Am J Cancer Res 2026;16(8):3710-3742
www.ajcr.us /ISSN:2156-6976/ajcr0175622

*Review Article*

# Mechanism and intervention strategy of thymus atrophy in cancer immunotherapy

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Received July 25, 2026; Accepted August 25, 2026; Epub August 25, 2026; Published August 30, 2026

**Abstract:** Thymic atrophy is a major determinant of T-cell dysfunction and immunosenescence, as it restricts de novo T-cell generation, reduces the T-cell receptor (TCR) repertoire, hastens peripheral T-cell aging, and disrupts central immune tolerance. It presents an enormous barrier to antitumor immune surveillance as well as current cancer immunotherapies such as immune checkpoint inhibitors (ICIs) and chimeric antigen receptor T-cell (CAR-T) therapy. In this review, we discuss how age, metabolic disorders, psychological stress, cancer pathology, and cancer-directed therapies converge upon thymic epithelial cells, stromal organization, and hematopoietic progenitor trafficking leading to structural and functional thymic decline. We then describe how thymopoiesis impairment reshapes tumor immunity and therapeutic responsiveness, and summarize noninvasive imaging methods for assessment of thymic health, and highlight new strategies for thymic regeneration or functional replacement through pharmacologic agents, cytokine and endocrine modulation, mRNA-based trophic factor delivery, cell replacement, tissue engineering, and thymus-centered CAR-T platforms. Despite significant translational challenges, rapid advances in thymic regeneration biology suggest that restoration of thymic function may be a rational host-directed approach to enhance the depth, duration, and safety of cancer immunotherapy.

**Keywords:** Thymus atrophy, cancer immunotherapy, thymic epithelial cells, T cell development

## Introduction

Cancer immunotherapy has transformed clinical oncology over the last decade. Although ICIs and CAR-T cell therapy have achieved remarkable responses in diseases that were previously difficult to treat, many patients remain unresponsive. The major driver of such heterogeneity comes from the functional state of the host immune system, especially its fitness, diversity, and responsiveness within the T-cell compartment [1-6]. T cells are critical players in antitumor immunity. Continuous generation of naive T cells maintains a diverse and flexible peripheral T-cell pool, while the breadth of the TCR repertoire determines whether the immune system recognizes tumor neoantigens and mounts an effective cytotoxic response [1, 7]. Because the thymus is the primary site of de novo T-cell development and TCR generation, thymic involution represents an important upstream contributor to age-associated immune remodeling, although peripheral immune maintenance and dysfunction are shaped by antigen exposure, chronic inflammation, infection, and tissue-specific immune environments [6].

The thymus is the primary lymphoid organ in which T cells differentiate, their TCR genes undergo recombination, and positive and negative selection occurs. Functional T cells are generated whereas autoreactive clones recognizing self-tissues at a high affinity are eliminated or redirected toward regulatory lineages within the thymus. This organ controls not only the diversity of the TCR repertoire but also the integrity of central immune tolerance [4, 7, 8]. Maintaining its function is essential for an effective immune monitoring of cancers.

Increasing evidence suggests that thymic status correlates with ICI efficacy and immune related adverse events (irAEs) [9, 10]. Both peripheral TCR diversity and recent thymic emi-

https://doi.org/10.62347/FDZV7570

grants (RTEs) correlate with objective response rate and progression free survival upon checkpoint blockade indicating that thymic health could be used as a host side biomarker of immunotherapy responsiveness [3, 11-13]. Thymic history of the donor T cell pool also plays an essential role during the production of CAR-T cells. T cells obtained from people who experienced thymic involution are typically highly differentiated with short telomeres and upregulation of exhaustion markers, compromising their ex-vivo expansion and in vivo persistence [2, 14]. On the other hand, cancer itself can alter the thymic microenvironment via systemic inflammation, cachexia, and treatment-related injury resulting in a malignant cycle where tumor burden damages the thymus, thymic failure weakens immunity, and immune collapse allows tumor progression [9, 10, 15].

For these reasons, thymic atrophy should be considered not merely a background feature of aging but a potentially modifiable host factor that may influence cancer immunity and immunotherapy outcomes. This review summarizes recent progress in understanding the mechanisms of thymic involution, the immunological consequences of thymic failure, Computed tomography (CT)-based and multimodal strategies for noninvasive thymic assessment, and emerging interventions including pharmacologic thymic regeneration, engineered thymic organoids, and intrathymic CAR-T engineering. The thymic reconstruction may become a synergistic target for improving the efficacy and safety of current and next-generation cancer immunotherapies.

## Structural and functional foundations of the thymus

The thymus is a highly specialized central lymphoid organ whose anatomical compartments and stromal cell networks create the microenvironment required for major histocompatibility complex (MHC) restriction, autoreactive T cell clearance and regulatory T cell (Treg) differentiation. Beyond intrathymic T cell developmental regulation, the thymus secretes a panel of endocrine peptides, and exert local control over thymopoiesis and enter systemic circulation to modulate anti-tumor immunity.

### *Thymic architecture and niches*

The thymus is located in the anterior mediastinum and is surrounded by a connective tissue capsule. It is divided into two major functional regions, the cortex and the medulla [16]. The cortex is enriched with immature double-negative ($CD4^-CD8^-$, DN) and double-positive ($CD4^+CD8^+$, DP) thymocytes, whereas the medulla contains more mature single-positive ($CD4^+$ or $CD8^+$, SP) thymocytes together with dendritic cells (DCs), macrophages, and specialized stromal cells. The blood-thymus barrier and perivascular spaces maintain a relatively insulated milieu for T-cell development [16].

Thymic epithelial cells (TECs) provide the main support structure for the thymic microenvironment. Cortical TECs (cTECs) and medullary TECs (mTECs) produce various chemokines including CCL19, CCL21, and CXCL12 and express Notch ligands such as DLL4, thereby regulating progenitor homing, expansion, and T-lineage specification [16]. This spatial organization allows TCR rearrangement, positive selection, and negative selection to occur in a tightly ordered sequence. Its disruption directly reduces the diversity of the peripheral T-cell pool and weakens the capacity of the immune system to detect and eliminate tumor cells [17].

### *Cellular composition and core immune functions*

Thymic stromal cells (TSCs) constitute a heterogeneous network that includes TECs, fibroblasts, endothelial cells, innate lymphoid cells, and antigen-presenting cells originating from the bone marrow. cTECs facilitate positive selection through the presentation of self-peptide-MHC complex to enable survival of those thymocytes with MHC restriction [16]. mTECs are the principal executors of central tolerance. Through high expression of the autoimmune regulator (AIRE), they drive ectopic expression of a broad panel of tissue-restricted antigens (TRAs), allowing the elimination of potentially autoreactive T cells to at the source [7, 8, 18].

Under the control of Notch signaling, bone marrow-derived hematopoietic progenitors progress through DN, DP, and SP stages [16]. Positive selection in the cortex is responsible for the establishment of MHC restriction, while

negative selection in the medulla destroys clones that exhibit a high affinity for self-antigens [7, 8]. Hassall's corpuscles are structures of medulla composed of terminally differentiated mTECs and represent a very specialized medullary environment involved in the induction of Tregs. In humans, Hassall's corpuscles produce thymic stromal lymphopoietin (TSLP), which allows dendritic cells in the close vicinity to present self-antigens to $CD4^+$ SP thymocytes that have already been positively selected, but remain self-reactive. Instead of clonal deletion, this interaction may lead to the expression of Foxp3 and drive the development of $CD4^+CD25^+Foxp3^+$ Treg lineage [19].

*Endocrine functions and immunoregulatory factors*

The thymus is classically considered an endocrine organ as well: TECs release thymosin alpha 1 (Tα1), thymosin beta 4 (Tβ4), thymopoietin, and other peptides that control local thymocyte development and circulates to exert systemic effects on immune function [20, 21].

The relevance of thymic peptides to immune responses has recently attracted interest. Tα1 enhances immune responses and reduces tumor burden in BALB/c mouse models [22]. While in clinical settings such as chronic hepatitis B, chronic hepatitis C, and non-small cell lung cancer, it shows an immunoadjuvant effect, improving interferon responses and survival in some cases [20]. Tβ4 is a peptide secreted from TECs; its precursor is broadly expressed in the nucleus and plays roles in nuclear function [23]. Tβ4 controls cytoskeleton dynamics via binding with G-actin. It was found to be expressed in certain types of tumor cells and has been associated with malignant properties and metastasis potential [20]. It stimulates hypothalamic release of luteinizing hormone-releasing factor, indicating participation of thymic peptides in neuroendocrine-immune crosstalk, which could indirectly affect immune activity within the tumor microenvironment [21].

In addition to Tα1 and Tβ4, another class of synthetic active fragments based on thymopoietin, namely thymopentin (TP5), has been identified, with potential for regulating T cell mediated immunity. Thympoietin is a 49-amino-acid thymic hormone, while TP5, consisting of residues 32-36 (Arg-Lys-Asp-Val-Tyr), is its active core. In transplantable tumor models, it delays tumor induced immunosuppression and partially normalizes immune function [21, 24].

## Etiology and pathological features of thymic atrophy

*Age-related thymic atrophy*

One of the most obvious manifestations of immunosenescence is age-related thymic involution. It manifests morphologically as reduced thymus size, blurring of the corticomedullary border, loss of epithelial space, and gradual adipose infiltration [25-29]. This decline is far from a simple linear attrition process; thymic output peaks shortly after birth before decreasing exponentially throughout life, eventually reaching a point at which the generation of new naive T cells becomes very limited [30].

Hormonal signaling plays an important role in driving involution. The surge of sex steroids at puberty impacts thymic structure and physiology; TECs express androgen receptors (ARs) and estrogen receptors (ERs) and are thus directly targeted by sex hormones [31]. Sex steroids strongly inhibit TEC proliferation in culture models such as the rat TEC line IT-45R1, possibly through protein kinase C [32]. Transcriptomics has revealed how sex hormones globally reprogram TEC gene expression, affecting their differentiation state and stromal functions [33].

An important consequence of sex steroid action is reduction of interleukin-7 (IL-7) production that occurs from TECs and is required for survival, proliferation, and TCR rearrangement of early DN thymocytes [34, 35]. During β-selection, IL-7 controls cell growth and differentiation, including the suppression of Bcl-6 [34]. While loss of IL-7 upon sex steroid suppression removes an essential survival signal. Anti-apoptotic pathways such as Bcl-2 weaken, or the restraint on proapoptotic molecules such as Bim is released, leading to increased apoptosis at this vulnerable time point [36]. Aging of the hematopoietic compartment, with decreased early T-cell progenitors and attenuated Notch signaling, contributes to this process.

Androgens may even directly induce thymocyte apoptosis. Testosterone in castrated male mice reduces thymic size within just 2-4 hours and

strongly implicates apoptotic mechanisms [37]. In thymic organ culture, dihydrotestosterone (DHT) increases thymocyte apoptosis, indicating a direct or microenvironment-mediated pro-apoptotic effect [37]. In parallel, other endocrine factors, growth hormone (GH) and the TEC-derived insulin-like growth factor 1 (IGF-1) axis decline with age, further impairing epithelial renewal and thymopoiesis [38-41].

However, the thymic stromal microenvironment itself is generally considered the dominant factor [42, 43]. Intrinsic reprogramming of TECs is another central mechanism of involution. The master transcription factor FOXN1 progressively decreases with age. Its reduction accelerates thymic degeneration, whereas TEC-specific overexpression of FOXN1 can delay involution [44-47]. At the epigenetic level, reduced miR-181a-5p and increased miR-125a-5p can promote involution by derepressing TGF-β signaling and suppressing FOXN1, respectively [48-50]. Wnt signaling, which TECs produce and respond to in an autocrine/paracrine manner, also declines with age [51]. Wnt4 positively regulates FOXN1 expression through the canonical β-catenin pathway within TECs [52], its loss promotes epithelial-to-mesenchymal transition (EMT), fibroblast accumulation, and adipogenic conversion. Deletion of β-catenin specifically in FoxN1$^+$ TECs results in severe thymic hypocellularity, delayed TEC maturation, and reduced expression of molecules critical for TEC function such as RANK and EphB2/3 [53].

Chronic inflammation acts as an additional contributing driver. Aged thymic tissue shows increased IL-1, IL-6, TNF-α, and related inflammatory mediators, which damage TECs through NF-kappaB and STAT3 signaling [54]. The NLRP3 inflammasome activation induces IL-1β maturation and enhances inflammatory cascades. NLRP3-deficient mice show a delayed degeneration of the thymus [55]. Another factor is oxidative stress, TECs are very sensitive to reactive oxygen species, lack of stromal catalase accelerates DNA damage [56, 57].

