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REVIEW

CD4$^+$ T cells in cancer: dual roles, exhaustion, and therapeutic breakthroughs

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ABSTRACT In recent years the crucial role of CD4$^+$ T cells in tumor immunomodulation has garnered increasing recognition. While conventional cancer immunotherapy research has predominantly focused on the cytotoxic function of CD8$^+$ T cells, emerging evidence has now shown that CD4$^+$ T cells enhance antitumor immunity by delivering co-stimulatory signals, secreting cytokines, and promoting cytotoxic T lymphocyte (CTL) activation and display unique immunoregulatory capabilities through direct tumor cell killing or remodeling of the tumor microenvironment. The high heterogeneity and functional plasticity of CD4$^+$ T cell subsets significantly influence clinical responses to immunotherapy with underlying mechanisms involving multi-level regulatory networks, including epigenetic modulation and metabolic reprogramming. Deciphering the functional heterogeneity of CD4$^+$ T cells and the interactions with the tumor microenvironment will provide essential mechanistic insights for next-generation immunotherapies, such as immune checkpoint inhibitors and chimeric antigen receptor T (CAR-T) therapies, thereby advancing personalized treatment paradigms.

KEYWORDS Mechanism of differentiation; dual role; T cell exhaustion; immunotherapeutic strategies; translational clinical applications

Introduction

With the emergence of immunotherapy as a promising cancer treatment strategy, CD4$^+$ T cells have attracted significant attention due to a critical role in mediating antitumor immune responses. As a key immunologic component that expresses the T cell receptor (TCR), CD4$^+$ T cells are known to perform cytokine-mediated helper functions and directly lyse target cells. CD4$^+$ T cells possess potent immunomodulatory capabilities with considerable therapeutic potential. CD4$^+$ T cells exhibit a broad range of antitumor effects, including a critical role in acquired immunity, promoting antibody production by B cells, enhancing CD8$^+$ T cell-mediated cytotoxic T lymphocyte (CTL) responses, and maintaining immune homeostasis[1,2]. Moreover, emerging research has further elucidated the functional mechanisms underlying cytotoxic CD4$^+$ T cell subsets in the context of cancer immunotherapy[3]. This manuscript begins with a discussion involving the biological fundamentals of CD4$^+$ T cells, including the activation mechanisms, subset heterogeneity, and differentiation regulatory networks within the tumor microenvironment (TME), to examine the dual roles in the context of cancer, encompassing the capacity to enhance anti-tumor immunity and the detrimental effects in tumor immune escape. Specifically, this review provides a comprehensive analysis of the exhaustion characteristics and mechanisms underlying CD4$^+$ T cells in the TME utilizing single-cell RNA sequencing (scRNA-seq) technology and highlights the interplay between immunologic tolerance and tumor-associated immune resistance. This review also underscores recent clinical advances in immunotherapy, including immunomodulatory strategies targeting CD4$^+$ T cells, with particular emphasis on the therapeutic potential of CD4$^+$ CTL subsets. Through these novel analyses, the review presents a comprehensive conceptual framework for the multifaceted roles of CD4$^+$ T cells in cancer immunotherapy, offering new perspectives for the development of future clinical applications and therapeutic strategies.

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The biological characteristics of CD4+ T cells

Activation mechanisms underlying CD4+ T cell-mediated antitumor immunity

Tumor immunotherapy induces durable antitumor responses by activating T cells, which begins with the release of tumor-associated antigens (TAAs) from dying tumor cells. These TAAs are presented to naïve T cells in tumor-draining lymph nodes (TDLNs), which initiate the activation of tumor-specific CD4+ T cells necessary for systemic antitumor immunity[4,5]. Naïve T cells are derived from hematopoietic stem cells in the bone marrow and mature in the thymus, where cytokines and stromal cells support T cell development[6]. T cell maturation is a multi-step process, during which the expression of a functional TCR is a pivotal event[7]. This TCR specifically recognizes peptide-major histocompatibility complex (MHC) complexes presented by antigen-presenting cells (APCs), such as conventional type 1 dendritic cells (cDC1s) and cDC2s. These APCs capture tumor antigens from the TME and migrate to TDLNs for antigen presentation, representing the first signal required for CD4+ T cell activation[8,9]. The second signal for activation is co-stimulation, which primarily occurs *via* CD28 receptor binding to CD80/CD86 molecules on APCs. This interaction activates signaling pathways, including PI3K/AKT/mTOR and NF-κB[10]. Moreover, the inducible co-stimulator (ICOS), a member of the CD28 family, and the ICOS ligand (ICOSL) enhance cytokine production, thereby contributing to T cell activation[11,12]. Co-stimulatory molecules in the tumor necrosis factor receptor (TNFR) superfamily, such as CD27, CD40, and OX40, also provide additional co-stimulation by binding to respective ligands, thereby exerting effects similar to the CD28 family[13-15]. This binding contributes to the activation of CD4+ T cells and CD8+ CTLs. A third signal, mediated by cytokines in the TME, is involved in influencing the differentiation of CD4+ T cells[16]. This process involves conventional signal transduction and nutrients, which act as signaling molecules that help regulate T cell function. Some studies suggest that nutrients may serve as a fourth signal licensing T cell immunity, although this concept has not gained widespread acceptance (**Box 1**).

Heterogeneous characteristics of CD4+ T cell subsets

CD4+ helper T (Th) cells represent a heterogeneous population that can differentiate into various subsets in response to cues from the TME. This plasticity enables CD4+ T cells to serve as central coordinators of antitumor immune responses. The classification of CD4+ T cell subsets originated from seminal studies involving Th1/Th2 cells[30]. Th1 and Th2 cells are defined by signature cytokine secretion profiles, as follows: Th1 cells primarily produce interferon-γ (IFN-γ), interleukin-2 (IL-2), and tumor necrosis factor-alpha (TNF-α); and Th2 cells are characterized by the production of IL-4, IL-5, and IL-13. The introduction of the Th1/Th2 paradigm marked a new phase in the study of CD4+ T cell subsets. To date, several conventional subsets have been identified within the TME,

Box 1 Nutrients: signal 4 in T cell immunity

Signaling pathways are crucial during CD4+ T cell activation and differentiation but after activation immune cells undergo metabolic reprogramming from oxidative phosphorylation (OXPHOS) to aerobic glycolysis. Recent studies emphasize the importance of nutrients in regulating T cell immune responses, influencing activation, differentiation, and antitumor immunity. In addition to signals 1–3, T cells utilize nutrient availability and metabolism to establish a distinct 'signal 4' through coordinated transport, sensing, and signaling[17-19]. Upon encountering tumor antigens, naïve T cells are activated *via* the TCR and CD28, initiating the PI3K/AKT/mTOR pathway. This pathway drives metabolic reprogramming, enhancing glycolytic factors, like MYC and hypoxia-inducible factor-1 alpha (HIF-1α), and increases glucose uptake through glucose transporters (GLUTs)[20]. GLUT1 deficiency impairs glucose uptake and glycolysis, affecting effector T cell survival and differentiation. However, regulatory T cells (Tregs) are less affected. Excessive GLUT1 expression disrupts Forkhead box protein P3 (FOXP3), destabilizing the Treg lineage[21]. Metabolic reprogramming also involves amino acids and fatty acids. Leucine and glutamine promote Th1 and Th17 differentiation by regulating DNA and histone methylation. Th17 cells are less dependent on glutamine but rely on leucine to suppress Th1 differentiation[22,23]. Tregs mainly depend on fatty acid oxidation (FAO) and exhibit increased AMP-activated protein kinase (AMPK) activity, supporting survival and stability[24]. Tumor cells consume nutrients and produce metabolic byproducts, like lactate, which accumulates in the TME. Lactate enhances Treg function and promotes Treg differentiation from naïve CD4+ T cells, while inhibiting Th1 and Th17 differentiation, contributing to an immunosuppressive environment that favors tumor progression[25]. Mechanistically, lactate decreases Th1 cell levels by inducing deacetylation and degradation of T-bet *via* sirtuin 1(SIRT1)[26]. Lactate also activates lactate dehydrogenase A (LDHA) in naïve CD4+ T cells, converting alpha-ketoglutaric acid (α-KG) to 2-hydroxyglutaric acid (2-HG), which inhibits ATP5B, suppressing mTOR, HIF-1α, and FOXP3 degradation. This cascade favors Treg differentiation and inhibits Th17 differentiation[27,28]. Recent studies have also explored the role of lactate in promoting Treg differentiation through a transforming growth factor-beta (TGF-β)-dependent mechanism[29]. These findings highlight the complex relationship between T cell metabolism and immune regulation, providing new opportunities for optimizing antitumor therapies by targeting nutrient pathways.

including Th1, Th2, Th17, Th9, Th22, follicular helper T (Tfh) cells, and Treg cells (**Figure 1**)[31]. These subsets form a broad and intricate network, underscoring the diverse and complex roles of $CD4^+$ T cells in the tumor context.

The advent of scRNA-seq and spatial transcriptomics has greatly advanced our understanding of $CD4^+$ T cell heterogeneity. scRNA-seq enables high-resolution profiling of individual T cells, uncovering distinct transcriptional states and functional potentials. Among commonly used platforms, 10× Genomics (Pleasanton, CA, US) offers high-throughput surface marker analysis, while Smart-seq2 (Illumina, San Diego, CA, US) provides greater sensitivity for low-abundance and full-length transcripts. These technologies have facilitated the discovery of novel $CD4^+$ T cell subsets and states in human cancers[32]. scRNA-seq revealed two distinct $CD4^+$ T cell subsets in bladder cancer with differing clinical significance, as follows: PD-1^{hi}CD200^{hi} cells exhibit pro-angiogenic properties and mediate therapeutic resistance *via* the UTP–P2RY6 axis; and PD-1^{hi}CD200^{low} cells are associated with improved clinical outcomes[33]. Integrated single-cell RNA and TCR sequencing also delineated $CD4^+$ CTLs, including granzyme B $(GZMB)^+$ and granzyme K $(GZMK)^+$ subsets, which are capable of killing MHC-II-restricted tumor cells[34,35]. scRNA-seq further facilitated the identification of rare immunosuppressive Treg populations in non-small cell lung cancer (NSCLC), such as tumor necrosis factor receptor superfamily member 9 $(TNFRSF9)^+$ Tregs, which overexpress inhibitory molecules, including layilin (LAYN) and correlate with poor prognosis[36]. Spatial transcriptomics enables the identification of distinct cell populations, while preserving spatial localization within tissue and providing critical data on the relationships between cellular function, phenotype, and anatomic context within the TME[37]. Spatial transcriptomics in pancreatic ductal adenocarcinoma revealed that chemokine (C-X-C motif) ligand 13 $(CXCL13)^+$ effector $CD4^+$ T cells were enriched at the tumor periphery. These $CXCL13^+$ $CD4^+$ T cells exhibited a more favorable response to immunotherapy compared to T cells within the tumor core[38]. Importantly, several of these transcriptionally distinct populations have shown potential as clinical biomarkers. For example, circulating $CXCR3^+$ $CCR6^+$ $CD4^+$ T cells have emerged as a non-invasive predictive indicator of response to anti-PD-1 therapy[39]. In summary, these findings highlight the profound heterogeneity and functional plasticity of $CD4^+$ T cells within the TME.

Differentiation regulatory networks of $CD4^+$ T cells in the TME

The differentiation of $CD4^+$ T cells is governed by specific stimulation conditions that modulate the expression of key transcription factors, thereby determining the developmental trajectory and subsequent production of cytokines. The diversity of the cytokine signal underlies the functional plasticity of $CD4^+$ T cells, driving the proliferation and polarization into distinct effector subsets. Naïve $CD4^+$ T cells can differentiate into various subsets depending on the signals encountered. This differentiation is not a rigid, linear progression but rather a dynamic and tightly regulated struggle that is orchestrated by master transcription factors and shaped by complex signaling networks and molecular interactions[40]. The differentiation of Th1 cells provides a classic paradigm of cytokine-directed Th cell specialization. This process requires two essential cytokines (IL-12 and IFN-γ)[41]. Upon cytokine stimulation, specific members of the signal transducer and activator of transcription (STAT) family are activated to drive lineage commitment[42]. The Th1 differentiation program involves the following: neutralization of IFN-γ (the characteristic cytokine of Th1 cells) *in vitro* significantly impairs Th1 differentiation in the IFN-γ/STAT1 pathway; the IL-12/STAT4 pathway cooperates with IFN-γ signaling; and synergistic induction of the major transcription factor, T-box expressed in T cells (T-bet) suggests core transcriptional regulation.

