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Thymosin α-1 in cancer therapy: Immunomodulatory mechanisms, clinical applications, and translational perspectives

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ABSTRACT

Cancer remains a leading cause of global mortality, underscoring the need for therapeutic strategies that not only target tumor cells but also enhance antitumor immunity. Thymosin α-1 (Tα1), a naturally occurring thymic peptide, has emerged as a promising immunomodulatory agent with potential applications in cancer therapy. Tα1 regulates both adaptive and innate immune responses by promoting T-cell maturation and T helper cell type 1 (Th1) polarization, enhancing natural killer cell activity, modulating dendritic cell function, and influencing tumor-associated macrophages and myeloid-derived suppressor cells. Through these actions, Tα1 may contribute to immune surveillance and modulation of the tumor microenvironment.

Preclinical and clinical studies suggested that Tα1 may possess antitumor activity and could enhance the efficacy of conventional and emerging cancer therapies, including chemotherapy, radiotherapy, and immune checkpoint inhibitors. Emerging evidence also indicates that Tα1 may improve immune infiltration in immunologically "cold" tumors and help mitigate certain immune-related adverse events associated with immunotherapy. Clinical investigations in malignancies, such as non-small cell lung cancer, hepatocellular carcinoma, and metastatic melanoma, have reported a favorable safety profile and potential therapeutic benefits, although the available evidence remains limited and heterogeneous.

This review summarizes the biological functions, immunoregulatory mechanisms, and therapeutic potential of Tα1 in oncology. We discuss recent advances in Tα1-based combination strategies, clinical observations, translational opportunities, and current limitations of the evidence. Furthermore, key challenges and future research directions are highlighted to provide an updated perspective on the role of Tα1 as a potential adjunctive immunomodulatory agent in modern cancer immunotherapy.

Abbreviations

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Abbreviation	Full Term
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AIDS	acquired immunodeficiency syndrome
ADV	oncolytic adenovirus
AI	artificial intelligence
BRMP	Biological Response Modifier Program
CTLA-4	cytotoxic T-lymphocyte-associated protein 4
$CD4^+$	cluster of differentiation 4-positive
$CD8^+$	cluster of differentiation 8-positive
DCR	disease control rate
DC	dendritic cell
DTIC	dacarbazine
ECOG	Eastern Cooperative Oncology Group
ELISA	enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCMV	human cytomegalovirus
HIV	human immunodeficiency virus
ICI	immune checkpoint inhibitor
IFN	interferon
IFN-α	interferon-alpha
IFN-γ	interferon-gamma
IKK	I-kappa B kinase
IL	interleukin
IL-2	interleukin-2
IL-3	interleukin-3
IL-6	interleukin-6
IL-8	interleukin-8
IL-10	interleukin-10
IL-10R	interleukin-10 receptor
IL-12	interleukin-12
LC-MS/MS	liquid chromatography–tandem mass spectrometry
MAPK	mitogen-activated protein kinase
MDSC	myeloid-derived suppressor cell
MHC	major histocompatibility complex
MHC-I	major histocompatibility complex class I
MHC-II	major histocompatibility complex class II
MyD88	myeloid differentiation primary response 88
NK	natural killer
NKT	natural killer T
NSCLC	non-small cell lung cancer
ORR	objective response rate
OS	overall survival
PD-1	programmed cell death protein-1
PD-L1	programmed death-ligand 1
PFS	progression-free survival
PTEN	phosphatase and tensin homolog
RCT	randomized controlled trial
RFS	recurrence-free survival
ROS	reactive oxygen species
TACE	transarterial chemoembolization
TAM	tumor-associated macrophage
TCR	T-cell receptor
TGF-β	transforming growth factor-beta
Th1	T helper cell type 1
Tβ4	thymosin beta 4
TIL	tumor-infiltrating lymphocyte
TIME	tumor immune microenvironment
TLR	Toll-like receptor
TLR-2	Toll-like receptor-2
TLR-9	Toll-like receptor-9
TME	tumor microenvironment
TMU	Taipei Medical University
TNF-α	tumor necrosis factor-alpha
TRAF	TNF receptor-associated factor
Treg	regulatory T cell
Tα1	thymosin alpha-1

1. Introduction

Recent global healthcare disruptions contributed to delays in cancer diagnosis and treatment, affecting timely patient management and continuity of care (Wei et al., 2023). The full impacts of these disruptions on late-stage cancer diagnoses and cancer-related mortality are expected to gradually emerge over the coming years. A recent modeling analysis projected an additional 4000–7000 deaths from colorectal cancer by 2040, depending on how quickly screening services recover (Van den Puttelaar et al., 2023). Importantly, the COVID-19 pandemic had disproportionate direct and indirect impacts including slower restoration of cancer screening programs, which is likely to further widen existing cancer disparities (Richman et al., 2023; Siegel et al., 2025).

The thymus is a primary lymphoid organ that plays a critical role in immune system development, including the maturation, differentiation, and regulation of immune cells, thereby maintaining immune homeostasis. Thymic epithelial cells constitute a key structural and functional component of the thymus, creating a specialized microenvironment that supports thymocyte development and releasing several biologically active peptides into the circulation that regulate thymocyte proliferation and differentiation. Age-associated thymic involution results in decreased production of thymus-derived peptides, which is associated with an elevated risk of cancer and other age-related disorders (Berthiaume et al., 1999; Thapa and Farber, 2019). Thymosin α-1 (Tα1) is an endogenous thymic peptide predominantly synthesized by thymic epithelial cells and was first isolated from thymic tissues by Goldstein and colleagues in 1977 (Goldstein et al., 1977). Although it is primarily localized in the thymus, Tα1 is also expressed by other organs, including the spleen, lungs, liver, and kidneys (Haritos et al., 1984).

Physiological concentrations of Tα1 are crucial for maintaining immune homeostasis throughout life. Circulating Tα1 is generated as a 28-amino-acid peptide derived from chromatin-associated protein, prothymosin (Low, 1979; Pica et al., 2018a) and is implicated in a variety of pathological conditions. Serum levels of Thymosin can be measured using a radioimmunoassay (McClure et al., 1982), enzyme-linked immunosorbent assay (Wada et al., 1988), or liquid chromatography-tandem mass spectrometry (Tuthill et al., 2000). Growing evidence suggests that reduced circulating Tα1 levels are associated with immune-related disorders, including hepatitis, systemic lupus erythematosus, rheumatoid arthritis, and sepsis (Pica et al., 2016a; Pica et al., 2018a; Sherman et al., 1991). In addition, Romani et al. (2017) reported that decreased sputum levels of Tα1 are linked to cystic fibrosis. These findings indicate that supplementation with exogenous Tα1 to restore physiological levels may offer therapeutic benefits.

Tα1 is an N-terminally acetylated acidic peptide consisting of 28 amino acids and has long been recognized for its ability to modulate, enhance, and restore immune function. Clinically, Tα1 has been applied to manage viral infections, immunodeficiency disorders, malignancies, and acquired immunodeficiency syndrome (Dominari et al., 2020a; Garaci, 2007; Garaci et al., 2007; Goldstein and Goldstein, 2009). Although the precise mechanisms underlying its therapeutic effects remain incompletely defined, they are generally attributed to its role in regulating immune responsiveness. Accumulating evidence supports the broad therapeutic potential of Tα1, and its molecular mechanisms have been extensively explored.

Thymopentin is a biologically active pentapeptide derived from thymosin II which represents the immunoreactive core of the thymosin polypeptide (Fabrizi et al., 2006; Singh et al., 1998). Clinically, thymosin-based therapeutics are available in multiple formulations, including thymosin injections, Tα1 injections, thymopentin injections, and enteric-coated tablets or capsules. These agents possess well-established immunomodulatory and immune-enhancing properties and are widely used to restore immune function in patients with immune suppression caused by sepsis and other systemic disorders (Feng et al., 2016; Liu et al., 2016; Matteucci et al., 2017). Importantly, thymosin drugs have also been extensively applied as therapeutic and adjuvant agents in cancer treatment (Garaci et al., 2015; Nevo et al., 2022). A growing body of clinical and mechanistic studies has demonstrated the efficacy of thymosin, either as monotherapy or in combination with

conventional treatments, for managing cancer, underscoring its considerable therapeutic potential (Costantini et al., 2019). Despite these encouraging findings, a comprehensive and systematic review of thymosin-based strategies in cancer therapy is currently lacking.

