

RESEARCH MONOGRAPH . COMPOUND PROFILE

Tufts

A spleen-dependent, IgG-derived tetrapeptide that activates phagocytic cells through neuropilin-1 receptor binding, stimulating macrophage phagocytosis, chemotaxis, and tumoricidal activity, and serving as the structural parent of the anxiolytic heptapeptide Selank.

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ABSTRACT

Tuftsins (L-threonyl-L-lysyl-L-prolyl-L-arginine) are endogenous tetrapeptides corresponding to residues 289 through 292 of the CH2 domain of the immunoglobulin G (IgG) heavy chain. First identified in 1970 by Victor A. Najjar and Keisuke Nishioka at the Tufts University School of Medicine during investigations of phagocytosis by polymorphonuclear granulocytes, the peptide derives its name from its institutional origin and has since become the prototype endogenous immunostimulatory peptide. Tuftsins are not synthesized *de novo* but are liberated from a larger IgG-associated polypeptide termed leukokinin through the sequential action of two proteases: tuftsin-endocarboxypeptidase, a splenic enzyme that cleaves the C-terminal bond of the tetrapeptide within the intact immunoglobulin, and leukokinase, a serine protease on the outer membrane of phagocytic cells that releases the active N-terminal tetrapeptide from the partially processed fragment. This two-step enzymatic liberation places the spleen as an obligate organ in tuftsin physiology and accounts for the well-documented tuftsin deficiency, impaired phagocytic function, and increased susceptibility to overwhelming bacterial infection observed following splenectomy.

The molecular pharmacology of tuftsins centers on their binding to neuropilin-1 (NRP1), a single-pass transmembrane glycoprotein that also serves as a co-receptor for vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF- β). Tuftsins bind the b1 domain of neuropilin-1 through a motif similar to the C-terminal sequence encoded by exon 8 of VEGF165, and signal through TGF- β receptor 1 (a co-receptor of NRP1).

via the canonical Smad3 phosphorylation pathway, with concurrent reduction in Akt phosphorylation. At the cellular level, tuftsin binding stimulates phagocytosis, pinocytosis, chemotaxis, respiratory burst (superoxide anion and hydrogen peroxide generation), antigen presentation, and tumoricidal activity in monocytes, macrophages, neutrophils, microglia, and Kupffer cells. Intracellular calcium serves as a critical second messenger in tuftsin-mediated phagocyte activation. In neuroinflammatory models, tuftsin promotes an anti-inflammatory M2 microglial phenotype shift, suppresses pro-inflammatory Th1 responses, upregulates Th2 responses, and expands regulatory T cell populations.

Pharmacokinetically, tuftsin is an extremely labile peptide in vivo, with a plasma half-life of approximately 16 minutes owing to rapid degradation by serum aminopeptidases and carboxypeptidases. Oral bioavailability is negligible because of gastric peptidase destruction. The rapid enzymatic clearance has been the principal barrier to clinical development. Initial clinical studies conducted at the Weizmann Institute of Science demonstrated that tuftsin is nontoxic in humans when administered intravenously at doses up to 5 mg per injection in patients with advanced malignancy; a Phase II study in 25 patients with various advanced cancers reported leucocytosis and increased natural killer activity with two partial responses among 16 evaluable patients, confirming biological activity but insufficient monotherapy efficacy.

The most significant translational legacy of tuftsin is the development of Selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro), a synthetic heptapeptide analog in which the tuftsin sequence is extended at the C-terminus with a Pro-Gly-Pro tripeptide motif that confers resistance to enzymatic degradation and facilitates

blood-brain barrier penetration. Selank was developed at the Institute of Molecular Genetics of the Russian Academy of Sciences and is approved in Russia as a nasal spray for generalized anxiety disorder and neurasthenia. Selank exhibits pronounced anxiolytic, nootropic, and immunomodulatory activity through mechanisms including allosteric modulation of GABA-A receptors and modulation of monoamine neurotransmitter metabolism, and does so without the sedation, tolerance, or dependence associated with benzodiazepines.

Beyond Selank, tuftsin has been developed as a targeting ligand for liposomal and nanoparticle drug delivery systems. Palmitoyl tuftsin grafted onto liposome surfaces enables selective binding to phagocytic cells and has demonstrated augmented antitumor efficacy of encapsulated cytotoxic agents (etoposide, doxorubicin, curcumin) against fibrosarcoma and Ehrlich ascites carcinoma in murine models. Tuftsin-bearing liposomes have also been used to deliver antimicrobial agents to macrophage-resident intracellular pathogens including *Leishmania* and *Plasmodium* species.

This monograph reviews the chemistry, enzymatic biogenesis, and structural biology of tuftsin; the neuropilin-1 receptor pharmacology and downstream signaling; the pharmacokinetic constraints and stabilization strategies; the preclinical pharmacology across immunostimulatory, anti-inflammatory, antitumor, and anti-infective applications; the clinical evidence base; sourcing and quality verification; reconstitution and handling; stack interactions; adverse events and safety; and a comparative assessment of five immunostimulatory peptide candidates (Selank, thymosin alpha-1, muramyl dipeptide, GM-CSF, and thymopentin) against tuftsin on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Common name: Tuftsin.

Systematic peptide name: L-Threonyl-L-lysyl-L-prolyl-L-arginine. The compound is universally referred to by its trivial name, which was coined by Victor A. Najjar in reference to Tufts University, where the peptide was discovered [1].

Abbreviations: TKPR (one-letter amino acid code); Thr-Lys-Pro-Arg (three-letter amino acid code).

Chemical Abstracts Service registry number: 9063-57-4 (free base). Additional CAS numbers appear in the literature for tuftsin analogs and conjugates: 63147-94-4 (4-aminophenylacetyl-tuftsin) and 75929-73-6 (tuftsin, Arg(3)-variant) [2].

International Union of Pure and Applied Chemistry name: (2S)-2-[(2S)-2-[(2S)-2-[(2S)-2-amino-3-hydroxybutanoyl]amino-6-aminohexanoyl]amino-5-(diaminomethylideneamino)pentanoyl]pyrrolidine-2-carboxylic acid. The IUPAC name is rarely used; the peptide three-letter and one-letter codes are standard in the pharmacological literature.

PubChem CID: 441388.

Protein Data Bank identifier: The tuftsin sequence corresponds to residues 289 through 292 of the CH2 domain of the human IgG1 heavy chain (UniProt P01857, positions 289-292 in the mature sequence numbering).

1.2 Structural class and chemistry

Tuftsin is a linear tetrapeptide of the sequence Thr-Lys-Pro-Arg. It belongs to the class of endogenous immunomodulatory peptides and is the shortest naturally occurring peptide with defined immunostimulatory activity. The peptide is not a free-standing gene product but is generated by enzymatic cleavage from a specific site within the Fc domain of immunoglobulin G [3].

Molecular formula: C₂₁H₄₀N₈O₆ (free base, neutral form).

Molecular weight: 500.59 g/mol (free base).

Exact mass: 500.3071 g/mol (monoisotopic, free base).

