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T cell dysfunction and therapeutic intervention in cancer

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Recent advances in immunotherapy have affirmed the curative potential of T cell-based approaches for treating relapsed and refractory cancers. However, the therapeutic efficacy is limited in part owing to the ability of cancers to evade immunosurveillance and adapt to immunological pressure. In this Review, we provide a brief overview of cancer-mediated immunosuppressive mechanisms with a specific focus on the repression of the surveillance and effector function of T cells. We discuss $CD8^+$ T cell exhaustion and functional heterogeneity and describe strategies for targeting the molecular checkpoints that restrict T cell differentiation and effector function to bolster immunotherapeutic effects. We also delineate the emerging contributions of the tumor microenvironment to T cell metabolism and conclude by highlighting discovery-based approaches for developing future cellular therapies. Continued exploration of T cell biology and engineering hold great promise for advancing therapeutic interventions for cancer.

Immunotherapy is often framed as a modern medical advancement, yet many current immunotherapy approaches are historically grounded in fundamental discoveries of cancer immunosurveillance. The concept of immunosurveillance emerged from Paul Ehrlich's work in the early 1900s and showed that aberrant cells commonly arise in humans but are often suppressed under most circumstances[1]. Lewis Thomas and Frank MacFarlane Burnet further refined this concept to suggest that the immune system, specifically lymphocytes, can recognize tumor-specific antigens and prohibit cancer growth[2]. While the immune system is protective against cancer, paradoxically it can also favor malignant outgrowth by altering tumor immunogenicity. The concept of 'cancer immunoediting' was first described by Robert Schreiber, whose seminal work established that functional T cells can suppress tumors but simultaneously select for tumor cells that are more capable of outgrowth in an immunocompetent host[3]. These core concepts of immune surveillance and editing help articulate the current challenges in describing mechanisms that limit modern immunotherapy. In addition, tumors are self-tissues, and powerful thymic and peripheral tolerance mechanisms restrict the potency of tumor-responsive T cell responses. These mechanisms effectively remove T cells with the highest binding avidity but routinely spare cells responding with lower avidity to non-mutated tumor antigen[4]. An exception might be neoantigens, against which higher-avidity T cells could be available. Current immunotherapy efforts rely on harnessing the pool of tumor-reactive T cells to provide targeted, durable protection against tumors. These approaches include cancer vaccines, inhibitory receptor blockade (immune checkpoint blockade (ICB)) and adoptive cell therapies such as chimeric antigen receptor (CAR) T cell therapy. In this Review, we focus on T cell-based immunotherapeutic approaches and the specific challenges to overcome cancer-driven immune evasion (Fig. 1).

The conceptualization and discovery that endogenous anti-tumor immune responses can control cancer have enabled tailored strategies that capitalize upon such inherent functions. ICB is a prominent example showing that targeting a pre-existing population of tumor-specific T cells can lead to durable tumor remission. This highly efficacious therapeutic approach arose from the discovery that lymphocytes express inhibitory receptors that limit their effector response. One such receptor, cytotoxic T lymphocyte antigen 4 (CTLA-4), is a co-inhibitory

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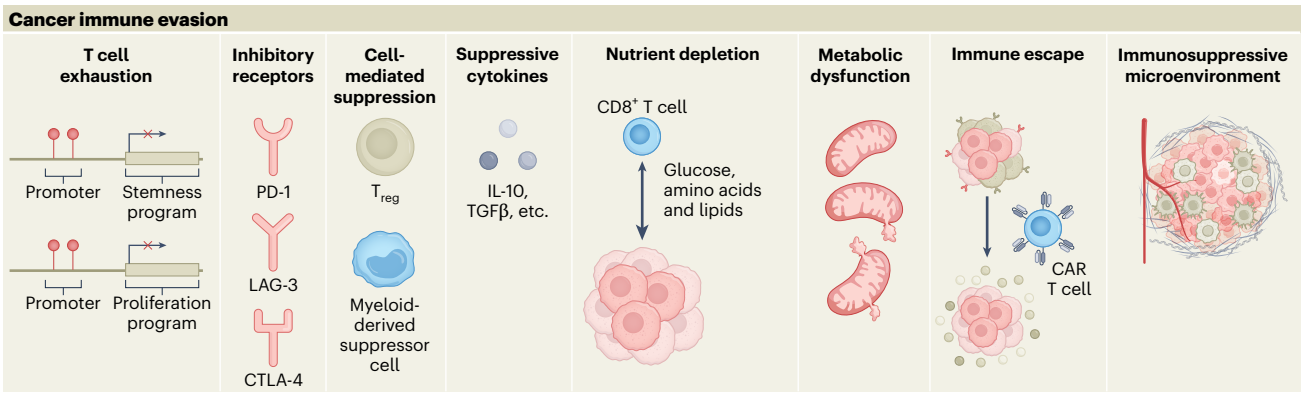

Fig. 1 | Cancer immune evasion. Several strategies exist for cancer to evade the immune system. These impediments include T cell exhaustion (by suppressing stemness and proliferation programs), upregulation of inhibitory receptors (for example, PD-1, CTLA-4 and LAG-3), cell-mediated repression (such as by T_{reg} cells), secretion of suppressive cytokines (for example, IL-10 and TGFβ), nutrient depletion (such as depletion of glucose and amino acids by tumor cells), metabolic dysfunction (for example, impaired mitochondrial fitness), immune escape (for example, loss of antigen on tumor cells) and an immunosuppressive tumor microenvironment.