Recently, it has been proposed that thymic involution may represent an evolutionary optimization of energy allocation rather than deteriorating [30]. High levels of thymic output at young ages create a diverse naive T cell pool while accumulating memory T cells with aging decreases the requirement for continuous production of new clones. Thymopoiesis is energetically demanding, and mathematical models based on Pontryagin's maximum principle suggest that the optimal strategy may be a high early-life peak followed by exponential or power-law decline, a pattern consistent with human TREC data [30].

*Obesity and metabolic-disease-associated thymic atrophy*

Lifestyle and metabolic factors measurably impact thymic health. In a deep-learning CT analysis of 27,612 asymptomatic adults, thymic health was associated with smoking, obesity, and physical activity [58]. People with better thymic function had about 50% less chance of dying from all causes during 12 years; mortality from lung cancer was about 50% lower, and cardiovascular disease mortality was reduced by roughly 60-90% [58]. Smoking intensity correlated negatively with thymic health. In terms of metabolism, high-density lipoprotein (HDL) was positively associated with thymic health, whereas triglycerides, fasting glucose, and blood pressure showed negative associations [58]. People with high concentrations of inflammatory cytokines in the plasma, including IL-6, IL-18, and oncostatin M, along with high C-reactive protein (CRP) were found to have poor thymus function, suggesting that chronic inflammation may be a potential cause of accelerated involution [58].

Obesity leads to thymic atrophy through complex mechanisms that cause abnormalities in T-cell development and immune surveillance [59, 60]. In diet-induced obese mice, thymic architecture deteriorates with reduced cortical and medullary cellularity, obliteration of cortico-medullary junctions, and increased perithymic adiposity [60]. Thymocyte counts decline significantly, accompanied by elevated apoptosis of CD4$^+$ and CD8$^+$ SP and DP populations. This damage results in functional deficiencies shown in decreased levels of signal-joint T-cell receptor excision circles (sjTRECs) in peripheral lymphocytes, suggesting the reduced number of recent thymic emigrants [60]. Mechanistically, obesity-induced chronic inflammation, characterized by higher IL-6 and TNF-α concentrations, leads to the apoptosis of thymocytes and affects the cytokine balance necessary for

proper thymopoiesis [59]. Adipokine dysfunction is another contributing factor, since hyperleptinemia in obesity is usually associated with leptin resistance and glucocorticoid effects, which counteract the positive role of leptin in maintaining thymic cellularity [59]. Moreover, increased aromatase activity in adipose tissue raises estrogen levels, and estrogen signaling through estrogen receptor α suppresses cortical thymocyte survival and differentiation. Although the insulin/IGF-1 axis may support thymic function, its potential benefits are overshadowed by concurrent metabolic disturbances, including insulin resistance and reduced growth hormone signaling [59]. In middle-aged people, there is an inverse correlation between TREC levels and body mass index, and obese individuals with or without type 2 diabetes exhibit significantly reduced thymic output compared with lean subjects [60].

In summary, these results provide evidence that obesity causes premature thymic aging by targeting multiple levels of the T cell generation pathway, including bone marrow precursors, intrathymic selection, and peripheral repertoire maintenance, thereby increasing susceptibility to infections and cancers [59, 60].

*Psychological stress-induced thymic atrophy*

Acute or chronic thymic atrophy induced by psychological stress occurs via the neuroendocrine-immune axis. Central mechanisms include activation of the hypothalamic-pituitary-adrenal axis with a persistent increase in glucocorticoids [61-63]. Glucocorticoids promote apoptosis of thymocytes, especially DP cells, and inhibit their proliferation, leading to decreased thymic weight and cellularity [62, 63]. In addition to their effects on thymocytes, glucocorticoids may secondarily injury the thymic stromal microenvironment [64].

This causal chain has been reproduced in multiple animal models. Physical stressors such as restraint, water immersion, and forced swimming, as well as psychological stressors such as anticipatory stress, can all induce significant thymic involution [61, 62, 65]. This effect may be prevented through adrenalectomy removing endogenous glucocorticoid production, glucocorticoid receptor antagonism using RU486, or inhibition of glucocorticoid synthesis via aminoglutethimide [65, 66]. These interventions confirm glucocorticoids as a core mediator of stress-induced thymocyte depletion.

The degree of thymic injury is dependent upon the type and severity of the stressor. Quantitative immunohistochemistry comparing physical water-immersion stress with psychological stress found that physical stress caused more severe thymic atrophy, including marked cortical thymocyte loss, blurred corticomedullary boundaries, and increased macrophage uptake of apoptotic bodies [62]. In addition, chronic stress increases local thymic inflammatory mediators like IL-6 and interacts with other immune cytokines like Interferon-gamma (IFN-γ) to compound microenvironmental damage [61, 67]. The resulting immunosuppression due to this stress may contribute to higher rates of infections, decreased vaccination response, and possibly an increased risk for tumors among those who are chronically stressed [67].

*Cancer-associated thymic atrophy*

Cancer-associated thymic atrophy is heterogeneous and appears to arise through mechanisms that depend on tumor type, disease stage, and host context. Some tumors predominantly disrupt early thymocyte differentiation or thymic epithelial cell homeostasis, whereas cachexia-associated models may remodel medullary stromal compartments and central tolerance. Tumor-derived inflammatory signals, infiltrating myeloid or dendritic-cell populations, nutritional and metabolic disturbances, and anticancer therapies may contribute to different degrees depending on the tumor model.

Cancer cachexia is likely one of the main drivers of thymic pathology [10, 68] (**Figure 1A**). The magnitude, temporal course, and underlying cellular mechanisms vary between tumor models. Mechanistic characterization is currently most detailed in hepatocellular carcinoma (HCC)-associated cachexia. Mouse models of HCC cachexia show marked atrophy of the thymus characterized by reduction in size, weight and total cellularity along with histology showing medullary degeneration [10]. Single-cell sequencing shows that stage II and III mature mTEC subsets are reduced in cachectic mice, accompanied by reduced AIRE and TRA expression. These changes directly weaken mTEC-mediated negative selection and dis-

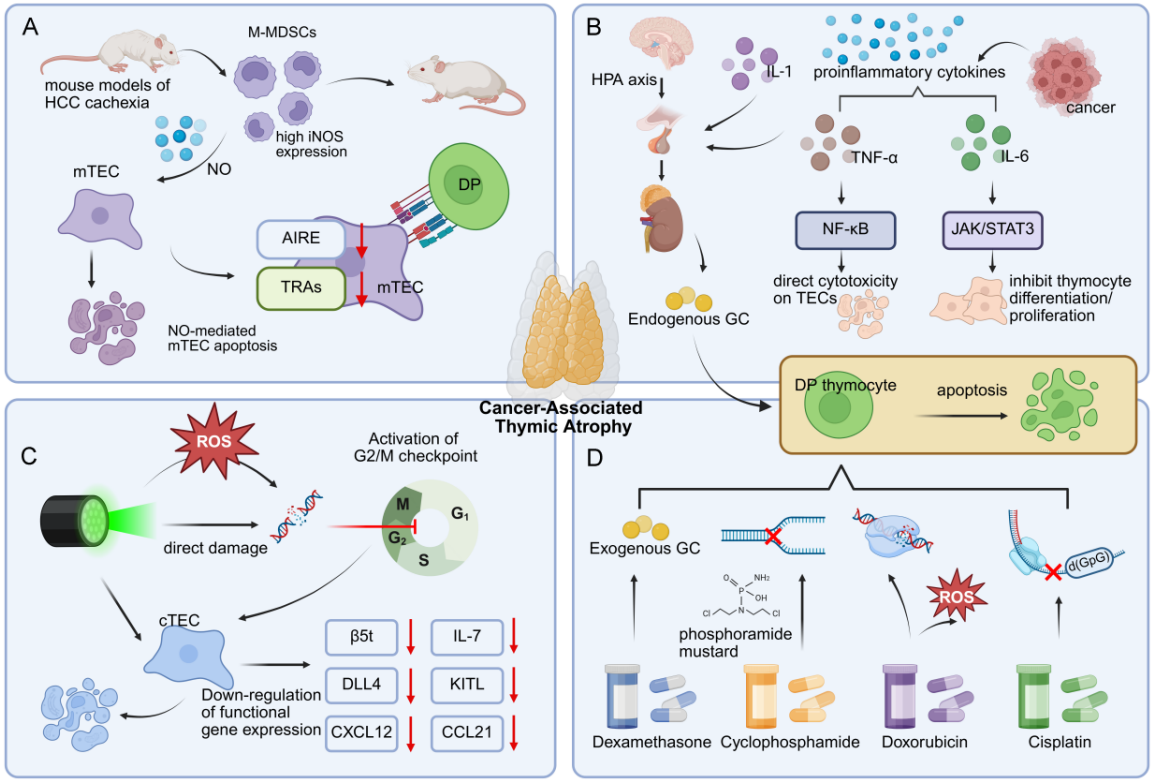


**Figure 1.** Cancer-associated thymic atrophy. A. Cancer cachexia. M-MDSCs are markedly enriched in the thymus of mice with HCC-associated cachexia. Through high iNOS expression, these M-MDSCs produce large amounts of NO and induce mTEC apoptosis. Reduced expression of AIRE and tissue-restricted antigens (TRAs) directly impairs mTEC-mediated negative selection and disrupts central immune tolerance. Injection of cachexia-derived M-MDSCs into tumor-free mice is sufficient to reproduce thymic atrophy and pathological multi-organ T-cell infiltration. B. Tumor-induced systemic inflammation. During the progression of cancer cachexia, multiple proinflammatory cytokines increase markedly. IL-6 suppresses thymocyte proliferation and differentiation through pathways such as JAK/STAT3. TNF-α activates NF-κB signaling, exerts direct toxicity on TECs, and cooperates with IL-1 to activate the hypothalamic-pituitary-adrenal axis, promote glucocorticoid release, and aggravate apoptosis of intrathymic DP thymocytes. C. Ionizing radiation (IR) acts directly on DNA to induce DNA double-strand breaks (DSBs) and also generates reactive oxygen species (ROS), which further damage DNA. DSBs activate the DNA damage response (DDR) and the G2/M checkpoint in TECs, arresting mitosis in G2 phase. IR also suppresses cTEC functional genes, including β5t, Il-7, DLL4, KitL, Cxcl12, Ccl21, and Ccl25. D. Cyclophosphamide is metabolized to phosphoramide mustard, which impedes DNA unwinding and replication-fork progression and induces DSBs. Doxorubicin stabilizes Topo II-DNA cleavage complexes, prevents religation of DNA ends, and sustains DNA breaks; its anthraquinone structure also undergoes redox cycling to generate ROS that oxidize DNA and damage lipids and proteins. Cisplatin forms d(GpG) adducts that distort the DNA double helix and block DNA and RNA polymerases, arresting replication and transcription. Dexamethasone, used as supportive therapy, acts as an exogenous glucocorticoid and induces a proapoptotic BCL-2-family program that activates mitochondrial apoptosis. DP cells are highly sensitive to all of these processes and are therefore severely depleted.

rupt central tolerance [10]. Apart from mTECs, single-cell profiling also reveals remodeling of the medullary fibroblast compartment, there is a reduction in mature medullary fibroblasts, accompanied by an accumulation of immature cell types. Such immature fibroblasts have poor capability of antigen processing and presentation along with low CCL19 production, affecting the CCR7-CCL19 axis, which is necessary for negative selection. In addition to that, TRA-related genes expression is diminished in these fibroblasts, further compromising central tolerance [9]. Cachexia-derived $CD45^+$ erythroid progenitor cells can induce $CD34^+$ progenitor death and impair maturation of thymic medullary fibroblasts, exacerbating the atrophic phenotype [9].

In the cachectic HCC model described above, monocytic myeloid-derived suppressor cells

(M-MDSCs) accumulate in the thymus. These cells have a high expression of inducible nitric oxide synthase (iNOS), produce nitric oxide, and induce mTEC apoptosis [10]. Adoptive transfer experiments show that cachexia-derived M-MDSCs can recapitulate thymic atrophy in tumor-free mice. In this model, these experiments define a cascade in which M-MDSC infiltration leads to NO-mediated mTEC apoptosis leading to decreased AIRE and TRA expression, defective negative selection and escape of autoreactive T cells. Preliminary clinical observations suggest its relevance for cancer patients [10].

Cachexia-associated thymic involution has also been observed in the Colon-26 (C26) adenocarcinoma model [69]. In the C26 cachexia model, thymic weight was markedly reduced to approximately 0.35-fold of the normal control value by day 21 after tumor inoculation [69]. An earlier study comparing cachexia-inducing and non-cachexia-inducing C26 subclones showed pronounced thymic contraction in mice bearing the cachexigenic clone 20, but not in those bearing the non-cachexigenic clone 5 [70]. The C26 model additionally provides temporal evidence that systemic immune alterations precede overt thymic mass loss. Circulating IL-6 and MCP-1 were elevated as early as day 3 after C26 inoculation, and CTLA-4 expression on $CD4^+$ T cells increased by day 7, whereas significant reductions in splenic $CD3^+$, $CD4^+$, and $CD8^+$ T-cell populations emerged from day 14 and significant thymic weight loss was detected at day 21 [69].