In this review, we provide a comprehensive overview of the role of thymosin in cancer therapy, with a particular focus on Tα1. We summarize current knowledge of its molecular mechanisms and immunomodulatory functions, including its effects on innate and adaptive immune responses, tumor immune surveillance, and the tumor microenvironment (TME). In addition, we discuss preclinical and clinical evidence supporting the therapeutic potential of thymosin in oncology, highlighting recent advances in translational research, combination strategies, and emerging applications. By integrating mechanistic insights with clinical progress, this review aims to elucidate the relevance of thymosin-based immunotherapy and identify future directions for its development in cancer treatment.

Tumor progression and therapeutic responsiveness are profoundly influenced by the tumor immune microenvironment (TIME), prompting increasing interest in therapeutic strategies capable of reversing immunosuppression and enhancing antitumor immunity (Lv et al., 2022; Sun et al., 2025; Wang et al., 2024). In this context, Tα1 has emerged as a promising immunomodulatory agent with potential applications in cancer therapy. Accumulating preclinical and early clinical evidence suggested that Tα1 may regulate both innate and adaptive immune responses through modulating dendritic cell (DC) maturation, enhancing T-cell differentiation and T helper cell type 1 (Th1) polarization, stimulating natural killer (NK) cell activity, and regulating immunosuppressive populations such as tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (Dominari et al., 2020a; Wei et al., 2023; Zhang et al., 2025). Through these coordinated immunological effects, Tα1 may contribute to improved immune surveillance and enhanced responsiveness to chemotherapy, radiotherapy, and immune checkpoint blockade (Xu et al., 2025). Nevertheless, despite these encouraging findings, much of the current evidence is derived from preclinical studies, retrospective analyses, and early-phase clinical investigations. Therefore, further well-designed prospective clinical studies are required to more clearly define the mechanistic role, therapeutic efficacy, and translational relevance of Tα1 within the evolving landscape of cancer immunotherapy.

Recent progress in cancer immunotherapy has emphasized the growing role of immunomodulatory agents as supportive components of conventional cancer treatments. Among these, thymosin-based therapies, particularly Tα1, have attracted increasing attention for their use in combination with chemotherapy, radiotherapy, immune checkpoint blockade, targeted therapies, cancer vaccines, and antiviral approaches to strengthen antitumor immunity and improve therapeutic efficacies. Recent findings also suggest that thymosin can modulate the TME, maintain immune balance, and reduce therapy-associated immunosuppression. Therefore, incorporating current mechanistic and translational evidence is important for understanding the expanding role of thymosin in contemporary oncology and its potential application in personalized cancer immunotherapy.

2. Biological activities and health benefits of thymosin α-1 (Tα1)

Tα1 acts as an agonist of Toll-like receptors (TLR-9 and TLR-2) in myeloid cells and DCs, which are key professional antigen-presenting cells (Romani et al., 2004a). Through activation of TLR signaling pathways, Tα1 enhances adaptive immune responses that are critical for host defense against viral, bacterial, and fungal infections, as well as malignancies, while also promoting immune activation (Romani et al., 2004a, 2007a; Wu et al., 2013). In addition, Tα1 was shown to upregulate the production of multiple immunoregulatory cytokines, including interleukin (IL)-2, IL-10, IL-12, interferon (IFN)-α, and IFN-γ (Tuthill et al., 2012). Its ability to stimulate T cell-dependent antibody responses further supports the development of Tα1 as an effective factor to enhance immunogenicity (Naylor et al., 2007).

Tα1 exhibits a broad spectrum of biological activities, ranging from immune modulation to direct antitumor effects. Its immunostimulatory functions are largely mediated by promoting T-cell maturation and differentiation into cluster of differentiation 4-positive ($CD4^+$) and $CD8^+$ T-cell subsets. Moreover, Tα1 directly activates NK cells and cytotoxic $CD8^+$ T cells, facilitating the elimination of virally infected and malignant cells. Concurrently, Tα1 suppresses proinflammatory mediators such as IL-1β and tumor necrosis factor (TNF)-α, thereby reducing excessive inflammatory responses, which is particularly beneficial in conditions such as chronic hepatitis and acute pancreatitis. Beyond cytokine regulation, Tα1 enhances the expression of major histocompatibility complex class I (MHC-I) molecules and viral antigens on target cells, while simultaneously inhibiting viral replication (Fig. 1) (Li et al., 2010a). Collectively, these findings highlight the multifaceted immunoregulatory mechanisms of Tα1, which acts on diverse immune targets to exert its broad immune-enhancing effects (Moody, 2007).

Tα1 has demonstrated significant inhibitory effects on tumor growth, supporting its application in treating multiple cancer types. Its antiproliferative activity was reported in metastatic models of lung and liver cancers. Studies by Moody et al. (Dominari et al., 2020a; Moody, 2007) further indicated that the antitumor efficacy of Tα1 is most pronounced in smaller-sized tumors. Mechanistically, Tα1 exerts its anticancer effects primarily through two complementary pathways: enhancement of host immune responses and direct reduction of tumor cell proliferation. In addition, Armutcu et al. (Armutcu et al., 2005; Dominari et al., 2020a) reported a protective role of Tα1 against oxidative stress, mediated through modulation of hepatic antioxidant enzymes, including superoxide dismutase and glutathione peroxidase (Dominari et al., 2020a).

As an endogenous thymic polypeptide, Tα1 plays a central role in regulating inflammation, immune activation, and immune tolerance. Its immunomodulatory effects are largely mediated through interactions with Toll-like receptors (TLRs), enabling the coordination of innate and adaptive immune responses. Owing to its broad activity across multiple immune cell types, Tα1 has been widely explored as a therapeutic agent for diseases characterized by immune dysregulation (Dominari et al., 2020a; Pica et al., 2018a) . Clinical trials investigating Tα1 demonstrated favorable outcomes in conditions such as DiGeorge syndrome, non-small cell lung cancer (NSCLC), hepatocellular carcinoma (HCC),

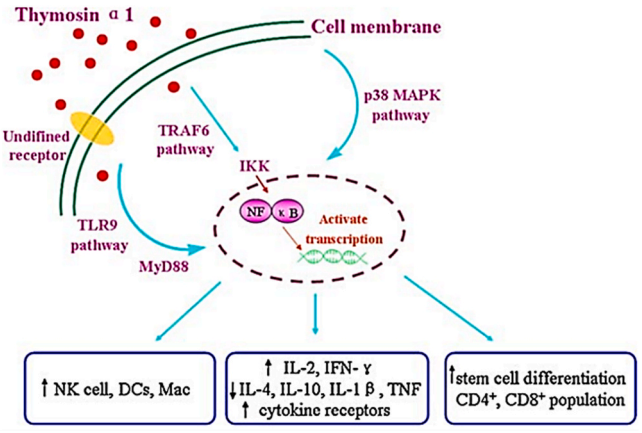

Fig. 1. Immunoregulatory mechanisms of Tα1. Schematic illustration of signaling pathways involved in Tα1-mediated immune modulation. Tα1 activates intracellular signaling cascades through receptor-associated pathways, including TLR9-MyD88-dependent signaling, leading to activation of p38 MAPK and IKK pathways and downstream immune responses in macrophages. TRAF, TNF receptor-associated factor; TLR9, Toll-like receptor 9; Mac, macrophage; p38 MAPK, p38 mitogen-activated protein kinase; IKK, I-kappa B kinase; MyD88, myeloid differentiation factor 88 (Li et al., 2010a). The copyright for this image was granted by Elsevier.

chronic hepatitis B and C, human immunodeficiency virus (HIV) infection, and melanomas (Chien et al., 1998a; Dominari et al., 2020a; Tuthill et al., 2010). Notably, Tα1, also known as thymalfasin (Zadaxin), a synthetic thymic peptide with established immunomodulatory activity which has been applied to treat malignant melanomas, chronic hepatitis B, HCC, and certain immune deficiency disorders (Dominari et al., 2020a), and thymosin beta 4 (Tβ4, also known as timbetasin) are thymus-derived peptide hormones with distinct chemical structures and biological functions. Both peptides originate from thymosin fraction 5 and are capable of modulating multiple immune-related processes in mammals. Tα1 primarily functions as an immunomodulatory peptide that enhances cell-mediated immunity and supports immune system restoration, particularly in thymus-deficient conditions. Due to its immune-enhancing properties, Tα1 has garnered significant clinical interest for treating several cancers, including HCC, lung cancer, and melanomas. In contrast, Tβ4 belongs to the family of actin-sequestering proteins and plays a critical role in regulating actin polymerization by maintaining intracellular pools of free G-actin monomers. Beyond its cytoskeletal functions, Tβ4 exhibits notable activity in response to viral infections and is currently being investigated as a potential therapeutic agent for influenza, HIV, and acquired immunodeficiency syndrome (AIDS).