receptor expressed by activated T cells that binds to the B7 ligand (specifically, CD80 or CD86) on the surface of antigen-presenting cells, thereby negatively regulating T cell activation and proliferation[5,6]. CTLA-4 is also crucial for regulatory T (T_{reg}) cell function under steady state and in the tumor microenvironment[7,8]. In addition to CTLA-4, the immune checkpoint receptor programmed death protein 1 (PD-1) and its ligand programmed death ligand 1 (PD-L1) were identified as targets whose blocking can reinvigorate T cell responses[9–11]. Several US Food and Drug Administration (FDA)-approved CTLA-4- and PD-1-blocking monoclonal antibodies are currently used to treat several types of cancer, including melanoma, non-small cell lung cancer, head and neck squamous cell carcinoma, renal cell carcinoma and Hodgkin's lymphoma. Combination therapy of anti-CTLA-4 (ipilimumab) and anti-PD-1 (nivolumab) monoclonal antibodies has a superior clinical response in patients with metastatic melanoma compared with administration of individual drugs[12]. New combinations of checkpoint inhibitors are also being explored. For example, lymphocyte-activation gene 3 (LAG-3) is a surface molecule that negatively regulates T cell proliferation and effector function, and dual inhibition of LAG-3 and PD-1 has demonstrated synergistic efficacy in preclinical models and in patients with advanced melanoma[13–15]. As opposed to ICB, which mostly reinvigorates endogenous populations of T cells, tumor-infiltrating lymphocyte (TIL) therapy involves isolation of tumor-specific lymphocytes from a patient's tumor, which are then expanded ex vivo and reinfused back into the patient[16]. Although the initial identification of TILs occurred decades ago, the FDA recently approved the first TIL therapy for melanoma, demonstrating the clinical potential of this therapeutic approach[17]. Despite the major advances of ICB and TIL therapies, mechanisms of tumor immune evasion limit their clinical benefits to a minority of patients, underscoring the need to better define tumor immunogenicity and microenvironmental suppressive mechanisms limiting T cell responses.

As an alternative to relying on endogenously derived tumor-specific T cell responses, engineering efforts have focused on synthetic receptors and other strategies that redirect T cells to recognize tumor antigens. One such approach is T cell receptor (TCR) therapy, which involves engineering T cells to specifically recognize tumor antigens in a major histocompatibility complex (MHC)-dependent manner. For example, TCR therapies targeting New York esophageal squamous cell carcinoma 1 (NY-ESO-1) have been successful in melanoma and synovial sarcoma trials[18]. Of note, the most common design is a CAR, which typically consists of an antigen-specific single-chain variable fragment, a hinge or transmembrane domain and a CD28–CD3ζ- or 4-1BB–CD3ζ-based intracellular signaling domain[19–22]. CD28ζ- or 4-1BBζ-specific CAR T cell products are remarkably active against $CD19^+$ B cell malignancies and $BCMA^+$ multiple myeloma, leading to their FDA approval[23–25]. However, limited CAR T cell persistence or loss of CAR T cell function over time contributes to relapse in about half of patients who initially achieve a complete response[26,27]. In addition to limited persistence, CAR T cells have impaired anti-tumor activity in patients with solid tumors and brain tumors, highlighting additional roadblocks in the highly immunosuppressive tumor microenvironment[28]. Next-generation CAR T cells described briefly in this Review are designed to address these issues through genetic engineering approaches[29–32]. Cancer vaccines are another critical tool in advancing cancer immunotherapy that establish de novo B cell and T cell responses against tumors (reviewed elsewhere[33,34]). Here, we focus our discussion on mechanisms associated with ICB and adoptive cell therapies. In this Review, we discuss the fundamental principles of immunosurveillance that enable the development of immunotherapy approaches and highlight current mechanistic barriers that can limit the clinical breadth and durability of their applications before describing strategies for therapeutic intervention.

T cell exhaustion in the tumor microenvironment

The therapeutic potential of T cells is mostly ascribed to their rapid proliferation, secretion of effector cytokines and potent cytotoxic capacity. While these abilities are essential for clearing virally infected cells and tumors, the effector response can also result in host immunopathology and is therefore tightly regulated by mechanisms that actively suppress effector functions over time. The progressive repression of T cell effector function, termed exhaustion, is broadly characterized by a reduced capacity to secrete effector cytokines and proliferate. T cell exhaustion was first documented during studies investigating T cell-associated mechanisms that enabled the persistence of lymphocytic choriomeningitis virus (LCMV) infection in mice (Fig. 2a). During chronic LCMV infection, virus-specific $CD8^+$ T cells are able to persist but with reduced effector function[35–37]. Further characterization of exhaustion in the LCMV model system revealed that the impairment of T cell effector function occurs over time, associated with a diminished proliferative capacity in the cellular pool[38]. During T cell activation and subsequent differentiation, T cells upregulate PD-1, and, accordingly, administration of an antibody that blocks the interaction with its ligand PD-L1 enables a subset of T cells to proliferate[39]. Development of T cell exhaustion can also occur in humans with chronic infection, including human immunodeficiency virus, hepatitis B virus or hepatitis C virus[40–43]. These studies not only identified a mechanism of T cell exhaustion through chronic