Physical properties: White to off-white lyophilized powder or crystalline solid. Freely soluble in water; soluble in dilute acetic acid and aqueous buffers at physiological pH. Sparingly soluble in organic solvents. The peptide is hygroscopic and should be stored desiccated. Melting point data for the free peptide are not routinely reported owing to decomposition on heating; thermal stability is limited above 60 degrees Celsius in aqueous solution.

Stereochemistry: All four amino acid residues are in the L-configuration. The proline residue introduces a rigid pyrrolidine ring that constrains the backbone conformation and contributes to the characteristic beta-turn structure that is critical for receptor recognition [4]. Solution nuclear magnetic resonance studies have characterized tuftsin as adopting a type I beta-turn centered on the Lys-Pro peptide bond, with the Thr and Arg residues occupying the *i* and *i*+3 positions of the turn. This conformational preference is maintained in aqueous solution and is believed to be the bioactive conformation.

Charge state at physiological pH: At pH 7.4, tuftsin carries a net positive charge of approximately +2, arising from the protonated alpha-amino group of threonine (+1), the protonated epsilon-amino group of lysine (+1), and the protonated guanidinium group of arginine (+1), offset by the single carboxylate of the C-terminal arginine (-1). The strongly cationic character contributes to electrostatic interactions with cell surface glycosaminoglycans and with the acidic binding cleft of neuropilin-1.

1.3 Synthesis

Tuftsin is routinely synthesized by standard solid-phase peptide synthesis (SPPS) using Fmoc (9-fluorenylmethyloxycarbonyl) chemistry on Wang or Rink amide resin [5]. The synthesis proceeds from the C-terminal arginine residue through sequential deprotection and coupling of proline, lysine, and threonine using standard coupling reagents (HBTU, HATU, or PyBOP with DIEA). Side-chain protecting groups include tert-butyl for threonine, Boc (tert-butyloxycarbonyl) for lysine, and Pbf (2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl) for arginine. Cleavage from the resin and global side-chain deprotection are accomplished with trifluoroacetic acid containing appropriate scavengers (triisopropylsilane, water,

ethanedithiol). The crude peptide is purified by reverse-phase high-performance liquid chromatography (RP-HPLC) using C18 columns with water-acetonitrile gradients containing 0.1 percent trifluoroacetic acid. Typical synthesis yields exceed 80 percent for this short tetrapeptide, and purity of greater than 95 percent is routinely achieved after a single HPLC purification. The compound is supplied as the trifluoroacetate salt after lyophilization; conversion to acetate or hydrochloride salt forms is accomplished by ion-exchange or repeated lyophilization from dilute acetic acid or hydrochloric acid.

Liquid-phase synthesis using carbodiimide (DCC or EDC) coupling and Boc chemistry was the original synthetic route employed in the Najjar laboratory and is still used in some industrial-scale preparations [6]. The liquid-phase route proceeds by fragment condensation (typically Boc-Thr-Lys(Z)-OH coupled to H-Pro-Arg(NO₂)-OMe, followed by deprotection and cyclization-free purification). Both routes produce material of equivalent pharmacological activity when purity is confirmed analytically.

2. Abstract

Tuftsins (Thr-Lys-Pro-Arg) are endogenous tetrapeptides corresponding to residues 289 through 292 of the CH2 domain of the human immunoglobulin G heavy chain. Discovered in 1970 by Victor A. Najjar and Keisuke Nishioka at Tufts University during studies on the mechanism of phagocytosis, tuftsins are the prototypical endogenous immunostimulatory peptides and the structural parent compound of the clinically approved anxiolytic heptapeptide Selank [1, 7].

Tuftsins are not synthesized *de novo* but are generated by sequential proteolytic processing of the IgG heavy chain by two enzymes: tuftsins-endothelypeptidase, a splenic enzyme, and leukokininase, a membrane-bound serine protease on phagocytic cells. This two-step biogenesis places the spleen as an essential organ in tuftsins physiology and explains the tuftsins deficiency, impaired phagocytic function, and increased infection susceptibility documented following splenectomy [8, 9].

The molecular pharmacology of tuftsins centers on its binding to neuropilin-1 (NRP1), where it signals through TGF- β receptor 1 via the canonical Smad3 phosphorylation pathway [10, 11]. At the cellular level, tuftsins stimulate phagocytosis, chemotaxis, respiratory burst, antigen presentation, and tumoricidal activity in cells of monocytic lineage including macrophages, neutrophils, microglia, and Kupffer cells [3, 12]. In neuroinflammatory models, tuftsins drive an anti-inflammatory M2 microglial phenotype, suppress Th1 responses, and expand regulatory T cell populations [13]. The peptide also exhibits antiviral activity through enhancement of macrophage-mediated viral clearance and has been investigated as a targeting ligand for liposomal drug delivery to macrophage-resident intracellular pathogens [14, 15].

Pharmacokinetically, tuftsins have a plasma half-life of approximately 16 minutes owing to rapid degradation by serum aminopeptidases [16]. This extreme lability has been the principal barrier to clinical translation. Initial clinical studies at the Weizmann Institute of Science demonstrated safety at intravenous doses of up to 5 mg in cancer patients, with biological activity confirmed by leucocytosis and increased natural killer activity, but insufficient monotherapy antitumor efficacy [17].

The most consequential translational development arising from tuftsin is Selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro), in which the C-terminal Pro-Gly-Pro extension confers enzymatic stability and blood-brain barrier penetration. Selank is approved in Russia for generalized anxiety disorder and exhibits anxiolytic, nootropic, and immunomodulatory activity without benzodiazepine-class dependence or sedation [18, 19]. Tuftsin has additionally been developed as a macrophage-targeting ligand for liposomal formulations of cytotoxic agents (etoposide, doxorubicin) and antimicrobials, with preclinical evidence of augmented antitumor and anti-infective efficacy [20, 21, 22].

This monograph reviews the chemistry, enzymatic biogenesis, neuropilin-1 receptor pharmacology, pharmacokinetics, preclinical and clinical evidence, sourcing, handling, and a comparative assessment of five immunostimulatory candidates against tuftsin.

3. Discovery and Development History

3.1 Origin

The discovery of tuftsin arose from an unexpected observation during research on phagocytosis by polymorphonuclear granulocytes at the Tufts University School of Medicine in Boston, Massachusetts. In 1970, Victor A. Najjar and Keisuke Nishioka reported the isolation of a phagocytosis-stimulating tetrapeptide from the gamma-globulin fraction of human serum [1]. The initial observation was that a specific fraction of leukophilic gamma-globulin, termed leukokinin, dramatically enhanced the phagocytic activity of polymorphonuclear leukocytes in vitro. Sequential enzymatic digestion and chromatographic purification of leukokinin yielded the active tetrapeptide Thr-Lys-Pro-Arg, which Najjar named "tuftsin" after the university. The discovery was published in a landmark series of papers between 1970 and 1975 that established the peptide sequence, the enzymatic generation from IgG, and the principal biological activities [1, 3, 6].

3.2 Characterization of the IgG origin

Najjar and colleagues subsequently mapped tuftsin to residues 289 through 292 of the CH2 domain of the human IgG1 heavy chain, within a larger fragment they termed leukokinin (approximately 40 residues from the Fc region) [3]. The localization within the Fc domain explained the leukophilic (white blood cell binding) property of the parent immunoglobulin and provided the first demonstration that a defined bioactive peptide could be generated by limited proteolysis of an immunoglobulin molecule. This finding predated the broader recognition of cryptic bioactive peptides derived from larger precursor proteins by nearly two decades.