Th1 cell differentiation is negatively regulated by Th2-polarizing cytokines, particularly IL-4 and the master transcription factor, GATA3. Recent studies have identified additional cytokine-mediated mechanisms that extend beyond classical differentiation pathways. For example, Wen et al. demonstrated that IL-16 activates GSK signaling suppresses glutaminase activity in Th1 cells, thereby preventing glutamine hydrolysis into glutamate and elevating intracellular glutamine levels. This metabolic reprogramming promotes antitumor Th1 differentiation and enhances the efficacy of PD-L1 blockade. Clinically, high IL-16 expression correlates with significantly improved survival in immune checkpoint blockade (ICB)-treated patients, positioning IL-16 as a predictive biomarker and a potential co-target for immunotherapy[43]. In addition to cytokines and lineage-specifying transcription factors, other regulatory elements modulate $CD4^+$ T cell fate. Interferon regulatory factors (IRFs), especially IRF-1 and IRF-2, are induced by IFN-γ and act as transcriptional repressors to inhibit Th2 differentiation[44]. An alternative IRF1 isoform, IRF1Δ7, impairs full-length IRF1 activity at the Il12rb1 promoter, resulting in diminished IFN-γ secretion by Th1 cells. IRF1Δ7 is enriched in the TME, the inhibition of which has been shown to potentiate Th1-mediated antitumor responses[45]. Runt-related transcription factor (RUNX) has key roles in T cell development by repressing $CD4^+$ T cell gene expression in naïve T cells, thereby favoring $CD8^+$ T cell lineage commitment. RUNX3 modulates CTL differentiation within the TME by limiting T-bet overexpression. This restraint on terminal differentiation helps preserve the memory potential of nascent CTLs[46,47]. The differentiation of Th1 cells is orchestrated by a T-bet-centered transcriptional network that integrates signals from STAT family proteins, RUNX factors, and other

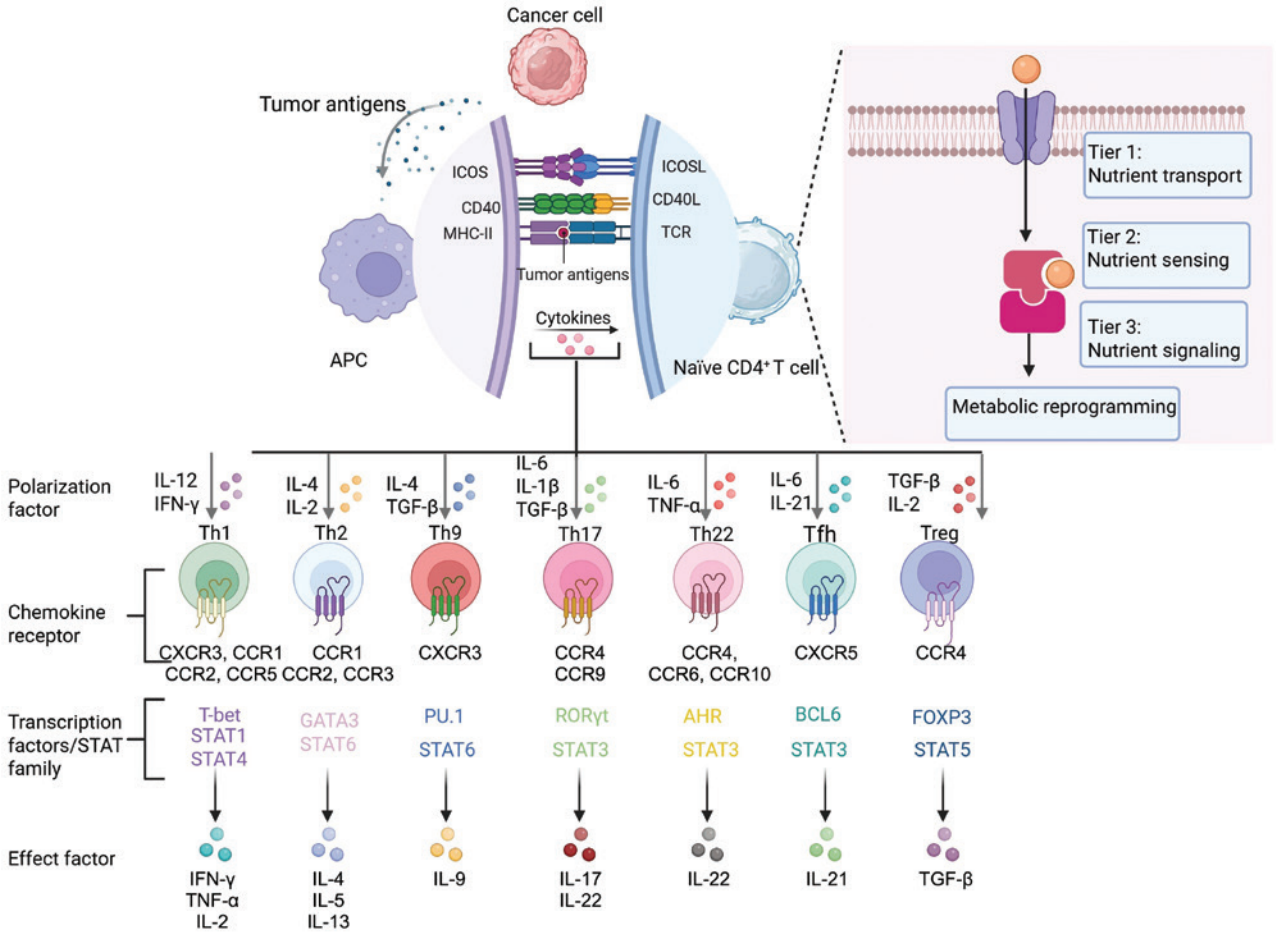


Figure 1 Activation and differentiation mechanisms underlying major CD4⁺ T cell effector subsets. APCs capture the tumor antigens presented by tumor cells and present the tumor antigens to the TCR on the surface of naïve T cells *via* the MHC-II, serving as the first signal for the activation of CD4⁺ T cells. The co-stimulatory signals between the ligands and receptors expressed on APCs and T cells serve as the second signal for CD4⁺ T cell activation, such as the interaction between CD40L\ICOSL expressed on CD4⁺ T cells and the homologous receptor CD40\ICOS on APCs. Cytokines in the TME act as the third signal to activate distinct signaling pathways in CD4⁺ T cells. The nutrients are transported, sensed, and signal pathways act as the fourth signal to initiate metabolic reprogramming. **Th1 cell differentiation**: polarization factors (IFN-γ and IL-12) promote the expression of chemokine receptors (CXCR3\CCR1\CCR2\CCR5) of the Th1 subgroup and promote the expression of the master transcription factor (T-bet) through STAT1/STAT4 and eventually secrete IFN-γ, TNF-α, and IL-2. **Th2 cell differentiation**: IL-2 and IL-4 promote the expression of chemokine receptors (CCR1\CCR2\CCR3) of the Th2 and promote the expression of the GATA3 through STAT6 and eventually secrete IL-4, IL-5, and IL-13. **Th9 cell differentiation:** IL-4 and TGF-β promote the expression of chemokine receptors (CXCR3) of the Th9 and promote the expression of the PU.1 through STAT6 and eventually secrete IL-9. **Th17 cell differentiation:** IL-6, IL-1β, and TGF-β promote the expression of chemokine receptors (CCR4\CCR9) of the Th9 and promote the expression of the RORγt through STAT3 and eventually secrete IL-17 and IL-22. **Th22 cell differentiation:** IL-6 and TNF-α promote the expression of chemokine receptors (CCR4\CCR6\CCR10) of the Th22 and promote the expression of the AHR through STAT3 and eventually secrete IL-22. **Tfh cell differentiation:** IL-6 and IL-21 promote the expression of chemokine receptors (CXCR5) of the Th22 and promote the expression of the BCL6 through STAT3 and eventually secrete IL-21. **Treg cell differentiation:** TGF-β promotes the expression of chemokine receptors (CCR4) of the Treg and promote the expression of the FOXP3 through STAT5 and eventually secrete TGF-β. AHR, aryl hydrocarbon receptor; APC, antigen-presenting cell; BCL6, B-cell lymphoma 6 protein; CCR1, C-C motif chemokine receptor 1; CD40L, CD40 ligand; CXCR3, C-X-C motif chemokine receptor 3; FOXP3, forkhead box P3; GATA-3, GATA binding protein 3; ICOS, inducible T-cell co-stimulator; IFN-γ, interferon gamma; IL, interleukin; MHC, major histocompatibility complex; PU.1, purine-rich box 1; RORγt, RAR-related orphan receptor gamma, isoform t; STAT, signal transducer and activator of transcription; T-bet, T-box transcription factor; TCR, T cell receptor; Tfh, follicular helper T cells; Th, T helper cell; TNF-α, tumor necrosis factor alpha; Treg, regulatory T cells. The figure was created with BioRender.com.

modulators. This regulatory framework ensures lineage commitment and functional competence of Th1 effector cells.

Multi-dimensional roles of CD4+ T cells in tumor immunology

Molecular basis of direct tumor cell cytotoxicity by CD4+ T cells

CD4+ CTLs

CD4+ CTLs are distinguished from conventional CD4+ T cells by an ability to directly lyse target cells, complementing the cytotoxicity of CD8+ T cells. Although CD4+ CTLs exhibit lower effector potency compared to CD8+ CTLs, CD4+ CTLs still mediate significant tumoricidal activity, particularly when present in large numbers within the TME. This functional compensation becomes especially important in cases of tumor MHC-I loss, in which CD8+ T cell responses are impaired[48]. A study by Chun et al. found no significant difference in CD8+ T cell infiltration between tumor and normal tissues. However, CD4+ T cells were more abundant in tumors and exhibited distinct compartmentalization. CD4+ CTL subsets were enriched in tumors with the following two specialized populations identified: GZMB+ (9% of FOXP3− CCR7− CD4+ T cells); and GZMK+ (16% of FOXP3− CCR7− CD4+ T cells). Bladder cancer cells maintained MHC-II expression, allowing recognition by CD4+ CTLs. Flow cytometry of 11 other bladder tumor specimens confirmed that CD4+ CTLs produced effector cytokines and underwent clonal expansion[49]. These tumor-infiltrating CD4+ T cells demonstrated cytotoxic activity, inducing tumor cell apoptosis. Moreover, the infiltration of CD4+ CTLs correlated with clinical responses to anti-PD-1 therapy, a finding replicated in NSCLC studies, underscoring the critical role of CD4+ CTLs in enhancing checkpoint immunotherapy efficacy[50]. CD4+ CTLs represent a unique subset of CD4+ T cells endowed with antigen-specific cytotoxic capacity. However, this population currently lacks definitive surface markers, making the identification of discriminative markers to distinguish CD4+ CTLs from other Th subsets an active area of investigation. CD4+ CTLs share key transcriptional regulators with Th1 cells, including T-bet and RUNX3 expression. Notably, although Th1-polarizing cytokines, like IL-12, can potentiate CD4+ CTL cytotoxicity, these Th1-associated features are dispensable in CD4+ CTL development and function. Analysis of CD4+ CTL origins revealed that the differentiation of CD4+ CTLs depends on IL-2. Preclinical murine melanoma models demonstrated that IL-2-induced BLIMP-1 expression in CD4+ T cells promotes CTL differentiation *via* STAT5, although BLIMP-1 deficiency only partially reduces the effector capacity of CD4+ CTLs[51]. IL-2 also upregulates CRTAM expression, enabling CD4+ T cells to acquire cytotoxicity through an Eomesodermin (EOMES)-dependent pathway. Targeting CRTAM enhances CD4+ CTL function in preclinical settings because CRTAM+ CD4+ CTLs exhibit stronger cytotoxic activity in murine TC-1 lung cancer models with the CRTAM+ CD4+ CTL infiltration closely correlated to reduced tumor size. However, the transient nature of CRTAM expression limits utility as a reliable marker. Overall, identifying precise and definitive markers remains a key focus in CD4+ CTL research[52,53]. CD4+ CTLs are widely present in humans and mice. CD4+ CTL differentiation is programmed according to environmental requirements and consequently produces different direct cell killing effects. CD4+ CTL can produce cytotoxicity through direct expression of GZM, perforin (PRF), and other granule-associated proteins (**Figure 2**)[54,55]. Studies in melanoma have shown that CD4+ CTLs mediate tumor cell apoptosis through the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) pathway[56].

Cytokines

IFN-γ has direct antiproliferative and pro-apoptotic effects by reprogramming genes involved in cell cycle regulation, proliferation, and metabolism. IFN-γ inhibits cell cycle progression, suppresses tumor proliferation, induces cell quiescence, promotes senescence, and triggers apoptosis *via* classical signaling pathways[57,58]. Immunohistochemical analysis of brain tumor samples has shown that IFN-γ reduces endothelial cell density and induces vascular disruption, leading to tumor necrosis. Angiogenesis is regulated by a balance of pro- and anti-angiogenic factors, with VEGF serving as a key pro-angiogenic molecule in tumors. Tumor-associated macrophages (TAMs) are major VEGF producers. Studies have shown that IFN-γ downregulates VEGF in brain tumors, suppressing angiogenesis and promoting vascular disruption and necrosis. Additionally, IFN-γ enhances monocyte/macrophage infiltration into tumors while inhibiting TAM differentiation, thereby reducing TAM polarization and VEGF expression, ultimately attenuating tumor angiogenesis[59-62].