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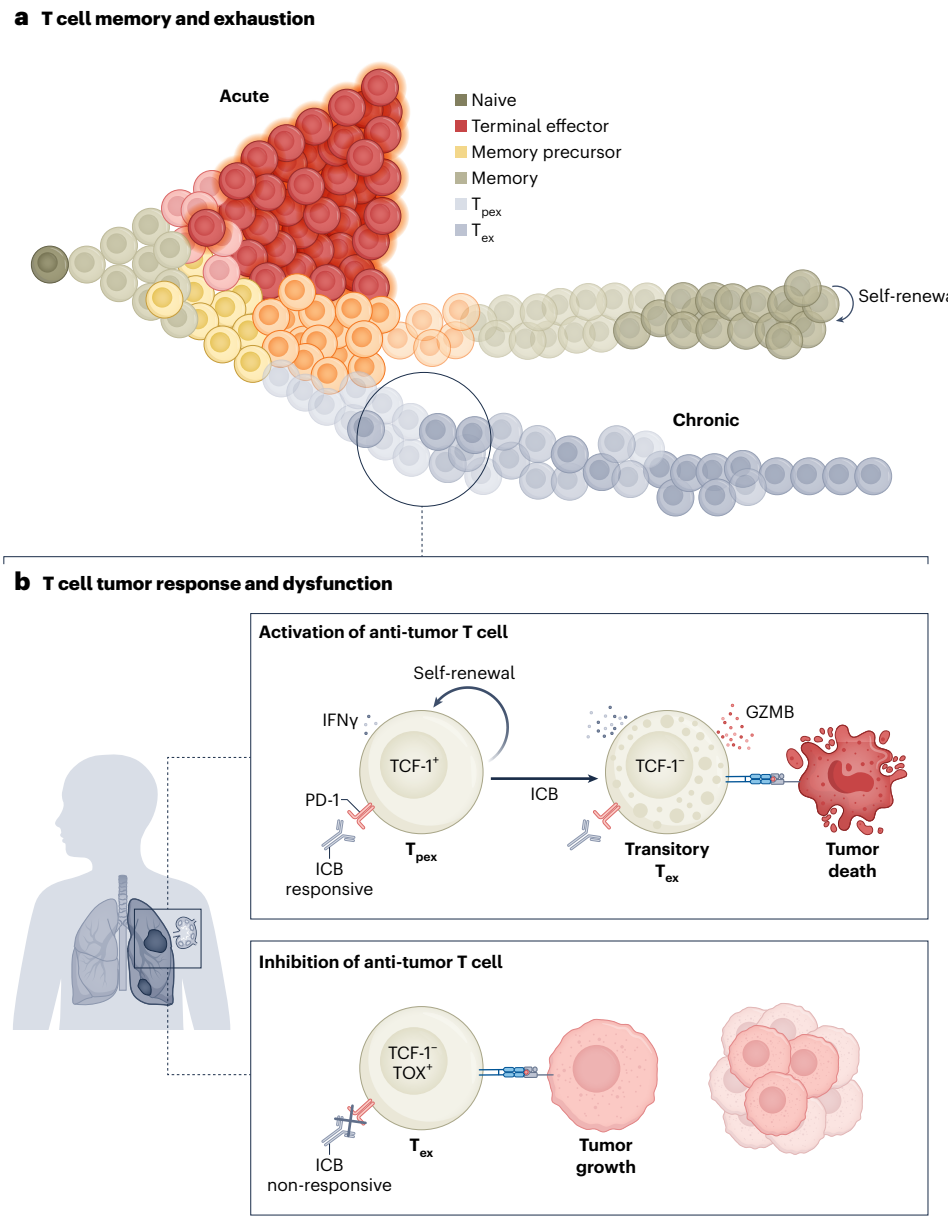

Fig. 2 | T cell differentiation and exhaustion. a, In settings of acute antigen exposure, T cells differentiate into terminal effectors or a long-lived memory population that is derived from memory precursor cells in the early stages of antigen exposure. If exposed to chronic antigen, T cells can acquire functional features of terminal exhaustion. Both T_{pex} cells and T_{ex} cells are found during T cell exhaustion. **b**, The tumor microenvironment shapes T cell heterogeneity and anti-tumor function. T_{pex} cells are enriched in lymph nodes, whereas, in tumors, stem-like T cells preferentially localize to the tertiary lymphoid structure and stem immunity hubs. The TCF-1$^+$ T_{pex} cell population can self-renew and, in response to ICB, mount a proliferative burst to differentiate toward TCF-1$^-$ transitory T_{ex} cells (expressing abundant IFNγ and granzyme B (GZMB)), whereas the T_{ex} cell population within the tumor lacks the ability to respond to ICB.

antigen exposure in mice and humans but also laid the foundation for uncovering immunological interventions to boost T cell function and a framework for understanding T cell dysfunction in the tumor microenvironment.

The description of T cell exhaustion has now evolved into a multifaceted, molecular definition that encompasses transcriptional and metabolic adaptations tied to discrete developmental kinetics. These alterations are derived in part from downstream signaling through the TCR, co-stimulatory receptors and inhibitory receptors at different stages of chronic antigen exposure. In addition to TCR interaction with its cognate antigen (signal 1), engagement of CD28 with either B7-1 (CD80) or B7-2 (CD86) co-stimulatory ligands on antigen-presenting cells provides the second signal for T cell activation and stimulates proliferation. TCR engagement and co-stimulation also upregulate CTLA-4 expression, which binds B7-1 and B7-2 with higher affinity than CD28, therefore blocking T cell activation[6]. In addition to CTLA-4, antigen-specific CD8$^+$ T cells also upregulate the inhibitory receptors PD-1, LAG-3 and T cell immunoglobulin and mucin domain 3 (TIM-3)[44,45]. A description of these inhibitory receptors and their function contributed to the seminal discovery that transient blockade of inhibitory signals could 'rejuvenate' a subset of suppressed cells[39]. Although this general concept of rejuvenation inspired many of the current therapeutic modalities, this model has now evolved to include a heightened transition of the precursor to an effector-like state rather than reversal of a terminally differentiated T cell[46]. Further mechanistic insights underlying inhibitory receptor blockade have now been described. For example, although it was initially thought that PD-1-mediated T cell inhibition occurs through interplay with TCR signaling, it has now been

shown that CD28 is the main target of PD-1 signaling and the presence of CD28 is essential for anti-PD-1 therapy[47,48].