In contrast to the mechanistically detailed HCC cachexia model, the cellular and molecular basis of thymic involution in C26-bearing mice remains less well defined. TEC subsets, AIRE/TRA expression, thymic-output markers such as T-cell receptor excision circles (TRECs), and TCR repertoire alterations were not assessed in the C26 study [69]. Thus, although HCC and C26 cachexia models share thymic involution as a phenotypic endpoint, they cannot currently be assumed to share the same proximal mechanism, particularly the M-MDSC-iNOS/NO-mTEC pathway demonstrated in HCC.

Tumor-induced systemic inflammation provides another mechanism of thymic injury [68] (**Figure 1B**). As cachexia progresses, levels of IL-6, TNF-α, and other proinflammatory cytokines rise. IL-6 activates Janus Kinase-Signal Transducer and Activator of Transcription 3 (JAK/STAT3) signaling and disrupts TEC homeostasis whereas TNF-α activates NF-kappa B, which is directly toxic to TECs, and cooperates with IL-1 to stimulate glucocorticoid release via the hypothalamic-pituitary-adrenal axis, leading to increased apoptosis of DP thymocytes and additional local tissue injury [68]. Tumor-induced peripheral activated T cells and M-MDSCs might also home abnormally into the thymus and exacerbate microenvironment disruption [10, 68].

Tumor-induced thymic atrophy is reflected by a reduction in organ weight and thymocyte cellularity [71]. Mice with tumors had T cell maturation defects marked by an increase in DN2 cells and decrease in DN3 and DN4 subsets together with downregulation of Notch1 and DLL4 signaling reflecting impaired lineage commitment. Concurrently, tumors induced CCR9-dependent accumulation of two different plasmacytoid dendritic cells (pDC) subsets [71]. Common dendritic cell progenitors (CDP)-derived pDCs captured and presented tumor antigens within the thymus resulting in clonal deletion of tumor-specific T cells whereas common lymphoid progenitors (CLP)-derived pDCs expanded and released type I interferon enhancing Qa2 expression on mature single-positive thymocytes suggesting aberrant maturation. Abrogation of CCR9 prevented pDC infiltration restoring T cell generation and alleviating these functional changes. Thus, tumors exploit thymic central tolerance pathways to suppress T cell output and modify the quality of newly exported T cells [71].

Cancer therapy itself is an important cause of thymic damage. Chemotherapeutic agents such as cyclophosphamide induce DNA damage and rapidly deplete apoptosis-prone DP thymocytes [68]. Radiation directly damages thymocytes and can cause long-lasting injury to stromal compartments, particularly mTECs, which are required for central tolerance [64, 68]. Such treatment-related damage contributes to prolonged T-cell immune deficiency, infection susceptibility, and relapse risk after hematopoietic cell transplantation [64].

Ionizing radiation (IR) used in radiotherapy acts directly on DNA to induce DNA double-strand breaks (DSBs) and also ionizes water mole-

cules to generate reactive oxygen species (ROS), which further damage DNA [72, 73]. DSBs trigger the DNA damage response (DDR) and activate the G2/M cell-cycle checkpoint in TECs, arresting mitosis in G2 phase (**Figure 1C**) [74, 75]. In addition to causing direct TEC injury, IR suppresses the expression of genes required for TEC function. Among all TEC subsets, cTECs are the most severely affected, with broad downregulation of nearly all highly expressed functional genes, including β5t, Il-7, DLL4, KitL, Cxcl12, Ccl21, and Ccl25 [72, 73].

The thymotoxic effects of chemotherapy are equally important (**Figure 1D**). Chemotherapeutic agents ultimately trigger DP thymocyte apoptosis through distinct signaling pathways. Cyclophosphamide and cisplatin elicit p53-dependent or p53-independent apoptotic cascades in response to DNA damage, whereas doxorubicin-induced oxidative stress activates the intrinsic mitochondrial pathway [76-79]. Dexamethasone, acting through glucocorticoid receptor GR/NR3C1, alters the balance of BCL-2 family members, thereby initiating mitochondrial outer membrane permeabilization [80]. DP cells exhibit inherent sensitivity to these signals due to their immature DNA repair capacity and high basal ROS levels, thereby lowering the apoptotic threshold [81, 82].

Although some of these immune-stimulating strategies can induce acute thymic involution, they remain important for enhancing antitumor immunity. High dose IL-2 and agonistic anti-CD40 treatment, which are intended to stimulate antitumor response, lead to a rapid increase in glucocorticoid production. In mice, strong systemic immunostimulation decreases thymocyte numbers by approximately 60% within 48 hours, which can be inhibited by adrenalectomy [83]. Elevated glucocorticoids and a decrease in T-cell receptor excision circles were found in patients with cancer who received high-dose IL-2, suggesting reduced thymic output [84]. These studies emphasize the need for balancing immune stimulation and thymus protection during cancer treatment.

### Imaging-based assessment of thymic health

Noninvasive assessment of thymic status is important for investigating the clinical relevance of thymic involution in cancer patients. Because direct evaluation of thymic tissue is generally impractical, imaging provides an accessible approach for characterizing residual thymic structure and fat replacement and for examining their associations with antitumor immune responses and treatment outcomes. CT is currently the most practical modality for adult thymic assessment, and quantitative and artificial intelligence-based approaches may improve the reproducibility of imaging-derived thymic phenotypes [17, 85].

The normal thymus is seen on CT as a triangle-shaped or bilobed anterior mediastinum that has a slightly larger left lobe and concave or straight margins [86]. With age, the gland undergoes progressive fatty replacement [87, 88]. In a CT analysis of 154 individuals without chest disease, the thymus was visible in 100% of individuals younger than 30 years, 73% of those aged 30-49 years, and only 17% of those older than 49 years [89]. Quantitative analysis of 194 trauma patients further showed that thymic density and volume decline with age, with no solid soft-tissue component visible in patients older than 54 years [87].

Using data from the Framingham Heart Study, Araki and colleagues developed a four-point thymic scoring system, ranging from complete fatty replacement (grade 0) to predominantly soft-tissue thymus (grade 3) [88]. The system showed excellent reproducibility, with intraobserver and interobserver weighted kappa values of 0.87 and 0.83 [88]. Thymic score was inversely associated with age, and women showed a 10- to 20-year delay in fatty replacement compared with men. Higher BMI and a history of smoking were also associated with more advanced fatty replacement [88].

Traditional visual scoring and manual measurements are limited by subjectivity. A self-supervised deep-learning system can automatically quantify thymic health on routine chest CT and output a continuous 0-100 score [85]. Higher thymic health scores have been shown to be associated with improved progression-free survival and overall survival in a pan-cancer cohort of 3,476 patients who had received ICI treatment, comparable to PD-L1 expression and tumor mutational burden [85]. Notably, for patients with PD-L1 expression less than 1%, high thymic health remained associated with a 44% reduction in progression risk, indicating that thymic assessment provides prognos-

tic information independent of tumor-centered biomarkers [85].

Percent thymic tissue (pTT) is another imaging metric designed to estimate residual functional thymic tissue [17]. It uses a Gaussian mixture model to decompose the CT attenuation histogram of the thymic region, estimating the probability that voxels represent thymic parenchyma rather than fat [17]. In two independent cohorts of locally advanced Non-Small Cell Lung Cancer (NSCLC), pTT was negatively associated with age and was lower in men than in women [17]. Among patients receiving chemoradiotherapy followed by durvalumab consolidation, higher pTT predicted distant-metastasis-free survival (aHR=0.51); and in stage IIIC patients, predicted durvalumab discontinuation due to treatment-related adverse events with an AUC of 0.80 [17]. These findings suggest that preserved thymic structure may reflect stronger host immune surveillance.

The clinical value of imaging depends on whether image-derived parameters reflect function. In the CT-based TRACERx prospective cohort of 464 resectable NSCLC patients, high thymic health scores were associated with higher peripheral sjTREC levels, greater TCR α- and β-chain diversity in blood and tumor tissue, and increased circulating T-cell abundance [85]. Plasma proteomics further showed enrichment of adaptive immune pathways among patients with higher thymic health [85]. These associations support the biological relevance of imaging-derived thymic health and suggest that structural preservation may correlate with residual thymopoietic activity.

Thymic assessment is particularly important in thymic epithelial tumors. These patients have impaired self-tolerance and are at increased risk of irAEs, especially myositis, myocarditis, and myasthenia gravis, during ICI treatment [90]. Baseline imaging should assess both tumor burden and the functional reserve of remaining normal thymic tissue in order to balance potential immunotherapy benefits with risks for toxicity [90].

While CT is fundamental, other modalities offer complementary data, including magnetic resonance imaging (MRI) and positron emission tomography (PET). The technique of chemical shift MRI can distinguish thymic parenchyma versus fat based on phase difference between water and fat protons [91]. When distinguishing thymic hyperplasia from thymic tumors, hyperplasia has an average chemical-shift ratio around 0.37 with significant signal loss on opposed-phase images while tumors have ratios ranging from 0.9 to 1.8 without signal loss [91]. Diffusion-weighted imaging and apparent diffusion coefficient mapping offer additional functionally relevant data, hyperplasia typically lacks diffusion restriction but malignant thymic tumors do so frequently [91]. Differentiation of thymic lesions by Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) is less accurate because normal young thymus, thymic hyperplasia, and thymoma all demonstrate increased FDG uptake [86]. However, new immuno-PET probes like radiolabeled antibodies that target CD8 may allow noninvasive monitoring of tumor infiltrating $CD8^+$ T cells [92].

These imaging-derived measures should currently be interpreted as structural or composite correlates of thymic status rather than as direct measurements of thymic immune function. Their associations with immunotherapy efficacy and toxicity suggest potential value as host-side biomarkers, but they are not yet validated for selecting or modifying anticancer treatment. Further prospective studies integrating imaging with functional measures of thymopoiesis will be required to define their clinical utility.

### Mechanisms by which thymic atrophy impairs antitumor immunity

Thymic atrophy is far from being merely an anatomical change; it triggers a cascade of immune dysfunctions that reshape antitumor immunity. The failure in naive T-cell output leads to reduced TCR diversity, limiting recognition of tumor neoantigens. Peripheral T cells compensate for this through homeostatic proliferation acquiring a senescent phenotype which can also remigrate back into the thymus leading to a feedback loop between peripheral and central immune aging. Autoreactive T cells that escape due to defective mTEC-mediated negative selection have both pathogenic and potential antitumor implications. Loss of thymic endocrine activity impairs systemic immune

regulation. These mechanisms represent the barriers on the host side caused by thymic involution.

*Failure of naive T-cell output and loss of TCR diversity*

The most immediately apparent immunological effect of thymic atrophy is a gradual loss of naive T cell output [93] (**Figure 2A**). Exponential declines in sjTRECs, as well as decreased numbers of $CD31^+$ naive $CD4^+$ T cells, indicate an impairment in generation of recent thymic emigrants [93]. TREC levels may decrease over 95% between age 25 and 60 [94]. Single-cell analysis of the aging human thymus reveals diminished proportions of DN and DP thymocytes with relatively increased SP cells and extrathymic innate immune cells suggesting reduced efficiency for intrathymic T-cell development [93].

This output failure impairs TCR repertoire diversity [95]. Mathematical models show that if thymic output drops below a critical threshold, there is a nonlinear collapse in repertoire diversity with loss of small immune clones. Although homeostatic proliferation maintains the overall count of naive T cells somewhat, it does not compensate for deleted clonotypes [95]. A sharp contraction in TCR diversity was observed by Naylor and colleagues around the seventh decade of life, from ~$2\times10^7$ to $2\times10^5$ clonotypes [94]. This erosion undermines the molecular foundation for recognition of new antigens such as tumor neoantigens [93].

Age-associated TCR loss is not only quantitative but also structural in nature [96]. High-throughput sequencing reveals that for both $CD4^+$ and $CD8^+$ naive T cell subsets, older people exhibit shorter TCR β-chain CDR3 loops, less NDN nucleotide insertions, and changes in physicochemical properties on the central part of CDR3 [96]. Measures like interaction strength or hydropathy decline, indicating preference for losing clones with strong interactions with self peptide-MHC during long term peripheral selection and immune responses [96]. The repertoire becomes more shared; the percentage of clonotypes shared among individuals increases, whereas private and highly diverse clonotypes disappear [96]. This leads to reduced capacity for the immune system to react against the complex antigenic landscape of tumors [96, 97].