3. Applications in oncologic patients

A growing body of evidence supports the therapeutic potential of Tα1 for managing various malignancies, including metastatic melanomas, lung cancer, and HCC (Costantini et al., 2019). In certain clinical settings and regions, Tα1 has been investigated and utilized as an adjuvant immunomodulatory therapy to help alleviate chemotherapy-associated immune suppression and immune dysfunction in cancer patients. Moreover, clinical studies have demonstrated that the addition of Tα1 to conventional chemotherapy or radiotherapy regimens significantly improves survival outcomes in patients with NSCLC. Given that NSCLC constitutes approximately 85% of all lung cancer cases and is typically characterized by limited responsiveness to chemotherapy, these findings underscore the value of Tα1 as an immune-supportive adjunct in oncologic treatment strategies (Dominari et al., 2020a).

3.1. Foundations of Tα1-Based cancer immunotherapy

The immunostimulatory properties of thymosin prompted early clinical investigations evaluating thymosin fraction 5 and Tα1 in patients with primary immunodeficiencies and various malignancies. The therapeutic rationale for employing thymosin in oncology was based on its ability to enhance host immune competence, thereby improving antitumor immune responses and reducing susceptibility to opportunistic infections. In addition, thymosin was proposed to alleviate the immunosuppressive effects commonly associated with conventional chemotherapy and radiotherapy. The first phase I clinical trial involving Tα1 was conducted at the National Cancer Institute, followed by additional studies supported by the Biological Response Modifier, which aimed to promote the development of biologically active agents with potential anticancer efficacy. Early BRMP-sponsored clinical studies demonstrated evidence of antitumor activity and immunological improvement, particularly in patients with a relatively limited tumor burden and those receiving localized radiotherapy, whereas patients with advanced-stage disease exhibited comparatively limited responses. Importantly, Tα1 administration was generally associated with a favorable safety and tolerability profile. Preclinical investigations further revealed that although Tα1 did not markedly enhance NK cell or macrophage-mediated tumoricidal activity, it significantly promoted T-cell-mediated immune responses both in vitro and in vivo, including antitumor effects in experimental B16 melanoma metastasis models (Asanuma et al., 1970; Goldstein, 2007; Goldstein et al., 1970a; Goldstein et al., 1977; Goldstein et al., 1966; Hooper et al., 1975; Law et al., 1968; Costantini et al., 2019).

3.2. Thymosin α1 and cancer: Evolution from early studies to current applications

Over the years, numerous preclinical and clinical studies have evaluated the therapeutic potential of Tα1 across multiple cancer types to better define its safety, immunological effects, and anticancer efficacy. Research has progressively expanded from the initial exploratory clinical studies to more-advanced translational and immunotherapeutic applications. In particular, investigations involving murine tumor models and human clinical studies extensively focused on melanomas, HCC, and NSCLC, where Tα1 demonstrated promising immunomodulatory and therapeutic benefits, especially when combined with chemotherapy, radiotherapy, cytokine therapy, or immune checkpoint blockade (Costantini et al., 2019). Table 1 presents representative preclinical and clinical studies highlighting the immunomodulatory and therapeutic applications of Tα1 across infectious diseases, cancer, inflammatory disorders, and immunocompromised conditions. Table 2 summarizes the major cancer treatment modalities and the therapeutic rationale for incorporating Tα1 into current treatment strategies.

4. Synergistic integration of Tα1 for cancer therapy

4.1. Immunomodulatory and adjuvant effects of Tα1 in chemotherapy

Accumulating evidence from fundamental and translational studies suggests that Tα1 possesses essential characteristics of an effective immunoadjuvant for correcting dysregulated immune responses, particularly in malignant diseases. Chemotherapy remains a cornerstone of cancer treatment, exerting its therapeutic effects primarily through inducing tumor cell death and inhibiting cancer progression. However, chemotherapy profoundly alters the TME, producing complex and often contradictory immunological effects. While certain chemotherapeutic agents enhance tumor immunogenicity and partially restore impaired immune surveillance, they can also induce an immunosuppressive TME that promotes tumor progression and therapeutic resistance (Tajbakhsh et al., 2021; Wei et al., 2023).

In this context, Tα1 has emerged as a potent immunomodulator capable of counteracting chemotherapy-induced immune dysfunction and suppression. Clinical studies demonstrated that administration of Tα1 during intervals between chemotherapy cycles yielded meaningful therapeutic benefits in patients with malignancies such as malignant melanomas, NSCLC (Costantini et al., 2019; Wei et al., 2023). In parallel, extensive preclinical investigations showed that combining Tα1 with chemotherapy produces enhanced antitumor efficacy across multiple experimental tumor models. For instance, co-administration of Tα1 and IL-2 following 5-fluorouracil treatment significantly reduced hepatic metastasis in rat models of colorectal cancer (Fig. 5) (Rasi et al., 1994; Silecchia et al., 1999; Garaci et al., 2000).

More recently, treatment with Tα1 after hyperthermic intraperitoneal chemotherapy markedly prolonged survival in mice bearing peritoneal metastases of colonic carcinoma, an effect attributed to the induction of a Th1-biased antitumor immune response and increased $CD8^+$ T-cell infiltration within visceral and omental metastatic lesions (Nevo et al., 2022). Similarly, the combination of Tα1 and gemcitabine demonstrated superior antitumor activity in nasal NK/T-cell lymphoma by reducing the Epstein-Barr virus load, enhancing proinflammatory cytokine expression, suppressing the epithelial-mesenchymal transition and tumor cell proliferation, and promoting autophagy and apoptosis (Chen et al., 2021; Wei et al., 2023). In addition, Wei et al. (2022) reported that Tα1 synergized with epirubicin chemotherapy to significantly augment both the abundance and functional activity of tumor-infiltrating T cells, leading to suppressed tumor growth in breast cancer models.

Table 1
Representative preclinical and clinical studies highlighting the immunomodulatory and therapeutic applications of thymosin α1 (Tα1) across infectious diseases, cancer, inflammatory disorders, and immunocompromised conditions. The table summarizes disease indications, major findings, and corresponding references demonstrating the diverse biological and clinical activities of Tα1.

Study Type	Disease/Application	Key Findings	Evidence Level	References
Preclinical	Human cancers (leukemia, NSCLC, melanoma, breast cancer)	Tα1 reduced tumor cell proliferation and induced apoptosis in multiple cancer cell lines	Preclinical (in vitro/in vivo)	Dominari et al. (2020a); Guo et al. (2015)
Clinical	Chronic hepatitis B	Tα1 monotherapy or combination with IFN-α improved immune responses and IL-2 receptor expression	Randomized clinical studies/Meta-analyses	Chien et al. (1998a); Dominari et al. (2020a); Li et al. (2010a)
Clinical	Sepsis	Significant reduction in mortality associated with multiple organ failure	Randomized Controlled Trial (RCT)	Dominari et al. (2020a); Li et al. (2015)
Clinical	HIV infection	Tα1 enhanced $CD4^+$ T-cell function, reduced viral load, and decreased oxidative tissue damage	Clinical studies	Dominari et al. (2020a); Li et al. (2010a)
Clinical	Advanced HIV disease	Tα1 significantly increased sjTREC levels, suggesting improved thymic immune recovery	Clinical observational study	Dominari et al. (2020a); Li et al. (2010a)
Clinical	Bone marrow transplantation/ *Pseudomonas* infection	Tα1 showed beneficial effects against opportunistic infections	Clinical observational study	Camerini and Garaci (2015); Dominari et al. (2020a)
Clinical	Mold infections	Tα1 demonstrated potential antifungal immunomodulatory activity	Clinical pilot study	Antachopoulos et al. (2012); Dominari et al. (2020a)
Clinical	Immunocompromised conditions	Tα1 enhanced cytokine production and immune responsiveness	Clinical studies	Dominari et al. (2020a); King and Tuthill (2016a)
Clinical	Psoriatic arthritis	Tα1 was proposed to regulate immune responses and reduce inflammation	Pilot clinical study	Dominari et al. (2020a); Pica et al. (2018a)
Clinical	Influenza vaccination	Tα1 improved vaccine-induced immune responses and immunogenicity	Randomized clinical studies	Carraro et al. (2012); Dominari et al. (2020a); Panatto et al. (2011)
Preclinical (in vivo)	Severe acute pancreatitis	Tα1 improved outcomes in a rat model by regulating peripheral T-cell numbers and serum cytokine levels.	Preclinical (in vivo)	Yao et al. (2007b)
Clinical	Cancer immunotherapy	Promising clinical responses reported in melanoma, head and neck carcinoma, lung cancer, breast cancer, and hepatocellular carcinoma	Early clinical evidence (Phase II/Retrospective studies)	Dominari et al. (2020a); Li et al. (2010a)
Clinical	Sepsis (randomized controlled trial (RCT))	A single-blind RCT showed approximately 9% lower mortality in Tα1-treated patients	Randomized Controlled Trial (RCT)	Dominari et al. (2020a); Romani et al. (2007a)
Clinical	Chronic hepatitis B	Tα1 improved serum ALT normalization and HBV DNA clearance	Randomized clinical studies	Dominari et al. (2020a); Li et al. (2010a); Sugahara et al. (2002)