These studies have prompted the in-depth characterization of exhausted T cells, thereby refining the definition beyond cell surface markers to a molecular description that includes heritable modifications in gene expression programs that account for long-lived changes in effector function, adhesion, chemotaxis and metabolic defects[44]. The transcriptional programming of T cell exhaustion involves a sequential process of epigenetic rearrangement that occurs throughout the genome. Transcriptional changes that accompany suppression of effector responses during chronic antigen stimulation of T cells are coupled to covalent modification of histones and DNA that reinforce exhaustion-associated gene expression programs[49,50]. In addition to covalent epigenetic modifications, changes in chromatin accessibility accompany the development of T cell exhaustion in viral and tumor models[51–53]. Building on these correlates of exhaustion, causality was established by showing that the progressive acquisition of epigenetic marks, specifically DNA methyltransferase 3A (DNMT3A)-mediated de novo DNA methylation, results in suppression of genes essential for effector function, homing and proliferation[50]. The resulting exhausted T cells are unable to control chronic LCMV infection and also become unresponsive to ICB, and deletion of *Dnmt3a* prevents acquisition of exhaustion-associated DNA methylation programs, enabling T cells to mount a proliferative burst in response to anti-PD-L1 therapy[50].

In addition to DNMT3A, targeting other epigenetic regulators can impair terminal differentiation and preserve function of T cells. For example, SUV39H1 marks chromatin through histone H3 lysine 9 trimethylation and has a critical function in the silencing of stem-associated and memory-associated genes during effector differentiation[54]. Consequently, SUV39H1-deficient $CD8^+$ T cells have increased proliferation and long-term survival. Another histone methyltransferase, the enzyme enhancer of zeste homolog 2 (EZH2), functions as part of the polycomb repressive complex (PRC2) to catalyze de novo histone H3 lysine 27 trimethylation (H3K27me3). Deposition of H3K27me3 occurs at memory-associated and survival-associated genes in terminally differentiated effector T cells and therefore limits their memory potential. Accordingly, EZH2 deficiency impairs epigenetic silencing of genes controlling T cell memory and survival[55]. These studies collectively establish an epigenetic mechanism for reinforcing T cell exhaustion and confirm exhausted T cells as a bona fide cell lineage.

The molecular definition of T cell exhaustion described above has been observed in mouse and human tumors. Longitudinal studies of mouse tumor-specific T cells in well-defined syngeneic and genetic model systems have revealed that molecular features of exhaustion coincide with dysfunction during the early stages of tumor formation or transplantation. These studies also show that, although initially this state is therapeutically reversible, persistent antigen exposure can cause permanent cell-intrinsic alterations that result in terminal T cell exhaustion[56]. T cell differentiation and exhaustion within the tumor microenvironment have also been described in humans and are associated with response to ICB[57]. Similar to the mouse studies, less-differentiated T cell populations were found to drive response to immunotherapy in humans. These results emphasize the importance of understanding the discrete functions attributed to each T cell exhaustion subset and provide a further rationale for examining T cell heterogeneity in the anti-tumor response.

Dissecting T cell heterogeneity

Despite a clonal origin, chronic stimulation of antigen-specific T cells results in expansion into a highly heterogeneous pool of cells within the tumor microenvironment. The diversity of T cells that develop in response to tumor antigen is mediated by several factors including anatomical location and duration of antigen exposure. T cells are spatially organized within the tumor, which contains specific hubs of interacting malignant and immune cells[58]. Therefore, tumor-intrinsic T cell subsets are present in varying quantities and have different anti-tumor capabilities. Evidence indicates that only a small percentage of intratumoral T cells are tumor specific, and there is an abundance of TILs that recognize non-tumor antigens[59]. However, tumor recognition by even a fraction of tumor-intrinsic $CD8^+$ T cells generates an interferon-γ (IFNγ) gradient that can affect a large fraction of the tumor mass[60]. Given their abundance and innate-like killing capacity, bystander T cells are now being targeted for therapeutic approaches[61].

T cell heterogeneity also has an important functional effect, with different T cell subsets responding variably to immunotherapy. For example, ICB is reliant on the ability of $CD8^+$ T cells to mount a proliferative burst in response to blockade of the inhibitory pathway. This proliferative response is mediated by the precursor exhausted T (T_{pex}) cell subset that expresses T cell factor 1 (TCF-1) and PD-1 (refs. 46,62–65) (Fig. 2b). T_{pex} cells also express thymocyte selection-associated high-mobility group box (TOX) protein, which is required for their generation during chronic antigen exposure[66–69]. High expression of TOX is also important for terminally exhausted T (T_{ex}) cell differentiation[69–71]. In addition, the transcription factor MYB is essential for preserving a subpopulation of T_{pex} cells (marked by CD62L expression) that maintain the repopulation capacity of T cells during chronic infection[72]. T_{pex} cells reside in lymph nodes and within tumors, where they are spatially localized to tertiary lymphoid structures[73] and stem immunity hubs[74]. This anatomical partitioning enables T_{pex} cells to retain stem-like properties, including the ability to self-renew and give rise to transitory T cells (expressing PD-1, TIM-3 and C–X_3–C motif chemokine receptor 1 (CX_3CR1)) that can then convert to terminally differentiated T cells marked by high expression of PD-1, TIM-3 and CD101 (refs. 75,76). Although these phenotypical markers for T_{pex} cells were derived in mouse systems of chronic viral infection, stem-like $CD8^+$ T cells have also been identified in humans and are responsible for mounting an immune response against human cancers. Specifically, T_{pex} cells from regional lymph nodes or stromal areas in patients with head and neck squamous cell carcinomas are clonally related to T_{ex} cells within the tumor[77,78]. After anti-PD-L1 treatment, T_{pex} cells undergo activation and differentiation into circulating intermediate exhausted $CD8^+$ T cells. Notably, the response to immunotherapy is impaired in metastatic lymph nodes, demonstrating the importance of intact cellular niches for clinical response as well as the negative impact of the tumor microenvironment on T cell function[77].