*Peripheral T-cell senescence and remigration to the thymus*

Compensatory homeostatic proliferation occurs in the periphery in response to reduced thymic output. This alters the composition of the T-cell compartment with contraction of the naive pool and accumulation of memory-phenotype cells over time [98, 99]. Among these expanded populations, senescence-associated $CD4^+$ T cells (SA-T cells) represent a distinct subset characterized by impaired TCR-driven proliferation, increased expression of cell cycle inhibitors such as Cdkn1a and Cdkn2b, telomere shortening, and enhanced senescence-associated β-galactosidase activity [98, 99] (**Figure 2B**). Although having low proliferative capacity, SA-T cells produce various inflammatory mediators including osteopontin, IFN-γ, CCL3, CCL4, etc., after being stimulated by their TCRs, thus developing a senescence-associated secretory phenotype [99]. The senescent $CD8^+$ T cells might express NK receptors such as NKG2D or NKG2A and exhibit antigen-independent cytotoxicity. Meanwhile, the senescent $CD4^+$ T cells could gain cytotoxic properties expressing granzyme B and granzyme K. They also lose lymph node homing capability and migrate into nonlymphoid tissues promoting tissue damage [100].

Inflammation leads to T-cell senescence. The cytokine TNF-α induces direct $CD4^+$ T cell senescence and terminal differentiation via p38 Mitogen-Activated Protein Kinase (p38 MAPK) signaling. Subsequently, these senescent T cells secrete further inflammatory mediators in a feed-forward inflammatory loop that maintains inflammaging [101]. Chronic inflammation impairs cytotoxic T-lymphocyte function and promotes thymic involution, contributing to the pathogenesis of age-related cardiovascular disease, cancer, and type 2 diabetes [101, 102].

Peripheral T cell aging and thymic degeneration are reciprocally linked. Mature peripheral T cells can migrate back into the thymus [103]. Activated T cells cross via high endothelial venules in the corticomedullary junction, mainly reside in the medulla, and may remain as tissue-resident memory-like cells [103]. Rarely

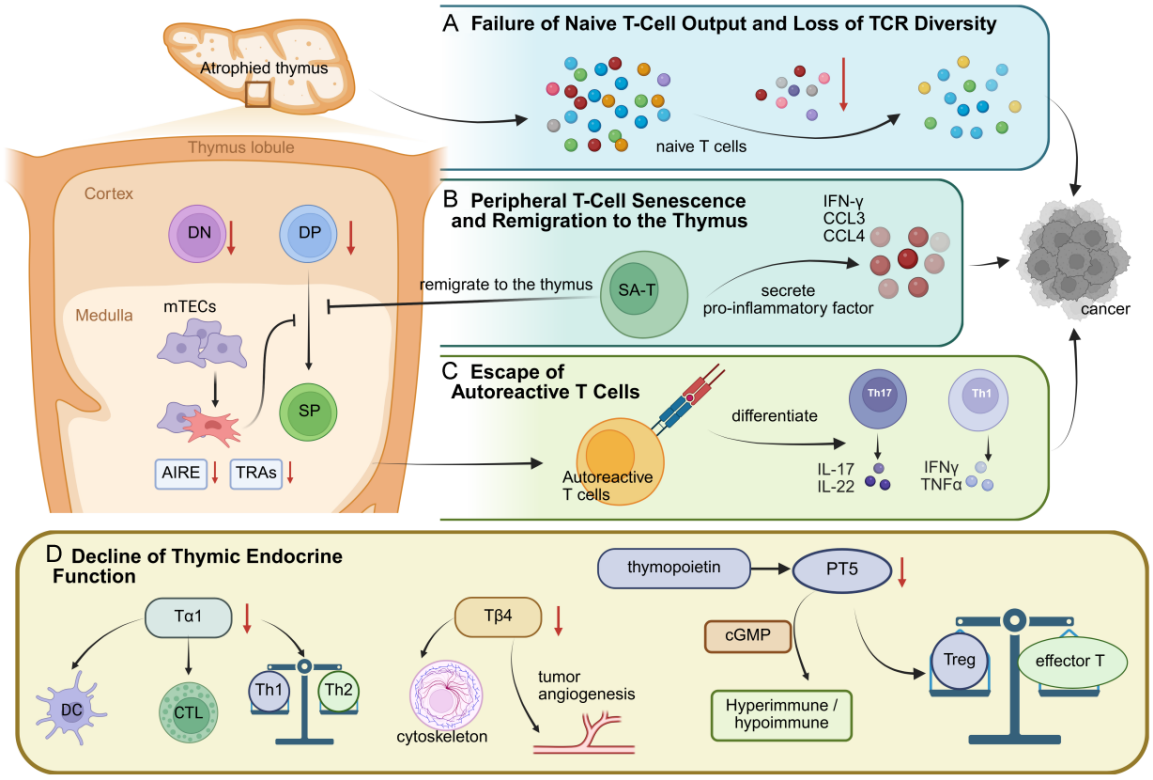


**Figure 2.** Mechanisms by which thymic atrophy impairs antitumor immunity. A. When the thymus shrinks, the most immediate fallout is a steady drop in the production of fresh, naive T cells. Without a constant stream of these new cells, the diversity of the T-cell receptor (TCR) library - or repertoire - takes a major hit. Instead of a vast, highly personalized library of cells tailored to the individual, the system becomes homogenized. We end up with a much more generic, shared set of receptors across different people, while losing the rare, highly specific ones. This loss of variety directly cripples the immune system's ability to spot and attack the highly diverse mutations found in tumors. Think of it as trying to crack a complex, evolving lock with only a handful of generic master keys. B. To compensate for this drop-off, the body coaxes existing peripheral T cells to divide and multiply. But this copy-of-a-copy approach (homeostatic proliferation) comes with a cost. Over time, it rewires the entire T-cell landscape: the pool of naive cells dries up, replaced by an accumulation of older, memory-like cells. These worn-out, senescent T cells (SA-T cells) don't just retire quietly. When stimulated, they actively secrete a cocktail of inflammatory signals - including osteopontin, IFN-γ, CCL3, and CCL4 - a state known as the senescence-associated secretory phenotype (SASP). Even worse, these senescent cells can migrate back to the thymic medulla, disrupting the critical quality-control process of negative selection. C. This breakdown in quality control gets worse inside the decaying thymus. The specialized cells responsible for screening new T cells (mTECs) drop in number and fail to mature properly. This disrupts the AIRE-dependent presentation of tissue-restricted antigens (TRAs) - essentially, the immune system's "do-not-attack" training list. Consequently, negative selection weakens, allowing self-reactive T-cell precursors to escape into circulation. Once these rogue cells encounter their matching antigen in the body, they quickly mature into active effector cells like Th1 or Th17, pumping out inflammatory cytokines and driving a chronic, low-grade local inflammation. D. Thymic atrophy is accompanied by a marked decline in thymic peptide hormones, including thymosin α1 (Tα1), thymosin β4 (Tβ4), and thymopentin (TP5). Tα1 deficiency weakens antigen presentation, reduces cytotoxic T-lymphocyte activity, and disrupts the Th1/Th2 balance, shifting the tumor microenvironment toward immunosuppression. Tβ4 is the major intracellular G-actin-sequestering peptide. Reduced Tβ4 disrupts cytoskeletal remodeling in immune effector cells, impairing their directed migration to tumors and formation of immune synapses. β-Thymosin isoforms differentially regulate angiogenesis: Tβ4 and Tα1 are proangiogenic, whereas Tβ9 and Tβ10 are inhibitory; an altered balance may favor tumor neovascularization. Loss of TP5, the synthetic pentapeptide corresponding to the active site of thymopoietin, further aggravates immune dysregulation. By modulating cyclic guanosine monophosphate (cGMP) in peripheral T cells, TP5 can normalize immune hyperactivity or deficiency caused by thymectomy or aging. Its loss in the tumor microenvironment may disturb the balance between Tregs and effector T cells.

do naive T cells penetrate the healthy adult thymus; however, they can do so during neonatal life, aging, or lymphopenia [103]. The percentage of remigrating mature T cells increases in mice from ~1-2% at six weeks of age to ~25% after one year, whereas remigrating Tregs

increase from 10% at 10 weeks to 90% by 50 weeks [103]. These are mostly CD44-high activated cells. Chemokine receptors regulate remigration, CCR7 limits their entry to the thymus and CCR6 and CXCR4 contribute to homing [103]. Remigrating cells usually have low expression of CD62L and high levels of CD69 and CD103 indicating a tissue resident phenotype [103].

In function, remigrating T cells are able to contribute to central tolerance through presentation of self or alloantigen to developing thymocytes for negative selection [103]. But they can also disrupt tolerance, for instance, $CD8^+$ OT-I cells remigrating into the thymus of RIP-mOVA mice will eliminate OVA-expressing dendritic cells and mTECs, disrupting negative selection and allowing generation of autoreactive T cells [103]. Remigrating Tregs can inhibit generation of new thymic Tregs dependent on IL-2, thus altering the intrathymic Treg pool [103].

Another hallmark of T-cell aging is mitochondrial dysfunction. Defective mitophagy in senescent $CD4^+$ T cells allows damaged mitochondria to accumulate; leaked mitochondrial DNA activates cGAS-STING signaling and inflammasomes, driving inflammatory phenotypes [104, 105]. In summary, thymic atrophy decreases naive T-cell output leading to increased homeostatic proliferation and peripheral T cell senescence, these senescent T cells enter again into the thymus competing for scarce stromal niches and cytokines interfering with negative selection suppressing new Treg generation forming a central-peripheral feedback loop deepening immunosenescence [100, 103, 104].

*Abnormal central tolerance and escape of autoreactive T cells*

Thymus mediates central immune tolerance through deletion of autoreactive T cells as well as development of thymic Tregs, a process dependent upon mTECs using AIRE to ectopically express thousands of TRAs [7, 18]. Since tolerance relies on an intact stromal microenvironment, processes that reduce mTEC numbers, maturation, or function, such as thymic atrophy, may impair selection fidelity [106].

Loss or defective mTEC differentiation in the atrophic thymus results in inadequate AIRE-mediated TRA presentation, thereby impairing negative selection [106] (**Figure 2C**). Autoreactive high affinity precursors targeting prostate, retinal, melanocyte, or other tissue-specific antigens can evade deletion and gain access to the periphery [7, 107]. Such cells may remain quiescent but reactive; upon encountering their cognate antigen during inflammation or injury, they differentiate into effector cells such as Th1 or Th17 that secrete IFN-γ and TNF-α, resulting in chronic low-grade autoimmunity [18, 107].

An important consequence of thymic dysfunction is a paradox: impaired antitumor immune surveillance coexists with relaxed central tolerance. On the one hand, thymic involution reduces de novo T-cell production and long-term renewal of the peripheral TCR repertoire, potentially narrowing the pool of T cells available for tumor recognition. In the TRACERx cohort, lower thymic health was also associated with lower sjTREC levels and reduced TCR diversity in blood and tumor samples, suggesting that diminished thymic activity may be linked to a less diverse immune-surveillance substrate. Patients with lower radiographic thymic health had higher risks of disease progression and death after immune checkpoint inhibitor treatment [85]. On the other hand, selective impairment of medullary negative selection may allow some high-affinity self-reactive clones to escape deletion. If the corresponding self-antigens are also expressed by tumor cells, these clones may recognize appropriately presented tumor-associated differentiation antigens and contribute to antitumor responses. This possibility is supported by experimental models. Transient Receptor Activator of Nuclear Factor-κB Ligand (RANKL) blockade in adult mice depleted mature AIRE-expressing mTECs and reduced TRA expression while largely preserving cortical architecture, total thymocyte numbers, and positive selection. This selective defect allowed melanoma-reactive T cells to escape deletion and improved survival after tumor challenge [108]. Similarly, Aire deficiency expanded melanoma-reactive T-cell populations and enhanced the antitumor activity of CTLA-4 blockade; subsequent studies showed synergy between Aire deficiency and immune-checkpoint inhibition across multiple tumor models, accompanied by expansion of highly tumor-reactive TCR clonotypes [109,

110]. These findings support the concept that impaired central tolerance can enhance antitumor immunity when T-cell generation is relatively preserved and negative selection against shared self/tumor antigens is selectively weakened.

These experimental models should not, however, be equated with naturally occurring thymic atrophy. In cancer-associated thymic injury, impaired thymopoiesis, stromal remodeling, defective negative selection, and peripheral immune suppression may occur simultaneously. In cachectic HCC mice, medullary stromal remodeling was associated with reduced expression of TRA-related genes, altered thymocyte clonotypes, impaired negative selection, and increased autoreactivity [9]. In the clinical component of the same study, higher serum levels of autoantibodies reactive against tumor tissues were associated with longer progression-free survival in patients receiving immune-checkpoint inhibitors (HR 0.389, 95% CI 0.158-0.960) [9]. Nevertheless, these clinical associations do not establish that the autoantibodies or corresponding antitumor responses originate from thymic dysfunction, nor do they demonstrate that cancer-associated thymic atrophy improves tumor control.