Table 2
Summary of major cancer treatment modalities, their associated limitations, and the therapeutic rationale for incorporating thymosin α-1 (Tα1) in cancer therapy. The table highlights the immunomodulatory, immune-restorative, and tumor microenvironment-regulating properties of Tα1 that may enhance therapeutic efficacy, reduce treatment-associated immunosuppression, and improve antitumor immune responses when used alone or in combination with existing cancer therapies.

Cancer Treatment Modality	Major Limitations	Potential Role/Rationale for Thymosin α-1 (Tα1)	Representative References
Chemotherapy	Systemic toxicity, immune suppression, drug resistance, tumor recurrence	Restores immune function, enhances T-cell activity, reduces chemotherapy-induced immunosuppression, improves antitumor responses	Costantini et al. (2019)
Radiotherapy	Damage to healthy tissues, inflammatory toxicity, immune dysregulation	Enhances immune recovery, supports antitumor immunity, modulates inflammatory responses	Costantini et al. (2019)
Immune Checkpoint Inhibitors (PD-1/PD-L1, CTLA-4)	Limited response rate, immune-related adverse events (IRAEs), "cold" tumors	Converts immunologically "cold" tumors into "hot" tumors, enhances TIL infiltration, may help mitigate IRAEs	Danielli et al. (2018); Espinar-Buitrago et al. (2023); Renga et al. (2020); Wei et al. (2023)
Cancer Vaccines	Poor immunogenicity, limited clinical efficacy, manufacturing complexity	Functions as an immunoadjuvant to enhance antigen presentation and T-cell activation	Graff and Chamberlain (2015); Kantoff et al. (2010); Quesada et al. (1986); Riley et al. (2019)
Cytokine Therapy (IL-2, IFN-α)	Severe systemic toxicity, limited durability of response	Improves immune tolerance and synergistically enhances cytokine-mediated antitumor immunity	Garaci et al. (2000)
Targeted Therapy	Acquired resistance, tumor heterogeneity, incomplete immune activation	Enhances immune surveillance and supports combination therapeutic efficacy	Costantini et al. (2019)
Bacterial Immunotherapy	Difficulty controlling immune responses, immune evasion by bacteria	Enhances tumor-specific immune activation and regulates tumor immune microenvironment	Chang et al. (2025)
Oncolytic Viral Therapy	Immunosuppressive tumor microenvironment, limited viral persistence	Reprograms tumor-associated macrophages and enhances $CD8^+$ T-cell responses	Liu et al. (2024)
CAR-T Cell Therapy	Cytokine release syndrome, limited efficacy in solid tumors, T-cell exhaustion	May improve T-cell persistence, reduce exhaustion, and enhance immune regulation	Costantini et al. (2019); Wei et al. (2023)
Combination Chemo-Immunotherapy	Overlapping toxicities, tumor immune escape, immunosuppression	Enhances synergistic antitumor immunity while supporting immune homeostasis	Costantini et al. (2019)

4.2. Immunotherapy-enhancing role of Tα1

Cancer immunotherapy based on immune checkpoint blockade has become a cornerstone for treating advanced malignancies. The first US Food and Drug Administration (FDA)-approved cancer immunotherapies were recombinant forms of the cytokine interferon (IFN)-α, which received approval in 1986 for treating hairy cell leukemia (Quesada et al., 1986). After almost a decade of largely unsuccessful vaccine

trials, the first effective therapeutic cancer vaccine, sipuleucel-T an autologous DC-based therapy was approved in 2010 for prostate cancer treatment. However, its widespread clinical application was limited due to manufacturing challenges and other associated issues (Graff and Chamberlain, 2015; Kantoff et al., 2010; Riley et al., 2019). The combination of chemotherapy and immune checkpoint inhibitors (ICIs) emerged as a promising strategy in cancer therapy by enhancing both direct tumor killing and immune activation. Chemotherapy can promote immunogenic cell death, increase antigen release, and modulate the TME, thereby improving the effectiveness of ICIs. Recent advances in artificial intelligence (AI), including multi-omics analysis, radiomics, and deep learning, have further supported the optimization of chemo-immunotherapy through improved predictions of treatment responses, biomarker identification, and patient stratification. Despite encouraging clinical outcomes, challenges such as therapeutic resistance, tumor heterogeneity, and treatment-related immunosuppression continue to limit broader clinical success (Muhammad et al., 2026a). Chang et al. (2025) demonstrated that bacterial immunotherapy possesses strong anticancer potential, although its success relies on understanding how bacteria evade immune defenses while simultaneously activating anti-tumor immunity within the TME. Using an engineered *Salmonella enterica* strain, they revealed that elevated IL-10 receptor (IL-10R) expression drives tumor-infiltrating immune cells into a tumor-specific IL-10R^{hi} state. This condition allows bacteria to evade phagocytosis, enhance IL-10-producing TAMs, and activate exhausted tumor-resident CD8$^+$ T cells, ultimately suppressing tumor growth, reducing metastasis, and preventing recurrence across multiple tumor models. Those authors further suggested that the IL-10Rˆhi state may represent a common feature among human tumors, providing new insights into bacterial immunotherapy and intratumoral immune modulation.

CTLA-4 was the immune checkpoint to be therapeutically targeted, with the humanized anti-CTLA-4 antibody, ipilimumab, demonstrating a landmark improvement in long-term survival for patients with metastatic melanoma (Schadendorf et al., 2015). Notably, clinical observations indicated that pretreatment with Tα1 enhanced OS in metastatic melanoma patients receiving ipilimumab, suggesting a synergistic interaction between Tα1 and anti-CTLA-4 therapy (Danielli et al., 2018).

Targeting the PD-1/PD-L1 axis has also yielded significant clinical success across a wide range of solid and hematological malignancies. However, durable responses to PD-1/PD-L1 blockade are limited to a certain subset of patients (Han et al., 2020), largely due to differences in the TIME. Based on PD-L1 expression and the presence of tumor-infiltrating lymphocytes (TILs), TIMEs are categorized into four distinct types: type I (PD-L1$^-$, no TILs), type II (PD-L1$^+$, TILs present), type III (PD-L1$^-$, TILs present), and type IV (PD-L1$^+$, no TILs) (Kim et al., 2022). Among these, the type II TIME is considered the most responsive to PD-1/PD-L1 blockade, while the remaining types are theoretically convertible into type II through therapeutic interventions. Strategies to achieve this conversion include modulating the TME, blockade of systemic immunosuppression, and enhancement of T cell-mediated immunity.

Emerging evidence suggests that Tα1 plays a critical role in reshaping the TIME toward a type II phenotype. Preclinical studies demonstrated that Tα1 increases TIL infiltration and strengthens tumor-specific immune responses in tumor-bearing models by upregulating MHC-I expression by tumor cells (Giuliani et al., 2000).In addition, Bo et al. (2018) reported that Tα1 significantly suppresses proliferation and migration in PD-L1-high-expressing NSCLC cells compared to PD-L1-low counterparts, supporting a synergistic effect between Tα1 and anti-PD-L1 therapy. Consistently, combined treatment with low-dose Tα1 and anti-PD-L1 antibodies significantly enhanced therapeutic efficacy in a lung metastasis melanoma model (King and Tuthill, 2015; Wei et al., 2023).