3.3 Elucidation of the two-enzyme liberation mechanism

The two-step enzymatic mechanism for tuftsin liberation was elucidated through a series of studies in the 1970s and 1980s [8, 23]. The first enzyme, tuftsin-endocarboxypeptidase, is localized predominantly in splenic tissue and cleaves the C-terminal peptide bond of the tuftsin sequence within the intact IgG molecule, generating a partially processed intermediate still attached to the immunoglobulin. The sec-

ond enzyme, leukokininase, is a membrane-bound serine protease on the surface of phagocytic cells (neutrophils, monocytes, macrophages) that cleaves the N-terminal bond to release the free active tetrapeptide. This two-enzyme model explained the clinical observation that splenectomized patients have markedly reduced circulating tuftsin levels and correspondingly impaired phagocytic function [8, 9].

3.4 Tuftsin deficiency syndromes

The recognition that tuftsin deficiency accompanies splenectomy led to a substantial clinical literature linking the peptide to post-splenectomy susceptibility to overwhelming bacterial infection. Spirer et al. (1980) documented decreased tuftsin concentrations in patients who had undergone both traumatic and elective splenectomy, with concentrations falling significantly in the days following operation [9]. Acquired tuftsin deficiency was subsequently documented in patients with sickle cell disease (functional asplenia), intestinal failure on long-term intravenous nutrition, and HIV/AIDS [24, 25, 26]. The tuftsin deficiency in AIDS patients was proposed as a contributing factor to the impaired macrophage function and increased susceptibility to opportunistic infections characteristic of the disease [26].

3.5 Development of tuftsin analogs

The recognition that tuftsin's extremely short plasma half-life limited its therapeutic utility drove the development of stabilized analogs. The most successful of these was Selank, developed in the 1990s at the Institute of Molecular Genetics of the Russian Academy of Sciences by N.F. Myasoedov and colleagues [18]. Selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro) incorporates the full tuftsin sequence at the N-terminus with a C-terminal Pro-Gly-Pro extension that shields the tetrapeptide from carboxypeptidase degradation and confers favorable pharmacokinetic properties including blood-brain barrier penetration. Selank was approved in Russia as an intranasal preparation for the treatment of generalized anxiety disorder and neurasthenia, making it the only tuftsin-derived compound to achieve regulatory approval for clinical use.

A second derivative, the T-peptide (a tuftsin-derived modified peptide with enhanced metabolic stability), was developed as an antitumor agent and studied in preclinical sepsis and cancer models, with demonstrated improvement in survival in murine sepsis-associated secondary infection models [27, 28].

3.6 Tuftsin as a drug delivery targeting ligand

Beginning in the 1990s, tuftsin was developed as a macrophage-targeting ligand for liposomal drug delivery systems. The pioneering work of Agrawal and Gupta demonstrated that palmitoyl tuftsin grafted onto liposome surfaces via a C-terminal acyl chain enables selective binding to phagocytic cells expressing tuftsin receptors, and that such tuftsin-bearing liposomes could deliver encapsulated drugs (amphotericin B, sodium stibogluconate, etoposide) to macrophage-resident intracellular pathogens and to tumor-associated macrophages with markedly enhanced therapeutic indices compared to free drug or non-targeted liposomes [20, 21, 22]. This drug delivery application remains the most active area of current tuftsin research, with recent reports of legumain protease-activated tuftsin-functionalized nanoparticles for dual-targeting of tumor-associated macrophages and cancer chemotherapy [29].

4. Mechanism of Action

Tuftsins are single-target receptor ligands with pleiotropic downstream effects on innate and adaptive immune function. Its molecular pharmacology is defined by binding to neuropilin-1 on phagocytic cells, with signal transduction through the TGF-beta pathway and through calcium-dependent second messenger systems.

4.1 Neuropilin-1 receptor binding

The identification of neuropilin-1 (NRP1) as the principal tuftsin receptor was established through a convergence of binding studies and functional pharmacology. Von Wronski et al. (2006) demonstrated that tuftsin (TKPR) binds to the b1 domain of neuropilin-1 through a motif similar to the C-terminal sequence (KPRR) encoded by exon 8 of vascular endothelial growth factor 165 (VEGF165) [30]. The binding is competitive with VEGF165 at the b1 domain, and anti-neuropilin-1 antibodies block tuftsin binding to NRP1-expressing cells. A higher-affinity tuftsin antagonist peptide, TKPPR, also binds selectively to neuropilin-1 and blocks VEGF binding [31].

Neuropilin-1 is a single-pass transmembrane glycoprotein expressed on macrophages, monocytes, dendritic cells, T cells, endothelial cells, neurons, and certain tumor cells. The extracellular domain comprises two CUB domains (a1, a2), two coagulation factor V/VIII homology domains (b1, b2), and a MAM (meprin, A5 protein, receptor protein tyrosine phosphatase mu) domain. The b1 domain contains the binding cleft for both VEGF and tuftsin. The intracellular domain of neuropilin-1 is short (approximately 40 amino acids) and lacks intrinsic kinase activity; signal transduction therefore depends on co-receptor association, principally with TGF-beta receptor 1, VEGF receptor 2, and plexin family members [10].

4.2 Signal transduction through the TGF-beta pathway

Nissen et al. (2013) demonstrated that tuftsin signals through neuropilin-1 via the transforming growth factor beta pathway [10]. Tuftsin binding to NRP1 on microglia promotes association of NRP1 with TGF-beta receptor 1, activates the latent form of TGF-beta 1, and triggers canonical Smad signaling: Smad3 is phosphorylated, translocates to the nucleus, and activates transcription of anti-inflammatory

gene programs. Concurrently, tuftsin reduces Akt phosphorylation, shifting the intracellular signaling balance from pro-inflammatory (NF-kappaB-driven) toward anti-inflammatory (Smad-driven) transcriptional programs.

4.3 Calcium-dependent phagocyte activation

A second, more rapid signal transduction pathway involves intracellular calcium mobilization. Studies using the calcium chelator Quin-2 and the calcium channel blocker verapamil demonstrated that tuftsin-stimulated superoxide anion release from monocytes and macrophages is dependent on intracellular calcium $[Ca^{2+}]_i$ elevation [32]. This calcium-dependent activation mediates the immediate phagocyte effector functions (respiratory burst, degranulation, chemotaxis) and operates on a timescale of seconds to minutes, considerably faster than the Smad-dependent transcriptional effects.

4.4 Cellular effector functions

The downstream cellular consequences of tuftsin receptor activation include:

Phagocytosis: Tuftsin enhances the phagocytic uptake of bacteria, fungi, and apoptotic cells by macrophages and neutrophils. The magnitude of phagocytosis enhancement is typically 2- to 4-fold over unstimulated controls at concentrations of 10 to 100 micromolar in vitro [3, 12].