Therefore, the net effect of thymic damage is likely to depend on the relative extent of impaired T-cell production and defective central selection. Global or severe thymic atrophy, characterized by substantial loss of thymopoietic capacity, would be expected to reduce recent thymic emigrants and repertoire renewal and thereby impair broad antitumor immune surveillance. By contrast, partial or selective disruption of medullary negative selection, particularly when overall T-cell generation remains intact, may release self-reactive clones capable of recognizing tumors that express shared tissue antigens. In the setting of checkpoint blockade, such clones may be further activated, but they may also increase the risk of autoimmunity and immune-related adverse events. Naturally occurring thymic atrophy is unlikely to represent a purely selective defect, because impaired T-cell production and defective selection often coexist. Thus, current evidence supports a context-dependent model in which thymic atrophy may quantitatively restrict repertoire renewal while qualitatively relaxing repertoire selection, the balance between impaired tumor surveillance, enhanced self/tumor reactivity, and autoimmune toxicity remains dependent on tumor antigen expression, immune context, and the pattern of thymic injury.

*Decline of thymic endocrine function and effects on the tumor microenvironment*

Thymic atrophy is accompanied by reduced secretion of thymic peptides, including Tα1, Tβ4, TP5, and other immunoregulatory factors [21, 111, 112] (**Figure 2D**). The endocrine decline correlates with alterations in the sex-hormone environment. Estrogen can influence Tβ4 expression, therefore, an imbalance between sex hormones and the thymus might exacerbate the loss of thymic peptides during involution [113]. Decreased thymic peptide production impairs peripheral immunity, which could disrupt neuroendocrine-immune feedback. Tβ4 stimulates the hypothalamus for the release of luteinizing hormone-releasing factor, implying a thymus-hypothalamic-pituitary signaling loop exists [114]. Loss of such a network might affect system-wide stress and metabolic states, thus indirectly influencing the tumor microenvironment.

In terms of immune modulation, a lack of thymic peptides leads to impaired adaptive immunity. Low concentrations of Tα1 are able to induce Lyt+ T-cell markers, promote helper T-cell activity, enhance macrophage migration inhibition factor activity, and increase E-rosette formation [111], activates Toll-like receptor pathways in dendritic cells, promotes DC maturation and cytokine production, favors Th1 immunity [112]. A lack of Tα1 leads to reduced antigen presentation, weakened cytotoxic T-lymphocyte activity, disturbed Th1/Th2 balance, and an immunosuppressive tumor microenvironment [111, 112].

Tβ4 is a major G-actin-sequestering peptide that regulates actin polymerization and depolymerization [20]. Reduction of Tβ4 might disrupt cytoskeleton rearrangements for effector immune cells, impeding their migration into tumors and formation of immune synapses [20]. Various β-thymosin isoforms exert different effects on angiogenesis, Tβ4 and Tα1 promote it while Tβ9 and Tβ10 are inhibitory, Im-

balance between them may facilitates tumor neovascularization [20]. Oxidated form Tβ4 sulfoxide exerts an anti-inflammatory effect which inhibits neutrophil chemotaxis and adhesion, its reduction could further disturb inflammatory homeostasis within the tumor microenvironment [20].

TP5, which is a synthetic active pentapeptide of thymopoietin, further participates in immune equilibrium. It exerts an immunonormalizing effect and reverses both immune hyperactivity or deficiency due to thymectomy or aging through modulation of cyclic GMP level in peripheral T cells [21]. Deficiency of TP5 in the tumor microenvironment could disturb the balance between Tregs and effector T cells [21]. Thymosin α 7 induces suppressor T cells, thus, lack of coordinated thymic peptide networks might result in a complex environment for tumor immune escape [111].

In sum, endocrine failure due to thymic atrophy not only affects intrathymic T-cell differentiation but it also changes peripheral immune effector functions as well as cytoskeletal dynamics, angiogenesis, inflammatory tone, and neuroendocrine regulation thus setting up a system conducive for tumor growth and metastasis [20, 21, 111, 112].

## Effects of thymic atrophy on immunotherapies

### *Effects on immune checkpoint inhibitors*

As the central organ of de novo T-cell development, the thymus may influence ICI responsiveness by shaping long-term T-cell regenerative capacity and repertoire renewal. Age-related immunosenescence and cancer cachexia both lead to thymic atrophy through overlapping mechanisms, together forming a host-side determinant of response and toxicity in checkpoint blockade. However, its contribution operates alongside - and may be outweighed in individual tumors by - tumor antigenicity, antigen presentation, pre-existing T-cell states, and immunosuppressive features of the tumor microenvironment.

Peripheral TCR diversity has been associated with ICI outcomes and may represent a candidate host-side biomarker. Multiple studies have identified associations between peripheral blood TCR characteristics and clinical outcome. Higher pretreatment TCR richness is associated with improved objective response rates in advanced or metastatic NSCLC [12, 13], while increased baseline peripheral T-cell diversity in patients receiving ipilimumab plus nivolumab is correlated with improved response and reduced toxicity risk [13]. In renal cell carcinoma, although baseline TCR diversity may not directly predict efficacy, there was a significant decrease in TCR diversity at one month post-treatment indicating expansion of specific clones which correlates with durable clinical benefit [115]. The dynamics suggest activation via systemic expansion of tumor-specific clones upon ICI therapy, which requires a sufficiently broad starting repertoire [3]. In relapsed or refractory acute myeloid leukemia, single-cell TCR sequencing reveals that responders to PD-1 blockade exhibit repertoires dominated by $CD8^+$ T-cell clonal expansion compared to nonresponders who display further repertoire contraction, these results suggest that thymic function and repertoire diversity may be candidates for predicting ICI benefit in acute myelocytic leukemia (AML) [116].

A restricted peripheral TCR repertoire may reduce the number of clonotypes capable of recognizing tumor antigens and expanding after checkpoint blockade [3, 11, 115]. Once inhibitory signaling through the PD-1/PD-L1 or CTLA-4 pathways is relieved, therapeutic benefit still depends on the presence of pre-existing, checkpoint-responsive tumor-reactive T-cell populations [117, 118]. Thus, limited repertoire breadth provides a biologically plausible explanation for reduced ICI responsiveness in some patients. However, this interpretation remains inferential and does not establish that thymic involution is the cause of primary resistance. Peripheral TCR diversity can also be shaped by antigen-driven clonal expansion, chronic infection, tumor burden, lymphopenia, homeostatic proliferation, and previous anticancer therapy.

Moreover, ICI efficacy does not depend exclusively on ongoing thymic generation of new T cells. Older patients, despite generally reduced thymic output at the population level, can still derive substantial benefit from checkpoint blockade [119]. One explanation is that ICIs can act on pre-existing T-cell populations, par-

ticularly TCF1$^+$PD-1$^+$ progenitor-exhausted CD8$^+$ T cells maintained in tumor-draining lymphoid tissues. These cells can self-renew and differentiate after checkpoint blockade, providing a source of effector cells without requiring continuous generation of new thymic emigrants [117]. This model indicates that ongoing adult thymic output is neither universally required nor sufficient for ICI efficacy. Rather, thymic function may modify the size and flexibility of the peripheral immune pool on which checkpoint blockade acts.

Thymic dysfunction should be positioned as one host-side component of a broader multistep network governing responsiveness to immune checkpoint blockade. Although reduced thymopoiesis may constrain long-term T-cell repertoire renewal and decrease the probability of maintaining or generating potentially tumor-reactive clonotypes, successful checkpoint blockade additionally requires sufficient tumor antigenicity, intact antigen processing and presentation, effective T-cell priming and trafficking, the presence of checkpoint-responsive T-cell states, and a tumor microenvironment permissive for effector function. Failure at any of these downstream steps may result in resistance irrespective of thymic reserve.

Tumor-intrinsic immune escape can therefore outweigh the contribution of thymic dysfunction in individual patients. Loss of β2-microglobulin or defects in human leukocyte antigen (HLA) class I antigen presentation can prevent recognition by cytotoxic T cells, whereas disruption of IFN-JAK signaling may permit tumor cells to escape interferon-mediated immune control [120]. Similarly, tumor-intrinsic WNT/β-catenin activation can impair dendritic-cell recruitment and promote T-cell exclusion [121]. At the T-cell level, checkpoint blockade preferentially expands stem-like or progenitor exhausted populations rather than uniformly reinvigorating terminally dysfunctional T cells [118]. Suppressive myeloid populations, regulatory T cells, and metabolic constraints such as hypoxia, nutrient competition, lactate accumulation, and adenosine signaling provide additional barriers that cannot be corrected simply by increasing thymic T-cell production.

The relative importance of thymic dysfunction is therefore likely to be context dependent. It may be more relevant in patients with advanced immune aging, profound treatment-induced lymphopenia, or extensive prior cytotoxic therapy, in whom peripheral T-cell replenishment and repertoire renewal are limited. Conversely, when ICI resistance is primarily driven by defective antigen presentation, T-cell exclusion, terminal exhaustion, myeloid suppression, or metabolic restriction, restoration of thymic function alone is unlikely to restore treatment sensitivity. The thymus is consequently best conceptualized as one upstream component of the host immune substrate - contributing to long-term T-cell supply, repertoire renewal, and the quality of central tolerance - rather than as a necessary, sufficient, or dominant determinant of ICI response.

*Effects on CAR-T cell therapy*

Conventional autologous CAR-T-cell therapy is generated from mature peripheral T cells collected from the patient and therefore does not directly depend on ongoing thymic activity. Nevertheless, thymic involution may indirectly influence CAR-T therapy by contributing to long-term remodeling of the peripheral T-cell compartment from which CAR-T products are derived. Age-associated reductions in thymic output are accompanied by contraction of naïve and less-differentiated T-cell populations and increasing reliance on peripheral maintenance of the existing T-cell pool [94]. Because the differentiation state of the starting T-cell population strongly influences subsequent CAR-T-cell expansion, persistence, and antitumor activity, reduced availability of less-differentiated T cells provides a biologically plausible link between long-term thymic decline and conventional CAR-T-cell fitness.

Clinical studies support the importance of the peripheral starting-cell phenotype, although they do not establish thymic atrophy as its direct cause. In patients with chronic lymphocytic leukemia receiving CD19 CAR-T cells, Fraietta et al. found that complete responses were associated with a higher pretreatment frequency of CD27$^+$CD45RO$^-$CD8$^+$ memory-like T cells, whereas CAR-T cells from nonresponders were enriched for transcriptional programs associated with effector differentiation, glycolysis, exhaustion, and apoptosis [122]. More recently, analysis of a CD22 CAR-T trial in B-cell acute lymphoblastic leukemia

showed that nonresponders had more differentiated and exhausted CAR-T-cell phenotypes and, importantly, that these differences could already be detected in the apheresis material before CAR-T manufacturing [123]. Experimental studies further demonstrate that CAR-T products generated from preselected naïve/stem-memory T cells exhibit greater expansion, persistence, and antitumor activity than products from unselected T cells, supporting a functional advantage of less-differentiated starting populations [124].

However, the peripheral T-cell phenotype in patients with cancer cannot be attributed specifically to thymic involution. Tumor burden, chronic antigenic stimulation, systemic inflammation, previous lymphotoxic therapies, peripheral homeostatic proliferation, and the manufacturing process itself can independently alter T-cell differentiation and exhaustion. Consistent with this complexity, chronological age alone does not necessarily predict poorer CAR-T efficacy: several contemporary clinical cohorts have reported comparable response and survival outcomes in older and younger patients receiving CD19 CAR-T therapy [125]. Because age is only an indirect correlate of thymic involution, these findings do not exclude a contribution of thymic decline, but they indicate that age-associated thymic loss is not sufficient by itself to determine CAR-T outcome.

Thus, current evidence supports a cautious distinction between peripheral T-cell fitness and thymic function. The quality of the mature peripheral T cells used for CAR-T manufacturing is an established determinant of therapeutic performance, whereas the contribution of thymic involution to this starting-cell phenotype remains biologically plausible but clinically unproven. Direct studies integrating thymic imaging, recent thymic emigrants or TREC-based measures with CAR-T manufacturing characteristics and clinical outcomes will be required to determine whether thymic status provides independent information beyond age, previous treatment, and disease-related immune dysfunction.

All of the strategies described above depend on ex vivo manufacturing and therefore remain constrained by the initial quality of the patient's T cells and their limited in vivo persistence. A more radical strategy involves directly injecting CAR-transduced hematopoietic stem cells (HSPCs) into the thymus, leveraging its microenvironment to drive T-lineage differentiation and continuously generate naive CAR-T cells in vivo [126]. Single-cell sequencing shows that the thymus can induce T-lineage transcriptional programs in HSPCs [126]. CAR-T cells arise from cells that have not undergone extensive ex vivo expansion, retain strong proliferative potential, and can be produced continuously as donor-derived T cells. Compared with conventional intravenous infusion of effector CAR-T cells, thymus-derived CAR-T cells show markedly prolonged persistence in peripheral blood.