Beyond its effects on adaptive immunity, PD-1/PD-L1 signaling was recently implicated in the regulation of innate immune functions, particularly macrophage-mediated efferocytosis. In advanced tumors, PD-1-expressing TAMs predominantly exhibit an M2-like phenotype with an impaired capacity to engulf apoptotic tumor cells. Disruption of the PD-1/PD-L1 axis was shown to restore efferocytic activity in these macrophages (Gordon et al., 2017; Wei et al., 2023). In parallel, innate immune checkpoints such as CD47 contribute to tumor immune evasion by preventing macrophage-mediated clearance of malignant cells. Blocking CD47 signaling restores efferocytosis and normalizes apoptotic tumor cell removal (Kojima et al., 2016). Importantly, previous work demonstrated that Tα1 modulates the phenotype of efferocytosing TAMs (Wei et al., 2022), suggesting that combining Tα1 with anti-CD47 therapy may synergistically enhance tumor clearance while promoting macrophage polarization toward an antitumor M1 state. This dual targeting strategy integrating innate and adaptive immune checkpoint inhibition represents a promising therapeutic avenue. Beyond checkpoint blockade, Tα1 has also been combined with oncolytic virotherapy: a Tα1-armed oncolytic adenovirus reversed virus-induced M2-like TAM polarization and enhanced CD8$^+$ T-cell-mediated antitumor immunity (Fig. 2) (Liu et al., 2024).

Despite their clinical success, immune checkpoint inhibitors are frequently associated with immune-related adverse events (IRAEs) affecting multiple organ systems, including the skin, gastrointestinal tract, endocrine organs, liver, lungs, eyes, and nervous system. These toxicities typically arise within 3–6 months of treatment initiation, with reported incidence rates of 70%–90% among patients receiving anti–CTLA-4 or anti–PD-1/PD-L1 therapy (Espinar-Buitrago et al., 2023). These findings suggest that Tα1 might not only augment checkpoint inhibitor efficacy but also mitigate IRAEs, further supporting its value as a complementary immunotherapeutic agent (Wei et al., 2023). A previous study by (Chen et al., 2023) demonstrated that the therapeutic

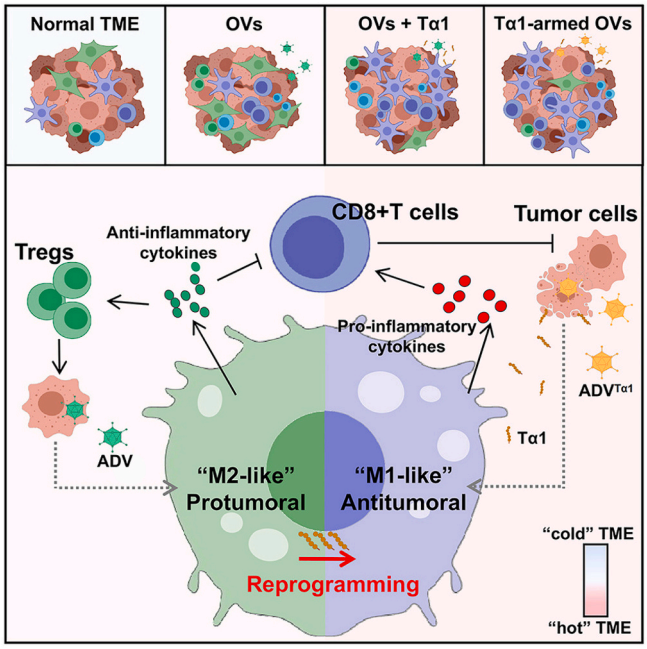

Fig. 2. Immunomodulatory effects of oncolytic adenovirus (ADV) and therapeutic reprogramming by Tα1. Schematic representation of the tumor microenvironment (TME) response to ADV and its modulation by Tα1. ADV induces immunosuppressive feedback by promoting M2-like tumor-associated macrophage (TAM) polarization and regulatory T cell (Treg) infiltration. Tα1 counteracts this effect by reprogramming M2-like TAMs toward an antitumor phenotype and enhancing CD8$^+$ T-cell-mediated immune responses. The engineered ADVTα1 platform integrated oncolytic activity with Tα1-mediated immunoregulation, thereby improving the antitumor efficacy and supporting the translational potential (Liu et al., 2024). The copyright for this image was granted by Elsevier.

efficacy of αCTLA-4 in mesenchymal-like glioblastomas was primarily dependent on $CD4^+$ T-cell-mediated immune responses rather than $CD8^+$ T cells. That study showed that αCTLA-4 treatment significantly prolonged survival and enhanced the infiltration of IFN-γ-producing $CD4^+$ T cells within the TME. Mechanistically, $CD4^+$ T cells directly interacted with microglia, promoting IFN-γ-dependent microglial activation and phagocytosis through the AXL/MER signaling pathway. Furthermore, microglia acted as antigen-presenting cells via MHC-II expression, thereby sustaining the $CD4^+$ T-cell response and contributing to tumor suppression. These findings highlighted the importance of the $CD4^+$ T cell–microglia immune circuit in mediating the antitumor effects of immune checkpoint blockade in glioblastomas (Fig. 3).

4.3. Adaptive immune response

Tα1 functions as an important regulator of adaptive immunity. Early work by Goldstein et al first identified its essential role in T-cell maturation (Ahmed et al., 1979). This was later supported by studies from Knutsen et al. (1999), who demonstrated that Tα1 promotes the differentiation of $CD34^+$ hematopoietic stem cells into $CD3^+CD4^+$ T cells. Subsequent investigations revealed that Tα1 stimulated the production of Th1-associated cytokines, including IL-2, IFN-γ, and IL-3, thereby reinforcing cell-mediated immune responses (Yao et al., 2007a).

In addition to enhancing Th1 polarization, Tα1-induced differentiation of precursor T cells toward the Th1 lineage was associated with improved neurogenesis, cognitive function, and antitumor immunity (Wang et al., 2017). Studies further reported that Tα1 promotes apoptosis of Treg cells in cecal ligation and puncture models, suggesting its capacity to alleviate immunosuppression under pathological conditions Notably, reduced expressions of exhaustion markers such as PD-1 and TIM3 by $CD8^+$ T cells were observed in Tα1-treated patients with severe disease, indicating enhanced T-cell functionality (Liu et al., 2020).

Beyond T-cell regulation, Tα1 indirectly promotes B-cell activation and differentiation into antibody-secreting plasma cells, leading to improved vaccine responsiveness (Ershler et al., 1984, 1985). More-recent evidence further suggested that Tα1 enhances the expansion of IL-10-producing regulatory B-cell subsets in peripheral blood mononuclear cells from patients with relapsing-remitting multiple sclerosis, highlighting its role in immune homeostasis (Giacomini et al., 2018). Consistent with its initial identification as a thymic peptide capable of restoring immune function in immunocompromised animal models, Tα1 has been extensively investigated at the cellular level (Fig. 4). Mechanistic studies have revealed that Tα1 regulates multiple intracellular signaling pathways involved in immune activation and modulation, thereby contributing to the enhancement of immune responses. Toll-like receptors (TLRs) constitute a family of pattern-recognition receptors that play a central role in innate immunity. Activation of TLRs by specific agonists can potentiate adaptive immune responses, thereby enhancing host defense against viral, bacterial, and fungal infections, as well as cancer. Furthermore, TLR stimulation contributes to the development of effective humoral immune responses, improving vaccine efficacy. Tα1 modulates TLR activity in both myeloid and plasmacytoid dendritic cells (DCs), key antigen-presenting cells of the immune system, leading to the activation of downstream signaling pathways and the production of cytokines that support antimicrobial and antitumor immunity (King and Tuthill, 2016a).