Tumor necrosis factor-α (TNF-α) is produced by CD4+ Th1 cells and exerts biological effects by binding to tumor necrosis factor receptor 1 (TNFR1) and TNFR2. TNF-α activates signaling pathways by binding to TNFR1, including MAPK and NF-κB, which transmit pro-apoptotic signals in tumor and endothelial cells, thereby disrupting the structure and function of tumor vasculature[63]. In addition, TNF-α has been shown to play a crucial role in regulating the cell cycle, influencing cell proliferation and growth[64]. Although research on cytokines has mainly focused on individual factors, the combined effects of TNF-α and IFN-γ in anti-tumor immunity and therapy have not been established. Studies have shown that the TNF-α and IFN-γ combination enhances tumor cell killing[65]. TNF-α and IFN-γ exhibit anti-tumor effects *in vitro* and *in vivo* with recombinant TNF-α and IFN-γ showing antiproliferative effects on pancreatic cancer cells[66]. TNF-α and

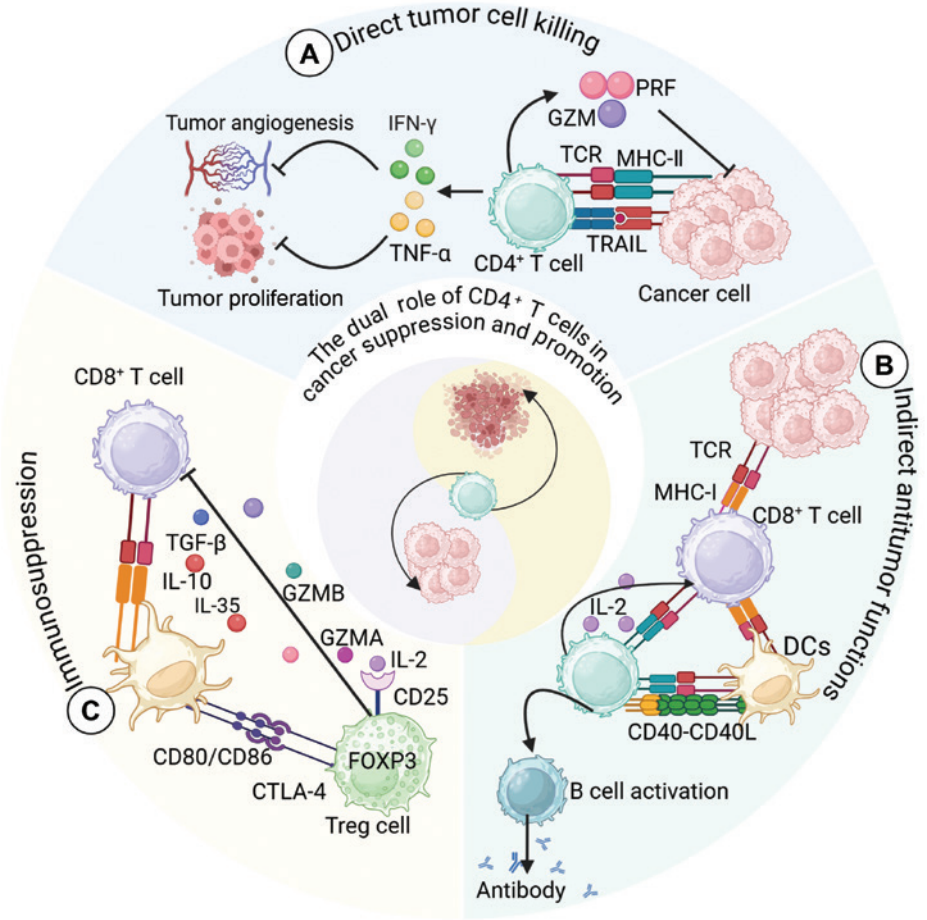


Figure 2 The dual role of CD4⁺ T cells in tumor immunity. CD4⁺ T cells have complex roles in tumor immunity, including direct killing of tumor cells, indirect antitumor functions, and immunosuppressive effects. (A) **Direct tumor cell killing.** CD4⁺ T cells directly interact with and kill cancer cells in an MHC-dependent manner or *via* the TRAIL pathway or by secreting GZM/PRF to induce tumor cell cytotoxicity. Furthermore, CD4⁺ T cells can secrete cytokines, such as IFN-γ or TNF-α, to inhibit tumor angiogenesis and the proliferation of tumor cells. (B) **Indirect antitumor functions.** CD4⁺ T cells secrete IL-2 to enhance the effector function of CD8⁺ T cells or promote CD8⁺ T cell activation through interactions with dendritic cells. Additionally, CD8⁺ T cells stimulate antibody production by activating B cells. (C) **Immunosuppression** FOXP3-expressing Treg cells inhibit or kill CD8⁺ T cells by competitively binding to co-stimulatory receptors (CD80/CD86) on dendritic cells through the expression of inhibitory receptors, such as CTLA-4, or by secreting cytokines, such as TGF-β, IL-10, IL-35, GZM, and PRF. Additionally, Treg cells can competitively acquire IL-2, thereby preventing IL-2 signaling in effector T cells. CTLA-4, cytotoxic T-lymphocyte antigen 4; DC, dendritic cell; GZM, granzyme; PRF, perforin; TGF-β, transforming growth factor beta; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

IFN-γ promote NF-κB-mediated apoptosis in colorectal cancer by enhancing Fas expression[67]. Nitric oxide and PI3K signaling are involved in the synergistic pro-apoptotic effects[68]. In addition, the TNF-α/IFN-γ combination induces tumor cell senescence by altering cell cycle regulation and inhibiting tumor growth through blood vessel normalization[69]. Studies on the mechanism of combined application of TNF-α and IFN-γ have revealed that IFN-γ enhances the sensitivity of endothelial cells to TNF, inducing selective killing of tumor endothelial cells by TNF and inhibiting tumor growth[70].

Synergistic enhancement mechanisms of CD4⁺ T cells on CD8⁺ T cell anti-tumor function

CD4⁺ T cells are essential in facilitating the proliferation and differentiation of CD8⁺ T cells. This helper function is exemplified by enhanced recruitment efficiency, increased proliferative capacity, and improved effector functions of CD8⁺ T cells within the TME.

Th cells activate CD8+ T cells

The maintenance of CD8+ T cell quantity and functional competence depends on IL-2-producing Th cells. Adoptive transfer experiments have demonstrated that Th cells isolated from IL-2$^{-/-}$ mice fail to induce tumor regression in tumor-bearing recipients. In contrast, Th cells derived from IL-2$^{+/+}$ mice effectively suppress tumor growth[71]. Bos et al. demonstrated that IL-2 produced by tumor-specific CD4+ T cells promotes CD8+ T cell proliferation and GZMB expression, while IFN-γ-induced chemokines from these CD4+ T cells accelerate CD8+ T cell recruitment. These findings collectively indicated that tumor-specific CD4+ T cells significantly enhance intratumoral CD8+ T cell recruitment, expansion, and effector function. Conversely, CD8+ T cells exhibit impaired survival and clonal expansion in the absence of CD4+ T cell help[72]. CX3CR1-expressing CD8+ T cell subset exhibits potent cytolytic activity. The development of this cytotoxic population is critically dependent on CD4+ T cell-derived IL-21. This developmental pathway can be therapeutically exploited to enhance the tumoricidal capacity of tumor-infiltrating CD8+ T cells[73]. Subsequent studies revealed that the basic leucine zipper ATF-like transcription factor (BATF), a downstream transcription factor of IL-21 signaling, has a critical role in the mechanism underlying IL-21-mediated CD4+ T cell help for CD8+ T cells. CD4+ T cells operating through the IL-21-BATF axis are essential for sustaining the effector function of tumor-infiltrating CD8+ T cells[74]. Espinosa-Carrasco et al. also noted that the effective activation and killing function of tumor-specific CD8+ T cells depend on the formation of a triad with CD4+ T cells and antigen-presenting cells because this "iron triangle" combination leads to reprogramming of intratumoral CD8+ T cells and even reversal of the dysfunction, thereby exerting cytotoxic effects and eliminating tumors[75]. In addition, it has been reported that CD8+ T cells directly receive help from CD4+ T cells *via* CD40-CD40L as the basis for CD8+ T cell memory generation[76].

CD4+ T cells amplify the antigen presentation process

CD4+ T cells assist in initiating the gene expression program of CD8+ T cells and enhancing CTL function through multiple molecular mechanisms. The antigen-specific contact between CD4+ T cells and DCs enables the DCs to optimize antigen presentation and deliver specific cytokines and co-stimulatory signals to CD8+ T cells. This process indirectly promotes the clonal expansion of CD8+ T cells and the differentiation into effector or memory T cells. As early as the 1980s, researchers discovered that DCs also require signals from CD4+ T cells, such as CD40-CD40L, to acquire an enhanced ability to activate naïve CD8+ T cells, a process referred to as "T cell help"[77]. Recent studies have suggested that activated CD4+ T cells enable human *ex vivo* cDC1s to induce CTL responses against tumor-associated antigens. These findings highlight the critical role of CD4+ T cells in supporting cDC1 function within the TME and suggest establishing cDC1 transcriptomic signatures as diagnostic markers for cancer[78]. Studies have demonstrated that this enhancement mechanism mediated by CD4+ T cells must collaborate with innate immune stimulation. Pretreatment with innate immune signaling endows DCs with the ability to more rapidly and efficiently express multiple co-stimulatory molecules or bioactive mediators by enhancing the activity of transcription factors (p65 and IRF1) after receiving CD40 stimulation from CD4+ T cells, which subsequently optimizes CD8+ T cell immune responses. Moreover, DCs that are primed by T cells enable CD8+ T cells to exhibit superior cytotoxic capabilities[79].

Borst et al.[80] systematically summarized three models of the roles of innate signals and CD4+ T cell helper signals in CTL priming: innate signals and CD4+ T cell helper signals are comparable with similar effects on CTL priming (redundancy model); CD4+ T cells amplify innate signals delivered to DCs in the second phase of CTL priming through helper function (amplification model); and CD4+ T cell helper signals and innate stimulation deliver distinct signals to DCs (complementary model). Each signal influences effector and memory differentiation pathways in unique ways, collectively determining the strength of effector and memory CTL responses.

These findings suggest that innate immune signals cannot replace CD4+ T cell help for optimal CTL quality, further highlighting the unique role of CD4+ T cells.

Regulatory pathways of CD4+ T cells in driving B cell humoral immune responses

The interaction between CD4+ T cells and B cells is primarily mediated by Tfh cells, a subset of CD4+ T cells that is characterized by high CXCR5 expression. Tfh cells support the formation of germinal centers (GCs), as well as the maturation, differentiation, and antibody production of B cells, thus having a critical role in maintaining immune homeostasis[81]. Cancer research has established a strong link between Tfh cell responses and anti-tumor immunity. Multiple studies have shown a positive correlation between the presence of Tfh and B cells and prolonged survival and improved prognosis in various tumors, particularly in breast cancer[82,83]. There are three potential mechanisms through which Tfh cells exert protective functions in infection and cancer.

- Promoting tertiary lymphoid structure (TLS) formation: Tfh cell responses significantly drive the formation of TLSs composed of B cells, T cells, natural killer (NK) cells, and APCs in chronically inflamed non-lymphoid organs. Mature intratumoral TLSs represent anti-tumor structures containing pro-inflammatory cytokines, activated complement cascades, and potent cytotoxic lymphocytes[84,85]. Tumor-infiltrating Tfh cells expressing high levels of CXCL13 and

IL-21 have a critical role in the formation of intratumoral TLSs in close association with CD8+ T and B cell infiltration, as well as prolonged survival in cancer patients[86]. Craft and colleagues identified germinal center B (GC B) and Tfh cells in lung adenocarcinoma (LUAD) tissue through single-cell sequencing. A stronger tumor-specific Tfh and GC B cell response was induced compared to a model without the antigen in a subcutaneous LUAD xenograft model with B cell antigen recognition, highlighting the importance of antigen recognition. Further studies showed that the effector function of CD8+ T cells was compromised in the absence of Tfh or GC B cells. The researchers suggested that IL-21 secreted by Tfh cells have a crucial role. Knocking out the IL-21 receptor reduced CD8+ T cell infiltration and accelerated tumor growth. The study concluded that tumor-specific B cells present tumor antigens and enhance CD8+ T cell function by promoting Tfh cell differentiation and increasing IL-21 production, thereby inhibiting tumor growth[87].