Thymic atrophy profoundly limits this strategy through both age-related and injury-induced changes. In the aging thymus, atypical age-associated thymic epithelial cells (aaTEC1 and aaTEC2) form high-density clusters devoid of thymocytes, directly replacing functional cortical and medullary spaces [127]. These aaTECs also act as sinks for regenerative signals such as Fibroblast Growth Factor (FGF) and bone morphogenetic (BMP), diverting trophic support away from conventional thymic epithelial cells, while FOXN1 downregulation drives differentiation away from functional TECs and toward aaTEC fate [127]. In acute injury from conditioning regimens, the Postn+ mesenchymal subset, which recruits early T cell progenitors via CCL19, is severely and persistently depleted [128]. This disrupts progenitor seeding into the thymus, and post-injury repair further exacerbates the problem by favoring aaTEC generation over functional epithelial regeneration [127, 128]. Collectively, these alterations impair engraftment, differentiation, and long-term CAR-T cell output from intrathymically injected HSPCs, necessitating concurrent thymic regenerative interventions to optimize therapeutic efficacy [127, 128].

Artificial thymic organoids offer another ex vivo engineering approach that enables differentiation of CAR-transduced human induced pluripotent stem cells (iPSCs) into functional CAR-T cells without causing tonic signaling or NK/ILC lineage diversion due to forced CAR expression [129]. Bioengineered thymic tissues derived from human pluripotent stem cell-derived thymic epithelial progenitor cells and custom extracellular matrices could potentially serve as a physiologically relevant niche for sustained CAR-T generation in the future [130].

Beyond the αβ T-cell-centered CAR-T strategies described above, γδ T cells are emerging as an alternative or complementary platform because they recognize tumors independently of MHC and possess broad antitumor activity. Their antitumor functions, including production of IFN-γ and TNF-α, are largely preprogrammed during thymic development. Thymic functional status may therefore directly influence the intrinsic functional quality of γδ T-cell products [131].

### Classic pharmacologic reconstruction of thymic function

Despite multiple forms of injury, the thymus retains endogenous regenerative potential, particularly in younger organisms [64]. However, regenerative capacity declines substantially with age. In the aged thymus, nonfunctional scar-like clusters of abnormal TECs limit recovery after injury [64]. Endogenous repair of the thymus depends on a network of molecular signals, among which RANKL and IL-22 are key drivers of TEC regeneration [132, 133]. Thymic peptides and their synthetic analogs may also act as immunomodulatory adjuvants in oncology [112, 134]. Endocrine interventions, including sex-steroid blockade and supplementation of the GH/IGF-1 axis, provide additional strategies for reversing age- or treatment-associated thymic atrophy [16, 135]. Although restoration of thymic structure and T-cell output represents an attractive approach to counteract age- or treatment-associated thymic atrophy, thymotropic interventions require particular caution in patients with cancer. Most regenerative pathways are not restricted to the thymus, and the same proliferative, survival, or differentiation signals may also act on malignant cells or alter tumor-associated immune responses. For example, RANKL and IL-22 have experimentally demonstrated tumor-promoting potential in selected RANK- or IL-22R-responsive malignancies, the risks associated with KGF and sex-steroid blockade depend strongly on receptor expression, tumor type, and anatomical context. The mechanisms, developmental status, potential therapeutic benefits, and tumor-associated safety concerns of the principal thymic restoration strategies discussed in Sections 7 and 8 are therefore summarized in **Table 1** [132, 136-154].

*RANKL pathway modulation*

The RANK-RANKL axis is both a cornerstone of central tolerance and a potent driver of thymic regeneration [134] (**Figure 3A**). RANKL expression increases after radiation or chemotherapy. RANKL directly stimulates TEC proliferation, and this effect can be blocked by osteoprotegerin [155]. RANKL increases anti-apoptotic Bcl-2 and Bcl-xL expression while reducing pro-apoptotic Bax, creating a survival-favorable microenvironment. It also enhances thymocyte-TEC adhesion and upregulates adhesion molecules such as ICAM-1 and VCAM-1, as well as thymopoietic factors such as IL-7 and GM-CSF [155].

In bone marrow transplantation models, RANKL is produced mainly by radiation-resistant $CD4^+$ thymocytes and lymphoid tissue inducer (LTi) cells. Neutralization of RANKL impairs TEC regeneration, whereas exogenous RANKL promotes recovery of cTECs, mTECs, and TEC-low populations enriched for epithelial progenitors [132]. In addition, RANKL drives lymphotoxin α expression by LTi cells that promote progenitor homing and de novo thymopoiesis via LTβR signaling. These effects are even present in aged mice indicating a translational potential for older recipients [132].

In cancer immunotherapy, transient blockade with denosumab, a humanized anti-RANKL antibody, can provide additional benefits by weakening mTEC-mediated negative selection, thereby releasing self-reactive T-cell clones capable of recognizing tumor-associated self-antigens. This enhances antitumor immunity and synergizes with ICIs, but requires careful safety management [134].

*IL-22*

IL-22, a member of the IL-10 cytokine family, is produced in the thymus mainly by $ROR\gamma t^+CCR6^+NKp46^-$ LTi cells (**Figure 3B**), and plays a central role in endogenous thymic repair [118, 121, 133, 138]. IL-22 is dispensable for steady-state thymopoiesis but is essential for recovery after irradiation or bone marrow transplantation. IL-22-deficient mice exhibit marked and persistent impairment of thymic regeneration after irradiation, lasting for as long as 98 days [118]. Exogenous IL-22 significantly improves thymic recovery after irradi-

**Table 1.** Thymic restoration strategies for cancer immunotherapy

| Strategy | Primary thymic mechanism | Developmental stage | Potential therapeutic benefits | Tumor-associated safety concerns | Ref |
|---|---|---|---|---|---|
| RANKL | Promotes TEC recovery/proliferation and LTα-dependent lymphoid progenitor homing after thymic injury | Preclinical | Restores TEC compartments, progenitor recruitment, and de novo thymopoiesis | Promotes progesterone-driven mammary epithelial proliferation and mammary tumorigenesis.<br>Increase the skeletal metastatic potential of breast cancer cells.<br>Accelerate bone metastasis of RANK-expressing melanoma and breast cancer cells. | [132, 136, 137] |
| IL-22 | Promotes TEC survival and proliferation following thymic injury | Preclinical<br>Irradiation/BMT models | Accelerates recovery of thymic cellularity and epithelial architecture | Acts on malignant epithelial cells expressing IL-22R.<br>Activates STAT3 and induces BCL-2, BCL-XL, Cyclin D1 and VEGF, promoting HCC survival, proliferation and metastasis. | [138, 139] |
| Tα1 | Promotes T-cell differentiation/function and enhances DC/T-cell responses | Clinical immunomodulator<br>Oncology trials | Improve immune competence and augment antitumor therapy | Studies found no stimulation of malignant-cell growth or oncogenic transformation.<br>In a randomized trial of 488 patients with metastatic melanoma, some efficacy signals favored Tα1-containing arms. | [140, 141] |
| TP5 | Immunomodulatory effects with reported enhancement of thymic architecture and T-cell function | Preclinical/early clinical immunomodulation | Restore T-cell responsiveness and counter tumor-associated immune suppression | No well-established direct tumor-promoting pathway has been demonstrated | [142, 143] |
| KGF | FGFR2IIIb/KGFR signaling promotes TECs and supports thymic epithelial recovery after injury | Preclinical thymic evidence<br>Clinical use primarily for epithelial protection | Protects TECs from cytotoxic injury and accelerates thymopoietic recovery | Risk is tumor-type and receptor dependent.<br>Stimulates clonal growth in 5 solid-tumor lines, including lung, gastric, colorectal and breast cancer, specifically in KGFR-expressing cells.<br>Stimulates proliferation of biliary tract cancer cells.<br>KGFR-positive biliary tumors were associated with poorer prognosis. | [144-147] |
| Sex-steroid blockade | Relieves sex-steroid-mediated inhibition of TECs and thymopoiesis, promoting thymic rebound | Preclinical<br>Early human/HSCT studies | Increases thymic output, naïve T-cell recovery, TRECs and TCR repertoire regeneration | Causes tumor flare, including worsening pain, obstruction and occasional spinal cord compression in metastatic prostate cancer.<br>Accelerate intracranial glioma growth through HPA-axis activation, glucocorticoid elevation and myeloid-mediated immunosuppression. | [148-150] |
| DFImRNA | Transiently restores age-depleted DLL1/Notch, FLT3L and IL-7 signaling, increasing lymphoid progenitors, thymopoiesis and immune reconstitution | Preclinical<br>Aged mice, including tumor/ICI models | Restores thymic output and TCR diversity and improves peripheral immune/DC function; enhances anti-PD-L1 efficacy in preclinical models | IL-7 is a direct growth factor for subsets of T-ALL and B-precursor ALL, inducing leukemic blast proliferation.<br>FLT3L can increase proliferation and exert anti-apoptotic effects in subsets of primary AML blasts, including FLT3-wild-type and FLT3-mutant samples.<br>Studies did not demonstrate a direct tumor-promoting DLL1 effect. | [151-154] |