Respiratory burst: Tuftsin stimulates the generation of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) by the NADPH oxidase system in phagocytes, augmenting the oxidative killing capacity. Importantly, this respiratory burst is induced without the requirement for particle phagocytosis, indicating that tuftsin directly activates the NADPH oxidase complex through receptor-mediated signaling [12].

Chemotaxis: Tuftsin enhances the directional migration of monocytes and macrophages toward sites of inflammation.

Antigen presentation: Tuftsin triggers the immunogenic function of macrophages, enhancing the processing and presentation of antigen to T lymphocytes. Najjar and Constantopoulos (1972) demonstrated that tuftsin augments the macrophage-dependent education of T cells [33].

Tumoricidal activity: Tuftsin activates macrophage-mediated tumor cell killing through enhanced production of tumor necrosis factor alpha (TNF-alpha), reactive oxygen intermediates, and nitric oxide [34].

Microglial polarization: In the central nervous system, tuftsin promotes the shift of microglia from a pro-inflammatory M1 phenotype to an anti-inflammatory M2 phenotype, with downregulation of TNF-alpha and IL-1-beta and upregulation of IL-10 and TGF-beta [13].

4.5 Anti-inflammatory and immunoregulatory effects

Tuftsin's anti-inflammatory activity, mediated through the NRP1-TGF-beta axis, includes suppression of pro-inflammatory Th1 responses, upregulation of Th2-specific responses, and expansion of immunosuppressive regulatory T cells (Tregs) [13, 35]. In the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis, tuftsin treatment attenuated clinical symptoms and promoted disease recovery through NRP1-dependent mechanisms; this recovery was abolished by anti-NRP1 antibody blockade, confirming the receptor specificity [35, 36].

4.6 Antiviral and anti-infective mechanisms

The antiviral activity of tuftsin operates indirectly through enhancement of macrophage-mediated viral clearance rather than through direct antiviral action. Tuftsin has been shown to augment macrophage killing of intracellular pathogens including *Leishmania* species, *Mycobacterium tuberculosis*, and *Plasmodium* species [14, 15, 22]. The mechanism is principally enhanced phagocytosis and enhanced intracellular killing through respiratory burst activation. Fridkin and colleagues demonstrated that tuftsin conjugated to azidothymidine (AZT) achieved targeted delivery to HIV-infected macrophages and inhibited reverse transcriptase activity and HIV antigen expression in vitro [37].

5. Pharmacokinetics and Routes of Administration

5.1 Endogenous generation and circulating levels

Tuftsins are generated from circulating IgG by a two-step enzymatic process. The first step occurs in the spleen, where tuftsin-endocarboxypeptidase cleaves the peptide bond C-terminal to arginine-292 within the CH2 domain of IgG. The resulting partially processed fragment remains attached to the immunoglobulin and circulates until encountering phagocytic cells, where the second enzyme, leukokinase, cleaves the peptide bond N-terminal to threonine-289 to liberate the free active tetrapeptide [8, 23]. Normal circulating tuftsin concentrations in healthy individuals are in the range of 200 to 400 nanograms per milliliter (0.4 to 0.8 micromolar), with concentrations subject to diurnal variation and to modulation by splenic function, IgG levels, and systemic inflammatory status [38].

5.2 Plasma half-life and degradation

Exogenous tuftsin administered intravenously is extremely labile, with a plasma half-life of approximately 16 minutes (0.27 hours) [16]. Degradation is mediated by ubiquitous serum aminopeptidases and carboxypeptidases that rapidly cleave the terminal amino acids of the unprotected tetrapeptide. The primary cleavage site is the Thr-Lys bond (N-terminal aminopeptidase activity), followed by C-terminal carboxypeptidase cleavage of the Arg residue. The resulting di- and tripeptide fragments are pharmacologically inactive.

The rapid enzymatic degradation imposes severe pharmacokinetic constraints on exogenous tuftsin administration. Drug accumulation is not possible regardless of dosing frequency, and any adverse reaction to exogenous tuftsin would be self-limiting within minutes. The clearance is predominantly enzymatic (serum peptidases) rather than renal or hepatic.

5.3 Oral bioavailability

Oral bioavailability of native tuftsin is effectively zero owing to complete degradation by gastric and intestinal peptidases prior to absorption. No oral formulation of native tuftsin has been developed for clinical use. Strategies to achieve oral delivery have focused on structural modifications (Selank, T-peptide) or on encapsulation in liposomal or nanoparticulate carriers [16, 27].

5.4 Distribution

Following intravenous administration, tuftsin distributes rapidly to well-vascularized organs. Studies with radiolabeled analogs in rats demonstrated preferential distribution to liver, kidneys, and heart, consistent with the high density of phagocytic cells (Kupffer cells) in the liver and the general perfusion-limited distribution of small peptides [39]. Central nervous system penetration of native tuftsin is limited by the blood-brain barrier and by rapid peripheral degradation. The C-terminal Pro-Gly-Pro extension in Selank was specifically designed to overcome this limitation.

5.5 Routes of administration

Intravenous: The principal route used in clinical studies. Intravenous bolus at doses of 0.5 to 5 mg produces measurable increases in circulating white blood cell counts and natural killer cell activity within 1 to 2 hours [17].

Intraperitoneal: Used in preclinical studies; produces peritoneal macrophage activation and increased peritoneal TNF-alpha production [34].

Subcutaneous: Used in some preclinical models; produces slower absorption and lower peak concentrations compared to intravenous administration, but the rapid degradation still limits the duration of effect to minutes.

Intranasal: Not applicable to native tuftsin owing to peptidase degradation in the nasal mucosa; however, the Selank derivative achieves effective intranasal delivery through its enhanced metabolic stability [18].

5.6 Pharmacokinetics of stabilized derivatives

The T-peptide (a tuftsin derivative with enhanced stability) has been characterized pharmacokinetically in beagle dogs, demonstrating a plasma half-life of 1.3 to 2.8 hours, representing approximately a 5- to 10-fold improvement over native tuftsin [27]. Selank, administered intranasally, has a metabolic half-life of approximately 3 to 5 minutes in rat blood plasma as the intact heptapeptide, but its principal pharmacologically active metabolite (the tuftsin tetrapeptide Thr-Lys-Pro-Arg) is detected for 7 to 10 minutes and accumulates in well-vascularized organs including the brain [39].

6. Preclinical Pharmacology

6.1 Immunostimulatory activity

The immunostimulatory activity of tuftsin has been characterized extensively in vitro and in vivo across multiple species. In vitro, tuftsin at concentrations of 1 to 100 micromolar enhances macrophage phagocytosis of bacteria (*Staphylococcus aureus*, *Escherichia coli*), fungi (*Candida albicans*), and tumor cells by 2- to 4-fold over unstimulated controls. The enhancement is dose-dependent and is blocked by anti-neuropilin-1 antibodies [3, 12, 30].

In vivo, intraperitoneal administration of tuftsin (1 to 10 mg/kg) in mice increases peritoneal macrophage phagocytic activity, elevates serum TNF-alpha concentrations, and augments natural killer cell cytotoxicity against YAC-1 target cells [34]. The immunostimulatory effect is preserved in euthymic but not in athymic (nude) mice for T cell-dependent endpoints, confirming the macrophage-to-T-cell immunogenic bridge.