- Enhancing B cell responses and antibody functions: Tfh cells promote B cell GC responses and the production of functional antibodies[88]. Effective anti-tumor immunity depends on antibody-mediated effector functions, such as antibody-dependent cellular cytotoxicity (ADCC), complement activation, and antibody-mediated tumor cell phagocytosis. Tumors with high levels of Tfh cells and mature TLSs typically exhibit a dense population of diverse B cells, plasma cells, and anti-tumor antibodies, which contribute to a robust anti-tumor immune response[89].
- Sustaining memory B cell generation: Tfh cells support the production of memory B cells, which are critical for rapid responses to reinfection and long-term immune protection[90].

Immunosuppressive transformation and pro-tumorigenic effects of tumor-associated CD4+ T cells

The pro-tumorigenic effects of CD4+ T cells have been observed across multiple subsets but are most pronounced in the Treg cell population, which represents the primary immunosuppressive subset of CD4+ T cells[65]. Treg cells precisely regulate immune responses to maintain immune tolerance and prevent chronic inflammation under normal physiologic conditions. However, in the context of cancer the role of Treg cells undergoes a shift. Specifically, in most tumors the presence of Treg cells is associated with poor clinical outcomes because Treg cells inhibit anti-tumor immunity, promote immune evasion, and enhance tumor growth and metastasis[91,92]. Interestingly, the opposite effect has been observed in some cancers, such as colorectal and ovarian cancer, primarily due to the high functional heterogeneity of Treg cells. Treg cells with anti-tumor functions typically express low levels of FOXP3 and secrete inflammatory cytokines[93-95]. Intratumoral FOXP3+ Treg cells are predominantly activated and highly proliferative compared to Treg cells in non-tumor tissues[96]. Several functionally distinct FOXP3+ Treg subsets have been identified, including quiescent Treg, effector Treg (eTreg), pro-inflammatory Treg, and T follicular regulatory (TFR) cells. Among these FOXP3+ Treg subsets, the highly suppressive eTreg subset, which expresses TNFRSF family members, such as OX40, GITR, and 4-1BB, is strongly correlated with resistance to immunotherapy. Four pro-tumorigenic mechanisms of Treg cells have been established.

- Inhibitory receptor-associated mechanisms: Treg cells exert a negative impact on the tumor immune process by expressing a range of co-inhibitory molecules at high levels, including CTLA-4, PD-1, LAG-3, TIM-3, and TIGIT[97]. CTLA-4 competes with the co-stimulatory receptor, CD28, on effector T cells for binding to CD80/CD86 on APCs, thereby inhibiting the co-stimulatory signals required for effector T cell activation[98]. In addition, CTLA-4 induces the internalization and degradation of CD80/CD86 through endocytosis and phagocytosis, preventing these ligands from activating T cell responses and ultimately resulting in T cell anergy[99,100].
- IL-2 sequestration: Treg cells constitutively express the high-affinity IL-2 heterotrimeric receptor containing the IL-2Rα chain, CD25. Compared to resting effector T cells that express low-affinity IL-2 receptors, Treg cells acquire more IL-2 through a process known as "IL-2 sequestration." This process prevents IL-2 signaling in effector T cells, leading to immune suppression[101,102].
- Immunosuppressive cytokine expression: Treg cells induce immunosuppression by producing immunosuppressive cytokines, such as IL-10, IL-33, IL-35 and TGF-β. TGF-β exerts its function on Th cells by inhibiting the transcription of pro-inflammatory cytokines, such as IFN-γ and GZMB, as well as Th cell subset master transcription factors, such as T-bet and GATA-3. TGF-β also suppresses the cytotoxic activity of CD8+ T cells and prevent the ability to traffic to tumours[103].
- Direct cytolysis: Treg cells can form membrane gap junctions through which Treg cells transfer cytolytic molecules (PRF, GZMA, and GZMB), leading to suppression through the killing of effector T cells[104,105].

Functional exhaustion of CD4+ T cells in the TME

The term "exhaustion" was first used to describe non-responsive antigen-specific CD8+ T cells with long-term persistence in mouse models of chronic viral infection. Despite expressing markers associated with T cell activation, these exhausted CD8+ T (CD8+ Tex) cells are unable to kill virally infected cells and fail to produce IFN-γ upon stimulation[106]. Currently, the understanding of CD8+ Tex has been expanded to encompass

impaired effector function, reduced proliferative capacity, elevated expression of negative inhibitory receptors (NIRs), and altered cellular programming[107].

T cell functional impairment significantly impacts physiologic balance and disease progression. However, the current definition of CD4+ T cell exhaustion remains incomplete and is mainly based on CD8+ T cell paradigms (**Figure 3**)[108]. A deeper understanding of shared and distinct features of CD4+ and CD8+ T cell exhaustion is crucial for clinical research (**Figure 3**). Evidence supports CD4+ T cell exhaustion in solid and hematologic malignancies that is characterized by upregulated NIRs (PD-1, CTLA-4, LAG-3, TIM-3, and TIGIT)[109-111]. Reduced marker expression correlates with treatment success in cancer therapy, while increased expression is associated with recurrence[112,113]. Chronic tumor exposure upregulates IRs and suppressive signals (e.g., PD-L1/indoleamine 2,3-dioxygenase 1 [IDO1]) in colorectal cancer, reducing effector molecules and increasing CXCL chemokines, which recruit more suppressive cells. Co-stimulatory signals, like CD86/ICOSL, induce exhaustion in CD4+ T cells and blocking these signals reduces CD4+ Tex. CD4+ T cell exhaustion is driven by direct cell contact and secreted factors in the tumor TME[114,115]. ICB, such as anti-PD-1, restores CD4+ T cell function, promotes clonal expansion, and reduces exhaustion markers, supporting PD-1/PD-L1 blockade in clinical applications[116].

However, upregulation of NIRs alone is insufficient to fully define CD4+ T cell exhaustion. Therefore, it is essential to investigate changes in epigenetics, transcriptomics, and metabolic patterns to gain a comprehensive understanding of CD4+ T cell exhaustion.

CD8+ Tex cells undergo extensive transcriptional and epigenetic reprogramming during chronic infection, disrupting genes related to TCR signaling, migration, and metabolism[117]. This Tex cell phenotype differs significantly from the effector

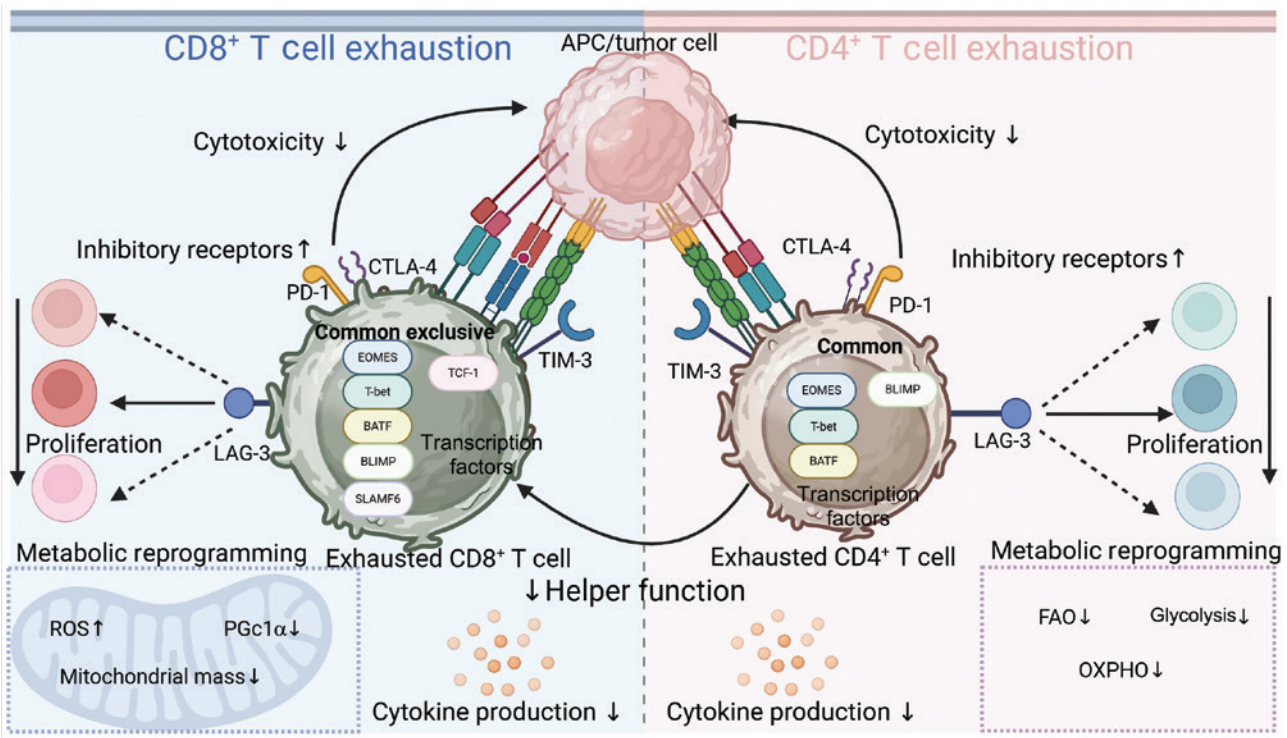


Figure 3 Comparison of characteristics between CD4+ T cell exhaustion and CD8+ T cells. Exhausted CD4+ and CD8+ T cells share the following characteristics. **Effector function:** Reduced cytotoxicity and helper function. **Inhibitory receptors:** Upregulation of inhibitory receptor expression, including PD-1, LAG-3, CTLA-4, and TIM-3. **Proliferation:** CD4+ T cell exhaustion and CD8+ T cells show reduced proliferative abilities. **Cytokines:** Decreased cytokine production. **Expression of the common transcription factors:** EOMES, T-bet, BATF, BLIMP1, and SLAMF6. Differences exist between exhausted CD4+ T cells and exhausted CD8+ T cells. **Exclusive transcription factors:** Exhausted CD8+ T cells express TCF-1. **Metabolic features:** Exhausted CD8+ T cells exhibit features of metabolic reprogramming of mitochondrial function, primarily characterized by upregulation of ROS and downregulation of PGC1α. Exhausted CD4+ T cells show downregulation of FAO, glycolysis, and OXPHOS. BATF, basic leucine zipper ATF-like transcription factor; BLIMP-1, B lymphocyte-induced maturation protein 1; EOMES, eomesodermin; FAO, fatty acid oxidation; LAG-3, lymphocyte-activation gene 3; OXPHOS, oxidative phosphorylation; PD-1, programmed death-1; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; ROS, reactive oxygen species; SLAMF6, signaling lymphocytic activation molecule family member 6; TIM-3, T cell immunoglobulin and mucin domain-containing protein 3.

and memory T cells generated during acute infection. Antigen-specific $CD4^+$ T cells exhibit a unique transcriptional profile in chronic viral infections, suggesting an independent differentiation pathway for $CD4^+$ T cell exhaustion. Although $CD4^+$ Tex cells differ from $CD8^+$ Tex cells, $CD4^+$ Tex cells share key exhaustion-associated transcription factors (e.g., Batf, Tbx21, Eomes, and Prdm1), indicating a potential common regulatory mechanism[118]. Tumor-infiltrating $CD4^+$ T cells lose TCF-1 and Slamf6 expression in melanoma, indicating transient or eventual exhaustion, which is partially reversible at the epigenetic level. PD-L1 blockade restores TCF-1 expression and downregulates TIM-3 and LAG-3[119].

Metabolic reprogramming is a key feature of $CD8^+$ Tex cells. Tex cells exhibit impaired glucose uptake and reduced mitochondrial quality within the TME, both of which are crucial for T cell function[17]. These metabolic defects correlate with the upregulation of NIRs, highlighting a link between T cell exhaustion and metabolic dysfunction. Persistent hypoxia in the TME stimulates peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α)-dependent mitochondrial reprogramming, which further impairs mitochondrial function. The accumulation of reactive oxygen species (ROS) causes cellular damage and accelerates the loss of Tex cell function[120]. Methionine depletion in the TME is identified as a key factor in $CD4^+$ T cell exhaustion. Tumors preferentially uptake methionine through high expression of the SLC43A2 transporter, leading to methionine deficiency in $CD4^+$ T cells. This uptake of methionine causes downregulation of H3K79me2 methylation and upregulation of PD-1 expression. PD-1 upregulation is associated with proteins involved in amino acid and fatty acid metabolism, such as AMPK. In addition, fatty acids and glucose accumulate, while lactate levels decrease, indicating inhibition of glycolysis and fatty acid oxidation[121]. Similar metabolic changes are observed in $CD39^{high}$ $PD\text{-}1^+$ $CD4^+$ Tex cells[122]. Furthermore, erythropoietin-induced $CD4^+$ T cell exhaustion is accompanied by reduced oxidative phosphorylation and the mTOR pathway[123]. While existing evidence highlights metabolic changes in $CD4^+$ Tex cells, a comprehensive understanding of exhaustion-induced metabolic reprogramming is still needed (**Table 1**).