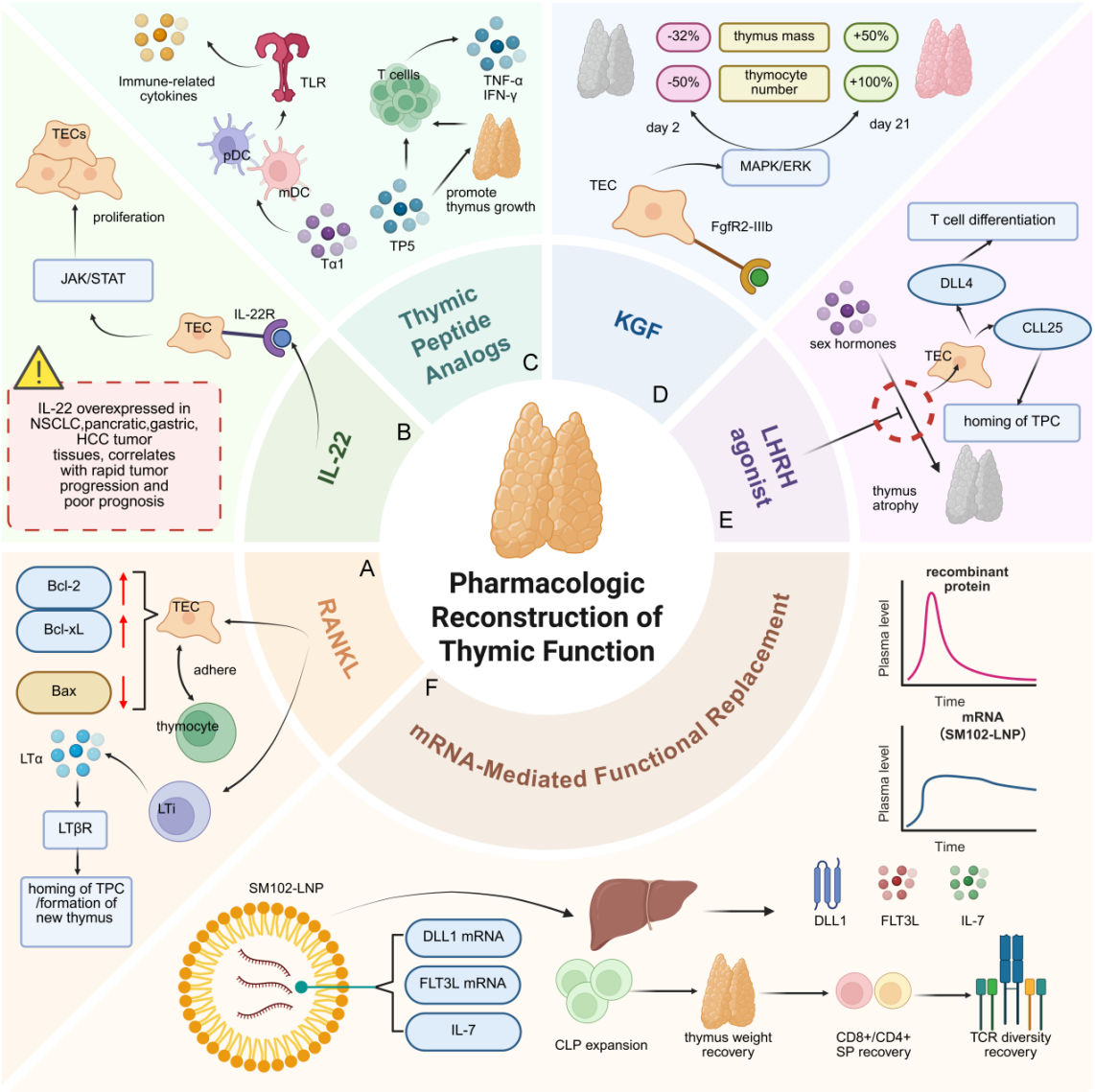


**Figure 3.** Pharmacologic reconstruction of thymic function. A. RANKL. RANKL increases the expression of the antiapoptotic genes Bcl-2 and Bcl-xL in TECs while reducing the proapoptotic gene Bax, thereby creating a survival-promoting microenvironment. It also enhances adhesion between thymocytes and TECs. In LTi cells, RANKL specifically induces LTα, which promotes thymic progenitor homing and de novo thymopoiesis through LTβR signaling. B. IL-22. IL-22 acts through IL-22R on cTECs and immature medullary TECs (mTEClo), promoting their proliferation and survival. However, IL-22 is upregulated in several tumor tissues and is associated with rapid tumor progression and poor prognosis. C. Thymic peptide analogs. Tα1 activates TLRs on pDCs and mDCs, initiating downstream signaling and promoting production of multiple immune-related cytokines. TP5 promotes thymus growth and regeneration, supporting de novo T cell development and maintaining a diverse T cell repertoire. Simultaneously, TP5 enhances T cell effector functions by augmenting the production of key pro-inflammatory cytokines, including TNF-α and IFN-γ, which are critical mediators of anti-tumor immunity. D. KGF. KGF activates MAPK/ERK in TECs, suppressing Wnt ligands → downregulates FOXN1 and DLL4 → attenuates Notch signaling, causing transient β-selection arrest. On day 2, thymus mass and cellularity decrease by 32% and 50%, respectively. By day 21, both rebound to +50% and +100% above controls, driven by sustained TEC expansion and long-term stromal remodeling. E. Sex-hormones. LHRH agonists can block sex-steroid-driven thymic atrophy. Sex-steroid withdrawal directly increases TEC expression of the chemokine CCL25, which promotes progenitor homing to the thymus, and the Notch ligand DLL4, which supports T-cell differentiation. F. mRNA-mediated functional replacement of thymic support. An mRNA combination encoding DLL1, FLT3L, and IL-7 (the DFI combination) is packaged in LNPs. After intravenous administration, mRNA-encoded FLT3L and IL-7 generate lower but more sustained concentrations, compared with target-protein expression peaking at approximately 48 hours and then rapidly declining. DFI expands age-depleted CLPs, enhances thymic T-cell generation, partially restores thymic weight and cellularity in aged mice, and restores TCR diversity.

ation and bone marrow transplantation in animal models [118, 121, 133, 138].

The regenerative circuit is initiated by depletion of $CD4^+CD8^+$ DP thymocyte, the reduction in DP cells induces $CD103^+$ dendritic cells to stimulate radiation-resistant LTi cells to produce IL-22 [133, 138]. IL-22 acts through IL-22R, which is expressed predominantly on TECs, and promotes the proliferation and survival of cTECs and immature medullary TECs (mTEClo). Binding of IL-22 to its receptor on TECs activates the canonical JAK/STAT pathway, expands cTEC and mTEC compartments, and accelerates reconstruction of corticomedullary architecture.

Despite this potent regenerative activity, the tumor-promoting potential of IL-22 must not be overlooked. IL-22 is upregulated in tumor tissues from patients with NSCLC, pancreatic cancer, gastric cancer, and HCC and is associated with rapid progression and poor prognosis. Its use as a thymic regenerative adjuvant therefore requires careful control of potential oncogenic risk [134].

*Thymic peptide analogs*

Tα1 participates in cell proliferation, chromatin remodeling, and nuclear transport [20]. By activating Toll-like receptors on pDCs and myeloid dendritic cells (mDCs), it initiates downstream signaling, promotes production of multiple immune-related cytokines, and regulates several immune-cell subsets [112] (**Figure 3C**). In preclinical tumor models, Tα1 combined with cyclophosphamide significantly prolonged survival and cured approximately 23% of tumor-bearing animals, this effect depended on T cells and IL-2 signaling. Furthermore, Tα1 promotes IDO-1 dependent tolerogenic immune pathways that prevent severe intestinal immunopathology caused by ICIs like anti-CTLA-4 antibodies [134].

Several clinical studies on various solid tumors suggest that Tα1 may provide benefit when combined with conventional therapy. A meta-analysis of 27 randomized controlled trials including 1,925 NSCLC patients showed that combination treatment using Tα1 and chemotherapy especially gemcitabine or vinorelbine plus cisplatin was associated with clinical benefit and good safety [134]. A retrospective analysis of 5,746 patients with R0-resected NSCLC demonstrated improvement of five-year disease-free survival (77.3% vs 57.9%), and overall survival (83.3% vs 65.6%) for those treated by Tα1, adjuvant Tα1 in small HCC after surgery led to an increase of five-year overall survival (82.9% vs 62.9%) and recurrence-free survival (53.3% vs 32.1%), Tα1 plus dacarbazine in metastatic melanoma resulted in increasing stable disease rate up to 26-37%, it also improved objective response versus controls and extended response duration [134].

TP5 exhibits strong thymopoietic effect through significant increases in thymic mass and cellularity in tumor-bearing mice, notably enhancing $CD4^+$, $CD8^+$, and $CD4^+CD8^+$ double-positive thymocyte population [142]. Administration of TP5 during cyclophosphamide-induced immunosuppression not only prevents severe thymic involution but also actively drives thymic recovery leading to increased thymic weight and T cell output relative to vehicle treatment [142]. Thus, TP5 has been shown to counteract cancer- and chemotherapy-induced thymic atrophy. One significant challenge associated with this thymus-directed therapy is its very brief plasma half-life (approximately 30 seconds) limiting prolonged exposure to the thymus [142]. As an alternative to improving the pharmacokinetics of TP5, another study developed palmitic acid-modified TP5 that self-assembles into nanoparticles providing greater stability and enhanced immunostimulatory activity though it was not tested on the thymus [156]. Such a formulation could be a feasible way to improve the bioavailability of TP5 for future thymic rejuvenation therapies [142, 156].

*Keratinocyte growth factor (KGF)*

KGF shows biphasic effects on thymopoiesis starting from temporary developmental arrest and then long-term expansion (**Figure 3D**). It was shown that KGF induces activation of the MAPK/ERK pathway in TECs which leads to suppression of expression of Wnt ligands like Wnt4, Wnt7a, and Wnt10a in a subset specific way [157], thereby marking dramatic downregulation of Foxn1, a master transcriptional regulator of TEC differentiation, at mRNA and protein level [157]. Reduction of Foxn1 further leads to diminished expression of its direct targets DLL4 encoding the essential Notch ligand

needed for early thymocyte progression, disrupting Notch signaling in DN2a to DN3a thymocytes thus suppressing the expression of key downstream genes like Bcl11b, Ptcra, Rag2. Disruption of pre TCR assembly leads to an arrest of thymocyte development at the beta selection checkpoint, resulting in significant reduction in post selection subsets. Ironically, this initial inhibition is counterbalanced by strong TEC proliferation via the same MAPK/ERK pathway eventually resulting in marked increase in thymic mass and thymocyte numbers by day 21 [157].

KGF's poor performance in clinics is partly attributed to its intrinsic pharmacokinetic limitations such as short half-life, fast diffusion out of the injection site, and lack of target specificity that lead to reduced bioavailability and limit sustained interaction with TECs [158]. Protein engineering strategies have been used to improve KGF retention in the extracellular matrix. One new strategy was designed through creating a fusion protein between KGF and the collagen binding domain (CBD), derived from Vibrio mimicus metalloprotease. The vibrioCBD KGF fusion showed significantly increased collagen binding affinity but retained full biological activity similar to native KGF. When applied to adipose derived stem cells cultured on collagen coated plates, the fusion protein induced keratinocyte differentiation markers at substantially lower concentrations than were required for unmodified KGF, demonstrating that CBD mediated tethering to collagen effectively increases local bioavailability and enhances KGF potency [158].

*Sex-hormone modulation*

Sex hormones are major drivers of age-related thymic involution (**Figure 3E**). Chemical sex-steroid blockade, such as treatment with luteinizing hormone-releasing hormone (LHRH) agonists, can reverse age- or chemotherapy-associated thymic atrophy. Sex-steroid withdrawal directly promotes TEC expression of CCL25 and DLL4, which supports progenitor homing to the thymus and T-cell differentiation, respectively [16]. In clinical studies, LHRH agonists accelerate T-cell reconstitution after bone marrow transplantation and increase peripheral TCR diversity [16].

**Novel functional replacement strategies**

*Novel mRNA-mediated thymic supports*

mRNA technology offers a cell-free strategy for thymic regeneration. Lipid nanoparticles (LNPs) can deliver mRNA encoding selected thymotropic factors, producing systemic, transient, and controllable protein expression without genomic integration (**Figure 3F**). Repeated dosing can maintain effect while avoiding risks associated with viral vectors [151]. Mapping signaling pathways in young and aged mice has shown that Notch signaling, especially DLL1-mediated cTEC-thymocyte interactions, and IL-7 signaling decline with age. FLT3L signaling in peripheral T cells is also markedly reduced [151]. These observations identify DLL1, FLT3L, and IL-7 as rational intervention targets.

Although recombinant proteins can promote thymic repair, single-factor protein therapy is limited by short half-life, frequent high-dose administration, and risk of systemic inflammation [16, 132, 138, 151]. Friedrich and colleagues therefore designed a three-factor mRNA combination encoding DLL1, FLT3L, and IL-7, referred to as the DFI combination [151]. These mRNAs were packaged into SM-102 ionizable LNPs. Following an intravenous injection, LNPs accumulate in the liver. In contrast to recombinant proteins that have a sharp peak of serum concentration, mRNA-encoded FLT3L and IL-7 show lower yet sustained levels. The protein expression peaks around 48 hours before it drops off quickly for transient and controlled supplementation [151].

The DFI therapy reverses aging-related immune decline via a multi-step process, DLL1 delivers essential Notch signaling for early T-lineage commitment, IL-7 ensures thymocyte survival and facilitates β-selection, whereas FLT3-L improves the thymic stromal microenvironment and drives dendritic cells' development. This regimen expands CLPs, which include $CCR9^+$ CLP that have thymic homing potential, and also increases thymic T-cell generation [151]. The treatment partially restores thymic weight and cellularity of aged mice, where after 28 days mature $CD4^+$ and $CD8^+$ SP thymocytes approach those observed in young mice. Increased peripheral TRECs pro-

vide molecular evidence for enhanced thymic output [151]. DFI also improves peripheral immune function by expanding conventional type 1 dendritic cells (cDC1s) in the spleen and upregulating H2-K1 and costimulatory molecules such as CD40, CD83, and CD86 [151]. In B16-OVA melanoma and MC38-OVA colon cancer models, DFI pretreatment increases the abundance and clonal diversity of tumor-infiltrating $CD8^+$ T-cells. Tumor-specific $CD8^+$ T cells show reduced expression of exhaustion markers including Havcr2, Lag3, and Pdcd1, and their TCR Shannon diversity increases [151]. In parallel with these thymic and peripheral immune changes, DFI synergizes with anti-PD-L1 therapy and induces complete tumor regression in 40% of aged mice bearing aggressive B16-OVA tumors [151].

Importantly, DFI induces two parallel forms of immune restoration: enhanced thymopoiesis and remodeling of peripheral immune compartments. The treatment increases thymic cellularity, thymocyte maturation, and peripheral TREC levels, consistent with improved thymic output, while simultaneously expanding dendritic-cell populations and altering peripheral T-cell states through the systemic actions of FLT3L and IL-7. Both processes could plausibly contribute to the increased tumor-infiltrating $CD8^+$ T-cell abundance, broader tumor-reactive TCR repertoire, and improved response to PD-L1 blockade observed after DFI treatment. Although the washout period used before tumor challenge reduces the likelihood that these effects reflect only acute exposure to the encoded factors, it cannot exclude persistent peripheral immune remodeling. Therefore, the current experiments do not fully distinguish the relative contributions of newly generated thymic T cells from thymus-independent peripheral immune effects to the enhanced antitumor response.

Resolving this distinction will require experimental approaches that selectively separate thymus-dependent from peripheral mechanisms. Adult thymectomy would test whether an intact thymus is required for the antitumor benefit of DFI, whereas inducible TEC-specific disruption of thymopoietic support could impair thymic T-cell generation while preserving the systemic actions of IL-7 and FLT3L. Lineage-tracing approaches could further determine whether newly generated thymic emigrants enter tumors, acquire tumor-reactive specificities, and contribute directly to tumor control. Tracking shared TCR clonotypes across the thymus, recent thymic emigrants, peripheral blood, and tumor-infiltrating lymphocytes would provide particularly strong evidence for a causal contribution of regenerated thymic output to antitumor immunity.

*Cryopreserved cultured thymus tissue implantation (cCTTI)*

Cultured allogeneic thymus tissue implantation (CTTI) is currently a standard treatment for congenital athymia, but its use is constrained by a narrow donor-recipient time window, requiring transplantation within 12-21 days after thymus procurement (**Figure 4A**). Preclinical studies in rats and pigs have shown that cultured thymic tissue can be cryopreserved and, after thawing, retain both viability and reconstructive potential [159].