The tumor microenvironment has been shown to reshape the chromatin landscape of tumor-infiltrating T cells to limit their transcriptional potential. Analysis of mouse tumor-specific T cells during tumor progression revealed that these T cells are initially in a therapeutically reprogrammable chromatin state that transitions to a fixed dysfunctional chromatin state in response to chronic exposure to tumor antigen[53]. Specific surface makers (such as CD101 and CD38) can be used to demarcate reprogrammable from non-reprogrammable PD-1^{hi} dysfunctional T cells within the heterogeneous population, and such markers are also expressed by human PD-1^{hi} tumor-infiltrating T cells from melanoma and non-small cell lung cancers[53]. The prolonged exposure of T cells to tumor antigen and the microenvironment can ultimately lead to an inability to control tumor growth[35,56,79]. Therefore, T cell heterogeneity observed initially during an anti-tumor response can progress toward exhaustion. After passing a 'point of no return', the T cells can no longer undergo reinvigoration, presenting a unique challenge for T cell-based immunotherapies.

In addition to ICB, adoptive cell therapy with ex vivo expanded TILs has been a promising approach to treat some types of cancers. Although curative for some patients, TIL-adoptive transfer is not universally successful. Therapeutic effects of TIL transfer require a pool of stem-like T cells that can self-renew and give rise to more terminally differentiated T cells to facilitate durable tumor control. Accordingly, TILs isolated from ICB responders retain a TCF-1^+CD39^-TIM3^- stem-like phenotype, while the acquisition of a more terminally differentiated

phenotype is associated with poor T cell persistence[80,81]. Removal of the terminally differentiated CD39$^+$TIM-3$^+$CD8$^+$ T cells from tumor infiltrates before anti-PD-1 treatment improves ex vivo tumor killing in an organoid model[57]. Consistent with these findings, T_{ex} cells have been shown to acquire suppressive activity in a CD39-dependent manner, and deletion of the gene encoding CD39 in endogenous CD8$^+$ T cells improves T cell anti-tumor function and enhances response to immunotherapy[82]. These results suggest a suppressive effect of exhausted T cells on the functional T cell population. ICB has been shown to oppose tumor-induced CD8$^+$ T cell suppression and provides an additional strategy to overcome T cell dysfunction[83].

Further complicating intrinsic T cell dysfunction, cancer has evolved mechanisms to circumvent a successful anti-tumor immune response. One such evasion strategy, cancer immunoediting, refers to the process by which tumor progression is shaped by the adaptive immune system[3]. T cell-mediated immunoediting can result in outgrowth of subclonal tumor cells that contributes to therapeutic resistance[84]. For example, neoantigen loss has been associated with resistance to ICB in patients with non-small cell lung cancer[85]. Furthermore, tumor progression is associated with terminal differentiation and dysfunction of intratumoral T cells[53]. The evolving tumor landscape has a role in acquired resistance to cancer treatment and leads to broader questions regarding extrinsic factors that can limit T cell responses.

Tumor microenvironmental effects on T cell metabolic fitness

T cells undergo metabolic reprogramming and adaptation during an immune response. Naive T cells are in a quiescent state and rely primarily on oxidative phosphorylation[86]. Upon antigen recognition, TCR ligation induces aerobic glycolysis and increases oxidative phosphorylation, which mediate metabolic reprogramming of effector T cell differentiation[87,88]. Co-stimulation through CD28 ligation enhances phosphoinositide 3-kinase (PI3K) and mammalian (or mechanistic) target of rapamycin complex 1 (mTORC1) activation, with mTORC1 serving as a signaling hub to coordinate T cell exit from quiescence and metabolic rewiring[89]. After antigen clearance, memory T cells again reacquire a quiescent state and use oxidative phosphorylation for their persistence; however, they retain the ability to rapidly engage glycolysis upon antigen rechallenge during a recall response[90]. By contrast, T cells in the tumor microenvironment are characterized by impaired mitochondrial fitness and functional capacity, which underlie their exhaustion and hypofunctional state. These alterations are associated with excessive levels of reactive oxygen species, which contribute to promoting exhaustion by several mechanisms, including altering expression of metabolic enzymes, inducing DNA damage and inhibiting phosphatase activity[91–94]. Therefore, to promote effective T cell anti-tumor function, it is important to restore mitochondrial fitness and function, such as by enforced expression of the transcription factors PGC-1α[95] or BATF[31,96,97] to circumvent T cell exhaustion. Moreover, altering specific pathways involved in mitochondrial metabolism can promote memory-like CD8$^+$ T cell differentiation and contribute to persistence and anti-tumor effects. Improved tumor control is achieved by deletion of genes encoding mitochondrial pyruvate carrier[98] or isocitrate dehydrogenase 2 (IDH2)[99] in T cells or by a glutamine antagonist[100]. Future work is warranted to ascertain the mechanistic basis and therapeutic potential of reprogramming T cell metabolism for improved anti-tumor effects.