Advances in $CD4^+$ T cell-directed tumor immunotherapy

Enhancing the function of $CD4^+$ T cells can improve CTL responses in cancer immunotherapy. This $CD4^+$ T cell enhancement can be achieved through several approaches. $CD4^+$ T cells have shown significant therapeutic potential in treating various diseases, including infections, autoimmune disorders, and cancer. Researchers are developing methods to modulate $CD4^+$ T cell activity to improve treatment outcomes for these conditions. Current therapeutic strategies targeting $CD4^+$ T cell responses can be broadly classified into passive immunotherapy (e.g., adoptive cell therapy [ACT]), ICB, and $CD4^+$ T cell-targeted vaccines (**Figure 4**). In addition, combination immunotherapies are increasingly used to overcome the limitations of individual treatments by enhancing efficacy or minimizing side effects.

Neoantigen-based $CD4^+$ T cell vaccine development strategies

Cancer vaccines, as a promising area in tumor immunotherapy, aim to activate the immune system, particularly T cells, to target tumor cells expressing tumor-specific antigens (TSAs). TSAs enhance immune recognition and attack tumors through four mechanisms: antigen release; antigen processing and presentation; T cell activation; and the cytotoxic activity of immune effector cells[125].

In vaccine design algorithms predict epitopes for binding to MHC-I molecules, activating $CD8^+$ T cells for direct tumor killing. However, vaccines often activate $CD4^+$ T cells instead, which bind to MHC-II molecules[126]. These $CD4^+$ T cells provide helper signals that enhance $CD8^+$ T cell cytotoxicity. Incorporating MHC-II epitopes in vaccines can boost $CD4^+$ T cell activation and amplify the immune response. It has been shown that $CD4^+$ T cell responses often persist longer than $CD8^+$ T cell responses, offering durable immune protection that helps prevent tumor recurrence[127]. Synthetic long peptides (SLPs), containing MHC class I and II epitopes, induce $CD8^+$ and $CD4^+$ T cell responses. While clinical trials of SLPs show a predominance of $CD4^+$ T cell induction, achieving better therapeutic efficacy may require strategies to enhance $CD8^+$ T cell responses for more effective tumor lysis[128,129].

Emerging evidence also highlights the importance of B cells in cancer vaccines. Activated B cells have a key role in priming and expanding T cells, especially $CD4^+$ T cells. A novel B-cell-targeting nanoparticle vaccine has shown promise in boosting B and T cell responses, leading to enhanced tumor suppression[130]. In combination immunotherapy neoantigen vaccines that stimulate both $CD4^+$ and $CD8^+$ T cells can overcome ICB resistance, remodel the TME, and expand T cell populations for sustained tumor suppression[131]. Future cancer vaccines can be designed to enhance $CD4^+$ T cell responses and overall immune activation by advancing an understanding of MHC-II epitopes and improving prediction tools, thereby improving tumor immunotherapy outcomes.

Immune checkpoint blockade in reprogramming $CD4^+$ T cell anti-tumor functions

ICB therapy targets NIRs on immune or cancer cells to restore effector functions. ICB enhances the ability of the immune system ability to target and eliminate tumor cells by disrupting

Table 1 Surface molecules, key transcription factors, and metabolic features of exhausted CD4+ T cells

Tumor type	Surface molecules	Key transcription factors	Metabolic characteristics
Bladder cancer[33]	CD200, PD1, CTLA4, LAG3, TIGIT, OX40	TCF, TOX	UTP/P2RY6 axis
Acute myeloid leukemia (AML)[114]	PD-1, CTLA-4, TIM-3, LAG-3	Blimp-1, T-bet	
Melanoma[121]	PD-1, CTLA-4 TIM-3, LAG-3	XBP1	Reduced levels of fatty acid and glucose metabolism
Head and neck cancer, cervical cancer, ovarian cancer[122]	CD39, PD-1, TIGIT, CTLA-4, TIM-3, CXCL13	TOX	Extracellular ATP is degraded to AMP
Triple-negative breast cancer[123]	CD39, CD69, CD44, CD103, PD-1, KLRG		Extracellular ATP is degraded to AMP
Gastric cancer[124]	CD39, PD-1, CTLA-4, TIGIT, GITR	IRF4, TOX	

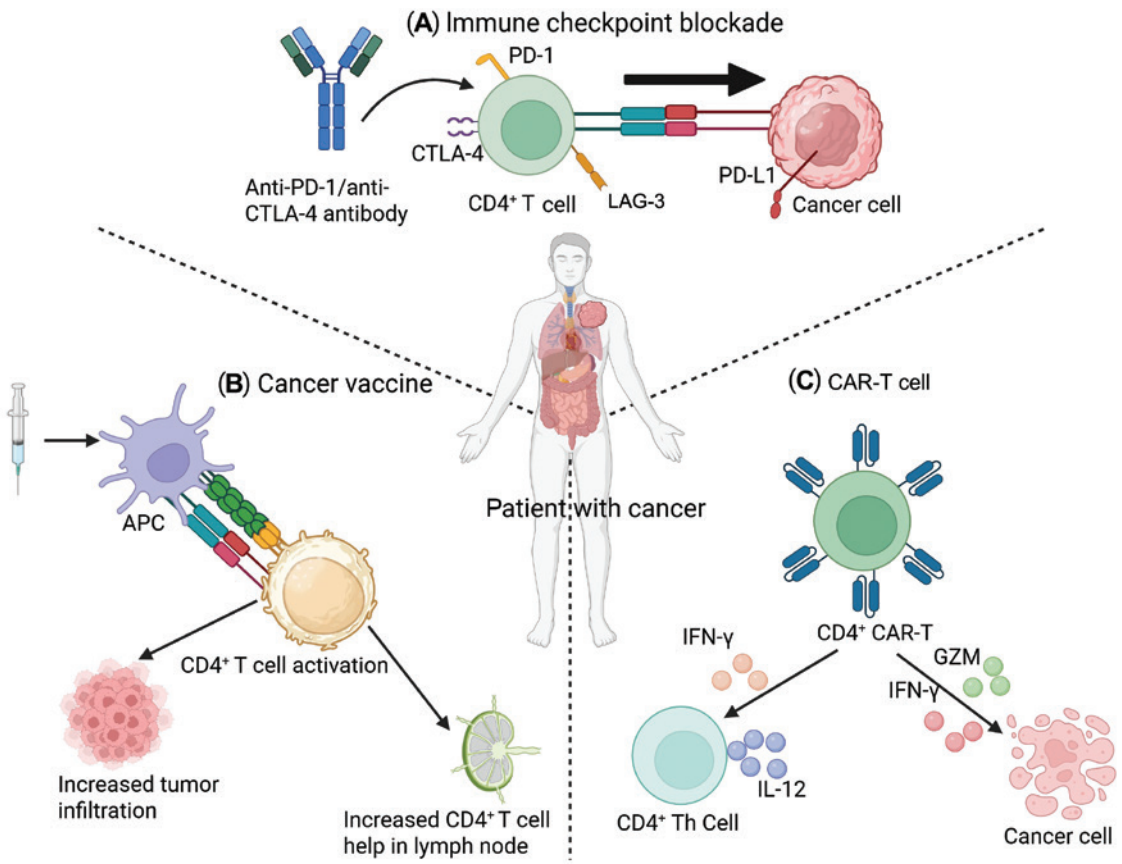


Figure 4 Clinical applications of CD4+ T cells. (A) **Immune checkpoint blockade.** Expression of inhibitory receptors on the surface of CD4+ T cells is primarily suppressed through antibodies targeting PD-1/CTLA-4, thereby enabling immune attack against cancer cells. (B) **Cancer vaccine.** Cancer vaccines activate CD4+ T cells through APC, promoting tumor cell infiltration and the supportive role of CD4+ T cells in lymph nodes. (C) **CAR-T cell.** CAR-T cells activate CD4+ Th cells through the secretion of IFN or induce apoptosis in cancer cells by secreting GZM and IFN-γ. CAR-T, chimeric antigen receptor T cell.

inhibitory signaling pathways and preventing tumor immune evasion[132]. Growing evidence indicates that the pre-treatment status of specific CD4+ T cell subsets has a critical role in determining the efficacy of ICB therapy[133]. CD4+ T cell immune function has a critical role in PD-L1/PD-1 blockade therapy, especially in NSCLC patients. The functional state

of $CD4^+$ T cells directly influences the treatment response. Patients with functional $CD4^+$ T cell immunity typically show a higher objective response rate (approximately 50%), whereas no significant response has been demonstrated in patients with impaired $CD4^+$ T cell function[134]. Notably, different ICB agents have varying effects on $CD4^+$ T cell dynamics. Anti-CTLA-4 antibodies promote the expansion and differentiation of $ICOS^+$ $CD4^+$ Th1-like subsets (FOXP3-negative) within the TME in mouse models of melanoma (B16) and colorectal adenocarcinoma (MC38), whereas anti-PD-1 therapy primarily expands exhausted $CD8^+$ TILs with minimal impact on $CD4^+$ TILs[135]. Several models have demonstrated that PD-1 blockade directly enhances tumor cell killing by promoting $CD4^+$ CTL differentiation[136].

A major limitation of ICIs is the primary resistance to monotherapy. To address this limitation, combination therapies targeting distinct immune checkpoints have been studied. The advantages of combination therapy lie in an ability to significantly enhance the immune system's response to tumors. Single-cell trajectory analysis demonstrated that combination therapy significantly promoted the differentiation of $CD4^+$ T cells from a naïve/central memory phenotype to a Th1 effector phenotype in a cohort of 20 patients with recurrent or metastatic head and neck squamous cell carcinoma. Anti-CTLA4 treatment activated $CD4^+$ T cells in the TDLNs, enhancing the migration to the tumor and functional differentiation. In contrast, monotherapy only expanded the pre-existing $CD8^+$ T cells within the tumor. Spatial transcriptomics revealed that T cell activation zones highly expressed IFN-γ, GZMB, and CXCL9/10 (T cell recruiting factors), co-occurring with antigen presentation signals from DCs. $CD4^+$ T cells act as "organizers" in the immune microenvironment, coordinating the interactions between $CD8^+$ T cells, DCs, and plasma cells, thereby promoting the formation of a functional immune response[137]. In addition to these established targets, anti-LAG-3 antibodies have emerged as a promising new therapy[138,139]. Relatlimab, the first Food and Drug Administration (FDA)-approved LAG-3 inhibitor, established LAG-3 as the third clinically validated immune checkpoint, after PD-1/PD-L1 and CTLA-4. A phase 2/3 randomized trial demonstrated that the combination of relatlimab and nivolumab (anti-PD-1) led to a 47.7% 12-month progression-free survival (PFS) rate in melanoma patients compared to 36% with nivolumab monotherapy. Based on these results, the FDA approved the fixed-dose combination, opdualag (relatlimab/nivolumab), in 2022 for unresectable or metastatic melanoma[139]. Rolig et al. explored the mechanism underlying anti-LAG-3 and anti-PD-1 combination therapy and reported that the combination of PD-1 and LAG-3 inhibitors could overcome resistance to PD-1 blockade in various mouse tumor models. Further investigation revealed that the therapeutic efficacy of this combination treatment was closely related to the instability of the Treg phenotype. Specifically, this instability was characterized by the loss of FOXP3 expression and the conversion of Tregs into effector T cells. Moreover, an increased proportion of unstable Tregs was significantly associated with a higher response rate and improved survival in patients receiving combination therapy in a cohort of 117 patients with metastatic melanoma. This finding suggests that the instability of the Treg phenotype may serve as a potential biomarker for predicting the response to immunotherapy[140].

Technologic breakthroughs and clinical translation in adoptive $CD4^+$ T cell therapy

ACT is an immunotherapy approach that involves collecting the patient's immune cells, genetically modifying or expanding the cells *in vitro* to enhance tumor-targeting capabilities, then reinfusing these activated cells back into the patient to eliminate tumor cells. ACT can be broadly classified into three types based on the delivered cell product: TILs; chimeric antigen receptor T (CAR-T) cells; and T cell receptor-engineered T (TCR-T) cells.