When cryopreserved cultured rat thymus tissue is implanted under the kidney capsule of congenitally athymic rats, or when porcine cCTTI is implanted into the quadriceps of thymectomized and T-cell-depleted pigs, grafts can engraft, reconstruct normal corticomedullary architecture, and generate peripheral naive T cells with diverse TCR repertoires [159]. Cryopreserved human thymic tissue also retains key quality-control markers after thawing, including CCL21 expression for progenitor recruitment, CK14+ TECs with regenerative potential, and proliferating Ki-67+ stromal cells [159].

Although cCTTI has not yet been directly applied to cancer patients, its core technology could eventually provide a route to thymic reconstruction in individuals with cancer-treatment-induced thymic failure. Clinical studies will be needed to test safety, feasibility, and immune benefit in this population.

*Cell replacement and thymus organ functional reconstruction*

Stem-cell- and tissue-engineering-based reconstruction of thymic function offers a highly promising strategy for overcoming T-cell immunodeficiency caused by thymic atrophy. The main idea is to create a functional thymic microenvironment in vitro/in vivo that supports

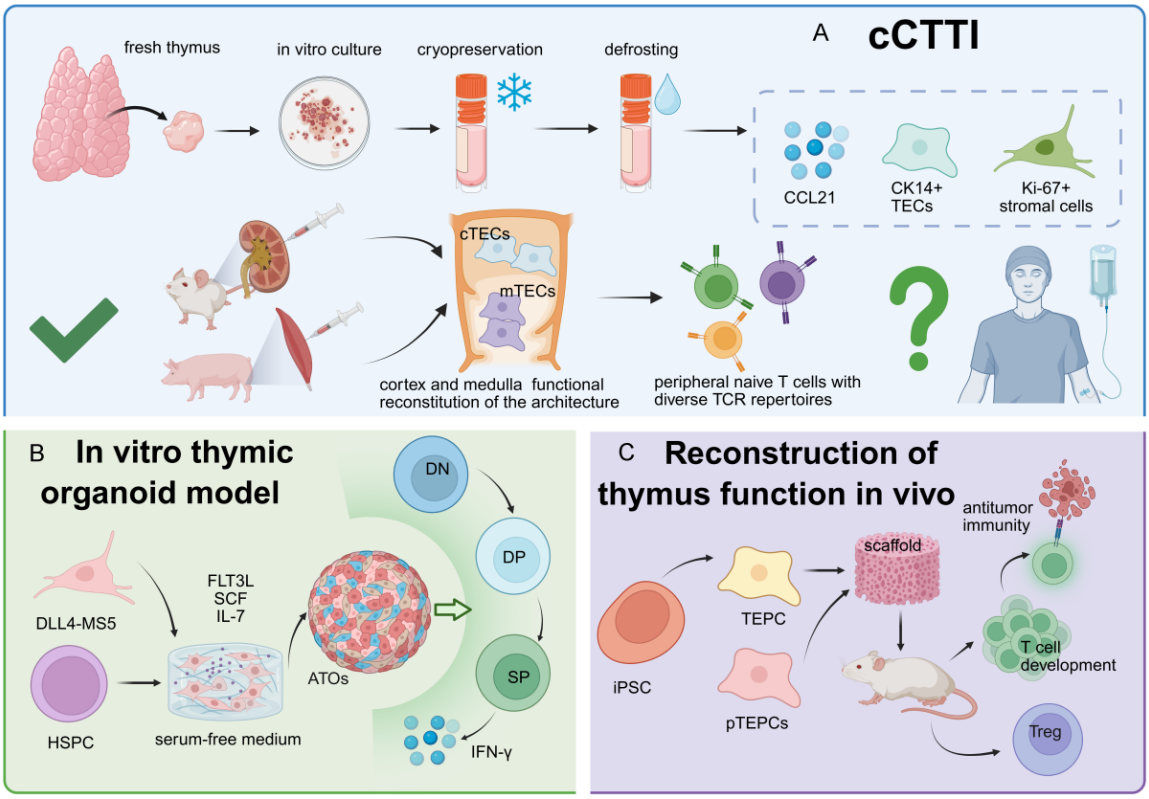


**Figure 4.** Cell replacement and thymus organ functional reconstruction. A. cCTTI. After thawing, cryopreserved human thymic tissue retains key quality-control features, including CCL21, CK14+ TECs, and actively proliferating Ki-67+ stromal cells. Implantation of rat cCTTI under the kidney capsule of congenitally athymic rats, or porcine cCTTI into the quadriceps of thymectomized and T-cell-depleted pigs, reconstructs corticomedullary architecture and generates naive T cells with diverse TCR repertoires. B. In vitro thymic organoid. ATOs are standardized three-dimensional culture systems in which HSPCs are aggregated with MS5 stromal cells engineered to express the Notch ligand DLL4 under serum-free, chemically defined conditions. ATOs reproduce key features of three-dimensional thymic architecture, support T-cell differentiation and positive selection, generate naive-phenotype $CD4^+$ and $CD8^+$ SP T cells, and produce functional responses to TCR stimulation, including secretion of cytokines such as IFN-γ. C. In vivo thymic reconstruction. iPSCs are directed toward thymic epithelial progenitor cells (TEPCs), combined with three-dimensional scaffolds constructed from decellularized native thymic matrix or synthetic biomaterials, and further differentiated into functional TECs. After transplantation into immunodeficient mice, iPSC-derived TEPC-scaffold constructs support host HSPC homing and engraftment, complete T-cell development, Treg generation, and reconstruction of antitumor immune function. In addition to iTECs, organoids generated by combining postnatal primary thymic epithelial stem/progenitor cells (pTEPCs) with three-dimensional scaffolds can also reconstruct a functional thymic microenvironment and support T-cell development.

directed HSPC differentiation, along with positive and negative selection, for T-cell regeneration and immune-repertoire rebuilding [160, 161].

Artificial thymic organoids (ATOs) are a key platform (**Figure 4B**). By aggregating HSPCs with MS5 stromal cells engineered to express DLL4 under serum-free, defined conditions, ATOs simulate three-dimensional thymic structure and support T-lineage commitment, differentiation, positive selection, and generation of naive $CD4^+$ and $CD8^+$ SP T cells [162]. ATOs can differentiate HSPCs from cord blood, bone marrow, or peripheral blood and can enforce TCR allelic exclusion in vitro, providing a route to tumor-specific TCR-T or CAR-T generation [160]. They also allow functional testing of HSPCs from patients with T-cell lymphopenia [129].

For in vivo reconstruction, induced pluripotent stem cell-derived thymic epithelial cells (iTECs) may eventually replace allogeneic cultured thymus tissue by enabling scalable and potentially HLA-matched thymic repair (**Figure 4C**).

iTECs can support positive and negative selection, generate diverse and centrally tolerant TCR repertoires, and produce Tregs in vivo [163]. Differentiation protocols use timed modulation of Activin A, BMP4, retinoic acid, FGF, and other pathways to guide iPSCs or embryonic stem cells (ESCs) into definitive endoderm, ventral pharyngeal endoderm, thymic epithelial progenitor cells (TEPCs), and ultimately functional TECs [130, 164]. Decellularized thymic matrix or synthetic biomaterials such as porous tantalum-coated carbon matrices, CellFoam, and collagen-polycaprolactone composite scaffolds can provide spatial and biochemical cues for TEPC engraftment, proliferation, and maturation [161, 165]. When iPSC-derived TEPCs are combined with three-dimensional scaffolds and transplanted into immunodeficient mice, host HSPCs can home to the graft and progress from DN to DP and SP stages, reconstructing a diverse and functional T-cell compartment [166, 167]. Generated T cells can mediate central tolerance, suppress allogeneic tumor growth, and mount TCR-dependent cytokine and proliferative responses, demonstrating functional adaptive immunity [167]. Postnatal primary thymic epithelial stem/progenitor cells (pTEPCs) also have self-renewal and multilineage potential and can generate both cTEC and mTEC lineages when incorporated into organoid scaffolds [163].

There exist several bottlenecks. The efficiency and stability of iPSC-to-TEPC differentiation, especially for mature/functional cTEC/mTEC subsets, are not optimal, Expression levels of important transcription factors like FOXN1 remain lower than in native thymic tissue [130, 161]. Long-term graft survival depends upon vascularization, whereas the use of xenogeneic materials such as mouse stromal cells or Matrigel poses a risk of immune rejection and pathogen contamination. Therefore, fully humanized, GMP-compatible systems are needed [161, 162, 165]. Patient-specific genetic backgrounds, including DiGeorge syndrome, CHD7 defects, or AIRE deficiency, may influence TEPC differentiation and require individualized optimization [164]. Finally, much functional validation still relies on mouse models, whose thymic microenvironment and T-cell developmental kinetics differ from those of humans. A fully humanized thymic organoid system remains the long-term objective [161, 167].

## Challenges and perspectives

Thymic atrophy is increasingly recognized as a key, and potentially reversible, determinant of antitumor immunity. Yet, translating this concept into clinical practice is no simple task. Currently, most evidence is derived from animal models, retrospective studies, or indirect markers. Because factors like age, obesity, cachexia, chronic inflammation, and prior cancer therapies affect both the thymus and patient outcomes, disentangling cause and effect remains challenging. Prospective trials are needed to determine whether thymic dysfunction drives immunotherapy resistance or merely reflects broader biological aging.

A major measurement hurdle also remains: we lack a standardized approach to assessing the thymus. CT scans can show physical size, and AI tools can score tissue structure, but these metrics do not necessarily indicate whether the organ is actually generating functional T cells or maintaining central tolerance. Conversely, blood-based markers such as TRECs or naive T-cell counts are easily skewed by peripheral cell division or chemotherapy-induced lymphopenia. To obtain a comprehensive picture, future evaluations must integrate imaging with molecular and cellular profiling, using standardized benchmarks adjusted for age and sex.

Furthermore, thymic restoration is not merely a numbers game; increasing organ size alone is insufficient. The thymus functions as a training ground; true regeneration requires restoring the entire developmental pipeline. This means ensuring proper progenitor homing, positive selection in the cortex, and negative selection in the medulla, alongside AIRE-dependent antigen expression and regulatory T-cell development. If this reconstruction is incomplete, T-cell numbers may increase without restoration of tolerance. For example, weakening negative selection may release tumor-reactive T cells, but it also carries the risks of unleashing autoimmunity and severe side effects. The goal should be controlled, balanced regeneration rather than indiscriminate expansion.

Safety represents another major challenge. Many agents used to stimulate regeneration - such as RANKL, IL-22, IL-7, FLT3L, growth factors, or sex-steroid blockers - can have systemic side effects or even promote tumor growth.

Avoiding these risks will require targeted delivery, short-term exposure, precise dosing, and long-term monitoring. Finally, timing poses a significant challenge. Generating new T cells is a slow process, lagging far behind the rapid reinvigoration of existing T cells achieved with checkpoint inhibitors. Identifying the optimal therapeutic window will be essential. Therefore, thymic restoration may be most relevant to long-term immune reconstitution, repertoire renewal, and durability of response, rather than to the immediate reinvigoration of pre-existing tumor-reactive T cells.

Future trials should incorporate functional immune endpoints, including recent thymic emigrants, TCR diversity, tumor-reactive clonotypes, infection susceptibility, immunotherapy response, CAR-T-cell persistence, and immune-related toxicity. Patient stratification according to thymic reserve, stromal injury, progenitor availability, metabolic status, and autoimmune risk will likely be necessary. Ultimately, combining thymic biomarkers with tumor-centered indicators may enable a more comprehensive precision-immunotherapy framework and establish thymic restoration as a strategy to improve treatment efficacy, durability, and safety.

## Conclusion

This review has systematically examined the mechanisms, immunological consequences, assessment methods, and therapeutic strategies associated with thymic atrophy in the setting of cancer immunotherapy. Thymic atrophy represents far more than an anatomical alteration. By constraining naive T-cell generation and TCR diversity, driving peripheral T-cell senescence, impairing central tolerance, and diminishing thymic endocrine support, it can influence the efficacy and durability of ICIs and CAR-T cell therapy.

Future investigations should prioritize standardized multimodal evaluation of thymic reserve, prospective clinical validation of thymus-associated biomarkers, and precisely targeted pharmacological, cellular, and tissue-engineering approaches to restore thymus function. Combining thymus-targeted interventions with tumor-centric immunotherapies may facilitate sustained immune reconstitution and improved therapeutic outcomes, positioning thymic regeneration as a valuable component of precision cancer immunotherapy.

## Acknowledgements

We thank Ms Jie Chen (National Key Laboratory of Immunity and inflammation) for technical assistance. This work was funded by the National Key Research and Development Program of China (2024YFC3607500), the National Natural Science Foundation of China (82572005), and Shanghai Oriental Talents Program (BJKJ2024045).

## Disclosure of conflict of interest

None.

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