The Bidirectional Interplay between T Cell–Based Immunotherapies and the Tumor Microenvironment

Alfredo Pherez-Farah[1], Gioia Boncompagni[1], Aleksey Chudnovskiy[2], and Giulia Pasqual[1,3]

ABSTRACT

T cell–based therapies, including tumor-infiltrating lymphocyte therapy, T-cell receptor–engineered T cells, and chimeric antigen receptor T cells, are powerful therapeutic approaches for cancer treatment. Whereas these therapies are primarily known for their direct cytotoxic effects on cancer cells, accumulating evidence indicates that they also influence the tumor microenvironment (TME) by altering the cytokine milieu and recruiting additional effector populations to help orchestrate the antitumor immune response. Conversely, the TME itself can modulate the behavior of these therapies within the host by either supporting or inhibiting their activity. In this review, we provide an overview of clinical and preclinical data on the bidirectional influences between T-cell therapies and the TME. Unraveling the interactions between T cell–based therapies and the TME is critical for a better understanding of their mechanisms of action, resistance, and toxicity, with the goal of optimizing efficacy and safety.

Introduction

Cellular immunotherapies are emerging as groundbreaking strategies in cancer treatment. These therapies encompass a variety of approaches, such as dendritic cell (DC) vaccines, cytokine-induced killer cells, NK cell–based strategies, and T cell–based immunotherapies, each harnessing distinct mechanisms to target and eliminate cancer cells (1). Among these, T cell–based approaches such as tumor-infiltrating lymphocyte (TIL) therapy, T-cell receptor–engineered T cells (TCR T), and chimeric antigen receptor T cells (CAR T) have gained substantial attention due to their clinical success, and several of them have been granted FDA approval for the treatment of certain malignancies in recent years (**Table 1**). Regardless of the specific approach, the main shared mechanism of action of T cell–based immunotherapies is the induction of cytotoxicity via the perforin/granzyme pathway; however, there is increasing evidence that their effects extend beyond the direct killing of tumor cells. For instance, they can modify the cytokine milieu and reshape the dynamics of the tumor microenvironment (TME) by recruiting and activating additional effector populations (2).

The TME is a highly coordinated network of immune cells, stromal cells, and vascular cells. The constant interplay between themselves, tumor cells, and other components of this bustling ecosystem determines the course of the disease by promoting antitumor responses or by favoring disease progression (3). The presence of therapeutic T cells adds an additional layer of complexity because they also integrate into this convoluted niche. Reciprocally, the TME influences the activity of therapeutic T cells, potentially supporting or inhibiting their activity. This phenomenon is particularly relevant because, despite their promise, T-cell therapies face substantial challenges that limit their clinical translation. Severe toxicities such as cytokine release syndrome (CRS) are frequent events in clinical settings (4). Additionally, limited efficacy due to T-cell exhaustion and reduced infiltration are major concerns, especially in solid malignancies (5). Unraveling the bidirectional communication between T cell–based therapies and the TME is crucial for a better understanding of their mechanisms of action, resistance, and toxicity, with the ultimate goal of optimizing their efficacy and safety. In this review, we will discuss preclinical and clinical research to provide an in-depth view of how these therapies influence, and are influenced by, the TME. For convenience, selected cell types will be discussed separately; nonetheless, in reality, these complex multicellular networks are inherently influenced by countless factors, including simultaneous interplay between several subsets of immune cells.

Influence of Lymphoid Cells in the TME on T Cell–Based Immunotherapies

Lymphoid populations in the TME

TILs are a heterogeneous group of lymphocytes that have migrated into the tumor site. Their presence is usually associated with a favorable immunotherapy response, underscoring their pivotal role in the antitumor response (6, 7). T cells, in particular, are the cornerstone of antitumor immunity, showcasing unparalleled functional versatility. Among T-cell subsets, $CD8^+$ cytotoxic T lymphocytes (CTL) execute direct cytotoxic functions, targeting and eliminating cancer cells (7, 8). On the other hand, $CD4^+$ T cells encompass a wide spectrum of activation states with distinct functions that influence the course of the disease (9). The Th1/Th2 balance is particularly relevant in cancer, as Th1 cells promote antitumor responses through the production of IFNγ and activation of CTLs, being therefore associated with a better immunotherapy response (10, 11). Additionally, the role of Th17 is context-dependent: in colorectal cancer, this type of response can support tumor growth and is associated with poor prognosis, whereas in other settings such as ovarian cancer, it is rather associated with an antitumor response (12, 13). Regulatory T cells (Treg), another crucial subset of $CD4^+$ T cells, inhibit antitumor

[1]Laboratory of Synthetic Immunology, Department of Surgery, Oncology and Gastroenterology, University of Padua, Padua, Italy. [2]The Francis Crick Institute, London, United Kingdom. [3]Veneto Institute of Oncology IOV IRCCS, Padua, Italy.

Corresponding Author: Giulia Pasqual, Department of Surgery, Oncology and Gastroenterology, University of Padua, via Gattamelata 64, Padua PD 35128, Italy. E-mail: giulia.pasqual@unipd.it

Cancer Immunol Res 2025;XX:XX–XX

doi: 10.1158/2326-6066.CIR-24-0857

Table 1. T-cell immunotherapies in clinical use.

Feature	TIL therapy (6, 137, 138)	CAR T-cell therapy (126, 139–145)	TCR T-cell therapy (146, 147)
Overview	TILs are isolated from the tumor, expanded *ex vivo*, and reinfused after lymphodepletion.	T cells are obtained from PBMCs, transduced with an antigen-specific CAR construct, expanded *ex vivo*, and reinfused after lymphodepletion.	T cells are collected from either PBMCs or TILs, transduced with an antigen-specific TCR, expanded *ex vivo*, and reinfused after lymphodepletion.
Advantages	Relies on the host's TCR repertoire with broad recognition of tumor-associated antigens and neoantigens.	MHC independency is useful in the context of HLA loss in cancer. CAR constructs can be engineered with several domains, on/off switches, and suicide genes to optimize efficacy and safety. Ongoing research on the generation of allogeneic "off-the-shelf" CAR T cells.	Can target intracellular antigens presented by MHC molecules.
Limitations	Requires access to tumor tissue, which can have great variability of TIL quality and quantity.	Limited to surface antigens. Potential for on-target/off-tumor effects due to shared antigen between tumor and healthy tissues. Difficult to predict and/or modulate antigen avidity and tonic signaling.	Requires precise epitope selection, TCR screening, and HLA matching. Potential for off-target/off-tumor effects due to TCR cross-reactivity.
Toxicities	CRS, neurotoxicity, and autoimmunity	CRS, neurotoxicity, cytopenias, and sepsis Recent reports of secondary malignancies, including CAR^+ leukemia.	CRS, neurotoxicity, and graft-vs-host disease
FDA status	Approved in 2024 for metastatic or unresectable melanoma previously treated with immunotherapy and/or kinase inhibitors. Several ongoing clinical trials for different malignancies.	First approved in 2017 for R/R pediatric B-cell ALL. Several other CD19-targeting CAR T cells have since been approved for other R/R B-cell malignancies such as DLBCL, MCL, FL, CLL, and ALL. Two anti-BCMA CAR T cells have been approved for R/R MM. Several ongoing clinical trials for other malignancies, including solid tumors.	Approved in 2024 for MAGE-A4–positive metastatic or unresectable synovial sarcoma. Several ongoing clinical trials for different malignancies.
Products in use	Lifileucel (Amtagvi)	Tisagenlecleucel (Kymriah) Axicabtagene ciloleucel (Yescarta) Brexucabtagene autoleucel (Tecartus) Lisocabtagene maraleucel (Breyanzi) Idecabtagene vicleucel (Abecma) Ciltacabtagene autoleucel (Carvykti) Obecabtagene autoleucel (Aucatzyl)	Afamitresgene autoleucel (Tecelra)

Abbreviations: ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; MM, multiple myeloma; PBMC, peripheral blood mononuclear cell; R/R, relapsed/refractory.

responses through various mechanisms, including suppression of effector T cells and modulation of antigen presentation, thereby promoting immune escape (14). B cells can also infiltrate the TME, in which they also play a dual role. Although they can foster an immunosuppressive TME by secreting cytokines such as IL10, IL35, and TGFβ, they can also produce tumor-specific antibodies and present tumor antigens to T cells, supporting adaptive antitumor immunity (15, 16). Interestingly, tumor-reactive antibodies can emerge from either autoreactive clones that show reactivity in their germline configuration or because of somatic hypermutation of nonbinding precursors (17). B cells are also fundamental constituents of tertiary lymphoid structures (TLS), which are associated with the presence of IgG class-switched plasma cells and predict favorable immunotherapy response (18–20). Lastly, NK cells are innately endowed with several tumor detection and destruction mechanisms, such as missing self-detection, recognition of NK group 2 member D (NKG2D) stress ligands, and antibody-dependent cellular cytotoxicity (21, 22). As we will discuss in detail in the next paragraphs, endogenous T cells, B cells, and NK cells in the TME can influence the outcome of T cell–based immunotherapies.

T cell–based immunotherapies can stimulate the endogenous T-cell response

The first observation linking T cell–based immunotherapies and lymphocytes populating the TME is their ability to stimulate the endogenous T-cell response. This statement holds true across all major types of T cell–based immunotherapies, namely adoptive T-cell therapy (ACT), TIL therapy, TCR T therapy, and CAR T therapy, and is supported by both preclinical and clinical evidence.

Preclinical data from different murine models has shown that ACTs recruit and activate host T cells as a part of the antitumor response (3, 23). In the TCR T and CAR T contexts, different mouse tumor models have consistently shown an increased infiltration and activation of endogenous T cells upon therapy (24–26). Moreover, through tetramer staining, several groups have independently demonstrated that CAR T-cell therapy promotes the expansion of endogenous tumor-specific T cells (2, 27). Additionally, Alizadeh and colleagues found that IL13Rα2 CAR T-cell therapy against murine glioblastoma promoted T cell–mediated immunologic memory generation. Briefly, tumor clearance in mice with previously robust TMEs rejected rechallenge with IL13Rα2–negative

tumors unlike those with less T cell–infiltrated TMEs. *Ex vivo*, T cells from CAR T–treated mice showed enhanced proliferation and cytotoxicity, and when transferred to new tumor-bearing mice, they controlled tumor growth better than T cells from untreated hosts (24). This observation is promising as it addresses the issue of antigen loss variants, a common drawback of CAR T-cell therapy.