CAR-T cell immunotherapy has achieved remarkable success in treating some hematologic malignancies with growing evidence supporting the critical role of $CD4^+$ CAR-T cells in sustaining therapeutic effects. Studies on CD19-CAR-T cell therapy in patients with chronic lymphocytic leukemia (CLL) have shown that $CD4^+$ CAR-T cells express a variety of cytotoxicity-associated genes and upregulate PRF and GZMA upon *in vitro* stimulation, exhibiting cytotoxic characteristics. These cells are predominant in the peripheral blood of patients who achieve long-term complete remission[141,142]. However, $CD4^+$ CAR-T cells are more effective in activating host immunity compared to $CD8^+$ CAR-T cells. $CD4^+$ CAR-T cells can promote the production of Th cells by generating cytokines, such as IFN-γ and IL-12, but have less direct tumor-killing ability[143]. In addition, Bousso and colleagues reported that the antitumor activity of $CD4^+$ CAR-T cells does not rely on cytotoxicity. Instead, $CD4^+$ CAR-T cells exert indirect, large-scale, and long-distance effects on tumor cells through the production of IFN-γ and selectively killing tumors that are sensitive to IFN-γ-induced apoptosis[144]. Although CAR-T therapy has shown remarkable efficacy in hematologic malignancies, CAR-T therapy application in solid tumors is hindered by the challenging TME, which is characterized by glucose deprivation, hypoxia, and lactic acid accumulation. These metabolic obstacles limit CAR-T cell energy supply, cause metabolic exhaustion, and reduce antitumor efficacy. Notably, recent studies have indicated that FOXP3 interacts with the mitochondrial fission protein (dynamin-related protein 1 [Drp1]) to remodel CAR-T cell metabolism, suppressing glycolysis and OXPHOS while promoting lipid metabolism. This adaptation improves CAR-T cell survival and sustained antitumor function in solid tumors, offering new theoretical and translational avenues for

CAR-T therapy in these settings[145]. CD4+ anti-TGF-β CART cells have also been shown to directly kill solid tumors by producing GZMB and IFN-γ and the ability to reduce T cell exhaustion. Relevant clinical trials are ongoing[146].

In addition, Kruse et al. showed that adoptive CD4+ T cell therapy, when combined with innate immune stimulation, reprograms monocyte populations in the TME, enabling indirect tumor killing[147]. This CD4+ T cell-mediated indirect antitumor mechanism provides a rationale for combining adoptive T cell therapy with innate immune stimulation to promote the regression of established tumors. The *in vitro* and *in vivo* experimental results of engineered CD4 TCR T cells targeting the conserved high-affinity TCR for NY-ESO-1 demonstrated significant tumor regression with no apparent off-target toxicity. This finding greatly expands the potential patient population who could benefit considering the widespread expression of the targeted antigen across various cancers[148].

Conclusions

With the continuous advances in cancer immunotherapy the regulation of CD4+ T cells has become a critical factor in improving therapeutic efficacy and overcoming immune resistance. In-depth research on the activation mechanisms, metabolic pathways, and exhaustion characteristics of CD4+ T cells is expected to uncover new therapeutic targets, thereby driving the clinical application of immunotherapy. This review discusses the critical role of CD4+ T cells in tumor immunity with a focus on the heterogeneity of CD4+ T cell subsets and the broad functional implications, as revealed based on scRNA-seq. By analyzing the dual role of CD4+ T cells in tumor immunity this article highlights the positive contributions to anti-tumor immunity but also points out the potential immunosuppressive effects in tumor progression.

However, research on CD4+ T cells is still underdeveloped in many areas compared to CD8+ T cells. For example, the transcriptional and epigenetic mechanisms underlying CD4+ Tex cells are not fully understood and the metabolic characteristics remain inadequately described. These gaps in research partially limit the efficacy of CD4+ CAR-T cells in treating solid tumors.

Metabolic reprogramming offers vast potential for further exploration in tumor immunity, especially in regulating immune responses. Therapeutic approaches targeting T cell metabolism may provide new breakthroughs for optimizing existing anti-tumor strategies. The combined application of metabolic inhibitors and immunotherapy may offer more effective ways to modulate CD4+ T cell anti-cancer mechanisms. Moreover, the advent of multiplexed error-robust fluorescence *in situ* hybridization (MERFISH) technology, which allows high-resolution detection of spatial heterogeneity in gene expression and provides robust support for future studies on the dynamic changes of CD4+ T cells and the role in the TME.

In conclusion, with the ongoing research into the functions and metabolic characteristics of CD4+ T cells, coupled with the application of emerging technologies, cancer immunotherapy is expected to achieve more precise and effective breakthroughs in the future.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

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Performed the analysis: Siwei Pan.
Wrote the paper: Yangyang Zhang, Jingli Xu.

References

1. Topchyan P, Lin S, Cui W. The role of CD4 T cell help in CD8 T cell differentiation and function during chronic infection and cancer. Immune Netw. 2023; 23: e41.
2. Bonilha CS. The synapse revisited: molecular networks of CD4 T cell–DC interactions. Int Immunopharmacol. 2025; 167: 115715.

3. Malyshkina A, Brüggemann A, Paschen A, Dittmer U. Cytotoxic $CD4^+$ T cells in chronic viral infections and cancer. Front Immunol. 2023; 14: 1271236.
4. Chopp L, Redmond C, O'Shea JJ, Schwartz DM. From thymus to tissues and tumors: a review of T-cell biology. J Allergy Clin Immunol. 2023; 151: 81-97.
5. Koukourakis MI, Giatromanolaki A. Tumor draining lymph nodes, immune response, and radiotherapy: towards a revisal of therapeutic principles. Biochim Biophys Acta Rev Cancer. 2022; 1877: 188704.
6. Cardenas MA, Kissick HT. Stem-like cells at the center of CD4 T cell differentiation. Trends Cell Biol. 2025. https://doi.org/10.1016/j.tcb.2025.06.004.
7. Dutta A, Zhao B, Love PE. New insights into TCR β-selection. Trends Immunol. 2021; 42: 735-50.
8. Wculek SK, Cueto FJ, Mujal AM, Melero I, Krummel MF, Sancho D. Dendritic cells in cancer immunology and immunotherapy. Nat Rev Immunol. 2020; 20: 7-24.
9. Yang K, Halima A, Chan TA. Antigen presentation in cancer – mechanisms and clinical implications for immunotherapy. Nat Rev Clin Oncol. 2023; 20: 604-23.
10. Lotze MT, Olejniczak SH, Skokos D. CD28 co-stimulation: novel insights and applications in cancer immunotherapy. Nat Rev Immunol. 2024; 24: 878-95.
11. Amatore F, Gorvel L, Olive D. Role of Inducible Co-Stimulator (ICOS) in cancer immunotherapy. Expert Opin Biol Ther. 2020; 20: 141-50.
12. Upadhyay S, Kaur B, Gabr MT. CD28 and ICOS in immune regulation: structural insights and therapeutic targeting. Bioorg Med Chem Lett. 2025; 127: 130310.
13. Thapa B, Kato S, Nishizaki D, Miyashita H, Lee S, Nesline MK, et al. OX40/OX40 ligand and its role in precision immune oncology. Cancer Metastasis Rev. 2024; 43: 1001-13.
14. Pazoki A, Dadfar S, Shadab A, Haghmorad D, Oksenych V. Soluble cd40 ligand as a promising biomarker in cancer diagnosis. Cells. 2024; 13: 1267.
15. Franzese O. Tumor microenvironment drives the cross-talk between co-stimulatory and inhibitory molecules in tumor-infiltrating lymphocytes: implications for optimizing immunotherapy outcomes. Int J Mol Sci. 2024; 25: 12848.
16. Sun L, Su Y, Jiao A, Wang X, Zhang B. T cells in health and disease. Signal Transduct Target Ther. 2023; 8: 235.
17. Xuekai L, Yan S, Jian C, Yifei S, Xinyue W, Wenyuan Z, et al. Advances in reprogramming of energy metabolism in tumor T cells. Front Immunol. 2024; 15: 1347181.
18. Raynor JL, Chi H. Nutrients: signal 4 in T cell immunity. J Exp Med. 2024; 221: e20221839.
19. Trefny MP, Kroemer G, Zitvogel L, Kobold S. Metabolites as agents and targets for cancer immunotherapy. Nat Rev Drug Discov. 2025; 24: 764-84.
20. Liu Y, Zhou Y, Zhang J, Li J, Zou L. Regulation of CD4+ T cell differentiation and function by glucose metabolism. Genes Immun. 2025; 26: 287-96.
21. Gerriets VA, Kishton RJ, Johnson MO, Cohen S, Siska PJ, Nichols AG, et al. Foxp3 and Toll-like receptor signaling balance T_{reg} cell anabolic metabolism for suppression. Nat Immunol. 2016; 17: 1459-66.
22. Chisolm DA, Savic D, Moore AJ, Ballesteros-Tato A, León B, Crossman DK, et al. CCCTC-binding factor translates interleukin 2-and α-ketoglutarate-sensitive metabolic changes in T cells into context-dependent gene programs. Immunity. 2017; 47: 251-67.e7.
23. Ma S, Ming Y, Wu J, Cui G. Cellular metabolism regulates the differentiation and function of T-cell subsets. Cell Mol Immunoly. 2024; 21: 419-35.
24. Gualdoni GA, Mayer KA, Göschl L, Boucheron N, Ellmeier W, Zlabinger GJ. The AMP analog AICAR modulates the Treg/Th17 axis through enhancement of fatty acid oxidation. FASEB J. 2016; 30: 3800-9.
25. Llibre A, Kucuk S, Gope A, Certo M, Mauro C. Lactate: a key regulator of the immune response. Immunity. 2025; 58: 535-54.
26. Comito G, Iscaro A, Bacci M, Morandi A, Ippolito L, Parri M, et al. Lactate modulates $CD4^+$ T-cell polarization and induces an immunosuppressive environment, which sustains prostate carcinoma progression via TLR8/miR21 axis. Oncogene. 2019; 38: 3681-95.
27. Zhang Y-T, Xing M-L, Fang H-H, Li W-D, Wu L, Chen Z-P. Effects of lactate on metabolism and differentiation of $CD4^+$ T cells. Mol Immunol. 2023; 154: 96-107.
28. Lopez Krol A, Nehring HP, Krause FF, Wempe A, Raifer H, Nist A, et al. Lactate induces metabolic and epigenetic reprogramming of pro-inflammatory Th17 cells. EMBO Rep. 2022; 23: e54685.
29. Rao D, Stunnenberg JA, Lacroix R, Dimitriadis P, Kaplon J, Verburg F, et al. Acidity-mediated induction of $FoxP3^+$ regulatory T cells. Eur J Immunol. 2023; 53: 2250258.
30. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol. 1986; 136: 2348-57.
31. Chraa D, Naim A, Olive D, Badou A. T lymphocyte subsets in cancer immunity: friends or foes. J Leukoc Biol. 2019; 105: 243-55.
32. An Q, Duan L, Wang Y, Wang F, Liu X, Liu C, et al. Role of $CD4^+$ T cells in cancer immunity: a single-cell sequencing exploration of tumor microenvironment. J Transl Med. 2025; 23: 179.
33. Wu C, Duan L, Li H, Liu X, Cai T, Yang Y, et al. $PD1^{hi}CD200^{hi}$ $CD4^+$ exhausted T cell increase immunotherapy resistance and tumour progression by promoting epithelial–mesenchymal transition in bladder cancer. Clin Transl Med. 2023; 13: e1303.
34. Sacher AG, St Paul M, Paige CJ, Ohashi PS. Cytotoxic $CD4^+$ T cells in bladder cancer—a new license to kill. Cancer Cell. 2020; 38: 28-30.
35. Lau D, Khare S, Stein MM, Jain P, Gao Y, BenTaieb A, et al. Integration of tumor extrinsic and intrinsic features associates with immunotherapy response in non-small cell lung cancer. Nat Commun. 2022; 13: 4053.
36. Hui Z, Zhang J, Zheng Y, Yang L, Yu W, An Y, et al. Single-cell sequencing reveals the transcriptome and TCR characteristics of pTregs and *in vitro* expanded iTregs. Front Immunol. 2021; 12: 619932.
37. Khaliq AM, Rajamohan M, Saeed O, Mansouri K, Adil A, Zhang C, et al. Spatial transcriptomic analysis of primary and metastatic