4.4. Innate immune response

In addition to its effects on adaptive immunity, Tα1 plays a critical role in modulating innate immune responses. Early studies demonstrated that Tα1 enhanced NK cell cytotoxic activity in multiple cancer models (Favalli et al., 1985; Serrate et al., 1987). Subsequent research primarily focused on its regulatory effects on DCs. Romani et al. (2004a) first proposed Tα1 as an immune adjuvant capable of activating DCs in 2004. They further demonstrated that Tα1 promotes functional maturation and IL-12 production in conventional DCs, while inducing IL-10 secretion by plasmacytoid DCs during fungal infections. This dual regulation facilitates a balanced immune response by coordinating

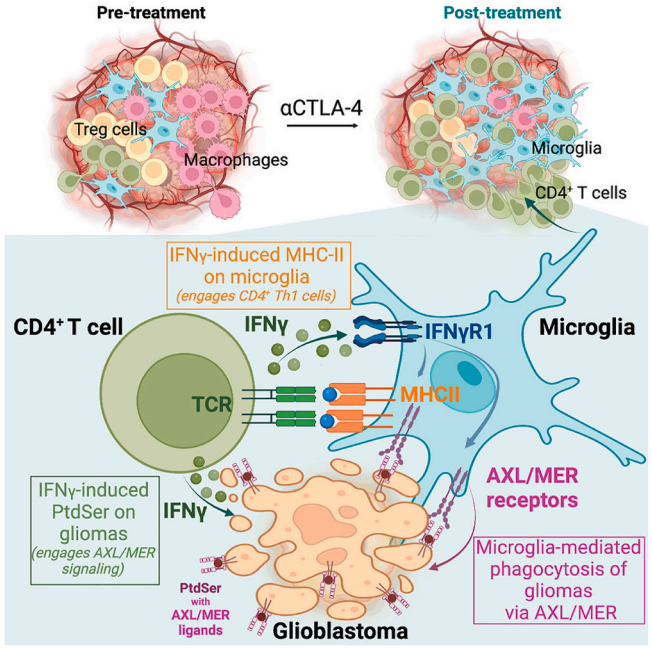

Fig. 3. Schematic illustration of the $CD4^+$ T cell-microglia immune circuit involved in αCTLA-4-mediated antitumor activity in mesenchymal-like glioblastomas. αCTLA-4 treatment enhanced the infiltration of IFN-γ-producing $CD4^+$ T cells, which directly interacted with microglia and promoted IFN-γ-dependent microglial activation and phagocytosis through the AXL/MER signaling pathway. Activated microglia further functioned as antigen-presenting cells via MHC-II expression, sustaining $CD4^+$ T-cell activation and contributing to tumor suppression (Chen et al., 2023). The copyright for this image was granted by Elsevier.

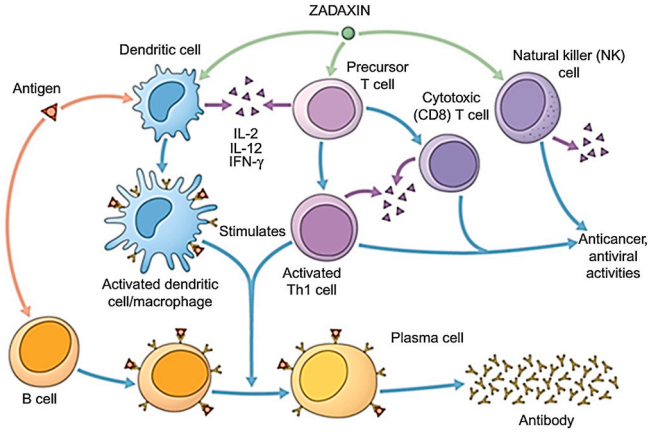

Fig. 4. Immune-stimulating mechanism of action of thymosin-α1 (Tα1). Tα1 enhances both innate and adaptive immunity by activating dendritic cells, macrophages, and natural killer cells, leading to improved antigen presentation and cytokine secretion. Tα1 promotes T-cell maturation and T helper cell type 1 (Th1) polarization, increasing IL-2, IFN-γ etc. Additionally, it modulates myeloid populations, including MDSCs, and reprograms tumor-associated macrophages toward an M1-like antitumor phenotype. These coordinated effects strengthen cytotoxic T-cell responses, restore immune surveillance, and enhance antitumor immunity (King and Tuthill, 2016a). The copyright for this image was granted by Elsevier.

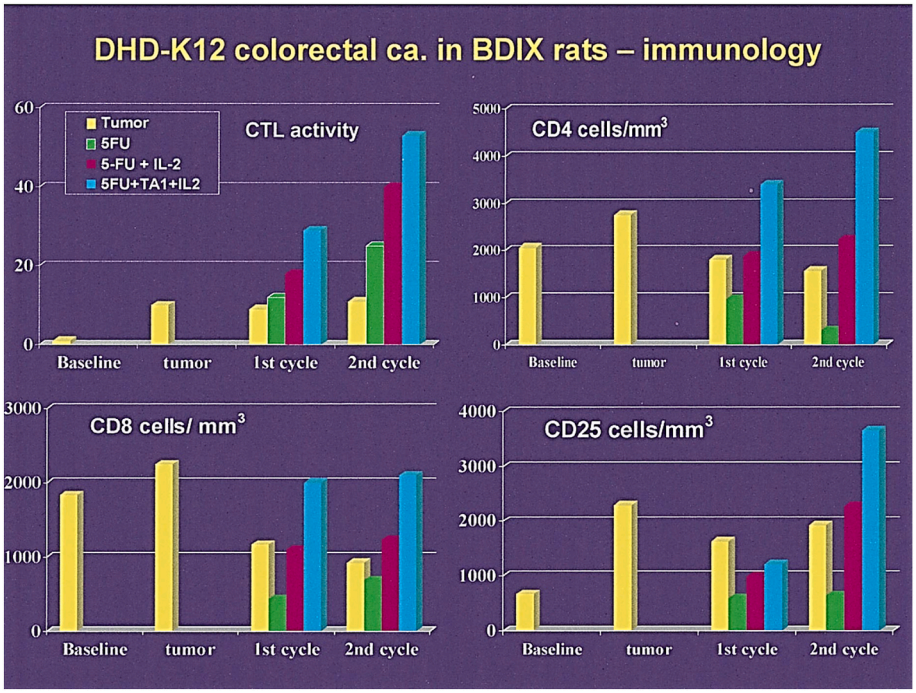

Fig. 5. Immunological changes induced by combined chemo-immunotherapy in BDIX rats bearing DHD-K12 colorectal carcinoma. Combined treatment markedly modulated the tumor immune microenvironment, enhancing antitumor immune responses. Therapy increased infiltration and activation of cytotoxic T lymphocytes while reducing immunosuppressive cell populations, leading to improved immune surveillance and tumor control. These findings indicated that chemo-immunotherapy synergistically reshaped the host's immunity to promote effective antitumor activity in this BDIX rat colorectal carcinoma model (Garaci et al., 2000). The copyright for this image was granted by Elsevier.

inflammation and immune tolerance through Th1 pathways, (Romani et al., 2004a, 2006).

Tα1 also exerts context-dependent effects on human monocyte-derived DCs during viral and bacterial infections. It enhances antiviral immunity by upregulating maturation markers such as CD40, CD80, and MHC class I and II molecules, as well as increasing the production of proinflammatory cytokines (IL-6, IL-8, and TNF-α) and IFN-α/IFN-γ. Conversely, during bacterial infections, Tα1 attenuates excessive inflammation by suppressing inflammatory signaling pathways in DCs (Bozza et al., 2007; Giacomini et al., 2015; Yao et al., 2007a).

Macrophages represent another key innate immune cell population regulated by Tα1. As early as 1989, Tα1 was shown to enhance antigen presentation by macrophages (Tzehoval et al., 1989). Subsequent studies demonstrated that Tα1 rapidly induces phagocytosis, enabling efficient killing of *Aspergillus niger* conidia within 30 min (Serafino et al., 2014). Moreover, Tα1 increases macrophage motility and chemotactic activity by promoting dynamic assembly and disassembly of podosomal structures, thereby strengthening primary immune responses against invading pathogens (Serafino et al., 2015). In tumor-bearing models, intraperitoneal administration of Tα1 significantly enhanced pinocytosis, phagocytosis, antigen presentation, and antitumor cytotoxicity of TAMs (Shrivastava et al., 2004).

Importantly, TAMs play a central role in the clearance of chemotherapy-induced apoptotic tumor cells through efferocytosis but are often polarized toward a pro-tumorigenic M2 phenotype, which facilitates tumor progression and metastasis. Therapeutic strategies aimed at reprogramming efferocytotic TAMs toward an antitumor M1-like antitumor phenotype have therefore gained increasing attention. In this regard, (Wei et al., 2023) demonstrated that Tα1 promotes macrophage plasticity by suppressing M2 markers such as IL-10 and CD206 while enhancing expressions of M1-associated markers including IL-6 and CD86. This phenotypic shift during efferocytosis triggers robust $CD4^+$ and $CD8^+$ T-cell-dependent antitumor immune responses. These findings highlight the therapeutic potential of targeting innate immune cells with Tα1 in cancer treatment (Wei et al., 2023).

Collectively, those studies illustrate the complex and multifaceted immunoregulatory functions of Tα1. Further investigations into the molecular mechanisms governing its coordination of adaptive and innate immune crosstalk are essential for optimizing its therapeutic application in cancer and immune-mediated diseases.