Human data confirm an increased recruitment of endogenous T cells after CAR T-cell therapy in hematologic malignancies (28–32). For instance, a multiplex immunofluorescence–based study conducted on post–anti-CD19 CAR T-cell treatment biopsies from patients with diffuse large B cell lymphoma (DLBCL) showed that responsive samples had a higher $CD3^+$ infiltrate driven by a relative increase of the $CD8^+/CD4^+$ ratio. Notably, although CAR T cells represented <5% of the TME, their presence correlated with an increased positivity for Ki-67, PD-1, and granzyme B in endogenous T populations in comparison with pretreatment biopsies or post-treatment samples lacking CAR T cells. Accordingly, *in situ* hybridization showed that non–CAR T cells frequently expressed IFNγ in the presence of CAR-positive cells (33). These findings suggest that CAR T-cell treatment promotes activation of endogenous T cells, which are in turn significant producers of IFNγ. It is worth noting that the role of IFNγ in CAR T-cell cytotoxicity is still an area of active research: Larson and colleagues performed a genome-wide CRISPR knockout screening in different human cancer cell lines to identify genes responsible for resistance to CAR T-cell therapy, finding that the loss of genes associated with the IFNγR signaling pathway conferred resistance to CAR T-cell therapy in solid tumors but not in hematologic malignancies (34). In addition, Wang and colleagues recently found that after locoregional delivery of IL13Rα2 CAR T cells against pediatric brain tumors, there was an infiltration of CAR-negative T cells with $CD8^+$ T memory, $CD8^+$ T tissue-resident memory, and $CD4^+$ T effector (Teff) phenotypes in the cerebrospinal fluid, whereas CAR^+ T cells were mostly proliferating and exhausted. Single-cell TCR sequencing revealed clonal expansion of CAR-negative $CD8^+$ Teff and activated $CD4^+$ T cells (Res Sq 2023.rs.3.rs-3454977/v1). Indeed, a CAR T cell–induced expansion of endogenous T-cell clones and intraclonal plasticity have been reported by several other groups both in the tumor niche and peripherally, indicating that CAR T cells not only enhance endogenous T-cell cytotoxicity but also promote the expansion of specific clones (28, 35, 36).

Despite this shared evidence that T cell–based immunotherapies can stimulate the endogenous T-cell response, differences might exist depending on the specific therapy. For instance, a study comparing multiple CAR constructs indicated that the effect of CAR T cells on TILs was dependent on CAR design. Notably, 4-1BB–based CARs exhibited larger proportions of effector and regulatory $CD4^+$ clusters in the TME compared with CD28-based designs (37). These findings put into evidence the complexity of the CAR T-cell influence on endogenous T cells due to a kaleidoscope of factors such as the CAR construct itself.

Finally, it is important to consider the role of preconditioning regimes in this context. Lymphodepletion with cyclophosphamide/fludarabine is essential for creating a suitable niche for ACT engraftment and expansion (38, 39). However, these agents can also strongly reshape the TME by overcoming immunosuppressive cues and reversing T-cell exclusion in cold tumors (40). The extent to which endogenous T-cell reconstitution after ACT is a result of treatment rather than homeostatic proliferation is not fully elucidated. Recently, Louie and colleagues reported that after infusion, CAR^+ and CAR^- T cells share a similar differentiation trajectory toward an NK-like phenotype, suggesting that CAR T-cell expansion is partially driven by a response of homeostatic signals induced by preconditioning. Moreover, it was hypothesized that the greater clonal expansion observed in CAR^- $CD8^+$ T cells could be due to certain clones surviving lymphodepletion rather than a consequence of treatment (36). These findings highlight the relevance of lymphodepletion in ACTs and its impact in the TME. Unfortunately, pretreatment with lymphodepleting agents is not a standardized practice in preclinical models, restricting our understanding on how these agents impact the dynamics of T-cell therapies in the TME.

The endogenous T-cell response can contribute to the efficacy of T cell–based immunotherapies

As discussed above, T cell–based immunotherapies can stimulate the endogenous T-cell response. Nevertheless, the direct contribution of the endogenous T-cell response to the observed therapeutic effect has not been comprehensively characterized. In a seminal work, Marigo and colleagues (41) used the EG.7 murine lymphoma model to show that a CD40/CD40L-mediated interaction between the host's $CD8^+$ cells and myeloid cells was essential for tumor eradication upon $CD8^+$ ACT. Of interest, interactions between endogenous $CD8^+$ T cells and myeloid cells have also been recently described by CODEX imaging of ACT-treated B16 tumors, suggestive of local tumor antigen presentation (3).

In the context of a murine model of CAR T-cell therapy, it has been shown that IFNγ sensing by endogenous cells is crucial for survival upon therapy (2, 24). In the same setting, endogenous T and NKT cells exhibit strong IFN transcriptional signatures. Even if these data do not allow to quantify the contribution of endogenous T cells to therapeutic efficacy, they support their potential role in tumor eradication (2). Additional research in the future will be essential to evaluate the specific contribution of endogenous T cells in different T cell–based immunotherapy settings.

Preexisting T cells in the TME influence the response to T cell–based immunotherapies

Another important observation linking T cell–based immunotherapies and lymphocytes populating the TME is that preexisting T cells in the TME can influence the response to T cell–based immunotherapies. Also in this case, this statement holds true across all major types of T cells–based immunotherapies, although in different ways. For clarity, in the following paragraphs we will discuss separately the influence mediated by conventional αβ T cells, Tregs, and γδ T cells.

Conventional αβ T cells

In the context of TIL therapy, Barras and colleagues reported differentially enriched baseline CTL signatures between responding and nonresponding patients with melanoma. Of relevance, responders showed an enrichment of progenitor-exhausted $CD8^+$ T cells and exhausted $CD8^+$ T cells (Tex) states, which also exhibited high TCR signaling, IFNγ activation, and CD28 costimulation signatures, suggesting tumor antigen recognition and DC interaction (42). Similarly, Bachireddy and colleagues profiled bone marrow (BM) transcriptomes of patients with relapsed chronic myeloid leukemia who either responded or did not respond to salvage donor lymphocyte infusion (DLI), another type of ACT. In this study, pre-DLI analyses showed that responders' T cells exhibited higher phenotypic diversity and enrichment of late differentiated programs with signatures similar to the Tex state. Matched post-DLI analyses showed expansion of different T-cell states, but clonotype

overlapping suggests that therapy led to the expansion of T cells already present in the TME (43). More recently, this same group carried out a similar setup in the setting of acute myeloid leukemia, finding disease-specific trends (bioRxiv 2024.02.09.579677). Collectively, these findings highlight the influence of TME composition and functional status on response to TIL therapy and suggest a positive contribution of preexisting progenitor-exhausted and Tex states for response to TIL therapy.

The presence of endogenous T-cell infiltrate and/or of specific T-cell states has also been associated with clinical outcomes to CAR T-cell therapy, even though clear common trends are not yet present. In the context of CAR T cells, Brown and colleagues recently reported that pretreatment CD3 levels of glioblastoma biopsies correlated with improved overall survival after IL13Rα2 CAR T-cell therapy, with two patients even achieving complete remission. This finding is particularly surprising, given that the proportion of grade 4 tumors was higher in the intratumoral CD3-high cohort (44). In a recent article with data from a phase 1 clinical study of GD2 CAR T cells against neuroblastoma and osteosarcoma, presence in the peripheral blood of a naïve $CD8^+$ $CCR7^+$ $CD45RA^+$ T-cell cluster and enrichment of the Th1-associated RUNX3 transcription factor at baseline were associated with an adequate CAR T-cell expansion, whereas a $CD8^+$ $CCR7^+$ $CD38^+$ terminally differentiated effector T-cell cluster was associated with poor expansion (45). Additionally, Scholler and colleagues correlated the immune contexture of CD19 CAR T cell–treated patients with DLBCL with disease prognosis, showing that in the TME, responders had an increase in T-cell activation and IFN-related genes. Interestingly, it was also observed that peak circulating levels of CAR T cells were associated with a lower expression of Tex genes in the TME (46). Moreover, Sworder and colleagues recently showed that mutations on TNFRSF14 were a common finding in poor CD19 CAR T-cell expanders. Through immune deconvolution of pretreatment samples, they observed that tumors with such mutation also exhibited increased levels of follicular helper T and $CD4^+$ resting memory T cells, suggesting that the tumor genome itself can influence CAR T-cell therapy kinetics and activity (47).

Adverse CAR T-cell outcomes can also be explained by the mutual influence of CAR T cells and endogenous T cells. Strati and colleagues recently found that in the BM of patients with DLBCL who developed CAR T cell–related prolonged cytopenia, there was an overrepresentation of a clonally expanded $CD8^+$ Teff cluster with high expression of GZMB and CX3CR1 and enrichment of IFNγ-associated pathways (48).

In sum, preexisting T-cell populations in the TME can impact both positively and negatively the response to T cell–based immunotherapies; future work will be instrumental to define in a disease-specific fashion the roles of distinct T-cell states in therapy response. We also estimate that the identification of shared prognostic features would strongly benefit shared analysis pipelines and nomenclature, which would make it possible to compare findings from different trials.

Tregs

There are several reports indicating a sustained reduction of peripheral Tregs after CAR T-cell therapy (49, 50). However, the role of preexisting Tregs in the TME prior to ACT is less understood. As expected, baseline Treg enrichment has been linked to poor CAR T-cell expansion (45). In line with this, lower pretreatment levels of Treg-related soluble factors in the TME have been associated with better clinical outcomes after CD19 CAR T-cell therapy (32). It is worth noting that decreased levels of Tregs in the TME have also been consistently associated with high-grade CAR T cell–induced neurotoxicity (46, 51), likely reflecting an impaired regulation of the infusion product, which is why in some of these reports, Treg density is paradoxically associated with better clinical outcomes(46). Supporting this, Jain and colleagues (52) found that the IFN signaling, which is enriched in patients with nondurable responses to CD19 CAR T cells, had a strong correlation with macrophages, neutrophils, and DCs but not Tregs. Furthermore, whereas murine and human research confirms an expected link between tumor relapse and Treg infiltration, Sworder and colleagues (25, 47) reported that relapsed tumors could be classified based on low or high CAR T-cell content, with the latter exhibiting significantly higher levels of Tregs, showcasing that resistance to CAR T-cell therapy is dependent on additional mechanisms beyond Treg involvement. Finally, in the context of TIL therapy, as shown by Barras and colleagues (42), patients with progressive disease after TIL therapy had the lowest baseline proportion of Tregs, and in fact, the Treg signature was lost in nonresponders after treatment, highlighting the complex behavior of this population in different T-cell immunotherapies.

γδ T cells

So far, we have presented information about conventional αβ T-cell subtypes; however, two recent transcriptomic reports have involved γδ T cells in the context of T-cell therapies. As for TIL therapy, Barras and colleagues (42) showed a pretreatment enrichment of highly proliferative γδ T cells in patients with progressive disease, suggesting a negative influence on therapy. Additionally, in the CAR T-cell scenario, these cells expand in peripheral blood after infusion (36). Although these data are descriptive, they suggest a possible role of this rare subtype in the biology of cellular immunotherapies.