6.2 Antitumor activity

Tuftsin exhibits antitumor activity in multiple murine tumor models through macrophage-mediated tumor cell killing. Direct antitumor activity (growth inhibition of tumor cells in culture) is modest; the principal mechanism is indirect through activation of tumor-associated macrophages. In the Ehrlich ascites carcinoma model, tuftsin administration (5 mg/kg intraperitoneally for 7 days) reduced tumor burden and prolonged survival [20]. The antitumor efficacy was substantially augmented when tuftsin was grafted onto liposomes co-encapsulated with cytotoxic agents: tuftsin-bearing liposomes loaded with etoposide produced significantly greater tumor weight reduction in the fibrosarcoma model than free etoposide or non-targeted liposomes [21]. Tuftsin-bearing liposomes co-encapsulating doxorubicin and curcumin efficiently inhibited Ehrlich ascites carcinoma growth in mice through p53-mediated apoptosis [20].

The T-peptide (tuftsin derivative) demonstrated anticancer activity in gastric tumor models, including inhibition of circulating gastric tumor cells through enhanced macrophage phagocytosis [28, 29].

6.3 Anti-infective activity

Tuftsins and tuftsins-bearing liposomes have demonstrated anti-infective efficacy against intracellular pathogens that reside within macrophages:

Leishmaniasis: Tuftsins-bearing liposomes loaded with sodium stibogluconate or miltefosine produced markedly enhanced antileishmanial efficacy in murine visceral leishmaniasis models compared to free drug, with no toxicity at doses of 5 mg/kg [22]. Prophylactic treatment with tuftsins-bearing liposomes enabled animals to resist subsequent *Leishmania* challenge.

Malaria: Pretreatment with tuftsins-bearing liposomes enhanced macrophage clearance of *Plasmodium*-infected erythrocytes and conferred resistance to malaria challenge in murine models [15].

Fungal infections: Tuftsins-bearing liposomal amphotericin B demonstrated enhanced antifungal efficacy against disseminated candidiasis in immunocompromised mice [22].

Tuberculosis: Tuftsins enhancement of macrophage respiratory burst has been shown to augment intracellular killing of *Mycobacterium tuberculosis* in in vitro macrophage infection models [14].

6.4 Neuroinflammatory and neuroprotective activity

In the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis, tuftsins treatment promoted an anti-inflammatory switch in microglia, attenuated clinical symptoms, and facilitated disease recovery [13, 35]. The mechanism involved NRP1-dependent TGF-beta signaling, microglial M2 polarization, suppression of Th1 pro-inflammatory responses, upregulation of Th2 responses, and expansion of regulatory T cells. The neuroprotective effect was abolished by anti-NRP1 antibodies or by genetic deletion of NRP1 on myeloid cells, confirming receptor specificity [36].

6.5 Antiviral activity

Tuftsins has been characterized as possessing antiviral activity through enhancement of macrophage-mediated viral clearance. Bar-Shavit et al. (1987) demonstrated that the structural basis of tuftsins's antiviral action involves stimulation of

the macrophage antiviral state, including enhanced interferon production and augmented viral particle phagocytosis [14]. Tuftsin-AZT conjugates demonstrated targeted antiviral delivery to HIV-infected macrophages in vitro [37].

6.6 Sepsis and critical care models

Tuftsin-derived T-peptide demonstrated prevention of cellular immunosuppression and improved survival in murine sepsis models [27]. More recently, Fcgamma-receptor-targeted tuftsin clusters were shown to rejuvenate immunosuppressed macrophages in a cecal slurry-induced septic mouse model with secondary pulmonary infection, improving bacterial clearance and survival rates [40].

7. Clinical Evidence Base

7.1 Phase I safety study

The initial clinical evaluation of tuftsin was conducted at the Weizmann Institute of Science in Israel. In a Phase I dose-escalation study, tuftsin was administered intravenously at a single dose of 0.96 mg/kg body weight to adult patients with advanced malignancy. The peptide was nontoxic, with no adverse events attributable to tuftsin at this dose. Biological activity was confirmed by transient leucocytosis and increased natural killer cell cytotoxicity in peripheral blood samples drawn at 2 and 6 hours post-administration [17].

7.2 Phase II cancer study

A Phase II study enrolled 25 patients with various advanced malignant diseases who received 5 mg of tuftsin intravenously two to three times per week. Of 16 evaluable patients, two demonstrated partial tumor responses. The study confirmed marked leucocytosis and increased natural killer activity following each administration, consistent with the immunostimulatory mechanism. No tuftsin-related toxicity was detected. The response rate was insufficient to support monotherapy development, but the biological activity data supported further investigation in combination regimens and in tuftsin-bearing drug delivery systems [17].

7.3 Tuftsin deficiency clinical studies

Multiple clinical studies have documented the consequences of tuftsin deficiency:

Post-splenectomy: Spirer et al. (1977) demonstrated that tuftsin concentrations fell significantly in the days following splenectomy, with post-splenectomy patients showing concentrations 60 to 80 percent lower than pre-operative levels [9]. The deficiency correlated with increased susceptibility to bacterial infections, particularly encapsulated organisms.

Intestinal failure: Bar-Shavit et al. (1998) documented impaired splenic function and tuftsin deficiency in patients with intestinal failure on long-term intravenous nutrition [25].

HIV/AIDS: Huang et al. (1991) reported tuftsin deficiency in symptomatic HIV-positive individuals, proposing a contribution to the impaired macrophage function characteristic of the disease [26].

Sickle cell disease: Functional asplenia in sickle cell disease is associated with reduced tuftsin levels and increased infection susceptibility, paralleling the post-splenectomy phenotype [24].

7.4 Selank clinical program

The clinical development of Selank (the tuftsin-derived heptapeptide) proceeded through the Russian regulatory system. Clinical trials demonstrated anxiolytic efficacy comparable to diazepam and phenazepam but without sedation, tolerance, or dependence. Selank was approved in Russia as a nasal spray (0.15 percent solution, administered intranasally at 75 micrograms per nostril three times daily) for the treatment of generalized anxiety disorder and neurasthenia [18, 19]. Clinical studies also demonstrated nootropic effects (enhanced cognitive function and memory) and immunomodulatory effects (modulation of cytokine and chemokine gene expression in splenic cells) [41, 42]. Selank has not been evaluated in FDA-regulated clinical trials and is not approved outside Russia.

7.5 SARS-CoV-2 research

A 2022 preprint from the Hazra laboratory reported computational and in vitro evidence suggesting that tuftsin may bind the SARS-CoV-2 spike protein and interfere with viral entry, positioning tuftsin as a potential natural antiviral molecule against COVID-19 [43]. These findings remain preliminary and have not been confirmed in clinical studies.

8. Sourcing and Quality Verification

Research-grade tuftsin is available from multiple peptide suppliers at greater than 95 percent purity by HPLC. Principal suppliers include Sigma-Aldrich (catalog number T2141), MedChemExpress (HY-P0233), Tocris, Bachem, GenScript, Cayman Chemical, and GlpBio. The compound is supplied as the trifluoroacetate or acetate salt in milligram quantities (typically 5 to 100 mg vials) as a lyophilized powder. Pricing ranges from approximately \$30 to \$150 per 5 mg vial depending on supplier and purity grade.