5. Combination therapy in human cancers

Encouraging preclinical findings prompted the clinical evaluation of Tα1-based combination regimens in cancer patients. To ethically assess the added benefit of chemo-immunotherapy compared to chemotherapy alone, two malignancies with poor responsiveness to conventional chemotherapy, namely NSCLC and advanced metastatic melanoma, were selected. In those studies, standard chemotherapy protocols were maintained, while immunotherapeutic agents were administered during intervals between chemotherapy cycles. The principal clinical outcomes of these trials are summarized below.

In patients with a stage III–IV metastatic melanoma, dacarbazine (DTIC) and IL-2, when used individually, exhibit limited antitumor efficacy. Given the ability of Tα1 to modulate IL-2 activity, a combination regimen consisting of DTIC, Tα1, and IL-2 was investigated. Forty-six patients with a measurable metastatic melanoma received DTIC (850 mg intravenously on day 1), Tα1 (2 mg subcutaneously on days 4–7), and IL-2 (18 million units/m^2 per day by continuous intravenous infusion on days 8–12), with treatment cycles repeated every 3 weeks. Among the 42 evaluable patients, objective responses were observed in 15 patients (36%), indicating promising antitumor activity. Median time to progression was 5.5 months, and median OS reached 11 months. Adverse events were mainly attributable to IL-2, and the combined regimen was generally well tolerated, with no overlapping toxicity between chemotherapy and immunotherapy (Garaci et al., 2000).

5.1. Clinical dosing regimens of thymosin α-1

A subsequent phase II open-label trial evaluated the feasibility, safety, and immunological effects of a triple-combination therapy consisting of Tα1, low-dose IFN, and dacarbazine (DTIC) in patients with an advanced metastatic melanoma (Rasi et al., 2000). Eligible patients had stage III or IV disease, an Eastern Co-operative Oncology Group (ECOG) performance status of 0–2, measurable unresectable metastases, and no prior chemotherapy or biotherapy. Of the 26 enrolled patients, 20 were evaluable for efficacy and toxicity. Treatment included DTIC (200 mg/m^2/day intravenously on days 1–4), followed by Tα1 (1 mg/day subcutaneously on days 8–11 and 15–18) and low-dose IFN (3 million IU intramuscularly on days 11 and 18), with cycles repeated every 28 days. Fifty percent of patients achieved objective responses, including five complete and five partial responses. Median survival was 11.5 months, with a median PFS of 5.5 months. Importantly, no additional toxicity was observed following immunotherapy. Immunological analyses revealed that DTIC alone significantly reduced NK cell activity and CD4$^+$ T-cell counts, whereas the addition of Tα1 and IFN preserved immune function and facilitated faster recovery of NK activity (Garaci et al., 2000).

Combination chemo-immunotherapy was also evaluated in patients with advanced NSCLC. In a phase II open study, patients received cisplatin (100 mg/m^2 on day 1) and etoposide (120 mg/m^2 on days 1–3), combined with Tα1 (1 mg/day subcutaneously on days 8–11 and 15–18) and low-dose IFN (3 million IU intramuscularly on days 11 and 18) (Garaci et al., 1995, 2000). Treatment cycles were repeated every 3 weeks, and the tumor response was assessed after two cycles. Among 56 evaluable patients, 24 objective responses were observed, including two complete and 22 partial responses, with a median survival of 12.6 months (Garaci et al., 2000). Although chemotherapy reduced NK cell activity and lymphocyte subsets, this immunosuppressive effect was markedly attenuated in patients receiving Tα1 and IFN compared to those treated with chemotherapy alone. These results support the therapeutic potential of combination chemo-immunotherapy in advanced NSCLC, although confirmation in randomized trials is warranted (Garaci et al., 2000).

Additional evidence of benefit was reported when Tα1 and low-dose IFN-α were combined with ifosfamide in NSCLC patients (Garaci et al., 2000; Salvati et al., 1996). This regimen resulted in major responses in 33% and minor responses in another 33% of patients, compared to a response rate of only 10% in patients treated with ifosfamide alone. Moreover, toxicity particularly hematologic toxicity was significantly reduced in the combination therapy group. While the low dose of IFN-α may partially account for the favorable tolerability, the reduction in chemotherapy-associated toxicity was largely attributed to the immunomodulatory effects of Tα1 (Garaci et al., 2000). Considerable variability also exists in dosing schedules and administration protocols. Clinical studies reported daily loading doses ranging 1.6–3.2 mg administered subcutaneously, while other protocols utilized intermittent schedules such as 1.6 mg twice weekly (Dominari et al., 2020a).

6. Clinical evaluation of Tα1 combined with radiotherapy and PD-1 inhibitors

Radiotherapy combined with immunotherapy has demonstrated increasing therapeutic potential in metastatic malignancies; however, treatment efficacy can be compromised by radiation-induced lymphocytopenia. Previous studies suggested that Tα1 may alleviate lymphocyte depletion and support immune recovery. In a retrospective study, 48 patients with advanced or refractory cancers received a 7-day loading dose of Tα1 (1.6 or 3.2 mg daily), followed by hypofractionated radiotherapy and PD-1 inhibitor treatment. Peripheral blood lymphocyte subsets, including T cells, B cells, and NK cells, were analyzed by flow cytometry before and after Tα1 administration. Results demonstrated significant increases in total T cells, CD4$^+$ T cells, and CD8$^+$ T cells following Tα1 treatment. Among 36 evaluable patients, the objective response rate (ORR) was 19.4%, while the disease control rate (DCR) reached 69.4%. Median PFS and OS were 5.1 and 9.6 months, respectively. Furthermore, only 4.2% of patients experienced grade $\geq$3 treatment-related adverse events, indicating a favorable safety profile. Overall, these findings suggested that a short-term Tα1 loading regimen may improve the immune status and support the therapeutic efficacy of combined radiotherapy and PD-1 blockade, although larger randomized studies with longer follow-up are required for further validation (Xu et al., 2025).

7. Thymosin α1 in the immune checkpoint inhibitor (ICI) era

The lack of large contemporary randomized controlled trials (RCTs) evaluating Tα1 in combination with ICIs is mainly attributed to historical treatment priorities, geographical fragmentation of studies, and the rapid shift toward targeted immunotherapies in modern oncology. Initially, Tα1 was primarily developed for infectious diseases and later investigated alongside chemotherapy or radiotherapy rather than ICIs. Consequently, research funding and clinical focus shifted toward newer immunotherapeutic combinations, limiting the development of large multicenter trials involving Tα1. Most recent studies investigating Tα1 with PD-1/PD-L1 inhibitors were mainly conducted in East Asia using retrospective or real-world study designs. Despite this limitation, accumulating preclinical and early clinical evidence suggests that Tα1 may synergistically enhance ICI therapy by reducing lymphopenia, promoting CD4$^+$ and CD8$^+$ T-cell recovery, reversing immunosuppressive TMEs, and may help mitigate IRAEs. Emerging studies in ovarian cancer and NSCLC have reported improved objective response rates, PFS, OS, and reduced treatment-related toxicities when Tα1 was combined with immunotherapy-based regimens. However, larger phase III multicenter RCTs are still required before Tα1 can be widely integrated into standard ICI-based cancer therapies (Bo et al., 2018; Liu and Lu, 2023a; Schulof et al., 1985; Wang et al., 2025; Wei et al., 2023).

8. Factors contributing to variability in thymosin α1 therapeutic outcomes

The clinical efficacy of Tα1 shows substantial heterogeneity due to differences in tumor biology, treatment combinations, dosing regimens, and clinical endpoints. Tα1 demonstrated stronger synergistic effects in certain solid tumors, such as NSCLC and HCC, particularly through modulation of the TIME and reversal of M2-like macrophage polarization. In contrast, some hematological malignancies, including multiple myelomas, showed comparatively limited therapeutic benefit. Since Tα1 primarily functions as an immunomodulatory adjunct rather than a direct cytotoxic agent, its efficacy is highly dependent on it being combined with therapies such as chemotherapy, radiotherapy, cytokines (e.g., IL-2 and IFN-α), or ICIs. Subcutaneous injections remain the predominant route of administration, although newer targeted delivery approaches are being explored to improve tumor localization and therapeutic distribution. Furthermore, study outcomes significantly differ depending on the selected clinical endpoints, which include objective tumor responses, PFS, OS, lymphocyte recovery, mitigation of treatment-induced lymphopenia, reduction of radiation pneumonitis, and improvements in cancer-related fatigue and quality of life (Bepler, 1994; Costantini et al., 2019; Garaci et al., 2003, 2024; Liu et al., 2022, 2024; Liu and Lu, 2023a; Peng et al., 2020a; Solmonese et al., 2025; Xu et al., 2025; Wei et al., 2023).