B cells can influence T cell–based immunotherapies by acting as antigen-presenting cells and providing costimulation

Because most CAR T cells in clinical use today target antigens that are also present in healthy B cells, researching their participation in this context is challenging. Additionally, because B cells are not typically considered major players in the TME, their interaction with other types of T-cell therapies has been poorly studied. One study reported that B cells prime antigen-specific $CD8^+$ T cells with enhanced antitumor immunity when *ex vivo* activated with TLR9 agonist CpG. Indeed, selective depletion of B cells from the CpG-treated activation culture negatively impacted the expansion of CTLs and almost completely abolished CTL persistence and antitumor efficacy *in vivo* (53). More recent works obtained from human data indicate that the B-cell lineage could be involved in therapy response. For instance, through interactome prediction, Maurer and colleagues reported that in DLI responders, plasma cells were part of a tumor-inhibiting multicellular network involving $CD4^+$ T, CTLs, and NKs. Moreover, through CODEX imaging, it was observed that prior to therapy, responders had a higher abundance of T- and B-cell niches (bioRxiv 2024.02.09.579677). Likewise, Barras and colleagues reported that in TIL responders, memory B cells displayed higher pretreatment IFN signaling and MHC II expression, suggesting a role in the modulation of local T cells; and indeed, responders exhibited higher frequencies of B–T doublets with tumor-reactive signatures, suggesting a costimulatory role. TLSs were also more abundant in the tumor stroma, leading to the hypothesis that B-cell contribution to the formation of TLSs could

be an additional mechanism through which T-cell immunotherapy is supported. Interestingly, although B cells were almost completely lost after treatment, in responders, the remaining memory B cells were predicted to interact with certain T-cell subsets such as T effector memory (TEM)-like, Tex, and ISG CD8$^+$ (42). Taken together, these results suggest that B cells might influence antitumor response upon T cell–based immunotherapies by providing costimulation, serving as local antigen-presenting cells (APC), and possibly supporting TLSs.

NK cells are recruited and activated in the TME upon T cell–based immunotherapies

Recent results indicate that NKs are recruited and activated in the tumor site upon T-cell therapy (2, 54). Nonetheless, they might be dispensable for the antitumor response in this context. For instance, Ramos and colleagues reported that cotreatment of CD19 CAR T cells and an NK-depleting antibody significantly improved mouse survival compared with CAR T-cell therapy alone. They propose that NK-derived IFNγ might decrease CAR T-cell efficacy by upregulating MHC-I molecules on tumor cells, leading to an engagement with NKG2A inhibitory molecules (22). In line with this, Textor and colleagues (55) showed that although NK cells infiltrated HER CAR T cell–treated ovarian tumors, NK depletion did not impact tumor rejection. Data on human studies are scarce and mostly descriptive, but they indicate that NK activation in the tumor site might hold prognostic value. Rade and colleagues (56) recently reported that in suboptimal responders to B-cell maturation antigen (BCMA) CAR T-cell therapy, there were fewer peripheral NK cells prior to infusion, as well as decreased activation of BM NK cells after treatment. Similarly, Maurer and colleagues reported that in acute myeloid leukemia, DLI responders had an expansion of GZMB, B3GAT1, FCGR3A NK clusters. Interactome prediction showed a response centered around cytolytic NKs and different CTLs, such as the ZNF683HI CD8$^+$ cluster. Interestingly, due to HLA-E/F and CD8A expression in this cross-talk, an antigen-presenting role of NK cells was proposed (bioRxiv 2024.02.09.579677). In sum, existing data fail to generate a consensus on the contribution of NK cells in the context of T cell–based immunotherapies but rather suggest that context-specific variables might define the role of NK cells.

Influence of Myeloid Cells in the TME on T Cell–Based Immunotherapies

Myeloid populations in the TME

Tumor-infiltrating myeloid cells (TIM) exhibit remarkable plasticity in the TME and are often the most abundant element. Frequently, they do not represent distinct populations but rather alternative activation states that are elicited in response to various cues such as tumor signals and therapeutic interventions. Indeed, in the context of T cell–based immunotherapies, they have been discussed based on their ontogenetic origin or their functional/transcriptional status. Such is the case for the M1/M2 and N1/N2 polarization of macrophages and neutrophils and the recently described mregDCs (57). Moreover, certain populations, mainly myeloid-derived suppressor cells (MDSC), represent a pathologically activated heterogenous group of cells originating from granulocytic or monocytic precursors rather than a single population (58). Because it is not possible to univocally convert functional definitions in ontogenetic lineages, we will discuss the contributions of different myeloid immune populations identified by both approaches, focusing our attention on monocytes, tumor-associated macrophages, monocyte-derived DCs (moDC), MDSCs, conventional DCs (cDC), and tumor-associated neutrophils (TANs). For convenience, we also include in this section plasmacytoid DCs (pDC), even if their myeloid or lymphoid origin is still a matter of debate. Finally, it is worth noting that some of these notations, such as M1/M2, are gradually being abandoned due to their failure to reflect the true complexity of such populations *in vivo*. Increasing multiomics evidence suggests that these classifications do not represent absolutely polarized cellular states but rather a more nuanced landscape (59, 60). However, because no official consensus currently exists, we will adhere to the nomenclature used in each specific study to avoid misinterpretation of the findings.

Key subsets of TIMs, including tumor-associated macrophages, TANs, and MDSCs, typically promote cancer progression by facilitating tumor growth, metastasis, and immune evasion (61). Other TIM populations like cDCs generally support antitumor immunity through antigen presentation and T-cell activation. Given their flexible nature, TIMs can be readily reprogrammed by different T-cell therapies to enhance their antitumor effect. However, the overwhelmingly immunosuppressive features of the TME prior to therapy can paradoxically hinder the efficacy of these treatments (61). Moreover, the plasticity that makes TIMs so adaptable also makes them challenging to study, as their constantly changing nature complicates efforts to characterize and target them.

Distinct monocyte populations are associated with variable CAR T-cell expansion and activity

Because monocytes differentiate into different populations in target tissues, they are not usually studied *per se* in the TME. Nonetheless, it is important to mention that higher blood levels of monocytes have consistently been associated with a less efficient CAR T-cell expansion (45, 62). Specific monocyte types might be responsible for this phenomenon. Kaczanowska and colleagues (45) reported that higher pretreatment levels of nonclassic CD14$^-$ CD16$^+$ CXCR3$^+$ monocytes were increased in good GD2 CAR T-cell expanders, whereas increased classical CD14$^+$ CD16$^-$ CXCR3$^-$ monocytes were associated with poor expansion. Coculturing GD2 CAR T cells with an osteosarcoma cell line and CXCR3-expressing THP-1 cells showed maintained IFNγ production as compared with untransduced THP-1 cells, suggesting that CXCR3$^+$ monocytes directly impact CAR T-cell activity. In line with this, it has been observed that BCMA CAR T cell–treated patients with multiple myeloma with better posttreatment outcomes had a lower proportion of BM CD14$^+$ myeloid cells (28, 56). Whereas the reasons for this association remain unclear, an enrichment of nonclassic CD16$^+$ monocytes has been recently reported to occur upon different T-cell therapies (30, 42). Through *in silico* prediction, Ledergor and colleagues (30) propose that in suboptimal responders, these cells engage with CD4$^+$ CAR T cells through the TGFβ axis, possibly driving them to exhaustion. Collectively, these observations support the notion that distinct monocyte populations are associated with variable CAR T-cell expansion and activity via a direct and/or indirect mechanism.

Macrophages are programmed toward an inflammatory state by T cell–based immunotherapies

A large body of work, carried out over the past few years, has identified macrophages as key players in the context of all types of T cell–based immunotherapies (63). In ACT, preclinical evidence overwhelmingly shows that an M1 polarization occurs as part of the

antitumor response. Importantly, this is true for both $CD8^+$ and $CD4^+$ ACTs. Marigo and colleagues (41) reported that $CD8^+$ ACT decreased $F4/80^+$ $ARG1^+$ M2 phenotype cells in the EG.7 TME. As for $CD4^+$ ACTs, Haabeth and colleagues (64) showed that Th1-polarized $CD4^+$ T cells eliminated multiple myeloma through a mechanism involving an IFNγ-mediated M1 shift of BM $F4/80^+$ cells. Accordingly, another group showed that in the B16 melanoma, $CD4^+$ ACT upregulated NOS2, IL1b, Cxcl10, and CD86 and decreased CD206 surface expression, confirming an M1 polarization (65). A similar observation was reported in the human biopsies of patients with synovial sarcoma treated with NY-ESO-1 TCR T cells, with decreased transcript and protein levels of CD163, another M2-associated molecule (66).

As for TIL therapy, the results from Barras and colleagues suggest that M1 polarization of the TME is not only a consequence of treatment but a requirement for antitumor response. Indeed, in responders, macrophages differentially expressed genes related to inflammatory processes, including antigen processing, complement synthesis, phagocytosis, and IFN signaling. Accordingly, ligandome analysis predicted a rich T cell–macrophage cross-talk, especially between $CD8^+$ Tex and IFN-stimulated macrophages, such as $CXCL9^+$ and $C1Q^+$, and to a lesser extent immunosuppressive macrophages such as $TREM2^+$ and $S100A8^+$. Moreover, T cell–myeloid doublets were enriched in CXCL9, suggesting that this particular subset is essential for supporting TIL engraftment. Post-treatment data showed that the $CD8^+$ T cell–$CXCL9^+$ macrophage interaction was a hallmark of response, overall suggesting a TIL-induced repolarization of TME macrophages (42).

With regard to CAR T cells, ample murine evidence also suggests an IFNγ-mediated shift of the M1/M2 ratio upon treatment (2, 24, 37, 55). Of relevance, these data come from very different tumor types, including blood, brain, and ovarian, showcasing that M1 polarization might be a common CAR T-cell mechanism regardless of the target tissue. Importantly, through a combination of $IFN\gamma^{-/-}$ CAR T cells or $IFN\gamma R^{-/-}$ hosts in a glioblastoma model, Alizadeh and colleagues confirm that IFNγ is responsible for such polarization. Of note, in this context, macrophage/microglia clusters exhibited higher expression of IFNγ-related genes as well as enrichment of the antigen processing and presentation machinery (24). This finding is in line with transcriptomic data from another murine glioma report in which macrophage/microglia clusters of CAR T cell–treated tumors displayed the largest and strongest set of predicted intercellular interactions, suggesting a relevant role in reshaping the TME upon therapy (37).

In humans, two groups independently reported that $CD163^+$ cells were higher in pretreatment biopsies of suboptimal CD19 CAR T-cell therapy responders, highlighting how the TME functional status contributes to T-cell therapy behavior (31, 32). Additionally, Faramand and colleagues (51) observed that patients who experienced severe CAR T cell–induced neurotoxicity exhibited higher TME macrophage scores, suggesting that they could serve as predictors of toxicity. In fact, the role of macrophages and microglia has long been proposed to be central in the pathophysiology CAR T-cell neurotoxicity (67). Findings from a phase I trial of GD2 CAR T cells targeting diffuse midline gliomas highlighted that the cerebrospinal fluid myeloid cell status and cytokine profile were highly influenced by the route of CAR T-cell administration. Specifically, intracerebroventricular infusion was linked to an IFN-dependent immune-activating signature during peak inflammation. In contrast, intravenous administration led to an immunosuppressive profile, characterized by MDSC-like signatures and previously described microglial states, such as disease-associated microglia and axon tract–associated microglia (68).