Investigators should confirm compound identity and purity on each lot through:

- Reverse-phase HPLC area-percent purity (target greater than 95 percent; greater than 98 percent preferred for dose-response studies)
- Mass spectrometry confirmation of molecular weight (expected $[M+H]^+$ of 501.31 for the protonated free base)
- Amino acid analysis to confirm composition (Thr:Lys:Pro:Arg in 1:1:1:1 ratio)
- Peptide content determination (net peptide content as a percentage of total weight, accounting for counterion, moisture, and residual solvent; typically 70 to 85 percent for TFA salt form)
- Endotoxin testing (Limulus amoebocyte lysate assay) for any preparation intended for cell culture or in vivo use

Custom synthesis of tuftsin at research scale (10 mg to 1 g) is available from peptide contract synthesis organizations (GenScript, CPC Scientific, LifeTein, Peptide Sciences) with typical turnaround times of 2 to 4 weeks and purity guarantees of greater than 95 percent. The short sequence (four residues) makes tuftsin straightforward to synthesize, and custom lots are routinely produced to greater than 98 percent purity.

Pharmaceutical-grade tuftsin is not commercially available, as no pharmaceutical product containing native tuftsin has been registered in any jurisdiction. Investigators requiring GMP-grade material for clinical research would need to commission custom GMP synthesis.

9. Reconstitution and Handling

Tuftsins as the lyophilized trifluoroacetate or acetate salt is freely soluble in water, phosphate-buffered saline, and aqueous buffers at physiological pH. Stock solutions are typically prepared at 1 to 10 mg/mL (2 to 20 millimolar) in sterile water, normal saline, or phosphate-buffered saline.

Reconstitution procedure: Add the desired volume of sterile solvent to the lyophilized vial, allow the powder to dissolve for 2 to 5 minutes at room temperature with gentle agitation, and verify complete dissolution visually. Do not vortex vigorously, as this may cause foaming and surface adsorption losses with dilute solutions. For concentrations below 0.1 mg/mL, addition of 0.1 percent bovine serum albumin or 0.01 percent polysorbate 20 as a carrier protein or surfactant is recommended to minimize nonspecific adsorption to glass and plastic surfaces.

Storage of stock solutions: Reconstituted tuftsins should be aliquoted into single-use volumes and stored at minus 20 degrees Celsius for short-term storage (up to 3 months) or minus 80 degrees Celsius for long-term storage (up to 12 months). Avoid repeated freeze-thaw cycles; stability data indicate measurable peptide degradation after 3 to 5 freeze-thaw cycles. Refrigerated storage (2 to 8 degrees Celsius) of aqueous solutions is acceptable for up to 7 days. Room temperature stability in aqueous solution is limited to approximately 24 hours owing to peptidase activity in non-sterile preparations and to non-enzymatic degradation (deamidation, oxidation) at ambient temperature.

Storage of lyophilized powder: Store desiccated at minus 20 degrees Celsius. The lyophilized material is stable for at least 2 years under these conditions. Protect from moisture. Light protection is not required but is reasonable practice.

Solvent compatibility: Tuftsins are compatible with water, saline, PBS, DMSO (dimethyl sulfoxide at up to 10 percent for DMSO-water mixtures), and cell culture media (DMEM, RPMI-1640). The peptide is not soluble in pure organic solvents (ethanol, methanol, acetonitrile) but is soluble in aqueous-organic mixtures.

Sterility: For cell culture or in vivo applications, reconstituted tuftsins should be sterile-filtered through 0.22 micrometer syringe filters. The peptide passes freely through standard cellulose acetate, PVDF, and PES membrane filters without signif-

ificant adsorptive losses at concentrations above 0.1 mg/mL.

10. Stack Interactions and Combinations

Tuftsins interact with several compound classes through its immunostimulatory mechanism and through pharmacodynamic synergism or antagonism at the phagocyte activation level. Investigators conducting research with tuftsins in combination regimens should consider the following interactions:

Immunosuppressants (corticosteroids, cyclosporine, tacrolimus): Corticosteroids and calcineurin inhibitors suppress macrophage activation and cytokine production through mechanisms that oppose tuftsins' immunostimulatory activity. Concurrent use would be expected to attenuate or abolish tuftsins-mediated phagocyte activation. The combination is pharmacodynamically antagonistic and should be avoided in immunostimulatory research protocols.

Cytotoxic chemotherapy agents: Tuftsins have been studied in combination with cytotoxic agents (etoposide, doxorubicin, cisplatin) in liposomal delivery systems. The combination produces pharmacodynamic synergism: tuftsins activate tumor-associated macrophage tumoricidal activity while the cytotoxic agent directly kills tumor cells [20, 21]. Myelosuppressive chemotherapy may impair the phagocyte population on which tuftsins depend, potentially limiting the benefit of concurrent (as opposed to sequential) administration.

Colony-stimulating factors (G-CSF, GM-CSF): Granulocyte and granulocyte-macrophage colony-stimulating factors expand the phagocyte population and may produce additive or synergistic effects with tuftsins on phagocyte-mediated immunity. The combination has not been formally characterized but is pharmacodynamically plausible.

Interferons (IFN-alpha, IFN-gamma): Interferon-gamma is the principal macrophage-activating cytokine and would be expected to produce additive effects with tuftsins on respiratory burst, antigen presentation, and tumoricidal activity. The combination may increase the risk of excessive inflammatory activation at high doses.

Antimicrobial agents (antileishmanials, antifungals, antimalarials): Tuftsin-bearing liposomal delivery of antimicrobial agents has demonstrated enhanced efficacy against intracellular pathogens. The interaction is pharmacokinetic (targeted delivery to infected macrophages) rather than pharmacodynamic at the receptor level [22].

Nonsteroidal anti-inflammatory drugs (NSAIDs): Cyclooxygenase inhibitors may modulate the downstream prostaglandin production associated with tuftsin-stimulated macrophage activation. The interaction is of uncertain clinical significance.

Other immunostimulatory peptides (thymosin alpha-1, thymopentin): Concurrent use with other immunostimulatory peptides may produce additive phagocyte activation. The risk of excessive immune stimulation is theoretically present but has not been characterized experimentally.

Selank (tuftsin derivative): Concurrent administration of native tuftsin with Selank would be pharmacologically redundant, as both act through the same neuropilin-1 receptor. Selank has superior pharmacokinetic properties and CNS penetration; native tuftsin adds no benefit to the Selank effect.

11. Adverse Events and Safety

11.1 Clinical safety profile

Tuftsins has demonstrated an exceptionally favorable safety profile across all published preclinical and clinical investigations. In the Phase I study, intravenous administration at 0.96 mg/kg produced no adverse events [17]. In the Phase II study, intravenous administration of 5 mg per injection two to three times weekly in 25 patients with advanced malignancy produced no detectable tuftsins-related toxicity [17]. Preclinical studies in mice, rats, and dogs at doses of 1 to 20 mg/kg intravenously and intraperitoneally revealed no toxic side effects but confirmed marked leucocytosis and increased natural killer cell activity [17].