pancreatic cancers highlights tumor microenvironmental heterogeneity. Nat Genet. 2024; 56: 2455-65.
38. Moncada R, Barkley D, Wagner F, Chiodin M, Devlin JC, Baron M, et al. Integrating microarray-based spatial transcriptomics and single-cell RNA-seq reveals tissue architecture in pancreatic ductal adenocarcinomas. Nat Biotechnol. 2020; 38: 333-42.
39. Kagamu H, Yamasaki S, Kitano S, Yamaguchi O, Mouri A, Shiono A, et al. Single-cell analysis reveals a CD4+ T-cell cluster that correlates with PD-1 blockade efficacy. Cancer Res. 2022; 82: 4641-53.
40. Zhu J. T helper cell differentiation, heterogeneity, and plasticity. Cold Spring Harb Perspect Biol. 2018; 10: a030338.
41. Meitei HT, Lal G. T cell receptor signaling in the differentiation and plasticity of CD4+ T cells. Cytokine Growth Factor Rev. 2023; 69: 14-27.
42. Saravia J, Chapman NM, Chi H. Helper T cell differentiation. Cell Mol Immunol. 2019; 16: 634-43.
43. Wen Z, Liu T, Xu X, Acharya N, Shen Z, Lu Y, et al. Interleukin-16 enhances anti-tumor immune responses by establishing a Th1 cell-macrophage crosstalk through reprogramming glutamine metabolism in mice. Nat Commun. 2025; 16: 2362.
44. Wang L, Zhu Y, Zhang N, Xian Y, Tang Y, Ye J, et al. The multiple roles of interferon regulatory factor family in health and disease. Signal Transduct Target Ther. 2024; 9: 282.
45. Bernard A, Hibos C, Richard C, Viltard E, Chevrier S, Lemoine S, et al. The tumor microenvironment impairs Th1 IFNγ secretion through alternative splicing modifications of *Irf1* Pre-mRNA. Cancer Immunol Res. 2021; 9: 324-36.
46. Korinfskaya S, Parameswaran S, Weirauch MT, Barski A. Runx transcription factors in T cells—what is beyond thymic development? Front Immunol. 2021; 12: 701924.
47. Wang D, Diao H, Getzler AJ, Rogal W, Frederick MA, Milner J, et al. The transcription factor Runx3 establishes chromatin accessibility of cis-regulatory landscapes that drive memory cytotoxic T lymphocyte formation. Immunity. 2018; 48: 659-74.e6.
48. Montauti E, Oh DY, Fong L. CD4+ T cells in antitumor immunity. Trends Cancer. 2024; 10: 969-85.
49. Oh DY, Kwek SS, Raju SS, Li T, McCarthy E, Chow E, et al. Intratumoral CD4+ T cells mediate anti-tumor cytotoxicity in human bladder cancer. Cell. 2020; 181: 1612-25.e13.
50. Iwahori K, Uenami T, Yano Y, Ueda T, Tone M, Naito Y, et al. Peripheral T cell cytotoxicity predicts the efficacy of anti-PD-1 therapy for advanced non-small cell lung cancer patients. Sci Rep. 2022; 12: 17461.
51. Śledzińska A, Vila de Mucha M, Bergerhoff K, Hotblack A, Demane DF, Ghorani E, et al. Regulatory T cells restrain interleukin-2- and Blimp-1-dependent acquisition of cytotoxic function by CD4+ T cells. Immunity. 2020; 52: 151-66.e6.
52. Takeuchi A, Badr MESG, Miyauchi K, Ishihara C, Onishi R, Guo Z, et al. CRTAM determines the CD4+ cytotoxic T lymphocyte lineage. J Exp Med. 2016; 213: 123-38.
53. Ducellier S, Demeules M, Letribot B, Gaetani M, Michaudel C, Sokol H, et al. Dual molecule targeting HDAC6 leads to intratumoral CD4+ cytotoxic lymphocytes recruitment through MHC-II upregulation on lung cancer cells. J Immunother Cancer. 2024; 12: e007588.
54. Oh DY, Fong L. Cytotoxic CD4+ T cells in cancer: expanding the immune effector toolbox. Immunity. 2021; 54: 2701-11.
55. Porakishvili N, Kardava L, Jewell AP, Yong K, Glennie MJ, Akbar A, et al. Cytotoxic CD4+ T cells in patients with B cell chronic lymphocytic leukemia kill via a perforin-mediated pathway. Haematologica. 2004; 89: 435-43.
56. Zhang G, Xu M, Zhang X, Ma L, Zhang H. TRAIL produced by SAM-1-activated CD4+ and CD8+ subgroup T cells induces apoptosis in human tumor cells through upregulation of death receptors. Toxicol Appl Pharmacol. 2021; 427: 115656.
57. Song M, Ping Y, Zhang K, Yang L, Li F, Zhang C, et al. Low-dose IFNγ induces tumor cell stemness in tumor microenvironment of non-small cell lung cancer. Cancer Res. 2019; 79: 3737-48.
58. Martínez-Sabadell A, Arenas EJ, Arribas J. IFNγ signaling in natural and therapy-induced antitumor responses. Clin Cancer Res. 2022; 28: 1243-9.
59. Xu J, Ding L, Mei J, Hu Y, Kong X, Dai S, et al. Dual roles and therapeutic targeting of tumor-associated macrophages in tumor microenvironments. Signal Transduct Target Ther. 2025; 10: 268.
60. Sun L, Kees T, Almeida AS, Liu B, He X-Y, Ng D, et al. Activating a collaborative innate-adaptive immune response to control metastasis. Cancer Cell. 2021; 39: 1361-74.e9.
61. Briesemeister D, Sommermeyer D, Loddenkemper C, Loew R, Uckert W, Blankenstein T, et al. Tumor rejection by local interferon gamma induction in established tumors is associated with blood vessel destruction and necrosis. Int J Cancer. 2011; 128: 371-8.
62. Sun T, Yang Y, Luo X, Cheng Y, Zhang M, Wang K, et al. Inhibition of tumor angiogenesis by interferon-γ by suppression of tumor-associated macrophage differentiation. Oncol Res. 2014; 21: 227-35.
63. Skartsis N, Ferreira LMR, Tang Q. The dichotomous outcomes of TNFα signaling in CD4+ T cells. Front Immunol. 2022; 13: 1042622.
64. Seung E, Xing Z, Wu L, Rao E, Cortez-Retamozo V, Ospina B, et al. A trispecific antibody targeting HER2 and T cells inhibits breast cancer growth via CD4 cells. Nature. 2022; 603: 328-34.
65. Speiser DE, Chijioke O, Schaeuble K, Münz C. CD4+ T cells in cancer. Nat Cancer. 2023; 4: 317-29.
66. Schmiegel WH, Caesar J, Kalthoff H, Greten H, Schreiber HW, Thiele HG. Antiproliferative effects exerted by recombinant human tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) on human pancreatic tumor cell lines. Pancreas. 1988; 3: 180-8.
67. Kimura M, Haisa M, Uetsuka H, Takaoka M, Ohkawa T, Kawashima R, et al. TNF combined with IFN-α accelerates NF-κB-mediated apoptosis through enhancement of Fas expression in colon cancer cells. Cell Death Differ. 2003; 10: 718-28.
68. Wright K, Kolios G, Westwick J, Ward SG. Cytokine-induced apoptosis in epithelial HT-29 cells is independent of nitric oxide formation. Evidence for an interleukin-13-driven phosphatidylinositol 3-kinase-dependent survival mechanism. J Biol Chem. 1999; 274: 17193-201.

69. Braumüller H, Wieder T, Brenner E, Aßmann S, Hahn M, Alkhaled M, et al. T-helper-1-cell cytokines drive cancer into senescence. Nature. 2013; 494: 361-5.
70. Huyghe L, Van Parys A, Cauwels A, Van Lint S, De Munter S, Bultinck J, et al. Safe eradication of large established tumors using neovasculature-targeted tumor necrosis factor-based therapies. EMBO Mol Med. 2020; 12: e11223.
71. Antony PA, Piccirillo CA, Akpinarli A, Finkelstein SE, Speiss PJ, Surman DR, et al. $CD8^+$ T cell immunity against a tumor/self-antigen is augmented by $CD4^+$ T helper cells and hindered by naturally occurring T regulatory cells. J Immunol. 2005; 174: 2591-601.
72. Bos R, Sherman LA. $CD4^+$ T-cell help in the tumor milieu is required for recruitment and cytolytic function of CD8+ T lymphocytes. Cancer Res. 2010; 70: 8368-77.
73. Zander R, Schauder D, Xin G, Nguyen C, Wu X, Zajac A, et al. $CD4^+$ T cell help is required for the formation of a cytolytic $CD8^+$ T cell subset that protects against chronic infection and cancer. Immunity. 2019; 51: 1028-42.e4.
74. Topchyan P, Xin G, Chen Y, Zheng S, Burns R, Shen J, et al. Harnessing the IL-21-BATF pathway in the $CD8^+$ T cell anti-tumor response. Cancers. 2021; 13: 1263.
75. Espinosa-Carrasco G, Chiu E, Scrivo A, Zumbo P, Dave A, Betel D, et al. Intratumoral immune triads are required for immunotherapy-mediated elimination of solid tumors. Cancer Cell. 2024; 42: 1202-16.e8.
76. Bourgeois C, Rocha B, Tanchot C. A role for CD40 expression on $CD8^+$ T cells in the generation of $CD8^+$ T cell memory. Science. 2002; 297: 2060-3.
77. Schoenberger SP, Toes RE, Van der Voort EI, Offringa R, Melief CJ. T-cell help for cytotoxic T lymphocytes is mediated by CD40–CD40L interactions. Nature. 1998; 393: 480-3.
78. Lei X, Khatri I, de Wit T, de Rink I, Nieuwland M, Kerkhoven R, et al. $CD4^+$ helper T cells endow cDC1 with cancer-impeding functions in the human tumor micro-environment. Nat Commun. 2023; 14: 217.
79. Gressier E, Schulte-Schrepping J, Petrov L, Brumhard S, Stubbemann P, Hiller A, et al. $CD4^+$ T cell calibration of antigen-presenting cells optimizes antiviral $CD8^+$ T cell immunity. Nat Immunol. 2023; 24: 979-90.
80. Borst J, Ahrends T, Bąbała N, Melief CJM, Kastenmüller W. $CD4^+$ T cell help in cancer immunology and immunotherapy. Nat Rev Immunol. 2018; 18: 635-47.
81. Gutiérrez-Melo N, Baumjohann D. T follicular helper cells in cancer. Trends Cancer. 2023; 9: 309-25.
82. Gu-Trantien C, Loi S, Garaud S, Equeter C, Libin M, De Wind A, et al. $CD4^+$ follicular helper T cell infiltration predicts breast cancer survival. J Clin Invest. 2013; 123: 2873-92.
83. Fujioka M, Fujioka S, Yoshitomi H, Hamanishi J, Suzuki H, Ukita M, et al. Oligoclonal expansion of IgG^+ B cells along with Tfh cell response is associated with a better outcome in endometrial cancer. Int Immunol. 2025:dxaf049.
84. Fridman WH, Meylan M, Petitprez F, Sun C-M, Italiano A, Sautès-Fridman C. B cells and tertiary lymphoid structures as determinants of tumour immune contexture and clinical outcome. Nat Rev Clin Oncol. 2022; 19: 441-57.
85. Radandish M, Mashhadi N, Aghayan AH, Taghizadeh M, Salehianfard S, Yahyazadeh S, et al. In-depth insight into tumor-infiltrating stromal cells linked to tertiary lymphoid structures and their prospective function in cancer immunotherapy. Exp Hematol Oncol. 2025; 14: 105.
86. Lin X, Ye L, Wang X, Liao Z, Dong J, Yang Y, et al. Follicular helper T cells remodel the immune microenvironment of pancreatic cancer via secreting CXCL13 and IL-21. Cancers. 2021; 13: 3678.
87. Cui C, Wang J, Fagerberg E, Chen P-M, Connolly KA, Damo M, et al. Neoantigen-driven B cell and CD4 T follicular helper cell collaboration promotes anti-tumor CD8 T cell responses. Cell. 2021; 184: 6101-18.e13.
88. Hou Y, Cao Y, He Y, Dong L, Zhao L, Dong Y, et al. SIRT3 negatively regulates TFH-cell differentiation in cancer. Cancer Immunol Res. 2024; 12: 891-904.
89. Garaud S, Zayakin P, Buisseret L, Rulle U, Silina K, De Wind A, et al. Antigen specificity and clinical significance of IgG and IgA autoantibodies produced *in situ* by tumor-infiltrating B cells in breast cancer. Front Immunol. 2018; 9: 2660.
90. Baumjohann D, Brossart P. T follicular helper cells: linking cancer immunotherapy and immune-related adverse events. J Immunother Cancer. 2021; 9: e002588.
91. Saleh R, Elkord E. $FoxP3^+$ T regulatory cells in cancer: prognostic biomarkers and therapeutic targets. Cancer Lett. 2020; 490: 174-85.
92. Stenström J, Hedenfalk I, Hagerling C. Regulatory T lymphocyte infiltration in metastatic breast cancer—an independent prognostic factor that changes with tumor progression. Breast Cancer Res. 2021; 23: 27.
93. Shan F, Somasundaram A, Bruno TC, Workman CJ, Vignali DAA. Therapeutic targeting of regulatory T cells in cancer. Trends Cancer. 2022; 8: 944-61.
94. Dikiy S, Rudensky AY. Principles of regulatory T cell function. Immunity. 2023; 56: 240-55.
95. Li Y, Zhang C, Jiang A, Lin A, Liu Z, Cheng X, et al. Potential anti-tumor effects of regulatory T cells in the tumor microenvironment: a review. J Transl Med. 2024; 22: 293.
96. Gao R, Shi G-P, Wang J. Functional diversities of regulatory T cells in the context of cancer immunotherapy. Front Immunol. 2022; 13: 833667.
97. Raffin C, Vo LT, Bluestone JA. T_{reg} cell-based therapies: challenges and perspectives. Nate Rev Immunol. 2020; 20: 158-72.
98. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, et al. CTLA-4 control over Foxp3+ regulatory T cell function. Science. 2008; 322: 271-5.
99. Qureshi OS, Zheng Y, Nakamura K, Attridge K, Manzotti C, Schmidt EM, et al. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. Science. 2011; 332: 600-3.
100. Tekguc M, Wing JB, Osaki M, Long J, Sakaguchi S. Treg-expressed CTLA-4 depletes CD80/CD86 by trogocytosis, releasing free PD-L1