Taken together, these data indicate that, across different types of T cell–based immunotherapy and malignancies, macrophages in the TME polarize toward a proinflammatory state upon therapy. In some cases, this shift relies on IFNγ sensing and associates with better response to therapy, suggesting a relevant macrophage contribution to antitumor response. However, this polarization can also be associated with adverse effects such as CRS and neurotoxicity, underscoring the complexity of macrophages and related populations in T cell–based therapies and the need for better understanding this critical balance.

moDCs can support adoptive cell therapy via antigen presentation and cytokine secretion

MoDCs are phenotypically and functionally related to both macrophages and conventional DCs, making their study challenging (69). Certain DC subtypes, such as TNF/inducible nitric oxide synthase (iNOS)–producing DCs (Tip-DC) have been identified as crucial pieces for effective ACT. Marigo and colleagues showed that $CD8^+$ ACT reshaped the myeloid TME through an expansion of $Ly6C^+$ $MHC\text{-}II^+$ $iNOS2^+$ cells, which were identified as Tip DCs on the basis of *ex vivo* production of TNF. This population was found to support $CD8^+$ ACTs through tumor antigen cross-presentation (41). Accordingly, another group observed an accumulation of monocyte-derived DC3s in the B16 model upon treatment, further showing that they promoted the proliferation of the $CD8^+$ ACT in an antigen-specific manner (70). In the CAR T-cell context, an *in vitro* study showed that the presence of monocyte-derived APCs in an acute lymphoblastic leukemia/CD19 CAR T-cell coculture drastically increased cytokine secretion in a contact-independent fashion. Interestingly, it was possible to establish the relative contribution of each component to the cytokine profile, finding that IFNγ was exclusively produced by CAR T cells, whereas IL6 was exclusively released by monocyte-derived APCs (71). This finding is particularly relevant because IL6 is the main cytokine involved in CRS, yielding valuable insight into the pathophysiology of this complication.

MDSCs associate with suboptimal CAR T-cell therapeutic response

Although MDSCs are classically divided into monocytic (M-MDSC) and granulocytic subtypes, most evidence in the T-cell therapy context focuses mostly on M-MDSCs. Clinical data from large cohorts of patients treated with CD19 CAR T cells against B-cell malignancies and GD2 CAR T cells against osteosarcoma and neuroblastoma consistently correlated circulating levels of MDSCs with suboptimal responses (5, 52, 72). In the TME, they interfere with CAR T-cell activity, as shown by Burga and colleagues (73) in a model of liver metastasis of colon cancer, in which MDSCs were found to reduce CAR T-cell proliferation through the PD-1/PD-L1 axis. Contrastingly, another report showed that in the B16 model, ACT-derived IFNγ was essential for tumor suppression while paradoxically being responsible for the recruitment of M-MDSCs into the tumor site. For this reason, additional drivers of M-MDSC migration were sought, identifying CCR2 as a key receptor. When growing tumors on $CCR2^{-/-}$ mice, the infiltration of M-MDSCs was drastically decreased but a compensatory increase of granulocytic MDSCs was observed, putting into evidence the plasticity of the myeloid TME and its complexity in the T-cell therapy response (54). In sum, not surprisingly, existing data support a negative

association between MDSCs and CAR T-cell therapy, with possible implications of the PD-1/PD-L1 axis.

Classic DCs support T cell–based immunotherapies by T-cell interaction

Considering their central role in adaptive immunity, it comes as no surprise that cDCs support ACT. Recently, Espinosa Carrasco and colleagues double-transduced B16 cells with $CD8^+$ and $CD4^+$ restricted antigens to demonstrate that the coadministration of $CD4^+$ T cells reversed $CD8^+$ T-cell dysfunction and enhanced ACT efficacy. Depletion of $CD11c^+$ cells confirmed that DCs were essential for this phenomenon. In order to mimic clonal evolution, they engineered B16 cells to express either the $CD8^+$ or $CD4^+$ antigen and injected a mixture, observing that tumor cells spatially organized by antigen expression, which lead to independent antigen presentation to either $CD8^+$ or $CD4^+$ T cells by different DCs. In the presence of both antigens, a single DC copresented antigens to both T cells through epitope linkage, forming DC-centered triads, whose frequency was associated with tumor regression (74). In line with this, Kruse and colleagues previously reported that $CD4^+$ ACT engaged in long-lasting, antigen-driven interactions with $CD11c^+$ cells in the B16 tumor margin, leading to IFNγ release and consequent engagement of iNOS-expressing antitumoral phagocytes (75). Human data from DLI-treated acute myeloid leukemia samples support this notion, showing that responders had an enrichment of BM colocalization niches composed of CTLs, T effector memory cells, and DCs (bioRxiv 2024.02.09.579677). Of note, this T cell/DC cross-talk seems to be a necessary feature of the TME for an adequate response to TIL therapy (42). It is worth noting that DC infiltration into the TME depends on the tumor genotype, as reported by Spranger and colleagues, who showed that ACT failed to infiltrate β-catenin–expressing tumors due to a defective CXCR3–CXCL9/10 axis caused by a poor infiltrate of $CD103^+$ DCs (76).

In sharp contrast, another group reported that depletion of pre-cDCs in B16 animals transplanted with Zbtb46DTR BM did not impact $CD8^+$ ACT expansion, IFNγ release, or tumor growth, suggesting that both cDC1 and cDC2 were dispensable for ACT (70). This study suggests that other $CD11c^+$ nonconventional DC populations, such as the previously discussed moDCs, might play a more relevant role in this scenario. Finally, in the CAR T-cell context, several murine models have shown a recruitment of DCs upon therapy, sometimes even clustering together with the CAR T cells (2, 25, 27, 28, 77). Once again, IFNγ seems to mediate these dynamics, as suggested by the results of Boulch and colleagues, who reported that not only did cDC1s exhibit a robust IFN signature but also that coculturing tumor cells, CAR T-cells, and DCs lead to an upregulation of IL12, a hallmark of IFNγ sensing (2).

pDCs

Type I IFN–producing pDCs play a minor role in the TME and are usually overlooked. However, Barras and colleagues (42) reported that in pre-TIL therapy samples, a positive clinical response signature was largely confined to pDCs. As for CAR T cells, murine data showed that they upregulated antigen-presenting machinery genes after CD19 CAR T-cell treatment (2). Although this is not a widely recognized function of this population, it has been previously reported to occur, opening the door to a potential role in regulating T-cell immunotherapies (78).

Neutrophils may limit therapeutic T-cell activity in the TME

Neutrophils are by far the most relevant granulocyte in the TME (61). As for their role in T-cell therapies, Glodde and colleagues showed that the mobilization of neutrophils into the melanoma TME limited ACT efficacy by inhibiting T-cell proliferation and IFNγ secretion. Of note, because IFNγ itself contributes to the immunosuppressive phenotype of TANs through the upregulation of PD-L1, the authors proposed that this response is driven by the ACT itself (79). Contrastingly, Hirschorn and colleagues demonstrated that in the B16 model, $CD4^+$ ACT directly reeducated TANs to promote a neutrophil extracellular trap (NET)-independent, iNOS-mediated elimination of antigen loss variants. Of relevance, neutrophil depletion exhibited a biphasic behavior, with initial tumor regression followed by accelerated regrowth (80). Therefore, these results do not overrule an immunosuppressive role of TANs but suggest that their function depends on TME cues that may change over time.

As for CAR T-cell therapy, data on neutrophil contribution are limited. Two groups have independently shown that neutrophils exhibit strong IFN signatures after treatment (2, 24). However, the functional repercussion of this observation is unknown. It is worth noting that, although outside the TME, circulating neutrophils hold prognostic value for CAR T-cell response (81, 82). Indeed, neutrophil peaks have been identified as pioneers of inflammatory TNF and IL6/JAK-STAT3 cascades in patients with CRS after CAR T-cell therapy (82). More recent data agree with this observation and propose a myeloperoxidase-mediated endothelial damage as a promoter of CRS (83). As for other granulocytes, limited research suggests that eosinophils appear to be needed for appropriate CAR T-cell responses. *In vivo* eosinophil depletion in a murine lymphoma model significantly reduced CD19 CAR T-cell infiltration into the tumor site and impaired their antitumor effect (84). This finding is in line with the reports that peripheral eosinophil counts are positively correlated with better clinical outcomes in CAR T-cell therapy, highlighting an increasingly recognized role of a relatively rare population in this context (84, 85).

Special Mention: Role of Nonimmune Cells of the TME in T Cell–Based Immunotherapy

Although immune cells are the hallmark of the TME, the presence of additional stromal cells such as cancer associated fibroblasts (CAF) and mesenchymal stem cells (MSC) cannot be neglected (86). In certain malignancies, CAFs are among the most abundant populations of the TME, in which they have an overwhelmingly protumorigenic role. Recent research shows that when in proximity, CAFs inhibit the expansion and cytokine production of BCMA CAR T-cells through the PD-1/PD-L1 axis (87). Likewise, MSCs have been reported to interfere with ACT and CAR T cells both by enhancing cancer cell resistance and by directly affecting their activity (88, 89). Unexpectedly, multiple leukemia models reveal that although their presence indeed reduced CAR T-cell proliferation and cytokine secretion and induced a senescent phenotype, the cytotoxic activity itself was unaffected (90, 91). Furthermore, the differential impact of MSCs in CAR T-cell subpopulations is poorly understood. For instance, whereas Towers and colleagues (90) reported a relative sparing of $CD8^+$ CAR T cells with preferential abrogation of $CD4^+$ CAR T-cell expansion, in a similar setup Zhang and colleagues (92) found the exact opposite trend. Finally, human data on CD19 CAR T cell–treated patients suggest that CAR T cell–induced pancytopenia is associated with the disruption of $CD271^+$ BM niche stromal cells, revealing a potential role of this population as a biomarker of adverse therapy outcomes (93).

Interestingly, both MSCs and CAFs have also been implicated in the regulation of T-cell metabolic state upon therapy. Upon activation, T cells require rapid energy production via glycolysis to support their proliferation and cytotoxic function against tumor cells. However, tumor cells compete with immune cells for essential nutrients such as glucose and amino acids, leading to nutrient depletion within the TME. This results in oxidative and mitochondrial stress in T cells, ultimately compromising their function. Recent studies indicate that restoring or enhancing metabolic function in T cells can enhance their survival and improve the efficacy of T cell–based therapies (94–98). For instance, Baldwin and colleagues (94) demonstrated that intercellular mitochondrial transfer from MSCs to T cells can restore mitochondrial fitness in exhausted T cells, which also seems to be associated with a reduction in the expression of exhaustion markers such as PD-1, LAG3, and TIGIT. Furthermore, Morotti and colleagues demonstrated that the release of immunosuppressive molecules, such as prostaglandins, by tumor cells impairs the efficacy of T cell–based therapies. Specifically, the prostaglandin E 2–EP2/EP4 axis disrupts TIL metabolism by altering the assembly of the IL2 receptor. Blocking this axis during TIL expansion restores IL2 sensitivity, and via the mTOR pathway, mitochondrial fitness is recovered, leading to enhanced T-cell proliferation (99).

These findings, despite not being comprehensive, suggest an inhibitory function of CAFs and a variable impact of MSCs on therapeutic T cells. Additional characterization of this cross-talk might be instrumental for the future engineering of CAR T cells with biological functions counteracting nonimmune cell negative conditioning.

