellular signal transduction rather than through epigenetic chromatin remodeling.

Development trajectory: Thymopentin was studied in clinical trials for rheumatoid arthritis, HIV infection, chronic hepatitis B, and primary immunodeficiency. It was registered in some markets (Italy, China) for immune-modulatory indications. Clinical development outside these markets was limited, and the compound is no longer in active development for new indications [22].

Novelty: Low. The thymopoietin-derived pentapeptide mechanism is well characterized and does not introduce novel concepts.

Effect size: Modest. Clinical trials in rheumatoid arthritis and HIV-related immunodeficiency reported moderate immune reconstitution effects but insufficient magnitude for broad clinical adoption.

Promising potential: Low. Development is essentially terminated in most jurisdictions.

Side-effect profile: Favorable. Clinical trials reported minimal adverse events, principally injection-site reactions and transient flu-like symptoms.

Overall validation: Moderate. The compound has clinical trial data and limited regulatory approval, but the clinical evidence base is small by contemporary standards and development is inactive.

Comparison to Thymalin: Thymopentin is a structurally defined single peptide with a conventional receptor-mediated mechanism. Thymalin has a broader range of reported clinical effects (immune restoration, geroprotection, hemostatic normalization) and a longer continuous clinical use history. Thymopentin has the advantage of Western clinical trial data, although both compounds lack robust ICH-standard validation.

12.4 Thymogen (L-Glutamyl-L-Tryptophan, EW Dipeptide)

Mechanism: Thymogen is the synthetic dipeptide Glu-Trp, identical to the principal bioactive constituent of Thymalin isolated by reversed-phase HPLC [3, 4]. The mechanism is therefore a subset of the Thymalin mechanism: DNA and histone binding, chromatin remodeling, and gene expression regulation of immune and hemostatic proteins. Thymogen modulates cAMP/cGMP balance in lymphocytes, stimulates T-cell differentiation, enhances phagocytosis, and has demonstrated anti-aging and anticarcinogenic effects in rats [4, 15]. Notably, the L-Glu-L-Trp stereoisomer (Thymogen) is immunostimulatory, while the D-Glu-D-Trp enantiomer (Thymodepressin) exhibits immunosuppressive properties, demonstrating stereospecific biological activity [23].

Development trajectory: Registered as a pharmaceutical in the Soviet Union in 1990, Thymogen is available in the Russian Federation as an injectable, an intranasal spray, and a topical cream. It has not been registered outside the Russian Federation and has not undergone Western regulatory evaluation [4].

Novelty: Low. Thymogen is a component of Thymalin and shares the same molecular mechanism.

Effect size: Comparable to Thymalin in immune restoration endpoints. In the carcinogenesis study, chronic Glu-Trp administration significantly reduced spontaneous tumor incidence and extended median lifespan in rats [15].

Promising potential: Moderate within the Russian pharmaceutical market; low in the international context due to absence of Western regulatory evaluation.

Side-effect profile: Excellent. Described as "practically non-toxic" in the Russian pharmacological literature, with minimal adverse events across decades of clinical use [4, 7].

Overall validation: Moderate. Pharmaceutical registration in Russia, decades of clinical use, but absence of ICH-standard clinical trial data and independent international replication.

Comparison to Thymalin: Thymogen is a single-component derivative of Thymalin. The advantage of Thymogen is structural precision and reproducibility (a defined synthetic dipeptide versus a heterogeneous extract). The advantage of Thymalin is the potentially synergistic contribution of the other constituent peptides (KE, EDP) not present in Thymogen. The choice between the two depends on whether the investigator values structural precision (Thymogen) or multicomponent activity (Thymalin).

12.5 Thymosin Beta-4 (TB-500)

Mechanism: Thymosin beta-4 is a 43-amino-acid peptide that is the principal actin-sequestering peptide in mammalian cells [24]. Although named for its original isolation from the thymus (as part of thymosin fraction 5), thymosin beta-4 is ubiquitously expressed and is not thymus-specific. Its principal biological activities are promotion of cell migration, angiogenesis, tissue repair, and wound healing, with immunomodulatory effects that are secondary to its tissue-repair function. The mechanism is fundamentally different from the thymic immunomodulatory peptides: thymosin beta-4 modulates actin dynamics and activates tissue-repair pathways rather than directly regulating T-cell differentiation.

Development trajectory: Thymosin beta-4 (as RGN-259 and RGN-352) was developed by RegeneRx Biopharmaceuticals for corneal wound healing, cardiac repair after myocardial infarction, and dermal wound healing. Phase 2 clinical trials were completed for corneal and cardiac indications with mixed results [24]. The compound is widely available as a research peptide (often marketed as TB-500) and is used in veterinary medicine for equine injury recovery.

Novelty: Moderate. The actin-sequestering and tissue-repair mechanism is distinct from conventional thymic immunomodulation and represents a different therapeutic paradigm within the "thymosin" nomenclature.

Effect size: Phase 2 clinical trials showed modest wound-healing effects. The tissue-repair mechanism is well validated in preclinical models.

Promising potential: Moderate. Active development continues for tissue-repair indications, but clinical results have not yet supported regulatory approval.

Side-effect profile: Favorable in clinical trials. Minimal systemic adverse events at studied doses [24].

Overall validation: Low to moderate. Phase 2 clinical data in wound healing; no regulatory approval; preclinical validation is strong for tissue repair.

Comparison to Thymalin: Thymosin beta-4 and Thymalin share nomenclatural heritage (both derived from thymus tissue) but are mechanistically and functionally distinct. Thymalin is an immunomodulatory agent acting through epigenetic regulation of immune gene expression; thymosin beta-4 is a tissue-repair agent acting through actin dynamics and angiogenesis. The compounds are not pharmacological alternatives for the same indication but could be complementary in settings requiring both immune restoration and tissue repair (such as post-surgical recovery).

12.6 Comparator summary

The five comparators occupy distinct positions in the thymic peptide and immunomodulatory peptide pharmacology space. **Thymosin alpha-1** is the most extensively validated thymic peptide globally, with regulatory approval in multiple jurisdictions, large-scale clinical trial data, and independent international replication; it represents the gold standard for thymic immunomodulation from a Western regulatory perspective. **Thymulin** is a structurally defined zinc-dependent nonapeptide

with strong preclinical characterization but no clinical validation. **Thymopentin** is a synthetic pentapeptide fragment with limited clinical data and largely terminated development. **Thymogen** is the synthetic dipeptide constituent of Thymalin with comparable pharmacology but greater structural precision. **Thymosin beta-4** is a tissue-repair peptide that is mechanistically distinct from the immunomodulatory thymic peptides.

Thymalin occupies a unique position in this comparator landscape: it is the only multicomponent extract among the comparators, containing multiple bioactive peptides with potentially synergistic epigenetic mechanisms. It has the longest continuous clinical use history of any thymic peptide preparation (more than 40 years in the Russian Federation) and the most extensive geroprotective evidence base (the 266-subject, 6- to 8-year prospective study). Its principal limitations are the absence of Western regulatory evaluation, the methodological limitations of the clinical evidence, and the inherent lot-to-lot variability of a heterogeneous extract. Investigators choosing between Thymalin and its comparators should weigh these considerations against the specific requirements of their research application: thymosin alpha-1 for regulatory-grade clinical work; Thymogen for structurally defined single-peptide epigenetic research; and Thymalin for multicomponent bioregulatory research with the longest historical clinical context.

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