11.2 Theoretical safety considerations

Excessive phagocyte activation: The principal theoretical concern with tuftsins administration is excessive macrophage activation leading to inflammatory tissue damage, cytokine storm, or autoimmune exacerbation. This has not been reported at experimental doses, and the extremely short half-life (approximately 16 minutes) provides an intrinsic safety margin: any adverse reaction to exogenous tuftsins would be self-limiting within minutes as the peptide is rapidly degraded by ubiquitous serum peptidases [16].

Autoimmune disease exacerbation: Tuftsins's immunostimulatory activity could theoretically exacerbate autoimmune conditions. However, the EAE studies demonstrated that tuftsins paradoxically promotes anti-inflammatory M2 polarization and regulatory T cell expansion, suggesting a net immunoregulatory rather than purely immunostimulatory effect in neuroinflammatory disease [13, 35]. Investigators should exercise caution when administering tuftsins in the context of active autoimmune disease.

Tumor promotion: Macrophage activation has been associated with both tumor suppression (M1 polarization) and tumor promotion (M2 polarization, angiogenesis) depending on the tumor microenvironment. Tuftsins's activation of neuropilin-1 (a VEGF co-receptor) raises the theoretical possibility of pro-angiogenic effects in

tumor settings. Published in vivo data have consistently demonstrated antitumor rather than tumor-promoting activity [20, 21], but the concern is noted for completeness.

11.3 Immunogenicity

Tuftsins as self-peptides derived from IgG are expected to have low immunogenicity. No anti-tuftsins antibody responses have been reported in clinical studies. The short length (four residues) is below the minimum size required for efficient MHC class II presentation and T-cell-dependent antibody responses.

11.4 Drug accumulation

Drug accumulation is not possible with native tuftsins regardless of dosing frequency, owing to the plasma half-life of approximately 16 minutes. The absence of accumulation eliminates the risk of delayed toxicity from chronic dosing.

11.5 Pregnancy and lactation

No reproductive toxicology studies of tuftsins have been published. The compound is an endogenous peptide present in the circulation of all healthy individuals, including pregnant women, as a product of IgG metabolism. Exogenous administration during pregnancy or lactation has not been studied and cannot be recommended in the absence of safety data.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five immunostimulatory candidates against tuftsin, 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 immunostimulatory peptides and biologics with substantive clinical or preclinical evidence in phagocyte activation, immune reconstitution, or innate immune enhancement, with priority on compounds that share tuftsin's mechanistic focus on macrophage activation.

12.1 Selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro)

Mechanism: Synthetic heptapeptide derived from tuftsin with C-terminal Pro-Gly-Pro extension. Retains tuftsin's immunomodulatory activity through NRP1 binding and additionally exhibits anxiolytic activity through allosteric modulation of GABA-A receptors and nootropic activity through modulation of BDNF expression and monoamine neurotransmitter metabolism [18, 19, 41].

Development trajectory: Developed at the Institute of Molecular Genetics of the Russian Academy of Sciences. Approved in Russia as a nasal spray for generalized anxiety disorder and neurasthenia. Not FDA-approved; not evaluated in FDA-regulated clinical trials. Available as a research peptide in Western markets.

Novelty: Moderate. The compound is a direct structural extension of tuftsin and shares the same receptor pharmacology. The novel contribution is the enhanced metabolic stability and CNS-directed pharmacology enabled by the Pro-Gly-Pro motif.

Effect size: Anxiolytic efficacy comparable to benzodiazepines (diazepam, phenazepam) in Russian clinical trials, without sedation or dependence [19]. Immunomodulatory effect sizes are modest and comparable to tuftsin in vitro.

Promising potential: Moderate. Approved in one jurisdiction. Limited adoption outside Russia owing to the absence of Western regulatory evaluation.

Side-effect profile: Favorable. No reports of sedation, tolerance, dependence, or withdrawal. No significant adverse events in published clinical studies.

Overall validation: Moderate. The clinical evidence is entirely from Russian studies with limited international peer review. The pharmacological rationale is strong.

Comparison to tuftsin: Selank is pharmacokinetically superior (enhanced stability, CNS penetration) and pharmacologically broader (additional GABA-A and monoamine mechanisms) than native tuftsin. Tuftsin remains the reference compound for fundamental NRP1-mediated immunostimulatory research, but Selank is the preferred molecule for any application requiring sustained systemic or CNS activity.

12.2 Thymosin alpha-1 (Thymalfasin)

Mechanism: 28-amino acid peptide originally isolated from thymic tissue (thymosin fraction 5). Acts through toll-like receptor 9 (TLR9) signaling on dendritic cells and macrophages, promoting dendritic cell maturation, cross-presentation of antigens, and Th1 immune responses. Enhances natural killer cell cytotoxicity and T cell proliferation [44].

Development trajectory: Developed by SciClone Pharmaceuticals (now acquired by Ascleptis Pharma). Approved in over 35 countries for the treatment of hepatitis B, hepatitis C (as adjunct to interferon), and as an immune adjuvant in cancer immunotherapy. Not FDA-approved in the United States; FDA orphan drug designation for hepatocellular carcinoma [44].

Novelty: Low. The compound has been studied since the 1970s. The mechanism (TLR9-mediated dendritic cell activation) is well characterized.

Effect size: Moderate. In hepatitis B, thymosin alpha-1 monotherapy produces HBeAg seroconversion rates of approximately 26 percent at 6 months, rising to approximately 35 to 40 percent at 12 months post-treatment. In combination with interferon, response rates are higher. In cancer immunotherapy, modest improvements in immune biomarkers and quality of life have been reported in multiple Phase II studies [44].

Promising potential: Moderate to high. Broad international registration for hepatitis B, ongoing studies in cancer immunotherapy and COVID-19.

Side-effect profile: Excellent. Injection site reactions are the principal adverse event. No systemic toxicity at therapeutic doses.

Overall validation: High. Extensive clinical experience in over 35 countries, large-scale clinical trials, and a well-characterized mechanism.

Comparison to tuftsin: Thymosin alpha-1 has substantially greater clinical validation and broader international regulatory acceptance than tuftsin. The mechanisms are complementary: tuftsin acts through NRP1 on macrophages, while thymosin alpha-1 acts through TLR9 on dendritic cells. Thymosin alpha-1 is a longer peptide (28 residues versus 4) with correspondingly more complex synthesis and higher cost.

12.3 Muramyl dipeptide (MDP; N-acetylmuramyl-L-alanyl-D-isoglutamine)

Mechanism: Minimal structural unit of bacterial peptidoglycan recognized by the innate immune receptor NOD2 (nucleotide-binding oligomerization domain 2). Activation of NOD2 triggers NF-kappaB signaling, inflammasome assembly (NLRP3), and production of pro-inflammatory cytokines (IL-1-beta, TNF-alpha, IL-6). MDP is the prototypical bacterial immunostimulant and the structural basis for numerous synthetic immunomodulatory analogs including murabutide and mifamurtide [45].

Development trajectory: MDP itself is too pyrogenic for systemic clinical use. The derivative mifamurtide (muramyl tripeptide-phosphatidylethanolamine, MTP-PE) was approved by the European Medicines Agency in 2009 for the treatment of osteosarcoma in combination with chemotherapy [46]. Murabutide (a non-pyrogenic derivative) has been studied as an immune adjuvant and anti-HIV agent.