Conclusions and Future Directions

In this review, we have presented evidence from preclinical research and clinical studies, highlighting the bidirectional interplay between T-cell immunotherapies and the TME. Based on the discussed research, we proposed possible immunologic mechanism linking immune cells in the TME and T cell–based immunotherapies (**Fig. 1**). We discuss how T-cell therapies reshape the TME through many mechanisms, including the recruitment and activation of endogenous T cells and the inflammatory polarization of the myeloid compartment. Importantly, many of these mechanisms are heavily reliant on T cell–derived IFNγ. Moreover, we discussed how the baseline composition and functional status of the TME can affect the kinetics and dynamics of T-cell therapies in the host, influencing therapy response and clinical outcomes. This information is crucial for the optimization of current T-cell therapy approaches as well as for the development of safer, more effective approaches.

Despite this growing interest, there still is a paucity of high-resolution single-cell multiomics datasets that reveal how the bidirectional influence of ACTs and the TME influence therapy outcomes. Capturing high-quality data at the single-cell level, other than being technically challenging and costly, is complicated in this setting due to the dynamic nature of both ACTs and the TME as well as the extreme intertumoral and intratumoral heterogeneity observed in cancer (100, 101). Due to this variability, functional signatures are often only partially consistent across studies. This partial overlap arises because different research settings address distinct questions with different methodologies. As a result, even well-defined signatures may not be universally reproducible, leading to an overidentification of cellular states and complicating the establishment of a universally accepted taxonomy. Moreover, high-throughput technologies continue to evolve, with novel techniques allowing for the simultaneous assessment of genomic, transcriptomic, proteomic, and metabolomic data coupled with spatial and temporal information (102, 103). Although these approaches individually offer irreplaceable insight, the integration of such complex multidimensional data and their translation into biological models and clinical implications is a difficult task (104).

For ACTs in particular, this is further accentuated because data can potentially be obtained from T cells at the moment of apheresis, from the infusion product after *ex vivo* manipulation, and based on the functional status they acquire in the host, each carrying distinct implications (105, 106). Once again, even when such data are successfully collected, generalization remains challenging. Not only do each patient's T cells exhibit inherent variability, but by this point, their fitness has also been substantially altered by extensive first- and second-line treatments (107). Further complicating matters, variations in manufacturing processes can yield CAR T-cell products that target the same antigen but with unique preinfusion phenotypes, driving the expansion of specific states within the host (108, 109). Each of these T-cell states could potentially engage and interact with the TME in diverse ways, adding yet another layer of complexity to treatment outcomes.

Still, in recent years, substantial efforts have focused on characterizing and linking T-cell states at different stages of the CAR T cell "life cycle" to specific clinical outcomes with the hope of finding trends to improve efficacy and safety (110–112). Although this topic warrants a review article of its own, some key findings are worth mentioning. For instance, several groups have independently associated naïve and stem cell–like memory phenotypes of apheresis material and CAR T-cell products to overall better and more sustained clinical responses (113–115). Similar signatures have also been found on long-lived CAR T cells after infusion (116, 117). Of particular relevance, the most recent paper from June's group has generated a comprehensive single-cell multiomics atlas from nearly 700,000 preinfusion CAR T cells from 82 patients with acute lymphoblastic leukemia who had been enrolled in the very first CAR T-cell clinical trials. Surprisingly, a type 2 functional status of the infusion products was associated with sustained therapeutic responses (118, 119). In contrast, Treg-like trajectories of CAR T cells have been consistently linked to impaired expansion and progressive disease (108, 120). These findings collectively stress the value of such research efforts in uncovering unforeseen information that could potentially guide future therapy improvements.

The scope of this review was limited to well-established preclinical models of ACT as well as currently approved T-cell immunotherapies. Looking ahead, emerging T-cell approaches based on unconventional subsets such as NKT and γδ T cells are expected to make a boom due to their unique properties, including classical MHC independence, expression of NKG2 receptors, and CD1d restriction (121, 122). In this context, recent advancements include the development of MHC-independent TCR receptors and double-chain chimeric synthetic TCR and antigen receptors as alternatives to address CAR T-cell limitations (123, 124).

Moreover, synthetic TILs and next-generation CAR T cells (e.g., TRUCKs, SUPRA CARs, tandem CARs, etc.) can be armored with a wide array of matrix enzymes, chemokines, and cytokines or even be used as microphamacies to deliver therapeutic agents to the TME. This enables active remodeling of the TME by recruiting and reprogramming various immune cell populations (125–129). Additionally, these therapies can be engineered to simultaneously

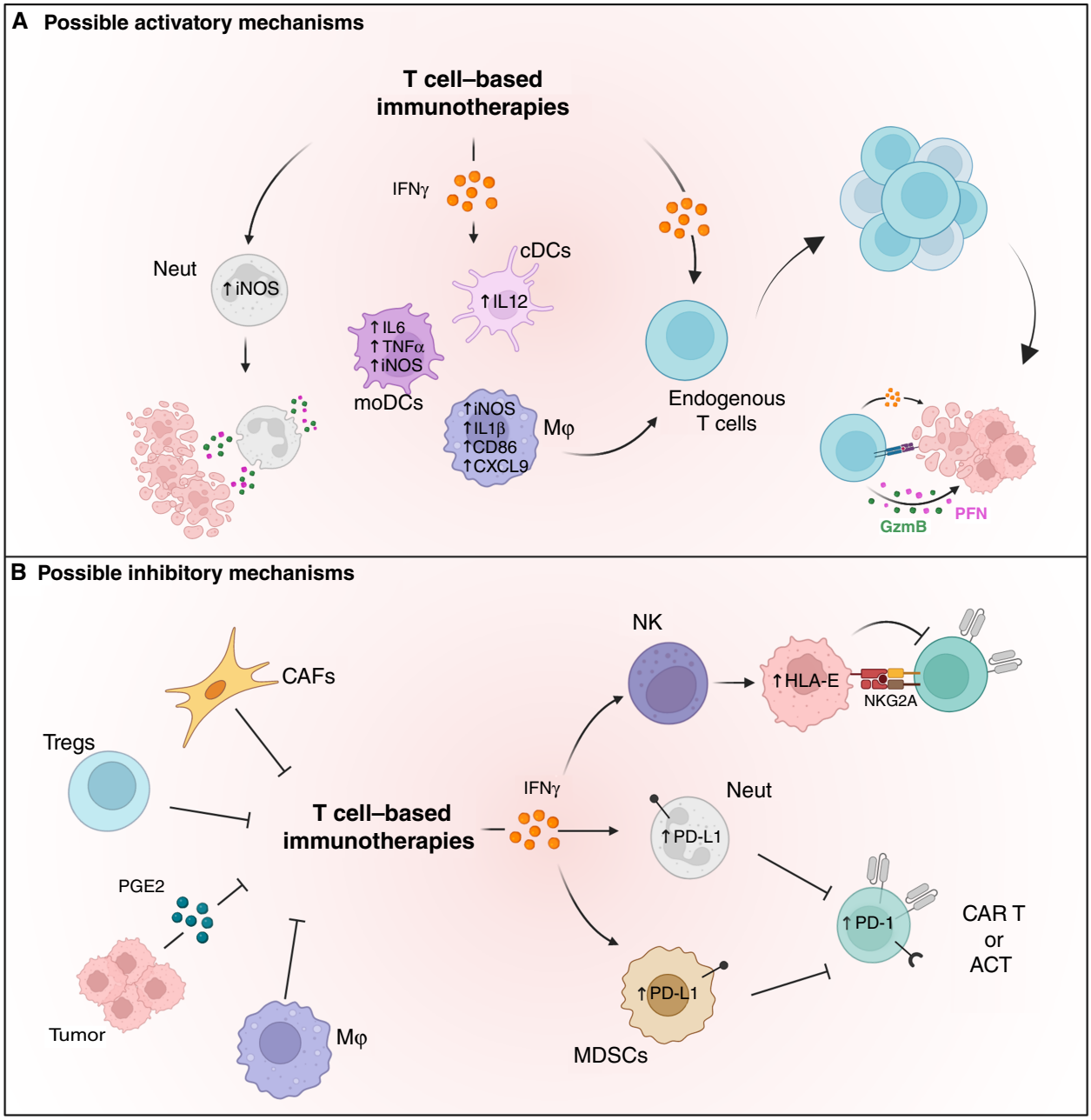

Figure 1.

Possible mechanisms linking immune and nonimmune cells in the TME and T cell–based immunotherapies **A,** Administration of T cell–based immunotherapies can induce in immune cells functional changes capable of supporting the antitumor response. Due to iNOS upregulation, neutrophils can kill more efficiently tumor cells; cDCs, moDCs, and macrophages can upregulate several proinflammatory mediators, supporting both therapeutic and endogenous T cells. Tumor-specific T-cell clones might undergo expansion and contribute to tumor eradication. **B,** Immune cells in the TME can also negatively modulate the antitumor response upon T cell–based immunotherapies. Preexisting macrophages (depending on their state) and Tregs could dampen the therapeutic response. Neutrophils and MDSCs exposed to IFNγ can upregulate PD-L1 and engage in inhibitory interactions with PD-1 expressed by therapeutic and endogenous T cells. NK cells, via IFNγ secretion, can lead to HLA-E upregulation by tumor cells, which can be recognized by the NKG2A/CD94 complex and inhibit NK and $CD8^+$ T-cell cytotoxic function. Additionally, nonimmune cells such as tumor cells themselves, via PGE2 release, and CAFs can limit the therapeutic efficacy of adoptively transferred T cells. ACT, adoptive cell therapy; GzmB, granzyme B; Mφ, macrophages; Neut, neutrophils; PFN, perforin; PGE, prostaglandin E.

target multiple antigens, including nontumoral targets within the TME, to optimize their infiltration, persistence, and functional activity (130–132).

Furthermore, combinations of T cell–based therapies with other strategies such as radiotherapy, chemotherapy, immune checkpoint blockade, cancer vaccines, and oncolytic viruses have

shown to markedly improve antitumor efficacy by sharply reshaping the TME (133–136). Finally, novel immunotherapeutic approaches, such as bispecific T-cell engagers and TCR mimics, also represent exciting strategies to leverage T cells for precise targeting of cancer cells (38). Although these agents are not T cells *per se*, exploring how their activity is influenced by the TME could also be beneficial for optimizing their therapeutic potential.

Overall, these promising advancements pave the way for a new era in cancer treatment, in which a deep understanding of the tumor, its microenvironment, and its host, coupled with increasingly innovative T-cell therapies, bring us one step forward in the race of personalized medicine.

Authors' Disclosures

No disclosures were reported.

Acknowledgments

This work was supported by the PRIN grant 20209Y5YFZ from the Italian Ministry of University and Research (G. Pasqual), by the grant GR-2019-12368485 from the Italian Ministry of Health (G. Pasqual), and by the European Research Council Starting Grant SYNVIVO 853179 (G. Pasqual). A. Pherez-Farah is supported by the European Union's Horizon 2020 Research and Innovation Program under the Marie Skłodowska-Curie grant agreement No 101034319.

Received September 2, 2024; revised November 6, 2024; accepted January 7, 2025; published first January 9, 2025.