Aside from such T cell-intrinsic regulation, the anti-tumor function of T cells is often restricted by nutrient deficits in the tumor microenvironment. Owing to their rapid rate of growth and proliferation, tumor cells often exhibit a unique metabolic profile, including increased uptake and metabolism of glucose and glutamine[101]. This altered cancer cell metabolism can directly affect T cell function by depriving T cells of glucose and glutamine, which are essential for T cell activation and function. Specifically, tumor and T cells can compete for glucose, and high glucose consumption by tumor cells restricts T cell function and metabolic rewiring, including attenuated mTORC1 and glycolytic activities[102,103]. Studies have now highlighted a complex interplay of nutrients in the tumor microenvironment, in which myeloid cells have the greatest degree of glucose uptake while cancer cells preferentially use glutamine[104]. This cancer cell-dependent glutamine restriction has a detrimental effect on the function of intratumoral type 1 conventional dendritic cells (cDC1s) that leads to impaired CD8$^+$ T cell anti-tumor function, and glutamine supplementation by intratumoral injection enhances anti-tumor immunity and overcomes resistance to ICB[105]. Moreover, other amino acids, such as arginine, methionine and serine, can be limiting in the tumor microenvironment, and such nutrient insufficiency impedes effective T cell anti-tumor immunity[106–108]. Beyond their metabolic effects, glucose and amino acids signal by a three-tiered process composed of nutrient transporters, protein sensors and transducers, thereby serving as signal 4 (that is, beyond the traditional immunological signals 1, 2 and 3) to license mTORC1 activation and T cell immunity[109,110]. Inosine, a nucleoside that can be derived from the microbiome, has been shown to have potent immunostimulatory effects on T cells that improve anti-tumor function[111–113]. Dietary nutrients also affect CD8$^+$ T cell function and anti-tumor immunity, including the stimulatory role of *trans*-vaccenic acid[114] and suppressive effects by the high-fat diet[115] and the artificial sweetener sucralose[116]. Other inhibitory metabolites can be produced by tumors such as lactic acid[117], cholesterol[118] and oxidized lipids[119] that can inhibit T cell function and anti-tumor immunity. For example, oxidized lipids and poly-unsaturated fatty acids can accumulate in the tumor microenvironment, and their increased uptake by CD8$^+$ T cells via the scavenger receptor CD36 leads to lipid peroxidation, ferroptosis and functional impairment of CD8$^+$ T cells. Blocking CD36 or inhibiting ferroptosis in CD8$^+$ T cells boosts their anti-tumor function, highlighting immunosuppressive effects of oxidized lipids in cancer[119,120]. Altogether, T cell anti-tumor immunity in the tumor microenvironment is shaped by nutrient or metabolite availability and composition, with both stimulatory and inhibitory factors and mechanisms, which offer opportunities for therapeutic intervention.

Emerging studies highlight the intricate interplay between immune signaling and metabolism. Immune signaling has long been known to regulate metabolism (for example, activation of mTORC1 and anabolic metabolism by TCR and co-stimulation), and more recent studies have revealed that chronic TCR signals act together with PD-1 and interleukin-10 (IL-10) to shape metabolic fitness, especially mitochondrial metabolism, of intratumoral CD8$^+$ T cells[91,92,121]. Conversely, growing evidence also highlights the signaling function of some metabolic intermediates, thereby forming bidirectional metabolic signaling crosstalk[86]. For example, phosphoenolpyruvate (PEP), a metabolic intermediate in glycolysis, is important for sustaining calcium and nuclear factor of activated T cells (NFAT) signaling by blocking the activity of sarcoendoplasmic reticulum calcium ATPase (SERCA), which mediates calcium reuptake in the endoplasmic reticulum[102]. Glucose deprivation results in defective PEP production, calcium–NFAT signaling and effector function, but increasing PEP production by overexpressing PEP carboxykinase 1 (PCK1) can boost T cell anti-tumor function[102]. As another example, glycolysis-derived intracellular ATP binds PI3K to modulate PI3K activity and downstream AKT and forkhead box O1 (FOXO1) signaling, which contributes to enhanced T cell function[122]. Additionally, D-2-hydroxyglutarate, which is an oncometabolite that drives tumorigenesis by acting as a competitive inhibitor of α-ketoglutarate-dependent dioxygenases, impairs CD8$^+$ T cell function by blocking the glycolytic enzyme lactate dehydrogenase (LDH)[123] or NFAT activation[124]. Future investigation of metabolite-mediated signal transduction, such as by systemically identifying metabolite–protein interactions[125], may lead to new insights into bidirectional metabolic signaling and actionable targets to bolster immune cell function for anti-tumor immunity.

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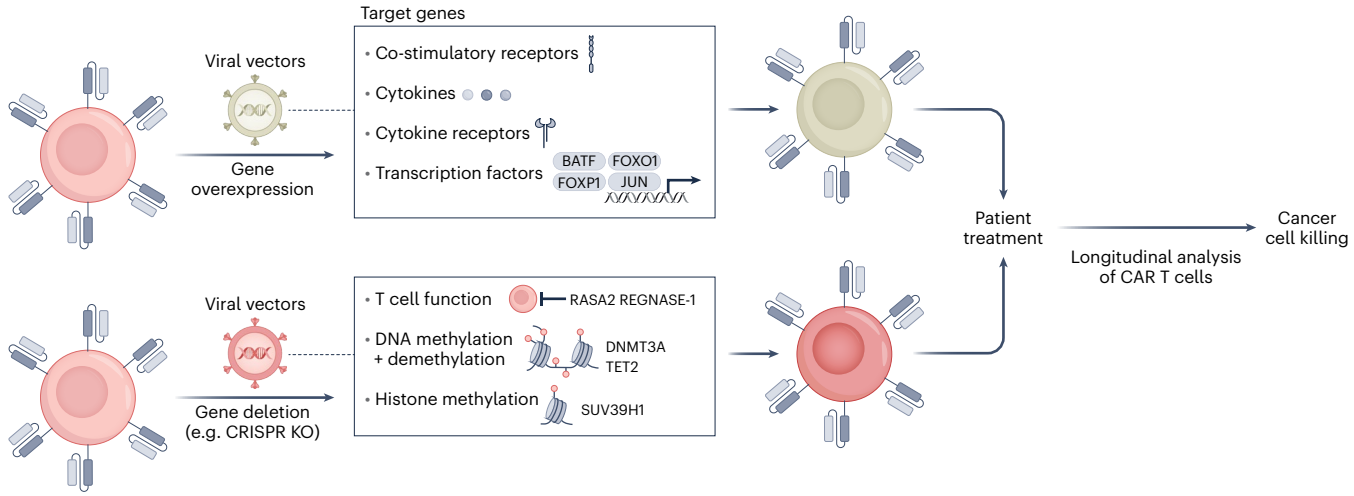