Novelty: Low. MDP has been studied since the 1970s.

Effect size: Mifamurtide in combination with standard chemotherapy improved 6-year overall survival in osteosarcoma from 70 percent to 78 percent in a randomized Phase III trial [46]. MDP itself produces strong macrophage activation in vitro but is too toxic for direct clinical use.

Promising potential: Low to moderate. Mifamurtide has a narrow registered indication (osteosarcoma). MDP research has largely shifted to structure-activity studies and vaccine adjuvant development.

Side-effect profile: MDP: pyrogenic, dose-limiting fever and rigors. Mifamurtide: fever, chills, tachycardia, nausea (common, self-limiting). The pyrogenicity of the MDP class is a significant limitation compared to tuftsin.

Overall validation: High for the MDP class (extensive literature, registered derivative mifamurtide). Low for MDP itself (too toxic for direct use).

Comparison to tuftsin: MDP activates a fundamentally different innate immune pathway (NOD2-inflammasome) than tuftsin (NRP1-TGF-beta). MDP is a more potent macrophage activator but with correspondingly greater toxicity (pyrogenicity). Tuftsin has a substantially superior safety profile and lacks the pyrogenic signal that limits MDP-class compounds.

12.4 Granulocyte-macrophage colony-stimulating factor (GM-CSF; sargramostim)

Mechanism: 127-amino acid glycoprotein cytokine that binds the GM-CSF receptor (CSF2RA/CSF2RB heterodimer) on myeloid progenitor cells, macrophages, and dendritic cells. Stimulates proliferation and differentiation of granulocyte and macrophage progenitors, activates mature macrophage effector functions, and enhances antigen presentation [47].

Development trajectory: Recombinant GM-CSF (sargramostim, Leukine) is FDA-approved for multiple indications including neutrophil recovery after autologous bone marrow transplantation, mobilization of hematopoietic progenitor cells, and treatment of acute radiation syndrome. It has been investigated as a cancer immunotherapy adjuvant (GVAX platform), as adjunct therapy in fungal infections, and in COVID-19 [47].

Novelty: Low. GM-CSF has been in clinical use since the early 1990s.

Effect size: Large. GM-CSF produces robust, measurable increases in neutrophil and monocyte counts and in macrophage activation markers. The absolute neutrophil count typically increases 5- to 10-fold within 3 to 5 days of treatment initiation.

Promising potential: High. FDA-approved, widely used, and actively investigated in new immunotherapy combinations.

Side-effect profile: Moderate. Common adverse events include injection site reactions, bone pain, fever, myalgia, and capillary leak syndrome at high doses. Rare serious events include pulmonary edema and splenic enlargement.

Overall validation: Very high. FDA-approved, decades of clinical experience, extensive literature.

Comparison to tuftsin: GM-CSF is a fundamentally different class of molecule (cytokine versus peptide) with orders-of-magnitude greater clinical validation. GM-CSF acts upstream of tuftsin (expanding the phagocyte population and activating macrophage effector programs), while tuftsin acts as a local activator of existing phagocytes through NRP1. GM-CSF has significant adverse events (bone pain, capillary leak) that tuftsin lacks. The two agents are mechanistically complementary.

12.5 Thymopentin (TP-5; Arg-Lys-Asp-Val-Tyr)

Mechanism: Synthetic pentapeptide corresponding to residues 32 through 36 of thymopoietin, a thymic hormone. Acts on T cell precursors to promote T cell maturation and differentiation. Enhances T cell proliferative responses to mitogens, augments natural killer cell activity, and modulates cytokine production (increased IL-2, IFN-gamma) [48].

Development trajectory: Approved in several countries (including China and Italy) for immunodeficiency conditions, chronic hepatitis B, and as an adjuvant in cancer immunotherapy. Not FDA-approved. Marketed as Timunox, Sintomodulina.

Novelty: Low. The compound has been studied since the 1980s.

Effect size: Moderate. In chronic hepatitis B, thymopentin in combination with interferon produces modestly higher seroconversion rates than interferon alone. In cancer immunotherapy, improvements in immune biomarkers (CD4/CD8 ratio, NK cell activity) have been reported in multiple uncontrolled studies.

Promising potential: Low to moderate. Limited to a few jurisdictions with approval. Development has been largely superseded by thymosin alpha-1 and by recombinant cytokine therapies.

Side-effect profile: Favorable. Injection site pain is the principal adverse event. No significant systemic toxicity.

Overall validation: Moderate. Approved in several countries with moderate clinical evidence.

Comparison to tuftsin: Thymopentin and tuftsin activate different arms of the immune system: thymopentin targets T cell maturation (adaptive immunity), while tuftsin targets macrophage activation (innate immunity). Both are short endogenous peptides with favorable safety profiles and limited pharmacokinetic properties. Tuftsin has a stronger mechanistic rationale for macrophage-directed applications; thymopentin has a stronger rationale for T cell-directed applications.

12.6 Comparator summary

The five comparator compounds span the spectrum of immunostimulatory mechanisms from innate macrophage activation (MDP, GM-CSF) through T cell maturation (thymosin alpha-1, thymopentin) to a tuftsin-derived dual immunostimulatory-anxiolytic agent (Selank). **GM-CSF** has the highest overall clinical validation as an FDA-approved cytokine with decades of clinical experience but carries significant adverse events and is a fundamentally different molecular class. **Thymosin alpha-1** has the broadest international registration among the peptide comparators and the strongest clinical evidence in hepatitis B and cancer immunotherapy. **MDP** (through its derivative mifamurtide) has EMA registration but is limited by pyrogenicity. **Thymopentin** is a T cell-directed peptide with modest evidence and limited geographical availability. **Selank** is the direct structural descendant of tuftsin with superior pharmacokinetics and additional CNS activity, but limited to Russian regulatory approval.

Tuftsin occupies a unique position as the endogenous prototype immunostimulatory tetrapeptide with the most favorable safety profile of any compound in the panel (no adverse events reported at any dose studied) and the shortest amino acid sequence of any bioactive peptide with defined immunological activity. Its principal limitation is the extremely short plasma half-life that has prevented clinical development as a standalone therapeutic agent. The two most productive translational pathways arising from tuftsin have been (a) the development of Selank as a stabilized, CNS-penetrant derivative with clinical approval for anxiety, and (b) the use of tuftsin as a macrophage-targeting ligand for liposomal and nanoparticle drug delivery systems. Investigators seeking macrophage-directed immunostimulation in a research context should consider native tuftsin for mechanistic studies, Selank

for applications requiring sustained or CNS activity, and tuftsin-bearing liposomes for targeted drug delivery to macrophage-resident pathogens or tumor-associated macrophages.

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This monograph is provided for research use only. Not for medical, diagnostic, or therapeutic purposes. Not for human consumption. Tuftsin is an endogenous peptide and is not approved as a pharmaceutical product by any regulatory authority. The tuftsin-derived compound Selank is approved only in Russia for generalized anxiety disorder; investigators outside Russia should treat Selank as an experimental research compound. Investigators should obtain analytical confirmation of identity and purity on every research-grade lot.