References

1. Han J, Zhang B, Zheng S, Jiang Y, Zhang X, Mao K. The progress and prospects of immune cell therapy for the treatment of cancer. Cell Transplant 2024;33:9636897241231892.
2. 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.
3. Hickey JW, Haist M, Horowitz N, Caraccio C, Tan Y, Rech AJ, et al. T cell-mediated curation and restructuring of tumor tissue coordinates an effective immune response. Cell Rep 2023;42:113494.
4. Brudno JN, Kochenderfer JN. Recent advances in CAR T-cell toxicity: mechanisms, manifestations and management. Blood Rev 2019;34:45–55.
5. Tumino N, Weber G, Besi F, Del Bufalo F, Bertaina V, Paci P, et al. Polymorphonuclear myeloid-derived suppressor cells impair the anti-tumor efficacy of GD2.CAR T-cells in patients with neuroblastoma. J Hematol Oncol 2021;14:191.
6. Monberg TJ, Borch TH, Svane IM, Donia M. TIL therapy: facts and hopes. Clin Cancer Res 2023;29:3275–83.
7. Whiteside TL. Tumor-infiltrating lymphocytes and their role in solid tumor progression. Exp Suppl 2022;113:89–106.
8. Okła K, Farber DL, Zou W. Tissue-resident memory T cells in tumor immunity and immunotherapy. J Exp Med 2021;218:e20201605.
9. Montauti E, Oh DY, Fong L. $CD4^+$ T cells in antitumor immunity. Trends Cancer 2024;10:969–85.
10. Rudqvist NP, Charpentier M, Lhuillier C, Wennerberg E, Spada S, Sheridan C, et al. Immunotherapy targeting different immune compartments in combination with radiation therapy induces regression of resistant tumors. Nat Commun 2023;14:5146.
11. Tay RE, Richardson EK, Toh HC. Revisiting the role of $CD4^+$ T cells in cancer immunotherapy-new insights into old paradigms. Cancer Gene Ther 2021;28:5–17.
12. Grivennikov SI, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D, et al. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. Nature 2012;491:254–8.
13. Marques HS, de Brito BB, da Silva FAF, Santos MLC, de Souza JCB, Correia TML, et al. Relationship between Th17 immune response and cancer. World J Clin Oncol 2021;12:845–67.
14. Li C, Jiang P, Wei S, Xu X, Wang J. Regulatory T cells in tumor microenvironment: new mechanisms, potential therapeutic strategies and future prospects. Mol Cancer 2020;19:116.
15. Downs-Canner SM, Meier J, Vincent BG, Serody JS. B cell function in the tumor microenvironment. Annu Rev Immunol 2022;40:169–93.
16. Fremd C, Schuetz F, Sohn C, Beckhove P, Domschke C. B cell-regulated immune responses in tumor models and cancer patients. Oncoimmunology 2013;2:e25443.
17. Mazor RD, Nathan N, Gilboa A, Stoler-Barak L, Moss L, Solomonov I, et al. Tumor-reactive antibodies evolve from non-binding and autoreactive precursors. Cell 2022;185:1208–22.e21.
18. Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, et al. B cells and tertiary lymphoid structures promote immunotherapy response. Nature 2020;577:549–55.
19. Weng Y, Yuan J, Cui X, Wang J, Chen H, Xu L, et al. The impact of tertiary lymphoid structures on tumor prognosis and the immune microenvironment in non-small cell lung cancer. Sci Rep 2024;14:16246.
20. Zhang Y, Liu G, Zeng Q, Wu W, Lei K, Zhang C, et al. CCL19-producing fibroblasts promote tertiary lymphoid structure formation enhancing anti-tumor IgG response in colorectal cancer liver metastasis. Cancer Cell 2024;42:1370–85.e9.
21. Barber A, Rynda A, Sentman CL. Chimeric NKG2D expressing T cells eliminate immunosuppression and activate immunity within the ovarian tumor microenvironment. J Immunol 2009;183:6939–47.
22. Ramos A, Koch CE, Liu-Lupo Y, Hellinger RD, Kyung T, Abbott KL, et al. Leukemia-intrinsic determinants of CAR-T response revealed by iterative in vivo genome-wide CRISPR screening. Nat Commun 2023;14:8048.
23. Goddard ET, Linde MH, Srivastava S, Klug G, Shabaneh TB, Iannone S, et al. Immune evasion of dormant disseminated tumor cells is due to their scarcity and can be overcome by T cell immunotherapies. Cancer Cell 2024;42:119–34.e12.
24. Alizadeh D, Wong RA, Gholamin S, Maker M, Aftabizadeh M, Yang X, et al. IFNγ is critical for CAR T cell-mediated myeloid activation and induction of endogenous immunity. Cancer Discov 2021;11:2248–65.
25. Zhao K, Ren C, Tang D, Zhao L, Chen X, Wang Y, et al. The altering cellular components and function in tumor microenvironment during remissive and relapsed stages of anti-CD19 CAR T-cell treated lymphoma mice. Front Immunol 2023;14:1101769.
26. Canè S, Barouni RM, Fabbi M, Cuozzo J, Fracasso G, Adamo A, et al. Neutralization of NET-associated human ARG1 enhances cancer immunotherapy. Sci Transl Med 2023;15:eabq6221.
27. Pituch KC, Miska J, Krenciute G, Panek WK, Li G, Rodriguez-Cruz T, et al. Adoptive transfer of IL13Rα2-specific chimeric antigen receptor T cells creates a pro-inflammatory environment in glioblastoma. Mol Ther 2018;26:986–95.
28. Dhodapkar KM, Cohen AD, Kaushal A, Garfall AL, Manalo RJ, Carr AR, et al. Changes in bone marrow tumor and immune cells correlate with durability of remissions following BCMA CAR T therapy in myeloma. Blood Cancer Discov 2022;3:490–501.
29. Jin J, Lin L, Meng J, Jiang L, Zhang M, Fang Y, et al. High-multiplex single-cell imaging analysis reveals tumor immune contexture associated with clinical outcomes after CAR T cell therapy. Mol Ther 2024;32:1252–65.
30. Ledergor G, Fan Z, Wu K, McCarthy E, Hyrenius-Wittsten A, Starzinski A, et al. CD4+ CAR T-cell exhaustion associated with early relapse of multiple myeloma after BCMA CAR T-cell therapy. Blood Adv 2024;8:3562–75.
31. Olson NE, Ragan SP, Reiss DJ, Thorpe J, Kim Y, Abramson JS, et al. Exploration of tumor biopsy gene signatures to understand the role of the tumor microenvironment in outcomes to lisocabtagene maraleucel. Mol Cancer Ther 2023;22:406–18.
32. Yan Z-X, Li L, Wang W, OuYang B-S, Cheng S, Wang L, et al. Clinical efficacy and tumor microenvironment influence in a dose-escalation study of anti-CD19 chimeric antigen receptor T cells in refractory B-cell non-Hodgkin's lymphoma. Clin Cancer Res 2019;25:6995–7003.