Fig. 3 | Rational design for future T cell therapies. To enhance the anti-tumor activity and persistence of CAR T cells, two main engineering approaches are used, including gene overexpression (for example, genes encoding co-stimulatory receptors, cytokines, cytokine receptors and transcription factors) and gene-editing technologies, such as CRISPR-mediated gene knockout (KO), to delete genes that encode molecules that suppress T cell function (such as RASA2 and REGNASE-1) or regulate DNA methylation (such as DNMT3A) or demethylation (such as TET2) and histone methylation (such as SUV39H1). After infusion into the patient, longitudinal analysis of CAR T cells is performed to monitor their persistence and anti-tumor response.

Immunometabolic regulation underlies T cell-based therapies. For example, CAR T cells expressing CD28 versus 4-1BB co-stimulatory domains display differential persistence, which is associated with their discrete metabolic alterations. Specifically, compared with CD28 domain-containing CAR T cells, 4-1BB domain-containing CAR T cells have better persistence, mitochondrial fitness and respiratory capacity[126]. Additionally, metabolic conditioning by culturing T cells with transient glucose restriction[127] or treatment with sodium bicarbonate[128] remodels their metabolism and improves anti-tumor function in adoptive cell therapy. Moreover, modulating metabolic enzyme expression in tumor-reactive T cells, such as by depleting IDH2 (refs. 99,129) or protein *O*-fucosyltransferase 1 (POFUT1)[130] or overexpressing proline dehydrogenase 2 (PRODH2)[131], results in better anti-tumor immunity from adoptive cell therapy. Immunometabolic regulation also functions in synergy with ICB and helps overcome therapeutic resistance to ICB. In particular, localized nutrient intervention in the tumor microenvironment is an emerging strategy to improve the therapeutic efficacy of ICB. For example, intratumoral methionine[107] or glutamine[105] injection or colonization of tumors with bacteria engineered to synthesize arginine[132] synergizes with ICB for improved anti-tumor immunity. A challenge in targeting the metabolic function of T cells is their cellular and metabolic heterogeneity in the tumor microenvironment. For example, precursor and terminally exhausted $CD8^+$ T cells show distinct metabolic profiles[133]. From this perspective, single cell-based CRISPR screens in vivo have enabled the identification of functional drivers in discrete tumor-specific T cell populations, the deletion of which rewires transcriptional, metabolic and proliferative activities[134]. Applications of these single cell-based functional genomics approaches promise to provide new insights and targets to improve T cell function and immunometabolism, even in the nutrient and metabolically challenging tumor microenvironment.

$CD4^+$–$CD8^+$ T cell crosstalk in anti-tumor immunity

Response to immunotherapy requires a coordinated immunological effort for successful $CD8^+$ T cell priming, activation and targeted tumor killing. These events are shaped by critical interactions between dendritic cells and $CD4^+$ T cells to foster the differentiation of tumor-targeting $CD8^+$ T cells. cDC1s are essential for response to anti-PD-L1 therapy, as they promote T_{pex} cell maintenance[135]. Mechanistically, cDC1s interact with T_{pex} cells via MHC-I to prevent overactivation in inflammatory environments[135]. Formation of T_{pex} cell-associated anatomical niches, which are mostly still undefined, prevents rapid differentiation of T_{pex} cells and thereby prolongs the therapeutic durability of ICB[135,136].

It has long been known that $CD4^+$ T cell help has a decisive effect on exhausted $CD8^+$ T cell populations. Even in the early description of T cell exhaustion in LCMV clone 13 infections in mice, it was recognized that the $CD8^+$ T cell response is more severely impaired after CD4 depletion[35]. Accordingly, a large proportion of these infections are now performed after CD4 depletion. The consequences of CD4 depletion on exhausted populations have since been elaborated upon. These studies have confirmed the known reduction in total $CD8^+$ T cell numbers following CD4 depletion but also revealed that this reduction results from a selective loss of terminally differentiated effector cells while the $TCF\text{-}1^+$ progenitor population was spared[137,138]. These observations from LCMV infection in mice seem to contrast those made from several tumor studies reporting that $CD4^+$ T cell removal increases anti-tumor activity[139–141]. The extent to which this effect results from removing T_{reg} cells as compared to conventional $CD4^+$ T cells is an open question. However, there is also strong evidence that $CD4^+$ T cells and, in particular, $CD4^+$ T cells directed against tumor-related antigens and neoantigens, in conjunction with their specific activation, assist the development of anti-tumor immunity[142,143]. These findings suggest a possible supportive function of $CD4^+$ T cells comparable with that in chronic infection, although the underlying mechanisms of how these cells support anti-tumor immunity are unclear. Similarly, the relevant $CD4^+$ T cell subsets are unknown, as are the molecules and cytokines through which this might occur. However, IL-2 and IL-21 are attractive and much-discussed candidates given their ability to expand specific populations of exhausted T cells[144–147]. Overall, despite all efforts to date, the biology of $CD4^+$ T cells and their effect on the exhausted $CD8^+$ T cell population is mostly unknown, but manipulating $CD4^+$ T cells has great potential to increase the efficacy of anti-tumor immunity.