on antigen-presenting cells. Proc Natl Acad Sci USA. 2021; 118: e2023739118.
101. Pipkin ME, Sacks JA, Cruz-Guilloty F, Lichtenheld MG, Bevan MJ, Rao A. Interleukin-2 and inflammation induce distinct transcriptional programs that promote the differentiation of effector cytolytic T cells. Immunity. 2010; 32: 79-90.
102. Boyman O, Sprent J. The role of interleukin-2 during homeostasis and activation of the immune system. Nat Rev Immunol. 2012; 12: 180-90.
103. Nixon BG, Gao S, Wang X, Li MO. TGFβ control of immune responses in cancer: a holistic immuno-oncology perspective. Nat Re Immunol. 2023; 23: 346-62.
104. Cao X, Cai SF, Fehniger TA, Song J, Collins LI, Piwnica-Worms DR, et al. Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance. Immunity. 2007; 27: 635-46.
105. Gondek D, Lu LF, Quezada SA, Sakaguchi S, Noelle RJ. Cutting edge: contact-mediated suppression by CD4+CD25+ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. J Immunol. 2005; 174: 1783-6.
106. Gallimore A, Glithero A, Godkin A, Tissot AC, Plückthun A, Elliott T, et al. Induction and exhaustion of lymphocytic choriomeningitis virus-specific cytotoxic T lymphocytes visualized using soluble tetrameric major histocompatibility complex class I-peptide complexes. J Exp Med. 1998; 187: 1383-93.
107. Baessler A, Vignali DAA. T cell exhaustion. Annu Rev Immunol. 2024; 42: 179-206.
108. Miggelbrink AM, Jackson JD, Lorrey SJ, Srinivasan ES, Waibl-Polania J, Wilkinson DS, et al. CD4 T-cell exhaustion.: does it exist and what are its roles in cancer? Clin Cancer Res. 2021; 27: 5742-52.
109. Dixon KO, Lahore GF, Kuchroo VK. Beyond T cell exhaustion: TIM-3 regulation of myeloid cells. Sci Immunol. 2024; 9: eadf2223.
110. Zhang P, Liu X, Gu Z, Jiang Z, Zhao S, Song Y, et al. Targeting TIGIT for cancer immunotherapy: recent advances and future directions. Biomark Res. 2024; 12: 7.
111. Aggarwal V, Workman CJ, Vignali DA. LAG-3 as the third checkpoint inhibitor. Nat Immunol. 2023; 24: 1415-22.
112. Nakano M, Ito M, Tanaka R, Yamaguchi K, Ariyama H, Mitsugi K, et al. PD-1+ TIM-3+ T cells in malignant ascites predict prognosis of gastrointestinal cancer. Cancer Sci. 2018; 109: 2986-92.
113. Yuan L, Xu B, Yuan P, Zhou J, Qin P, Han L, et al. Tumor-infiltrating CD4+ T cells in patients with gastric cancer. Cancer Cell Int. 2017; 17: 114.
114. Ozkazanc D, Yoyen-Ermis D, Tavukcuoglu E, Buyukasik Y, Esendagli G. Functional exhaustion of CD4+ T cells induced by co-stimulatory signals from myeloid leukaemia cells. Immunology. 2016; 149: 460-71.
115. Stachowiak M, Becker WJ, Olkhanud PB, Moreno PA, Markowicz S, Berzofsky JA, et al. Cancer cells accelerate exhaustion of persistently activated mouse CD4+ T cells. OncoImmunology. 2025; 14: 2521392.
116. Tracy SI, Venkatesh H, Hekim C, Heltemes-Harris LM, Knutson TP, Bachanova V, et al. Combining nilotinib and PD-L1 blockade reverses CD4+ T-cell dysfunction and prevents relapse in acute B-cell leukemia. Blood. 2022; 140: 335-48.
117. Doering TA, Crawford A, Angelosanto JM, Paley MA, Ziegler CG, Wherry EJ. Network analysis reveals centrally connected genes and pathways involved in CD8+ T cell exhaustion versus memory. Immunity. 2012; 37: 1130-44.
118. Crawford A, Angelosanto JM, Kao C, Doering TA, Odorizzi PM, Barnett BE, et al. Molecular and transcriptional basis of CD4+ T cell dysfunction during chronic infection. Immunity. 2014; 40: 289-302.
119. Fu J, Yu A, Xiao X, Tang J, Zu X, Chen W, et al. CD4+ T cell exhaustion leads to adoptive transfer therapy failure which can be prevented by immune checkpoint blockade. Am J Cancer Res. 2020; 10: 4234-50.
120. Scharping NE, Rivadeneira DB, Menk AV, Vignali PDA, Ford BR, Rittenhouse NL, et al. Mitochondrial stress induced by continuous stimulation under hypoxia rapidly drives T cell exhaustion. Nat Immunol. 2021; 22: 205-15.
121. Pandit M, Kil Y-S, Ahn J-H, Pokhrel RH, Gu Y, Mishra S, et al. Methionine consumption by cancer cells drives a progressive upregulation of PD-1 expression in CD4 T cells. Nat Commun. 2023; 14: 2593.
122. Balança C-C, Salvioni A, Scarlata C-M, Michelas M, Martinez-Gomez C, Gomez-Roca C, et al. PD-1 blockade restores helper activity of tumor-infiltrating, exhausted PD-1^{hi}CD39+ CD4 T cells. JCI Insight. 2021; 6: e142513.
123. Bessoles S, Chiron A, Sarrabayrouse G, De La Grange P, Abina AM, Hacein-Bey-Abina S. Erythropoietin induces tumour progression and CD39 expression on immune cells in a preclinical model of triple-negative breast cancer. Immunology. 2024; 173: 360-80.
124. Duan ZQ, Li YX, Qiu Y, Shen Y, Wang Y, Zhang YY, et al. CD39 expression defines exhausted CD4+ T cells associated with poor survival and immune evasion in human gastric cancer. Clin Transl Immunology. 2024; 13: e1499.
125. Zaidi N, Jaffee EM, Yarchoan M. Recent advances in therapeutic cancer vaccines. Nat Rev Cancer. 2025; 25: 517-33.
126. Alspach E, Lussier DM, Miceli AP, Kizhvatov I, DuPage M, et al. MHC-II neoantigens shape tumour immunity and response to immunotherapy. Nature. 2019; 574: 696-701.
127. Huff AL, Longway G, Mitchell JT, Andaloori L, Davis-Marcisak E, Chen F, et al. CD4 T cell-activating neoantigens enhance personalized cancer vaccine efficacy. JCI Insight. 2023; 8: e174027.
128. Melief CJ, Van Der Burg SH. Immunotherapy of established (pre) malignant disease by synthetic long peptide vaccines. Nat Rev Cancer. 2008; 8: 351-60.
129. Braun DA, Moranzoni G, Chea V, McGregor BA, Blass E, Tu CR, et al. A neoantigen vaccine generates antitumour immunity in renal cell carcinoma. Nature. 2025; 639: 474-82.
130. Li C, Clauson R, Bugada LF, Ke F, He B, Yu Z, et al. Antigen-clustered nanovaccine achieves long-term tumor remission by promoting B/CD 4 T cell crosstalk. ACS Nano. 2024; 18: 9584-604.

131. Dolina JS, Lee J, Brightman SE, McArdle S, Hall SM, Thota RR, et al. Linked $CD4^+$/$CD8^+$ T cell neoantigen vaccination overcomes immune checkpoint blockade resistance and enables tumor regression. J Clin Invest. 2023; 133: e164258.
132. Topalian SL, Forde PM, Emens LA, Yarchoan M, Smith KN, Pardoll DM. Neoadjuvant immune checkpoint blockade: a window of opportunity to advance cancer immunotherapy. Cancer Cell. 2023; 41: 1551-66.
133. Carlino MS, Larkin J, Long GV. Immune checkpoint inhibitors in melanoma. Lancet. 2021; 398: 1002-14.
134. Zuazo M, Arasanz H, Fernández-Hinojal G, García-Granda MJ, Gato M, Bocanegra A, et al. Functional systemic CD4 immunity is required for clinical responses to PD-L1/PD-1 blockade therapy. EMBO Mol Med. 2019; 11: e10293.
135. Wei SC, Levine JH, Cogdill AP, Zhao Y, Anang N-AAS, Andrews MC, et al. Distinct cellular mechanisms underlie anti-CTLA-4 and anti-PD-1 checkpoint blockade. Cell. 2017; 170: 1120-33.e17.
136. Nagasaki J, Togashi Y, Sugawara T, Itami M, Yamauchi N, Yuda J, et al. The critical role of $CD4^+$ T cells in PD-1 blockade against MHC-II-expressing tumors such as classic Hodgkin lymphoma. Blood Adv. 2020; 4: 4069-82.
137. Franken A, Bila M, Mechels A, Kint S, Van Dessel J, Pomella V, et al. $CD4^+$ T cell activation distinguishes response to anti-PD-L1+anti-CTLA4 therapy from anti-PD-L1 monotherapy. Immunity. 2024; 57: 541-58.e7.
138. Hodi FS, Chiarion-Sileni V, Gonzalez R, Grob J-J, Rutkowski P, Cowey CL, et al. Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. Lancet Oncol. 2018; 19: 1480-92.
139. Long GV, Stephen Hodi F, Lipson EJ, Schadendorf D, Ascierto PA, Matamala L, et al. Overall survival and response with nivolumab and relatlimab in advanced melanoma. NEJM Evid. 2023; 2: EVIDoa2200239.
140. Rolig AS, Peng X, Sturgill ER, Holay N, Kasiewicz M, Mick C, et al. The response to anti-PD-1 and anti-LAG-3 checkpoint blockade is associated with regulatory T cell reprogramming. Sci Transl Med. 2025; 17: eadk3702.
141. Melenhorst JJ, Chen GM, Wang M, Porter DL, Chen C, Collins MA, et al. Decade-long leukaemia remissions with persistence of $CD4^+$ CAR T cells. Nature. 2022; 602: 503-9.
142. Cimons JM, DeGolier KR, Burciaga SD, Yarnell MC, Novak AJ, Rivera-Reyes AM, et al. T-bet overexpression enhances CAR T cell effector functions and antigen sensitivity. J Immunother Cancer. 2025; 13: e010962.
143. Boulch M, Cazaux M, Loe-Mie Y, Thibaut R, Corre B, Lemaître F, et al. A cross-talk between CAR T cell subsets and the tumor microenvironment is essential for sustained cytotoxic activity. Sci Immunol. 2021; 6: eabd4344.
144. Boulch M, Cazaux M, Cuffel A, Guerin MV, Garcia Z, Alonso R, et al. Tumor-intrinsic sensitivity to the pro-apoptotic effects of IFN-γ is a major determinant of $CD4^+$ CAR T-cell antitumor activity. Nat Cancer. 2023; 4: 968-83.
145. Niu C, Wei H, Pan X, Wang Y, Song H, Li C, et al. Foxp3 confers long-term efficacy of chimeric antigen receptor-T cells via metabolic reprogramming. Cell Metab. 2025; 37: 1426-41.e7.
146. Zheng D, Qin L, Lv J, Che M, He B, Zheng Y, et al. $CD4^+$ anti-TGF-β CAR T cells and $CD8^+$ conventional CAR T cells exhibit synergistic antitumor effects. Cell Rep Med. 2025; 6: 102020.
147. Kruse B, Buzzai AC, Shridhar N, Braun AD, Gellert S, Knauth K, et al. $CD4^+$ T cell-induced inflammatory cell death controls immune-evasive tumours. Nature. 2023; 618: 1033-40.
148. Saillard M, Cenerenti M, Reichenbach P, Guillaume P, Su Z, Hafezi M, et al. Engineered CD4 TCR T cells with conserved high-affinity TCRs targeting NY-ESO-1 for advanced cellular therapies in cancer. Sci Adv. 2025; 11: eadu5754.

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