33. Chen PH, Lipschitz M, Weirather JL, Jacobson C, Armand P, Wright K, et al. Activation of CAR and non-CAR T cells within the tumor microenvironment following CAR T cell therapy. JCI Insight 2020;5:e134612.
34. Larson RC, Kann MC, Bailey SR, Haradhvala NJ, Llopis PM, Bouffard AA, et al. CAR T cell killing requires the IFNγR pathway in solid but not liquid tumours. Nature 2022;604:563–70.
35. Hegde M, Joseph SK, Pashankar F, DeRenzo C, Sanber K, Navai S, et al. Tumor response and endogenous immune reactivity after administration of HER2 CAR T cells in a child with metastatic rhabdomyosarcoma. Nat Commun 2020;11:3549.
36. Louie RHY, Cai C, Samir J, Singh M, Deveson IW, Ferguson JM, et al. CAR^+ and CAR^- T cells share a differentiation trajectory into an NK-like subset after CD19 CAR T cell infusion in patients with B cell malignancies. Nat Commun 2023;14:7767.
37. Haydar D, Ibañez-Vega J, Crawford JC, Chou C-H, Guy CS, Meehl M, et al. CAR T-cell design-dependent remodeling of the brain tumor immune microenvironment modulates tumor-associated macrophages and anti-glioma activity. Cancer Res Commun 2023;3:2430–46.
38. Klebanoff CA, Khong HT, Antony PA, Palmer DC, Restifo NP. Sinks, suppressors and antigen presenters: how lymphodepletion enhances T cell-mediated tumor immunotherapy. Trends Immunol 2005;26:111–7.
39. Williams KM, Hakim FT, Gress RE. T cell immune reconstitution following lymphodepletion. Semin Immunol 2007;19:318–30.
40. Murad JP, Tilakawardane D, Park AK, Lopez LS, Young CA, Gibson J, et al. Pre-conditioning modifies the TME to enhance solid tumor CAR T cell efficacy and endogenous protective immunity. Mol Ther 2021;29:2335–49.
41. Marigo I, Zilio S, Desantis G, Mlecnik B, Agnellini AHR, Ugel S, et al. T cell cancer therapy requires CD40-CD40L activation of tumor necrosis factor and inducible nitric-oxide-synthase-producing dendritic cells. Cancer Cell 2016;30:377–90.
42. Barras D, Ghisoni E, Chiffelle J, Orcurto A, Dagher J, Fahr N, et al. Response to tumor-infiltrating lymphocyte adoptive therapy is associated with preexisting $CD8^+$ T-myeloid cell networks in melanoma. Sci Immunol 2024;9:eadg7995.
43. Bachireddy P, Azizi E, Burdziak C, Nguyen VN, Ennis CS, Maurer K, et al. Mapping the evolution of T cell states during response and resistance to adoptive cellular therapy. Cell Rep 2021;37:109992.
44. Brown CE, Hibbard JC, Alizadeh D, Blanchard MS, Natri HM, Wang D, et al. Locoregional delivery of IL-13Rα2-targeting CAR-T cells in recurrent high-grade glioma: a phase 1 trial. Nat Med 2024;30:1001–12.
45. Kaczanowska S, Murty T, Alimadadi A, Contreras CF, Duault C, Subrahmanyam PB, et al. Immune determinants of CAR-T cell expansion in solid tumor patients receiving GD2 CAR-T cell therapy. Cancer Cell 2024;42:35–51.e8.
46. Scholler N, Perbost R, Locke FL, Jain MD, Turcan S, Danan C, et al. Tumor immune contexture is a determinant of anti-CD19 CAR T cell efficacy in large B cell lymphoma. Nat Med 2022;28:1872–82.
47. Sworder BJ, Kurtz DM, Alig SK, Frank MJ, Shukla N, Garofalo A, et al. Determinants of resistance to engineered T cell therapies targeting CD19 in large B cell lymphomas. Cancer Cell 2023;41:210–25.e5.
48. Strati P, Li X, Deng Q, Marques-Piubelli ML, Henderson J, Watson G, et al. Prolonged cytopenia following CD19 CAR T cell therapy is linked with bone marrow infiltration of clonally expanded IFNγ-expressing CD8 T cells. Cell Rep Med 2023;4:101158.
49. Guarini A, Radice G, Peragine N, Buracchi C, De Propris MS, Di Rocco A, et al. Long-term host immune modulation following tisagenlecleucel administration in patients with diffuse large B-cell lymphoma and B-lineage acute lymphoblastic leukemia. Cancers (Basel) 2023;15:2411.
50. Qi C, Xie T, Zhou J, Wang X, Gong J, Zhang X, et al. CT041 CAR T cell therapy for Claudin18.2-positive metastatic pancreatic cancer. J Hematol Oncol 2023;16:102.
51. Faramand R, Jain M, Staedtke V, Kotani H, Bai R, Reid K, et al. Tumor microenvironment composition and severe cytokine release syndrome (CRS) influence toxicity in patients with large B-cell lymphoma treated with axicabtagene ciloleucel. Clin Cancer Res 2020;26:4823–31.
52. Jain MD, Zhao H, Wang X, Atkins R, Menges M, Reid K, et al. Tumor interferon signaling and suppressive myeloid cells are associated with CAR T-cell failure in large B-cell lymphoma. Blood 2021;137:2621–33.
53. Smith AS, Knochelmann HM, Wyatt MM, Rangel Rivera GO, Rivera-Reyes AM, Dwyer CJ, et al. B cells imprint adoptively transferred $CD8^+$ T cells with enhanced tumor immunity. J Immunother Cancer 2022;10:e003078.
54. Hosoi A, Matsushita H, Shimizu K, Fujii S, Ueha S, Abe J, et al. Adoptive cytotoxic T lymphocyte therapy triggers a counter-regulatory immunosuppressive mechanism via recruitment of myeloid-derived suppressor cells. Int J Cancer 2014;134:1810–22.
55. Textor A, Listopad JJ, Wuhrmann LL, Perez C, Kruschinski A, Chmielewski M, et al. Efficacy of CAR T-cell therapy in large tumors relies upon stromal targeting by IFNγ. Cancer Res 2014;74:6796–805.
56. Rade M, Grieb N, Weiss R, Sia J, Fischer L, Born P, et al. Single-cell multiomic dissection of response and resistance to chimeric antigen receptor T cells against BCMA in relapsed multiple myeloma. Nat Cancer 2024;5:1318–33.
57. Maier B, Leader AM, Chen ST, Tung N, Chang C, LeBerichel J, et al. A conserved dendritic-cell regulatory program limits antitumour immunity. Nature 2020;580:257–62.
58. Veglia F, Sanseviero E, Gabrilovich DI. Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. Nat Rev Immunol 2021;21:485–98.
59. Cao F, Liu Y, Cheng Y, Wang Y, He Y, Xu Y. Multi-omics characteristics of tumor-associated macrophages in the tumor microenvironment of gastric cancer and their exploration of immunotherapy potential. Sci Rep 2023;13:18265.
60. Guimarães GR, Maklouf GR, Teixeira CE, de Oliveira Santos L, Tessarollo NG, de Toledo NE, et al. Single-cell resolution characterization of myeloid-derived cell states with implication in cancer outcome. Nat Commun 2024;15:5694.
61. Hinshaw DC, Shevde LA. The tumor microenvironment innately modulates cancer progression. Cancer Res 2019;79:4557–66.
62. Stroncek DF, Ren J, Lee DW, Tran M, Frodigh SE, Sabatino M, et al. Myeloid cells in peripheral blood mononuclear cell concentrates inhibit the expansion of chimeric antigen receptor T cells. Cytotherapy 2016;18:893–901.
63. van Elsas MJ, Middelburg J, Labrie C, Roelands J, Schaap G, Sluijter M, et al. Immunotherapy-activated T cells recruit and skew late-stage activated M1-like macrophages that are critical for therapeutic efficacy. Cancer Cell 2024;42:1032–50.e10.
64. Haabeth OAW, Hennig K, Fauskanger M, Løset GÅ, Bogen B, Tveita A. CD4+ T-cell killing of multiple myeloma cells is mediated by resident bone marrow macrophages. Blood Adv 2020;4:2595–605.
65. Eisel D, Das K, Dickes E, König R, Osen W, Eichmüller SB. Cognate interaction with $CD4^+$ T cells instructs tumor-associated macrophages to acquire M1-like phenotype. Front Immunol 2019;10:219.
66. Gyurdieva A, Zajic S, Chang YF, Houseman EA, Zhong S, Kim J, et al. Biomarker correlates with response to NY-ESO-1 TCR T cells in patients with synovial sarcoma. Nat Commun 2022;13:5296.
67. Yáñez L, Sánchez-Escamilla M, Perales MA. CAR T cell toxicity: current management and future directions. Hemasphere 2019;3:e186.
68. Majzner RG, Ramakrishna S, Yeom KW, Patel S, Chinnasamy H, Schultz LM, et al. GD2-CAR T cell therapy for H3K27M-mutated diffuse midline gliomas. Nature 2022;603:934–41.
69. Engelhardt JJ, Boldajipour B, Beemiller P, Pandurangi P, Sorensen C, Werb Z, et al. Marginating dendritic cells of the tumor microenvironment cross-present tumor antigens and stably engage tumor-specific T cells. Cancer Cell 2012;21:402–17.
70. Diao J, Gu H, Tang M, Zhao J, Cattral MS. Tumor dendritic cells (DCs) derived from precursors of conventional DCs are dispensable for intratumor CTL responses. J Immunol 2018;201:1306–14.
71. Singh N, Hofmann TJ, Gershenson Z, Levine BL, Grupp SA, Teachey DT, et al. Monocyte lineage-derived IL-6 does not affect chimeric antigen receptor T-cell function. Cytotherapy 2017;19:867–80.
72. Bourbon E, Sesques P, Gossez M, Tordo J, Ferrant E, Safar V, et al. HLA-DR expression on monocytes and outcome of anti-CD19 CAR T-cell therapy for large B-cell lymphoma. Blood Adv 2023;7:744–55.
73. Burga RA, Thorn M, Point GR, Guha P, Nguyen CT, Licata LA, et al. Liver myeloid-derived suppressor cells expand in response to liver metastases in mice and inhibit the anti-tumor efficacy of anti-CEA CAR-T. Cancer Immunol Immunother 2015;64:817–29.
74. 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.