Engineering to overcome tumor immune suppression

The immunosuppressive tumor microenvironment contributes to tumor growth and treatment resistance, thereby promoting cancer

development and progression. As discussed, recent advances have better characterized the mechanisms by which immunosuppression is induced by immune and metabolic dysregulation. Therefore, future T cell-based immunotherapy efforts are focused on improving T cell function in the setting of chronic antigen exposure as well as an immunosuppressive tumor microenvironment.

Limited efficacy of current CAR T cell approaches for B cell malignancies and multiple myeloma is often associated with suboptimal persistence: CAR T cells are able to induce an initial complete response, but lack of T cell persistence is associated with tumor recurrence. For example, in one study, patients with large B cell lymphoma who received CD19 CAR T cell therapy and had a complete response exhibited a threefold higher frequency of memory-like $CD8^+$ T cells in the infusion product than patients with a partial response or disease progression[148]. Consistent with these findings, TCF-1 expression is associated with more naive-like and early memory CD19 CAR T cells, which are maintained in patients with long-term CAR T cell persistence[149]. In addition to limited persistence, suboptimal initial anti-tumor activity presents a roadblock for CAR T cell treatment of solid tumors and brain tumors.

Two complementary engineering approaches are being pursued to enhance the anti-tumor activity and persistence of CAR T cells (Fig. 3). The first approach relies on overexpression of molecules, whereas the second approach takes advantage of gene-editing technologies, such as CRISPR, to delete genes that encode negative regulators of T cell function. Examples of the first approach include augmenting signals 2 and 3 of T cell activation in CAR T cells by transgenic expression of chimeric co-stimulatory receptors, secretory or membrane-bound cytokines, native and chimeric cytokine receptors, cytokine switch receptors or constitutive active cytokine receptors[150]. In this regard, a broad array of cytokine signaling pathways are being explored, including phosphorylation of signal transducer and activator of transcription 5 (STAT5) (for example, IL-15 (ref. 151) and IL-2 (ref. 152)) or STAT4 (for example, IL-12 (ref. 30)) or activating MyD88 (ref. 153) (for example, IL-18). Likewise, forced expression of transcription factors (for example, JUN[154]) to promote T cell stemness enhances CAR T cell function. Additional studies have also shown that the transcription factors FOXO1 and FOXP1 regulate a stem-like phenotype in CAR T cells and can be exploited to enhance CAR T cell anti-tumor ability[155–157]. Examples for the second approach include deleting genes encoding proteins that limit T cell function (for example, RASA2 (ref. 158) and REGNASE-1 (ref. 31)) or regulate T cell fate through DNA methylation or demethylation (for example, DNMT3A[29] and TET2 (ref. 159,160)) or histone methylation (for example, SUV39H1 (ref. 161)). Targeting these regulators of CAR T cell function is a promising approach that could be used to enhance cellular therapies in the future. However, rare genetic modifications that contribute to CAR T cell malignancies have been reported[162,163]. These uncommon events highlight the need to perform detailed safety studies for each individual genetic modification that is intended to augment the anti-tumor activity and persistence of CAR T cells.

Conclusions and future perspectives

The concepts of cancer immunosurveillance and cancer immunoediting can be summarized by the 'cancer-immunity cycle'[164,165]. The generation of cancer immunity produces immune-stimulatory factors that can broaden T cell responses, which may in turn amplify inhibitory and regulatory feedback mechanisms to dampen the immune response. This pattern of immune response and cancer-mediated immunosuppression can lead to both intrinsic and extrinsic T cell suppression and highlights the need for innovative T cell immunotherapy approaches to break the cycle.

Maintaining a long-lived population of T cells with sustained function over time is of pivotal importance for future strategies to improve immunotherapy. One report described iteratively stimulated T cells that were adoptively transferred into mice over the course of a decade[166]. Upon stimulation, these T cells were able to undergo a massive proliferative burst followed by a contraction phase that resulted in a pool of quiescent long-lived memory cells. Importantly, these T cells did not experience malignant transformation, despite undergoing rapid bursts of extensive proliferation. Additional efforts to better characterize the unique ability of mature T cells to maintain effector and proliferative potential are essential to further the advancement of long-lived T cell-based immunotherapies.

Investigation into human T cell longevity can be evaluated in patients receiving CAR T cell therapy. While patients are infused with a heterogeneous population of cells, specific subsets of CAR T cells experience outgrowth over time. Characterization of the remaining T cells could inform us about the molecular mechanisms enabling persistence of these T cells. Furthermore, additional targets could be identified by tracking CAR T cell proliferation after infusion and identifying survival-associated variants within the long-lived CAR T cell population. These patient-identified regulators of T cell durability could be used to engineer the next generation of T cell-based cellular therapies.

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Author contributions

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Competing interests

C.C.Z. declares patents related to epigenetic biomarkers and methods for enhancing CAR T cell function. S.G. is co-inventor on patents or patent applications in the fields of T cell and gene therapy for cancer, is a member of the scientific advisory board of Be Biopharma and CARGO and the Data and Safety Monitoring Board of Immatics and has received honoraria from Tessa Therapeutics within the last year. H.C. is a co-inventor on patents or patent applications in the field of immunotherapy and consults or consulted for Kumquat Biosciences, Chugai Pharmaceuticals, Ono Pharmaceutical and TCura Bioscience. D.Z. does not declare any competing interests.

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