9. Mechanistic plausibility versus proven clinical benefits of thymosin α-1

Tα1 demonstrates a strong distinction between mechanistic plausibility and proven clinical benefits, highlighting the difference between biological activity observed in experimental systems and consistent

therapeutic efficacy demonstrated in human clinical trials. Preclinical studies showed that Tα1 functions as a potent immunomodulator by promoting $CD4^+$ and $CD8^+$ T-cell maturation, enhancing NK cell and cytotoxic T lymphocyte activities, modulating cytokine production through increased IL-2 and IFN-γ expressions, and reversing immune exhaustion and lymphopenia induced by disease or therapy. These mechanisms provide strong theoretical support for its application in immune-related disorders and cancer immunotherapy. Emerging studies in immuno-oncology, particularly in NSCLC and ovarian cancer, suggest that Tα1 may enhance the efficacy of chemotherapy and ICIs by improving objective response rates and PFS. This discrepancy reflects the complexity of the human immune system, differences in pharmacokinetics and dosing, and the context-dependent nature of Tα1 activity, where its effects may substantially differ between healthy, immunosuppressed, and hyperinflammatory states. Consequently, while several applications of Tα1 are clinically validated, many broader or off-label uses remain primarily supported by mechanistic and theoretical evidence rather than large-scale randomized clinical trials (Bersanelli et al., 2021; Garaci et al., 2024; Mao, 2023; Tian et al., 2025; Li et al., 2010a; Pica et al., 2016a).

10. Limitations, clinical considerations, and risk-benefit balance of thymosin α-1-based combination therapy

Recent clinical evidence highlights the complexity of combining additional therapeutic agents with established treatment regimens, where improvements in intermediate clinical outcomes do not always translate into long-term survival benefits. For example, a real-world comparative study and network meta-analysis evaluating the addition of bevacizumab to first-line chemo-immunotherapy demonstrated a significant improvement in PFS, particularly in selected patient subgroups, including PD-L1-negative patients, older individuals, and those with brain metastases or multiple metastatic sites. However, despite these favorable disease-control outcomes, no significant improvement in OS was observed. Moreover, the intensified treatment strategy was associated with substantially increased rates of grade 3–5 adverse events, serious toxicities, and treatment discontinuation. These findings underscore the importance of carefully balancing therapeutic efficacy with treatment-related risks and emphasize that improvements in immunologic or short-term clinical responses might not consistently yield durable survival advantages. Such observations further support the need for adequately powered prospective studies and more-precise patient stratification to identify populations that may truly benefit from combination therapeutic strategies while minimizing unnecessary toxicity (Chen et al., 2026).

Recent evidence suggests that the clinical benefits of Tα1-based combination therapies should be interpreted with caution, particularly in the rapidly evolving landscape of modern cancer immunotherapy. Although earlier chemo-immunotherapy studies demonstrated encouraging improvements in immune restoration and clinical outcomes, many of those investigations were conducted prior to the widespread integration of contemporary PD-1/PD-L1 immune checkpoint blockade into standard oncology practice. Therefore, the direct translational relevance of older studies to current therapeutic settings remains limited. Furthermore, several available studies evaluating Tα1 combination therapies were retrospective in nature or involved relatively small patient cohorts, which may have introduced selection bias, treatment heterogeneity, and other study design limitations that could have influenced interpretations of therapeutic benefits. Importantly, while Tα1 consistently exhibits immunomodulatory activity through enhancement of T-cell maturation and differentiation, promotion of DC antigen presentation, and restoration of immune surveillance, these immunologic improvements do not always translate into consistent or durable survival benefits across all malignancies. Emerging evidence also indicates that the efficacy of Tα1 may be tumor-type-specific rather than universally applicable as a pan-cancer therapeutic strategy. Although emerging clinical observations suggest potential benefits of combining Tα1 with PD-1/PD-L1 blockade, the available evidence remains limited, and adequately powered prospective randomized trials are still required to establish its clinical efficacy. Additionally, despite increasing interest in Tα1-based immunomodulatory strategies, there remains a lack of adequately powered prospective clinical trials evaluating Tα1 in combination with current standard-of-care PD-1/PD-L1 regimens across different cancer types. Therefore, although Tα1-based combination therapy represents a promising adjunctive immunotherapeutic approach, further large-scale, randomized, and mechanistically guided clinical studies are still required to clarify its true clinical utility, optimal therapeutic settings, and long-term survival impacts in modern oncology practice (Akateh et al., 2019; Altekruse et al., 2012; Zhu et al., 2020).

11. Conclusions and future perspectives

Tα1 has emerged as a multifunctional immunomodulatory peptide with significant therapeutic potential in cancer treatment. Accumulating evidence from preclinical and clinical studies has demonstrated that Tα1 plays a central role in regulating both adaptive and innate immune responses, including enhancement of T-cell and natural killer cell activity, modulation of dendritic cell function, reprogramming of tumor-associated macrophages, and modulation of myeloid-derived suppressor cells. Through these coordinated mechanisms, Tα1 may help remodel the tumor immune microenvironment and enhance anti-tumor immune surveillance.

Importantly, Tα1 has shown encouraging benefits as an adjuvant in combination with conventional chemotherapy and modern immunotherapy, improving therapeutic efficacy while potentially mitigating treatment-induced immunosuppression and immune-related adverse events. Clinical evidence across multiple malignancies, including non-small cell lung cancer and metastatic melanomas, highlights its capacity to enhance response rates, preserve immune function, and improve patient outcomes. Moreover, its favorable safety profile and regulatory approval for specific indications underscore its translational relevance.

In addition, Tα1 was reported to reduce chemotherapy-induced neurotoxicity and myelosuppression. Beyond oncology, Tα1 also improves outcomes in sepsis and chronic hepatitis B infection by enhancing immune recovery and reducing inflammatory complications. Overall, Tα1 is considered a well-tolerated adjunctive therapy with considerable potential in both cancer and infectious disease management (Peng et al., 2020a; Wang et al., 2025; Wen et al., 2025; Dominari et al., 2020a).

In conclusion, Tα1 represents a promising immunotherapeutic agent that bridges innate and adaptive immunity and complements existing cancer treatment strategies. Continued mechanistic investigations, biomarker-guided patient selection, and well-designed randomized clinical trials are warranted to fully define its role in precision oncology and establish Tα1 as an integral component of next-generation cancer immunotherapy.

Despite substantial progress in elucidating the immunomodulatory and antitumor functions of Tα1, several important challenges and opportunities remain for its broader clinical translation in cancer therapy. Future research should focus on defining optimal dosing regimens, treatment timing, and patient stratification strategies to maximize the therapeutic benefits of Tα1, particularly in combination with chemotherapy, immune checkpoint inhibitors, and emerging immunotherapies. Given its capacity to remodel the tumor immune microenvironment by enhancing tumor-infiltrating lymphocytes, reprogramming tumor-associated macrophages, and reducing myeloid-derived suppressor cell-mediated immunosuppression, Tα1 holds promise as a key agent for converting immunologically “cold” tumors into “hot” tumors responsive to immunotherapy. In addition, its potential to mitigate immune-related adverse events associated with immune checkpoint blockade suggests a potential dual role in enhancing therapeutic efficacy and modulating treatment-associated toxicity.

Future well-designed randomized clinical trials, coupled with biomarker-driven approaches and mechanistic studies, will be essential to validate these benefits, clarify its role in innate-adaptive immune crosstalk, and establish Tα1 as a versatile component of next-generation cancer immunotherapy regimens.

CRediT authorship contribution statement

Sih-Chi Chuang: Writing – review & editing, Writing – original draft, Conceptualization. **Pei-Chia Hung:** Writing – original draft, Conceptualization. **Lekshmi Rethi:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Yung-Wei Lin:** Writing – review & editing, Writing – original draft, Methodology. **Hoa Duc Chau:** Writing – review & editing, Writing – original draft, Data curation. **Van Khiet Pham:** Writing – review & editing, Writing – original draft, Methodology. **Anh Thuy Vo:** Writing – review & editing, Writing – original draft, Methodology. **Hieu Trung Nguyen:** Writing – original draft. **Kai-Yi Tzou:** Writing – original draft, Visualization. **Andrew E.-Y. Chuang:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Conceptualization.

Consent for publication

The manuscript was approved for publication by all the authors who participated in the study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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