75. 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.
76. Spranger S, Dai D, Horton B, Gajewski TF. Tumor-residing Batf3 dendritic cells are required for effector T cell trafficking and adoptive T cell therapy. Cancer Cell 2017;31:711–23.e4.
77. Cheadle EJ, Sheard V, Rothwell DG, Bridgeman JS, Ashton G, Hanson V, et al. Differential role of Th1 and Th2 cytokines in autotoxicity driven by CD19-specific second-generation chimeric antigen receptor T cells in a mouse model. J Immunol 2014;192:3654–65.
78. Villadangos JA, Young L. Antigen-presentation properties of plasmacytoid dendritic cells. Immunity 2008;29:352–61.
79. Glodde N, Bald T, van den Boorn-Konijnenberg D, Nakamura K, O'Donnell JS, Szczepanski S, et al. Reactive neutrophil responses dependent on the receptor tyrosine kinase c-MET limit cancer immunotherapy. Immunity 2017;47:789–802.e9.
80. Hirschhorn D, Budhu S, Kraehenbuehl L, Gigoux M, Schröder D, Chow A, et al. T cell immunotherapies engage neutrophils to eliminate tumor antigen escape variants. Cell 2023;186:1432–47.e17.
81. Rejeski K, Perez A, Iacoboni G, Blumenberg V, Bücklein VL, Völkl S, et al. Severe hematotoxicity after CD19 CAR-T therapy is associated with suppressive immune dysregulation and limited CAR-T expansion. Sci Adv 2023;9:eadg3919.
82. Yang S, Xu J, Dai Y, Jin S, Sun Y, Li J, et al. Neutrophil activation and clonal CAR-T re-expansion underpinning cytokine release syndrome during ciltacabtagene autoleucel therapy in multiple myeloma. Nat Commun 2024;15:360.
83. Flora C, Olesnavich M, Zuo Y, Sandford E, Madhukar R, Rozwadowski M, et al. Longitudinal plasma proteomics in CAR T-cell therapy patients implicates neutrophils and NETosis in the genesis of CRS. Blood Adv 2024;8:1422–6.
84. Jia Q, Qin D, He F, Xie Q, Ying Z, Zhang Y, et al. Peripheral eosinophil counts predict efficacy of anti-CD19 CAR-T cell therapy against B-lineage non-Hodgkin lymphoma. Theranostics 2021;11:4699–709.
85. Dang X, Ye S, Zhou L, Lu Y, Li P, Liang A, et al. Prognostic impact of peripheral eosinophil counts in patients with diffuse large B-cell lymphoma receiving chimeric antigen receptor T-cell therapy. Cytotherapy 2023;25:573–7.
86. Mayer S, Milo T, Isaacson A, Halperin C, Miyara S, Stein Y, et al. The tumor microenvironment shows a hierarchy of cell-cell interactions dominated by fibroblasts. Nat Commun 2023;14:5810.
87. Sakemura R, Hefazi M, Siegler EL, Cox MJ, Larson DP, Hansen MJ, et al. Targeting cancer-associated fibroblasts in the bone marrow prevents resistance to CART-cell therapy in multiple myeloma. Blood 2022;139:3708–21.
88. de Haart SJ, van de Donk NWCJ, Minnema MC, Huang JH, Aarts-Riemens T, Bovenschen N, et al. Accessory cells of the microenvironment protect multiple myeloma from T-cell cytotoxicity through cell adhesion-mediated immune resistance. Clin Cancer Res 2013;19:5591–601.
89. Holthof LC, van der Schans JJ, Katsarou A, Poels R, Gelderloos AT, Drent E, et al. Bone marrow mesenchymal stromal cells can render multiple myeloma cells resistant to cytotoxic machinery of CAR T cells through inhibition of apoptosis. Clin Cancer Res 2021;27:3793–803.
90. Towers R, Trombello L, Fusenig M, Tunger A, Baumann A-L, Savoldelli R, et al. Bone marrow-derived mesenchymal stromal cells obstruct AML-targeting CD8$^+$ clonal effector and CAR T-cell function while promoting a senescence-associated phenotype. Cancer Immunol Immunother 2024;73:8.
91. Zanetti SR, Romecin PA, Vinyoles M, Juan M, Fuster JL, Cámos M, et al. Bone marrow MSC from pediatric patients with B-ALL highly immunosuppress T-cell responses but do not compromise CD19-CAR T-cell activity. J Immunother Cancer 2020;8:e001419.
92. Zhang R, Mao W, Niu L, Bao W, Wang Y, Wang Y, et al. NSC-derived exosomes enhance therapeutic effects of NSC transplantation on cerebral ischemia in mice. Elife 2023;12:e84493.
93. Kitamura W, Asada N, Naoi Y, Abe M, Fujiwara H, Ennishi D, et al. Bone marrow microenvironment disruption and sustained inflammation with prolonged haematologic toxicity after CAR T-cell therapy. Br J Haematol 2023;202:294–307.
94. Baldwin JG, Heuser-Loy C, Saha T, Schelker RC, Slavkovic-Lukic D, Strieder N, et al. Intercellular nanotube-mediated mitochondrial transfer enhances T cell metabolic fitness and antitumor efficacy. Cell 2024;187:6614–30.e21.
95. Li W, Pan X, Chen L, Cui H, Mo S, Pan Y, et al. Cell metabolism-based optimization strategy of CAR-T cell function in cancer therapy. Front Immunol 2023;14:1186383.
96. Rial Saborido J, Völkl S, Aigner M, Mackensen A, Mougiakakos D. Role of CAR T cell metabolism for therapeutic efficacy. Cancers (Basel) 2022;14:5442.
97. Scharping NE, Menk AV, Moreci RS, Whetstone RD, Dadey RE, Watkins SC, et al. The tumor microenvironment represses T cell mitochondrial biogenesis to drive intratumoral T cell metabolic insufficiency and dysfunction. Immunity 2016;45:374–88.
98. Zhang M, Jin X, Sun R, Xiong X, Wang J, Xie D, et al. Optimization of metabolism to improve efficacy during CAR-T cell manufacturing. J Transl Med 2021;19:499.
99. Morotti M, Grimm AJ, Hope HC, Arnaud M, Desbuisson M, Rayroux N, et al. PGE^2 inhibits TIL expansion by disrupting IL-2 signalling and mitochondrial function. Nature 2024;629:426–34.
100. Brady L, Kriner M, Coleman I, Morrissey C, Roudier M, True LD, et al. Inter- and intra-tumor heterogeneity of metastatic prostate cancer determined by digital spatial gene expression profiling. Nat Commun 2021;12:1426.
101. Tabata M, Sato Y, Kogure Y, McClure MB, Oshikawa-Kumade Y, Saito Y, et al. Inter- and intra-tumor heterogeneity of genetic and immune profiles in inherited renal cell carcinoma. Cell Rep 2023;42:112736.
102. Kirschenbaum D, Xie K, Ingelfinger F, Katzenelenbogen Y, Abadie K, Look T, et al. Time-resolved single-cell transcriptomics defines immune trajectories in glioblastoma. Cell 2024;187:149–65.e23.
103. Vandereyken K, Sifrim A, Thienpont B, Voet T. Methods and applications for single-cell and spatial multi-omics. Nat Rev Genet 2023;24:494–515.
104. Miao Z, Humphreys BD, McMahon AP, Kim J. Multi-omics integration in the age of million single-cell data. Nat Rev Nephrol 2021;17:710–24.
105. Goldberg L, Haas ER, Vyas V, Urak R, Forman SJ, Wang X. Single-cell analysis by mass cytometry reveals CD19 CAR T cell spatiotemporal plasticity in patients. Oncoimmunology 2022;11:2040772.
106. Li X, Henderson J, Gordon MJ, Sheikh I, Nastoupil LJ, Westin J, et al. A single-cell atlas of CD19 chimeric antigen receptor T cells. Cancer Cell 2023;41:1835–7.
107. Künkele A, Brown C, Beebe A, Mgebroff S, Johnson AJ, Taraseviciute A, et al. Manufacture of chimeric antigen receptor T cells from mobilized cyropreserved peripheral blood stem cell units depends on monocyte depletion. Biol Blood Marrow Transplant 2019;25:223–32.
108. Haradhvala NJ, Leick MB, Maurer K, Gohil SH, Larson RC, Yao N, et al. Distinct cellular dynamics associated with response to CAR-T therapy for refractory B cell lymphoma. Nat Med 2022;28:1848–59.
109. Monfrini C, Stella F, Aragona V, Magni M, Ljevar S, Vella C, et al. Phenotypic composition of commercial anti-CD19 CAR T cells affects in vivo expansion and disease response in patients with large B-cell lymphoma. Clin Cancer Res 2022;28:3378–86.
110. Deng Q, Han G, Puebla-Osorio N, Ma MCJ, Strati P, Chasen B, et al. Characteristics of anti-CD19 CAR T cell infusion products associated with efficacy and toxicity in patients with large B cell lymphomas. Nat Med 2020;26:1878–87.
111. Rezvan A, Romain G, Fathi M, Heeke D, Martinez-Paniagua M, An X, et al. Identification of a clinically efficacious CAR T cell subset in diffuse large B cell lymphoma by dynamic multidimensional single-cell profiling. Nat Cancer 2024;5:1010–23.
112. Wilson TL, Kim H, Chou C-H, Langfitt D, Mettelman RC, Minervina AA, et al. Common trajectories of highly effective CD19-specific CAR T cells identified by endogenous T-cell receptor lineages. Cancer Discov 2022;12:2098–119.
113. Chen GM, Chen C, Das RK, Gao P, Chen C-H, Bandyopadhyay S, et al. Integrative bulk and single-cell profiling of premanufacture T-cell populations reveals factors mediating long-term persistence of CAR T-cell therapy. Cancer Discov 2021;11:2186–99.
114. Filosto S, Vardhanabhuti S, Canales MA, Poiré X, Lekakis LJ, de Vos S, et al. Product attributes of CAR T-cell therapy differentially associate with efficacy and toxicity in second-line large B-cell lymphoma (ZUMA-7). Blood Cancer Discov 2024;5:21–33.
115. Wang Y, Tong C, Lu Y, Wu Z, Guo Y, Liu Y, et al. Characteristics of premanufacture CD8$^+$ T cells determine CAR-T efficacy in patients with diffuse large B-cell lymphoma. Signal Transduct Target Ther 2023;8:409.
116. Anderson ND, Birch J, Accogli T, Criado I, Khabirova E, Parks C, et al. Transcriptional signatures associated with persisting CD19 CAR-T cells in children with leukemia. Nat Med 2023;29:1700–9.

117. Fraietta JA, Lacey SF, Orlando EJ, Pruteanu-Malinici I, Gohil M, Lundh S, et al. Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. Nat Med 2018;24:563–71.
118. Bai Z, Feng B, McClory SE, de Oliveira BC, Diorio C, Gregoire C, et al. Single-cell CAR T atlas reveals type 2 function in 8-year leukaemia remission. Nature 2024;634:702–11.
119. Bai Z, Woodhouse S, Zhao Z, Arya R, Govek K, Kim D, et al. Single-cell antigen-specific landscape of CAR T infusion product identifies determinants of CD19-positive relapse in patients with ALL. Sci Adv 2022;8:eabj2820.
120. Good Z, Spiegel JY, Sahaf B, Malipatlolla MB, Ehlinger ZJ, Kurra S, et al. Post-infusion CAR T^{Reg} cells identify patients resistant to CD19-CAR therapy. Nat Med 2022;28:1860–71.
121. Gao Z, Bai Y, Lin A, Jiang A, Zhou C, Cheng Q, et al. Gamma delta T-cell-based immune checkpoint therapy: attractive candidate for antitumor treatment. Mol Cancer 2023;22:31.
122. Hadiloo K, Tahmasebi S, Esmaeilzadeh A. CAR-NKT cell therapy: a new promising paradigm of cancer immunotherapy. Cancer Cell Int 2023;23:86.
123. Chang J-F, Landmann JH, Chang T-C, Selli ME, Tenzin Y, Warrington JM, et al. Rational protein engineering to enhance MHC-independent T-cell receptors. Cancer Discov 2024;14:2109–21.
124. Liu Y, Liu G, Wang J, Zheng Z-Y, Jia L, Rui W, et al. Chimeric STAR receptors using TCR machinery mediate robust responses against solid tumors. Sci Transl Med 2021;13:eabb5191.
125. Altvater B, Kailayangiri S, Spurny C, Flügge M, Meltzer J, Greune L, et al. CAR T cells as micropharmacies against solid cancers: combining effector T-cell mediated cell death with vascular targeting in a one-step engineering process. Cancer Gene Ther 2023;30:1355–68.
126. Hong M, Clubb JD, Chen YY. Engineering CAR-T cells for next-generation cancer therapy. Cancer Cell 2020;38:473–88.
127. Jiménez-Reinoso A, Nehme-Álvarez D, Domínguez-Alonso C, Álvarez-Vallina L. Synthetic TILs: engineered tumor-infiltrating lymphocytes with improved therapeutic potential. Front Oncol 2020;10:593848.
128. Johnson LR, Lee DY, Eacret JS, Ye D, June CH, Minn AJ. The immunostimulatory RNA RN7SL1 enables CAR-T cells to enhance autonomous and endogenous immune function. Cell 2021;184:4981–95.e14.
129. Miao L, Zhang J, Huang B, Zhang Z, Wang S, Tang F, et al. Special chimeric antigen receptor (CAR) modifications of T cells: a review. Front Oncol 2022;12:832765.
130. Cho JH, Collins JJ, Wong WW. Universal chimeric antigen receptors for multiplexed and logical control of T cell responses. Cell 2018;173:1426–38.e11.
131. Han X, Wang Y, Wei J, Han W. Multi-antigen-targeted chimeric antigen receptor T cells for cancer therapy. J Hematol Oncol 2019;12:128.
132. Kankeu Fonkoua LA, Sirpilla O, Sakemura R, Siegler EL, Kenderian SS. CAR T cell therapy and the tumor microenvironment: current challenges and opportunities. Mol Ther Oncolytics 2022;25:69–77.
133. Cherkassky L, Morello A, Villena-Vargas J, Feng Y, Dimitrov DS, Jones DR, et al. Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. J Clin Invest 2016;126:3130–44.
134. Gao X, Liu J, Sun R, Zhang J, Cao X, Zhang Y, et al. Alliance between titans: combination strategies of CAR-T cell therapy and oncolytic virus for the treatment of hematological malignancies. Ann Hematol 2024;103:2569–89.
135. Kim SH, Cho E, Kim YI, Han C, Choi BK, Kwon BS. Adoptive immunotherapy with transient anti-CD4 treatment enhances anti-tumor response by increasing IL-18$R\alpha^{hi}$ $CD8^+$ T cells. Nat Commun 2021;12:5314.
136. Sim AJ, Jain MD, Figura NB, Chavez JC, Shah BD, Khimani F, et al. Radiation therapy as a bridging strategy for CAR T cell therapy with axicabtagene ciloleucel in diffuse large B-cell lymphoma. Int J Radiat Oncol Biol Phys 2019;105:1012–21.
137. Kwong MLM, Yang JC. Lifileucel: FDA-approved T-cell therapy for melanoma. Oncologist 2024;29:648–50.
138. Mullard A. FDA approves first tumour-infiltrating lymphocyte (TIL) therapy, bolstering hopes for cell therapies in solid cancers. Nat Rev Drug Discov 2024;23:238.
139. Chekol Abebe E, Yibeltal Shiferaw M, Tadele Admasu F, Asmamaw Dejenie T. Ciltacabtagene autoleucel: the second anti-BCMA CAR T-cell therapeutic armamentarium of relapsed or refractory multiple myeloma. Front Immunol 2022;13:991092.
140. Chen J, Qiu S, Li W, Wang K, Zhang Y, Yang H, et al. Tuning charge density of chimeric antigen receptor optimizes tonic signaling and CAR-T cell fitness. Cell Res 2023;33:341–54.
141. Depil S, Duchateau P, Grupp SA, Mufti G, Poirot L. Off-the-shelf' allogeneic CAR T cells: development and challenges. Nat Rev Drug Discov 2020;19:185–99.
142. Elsallab M, Ellithi M, Lunning MA, D'Angelo C, Ma J, Perales MA, et al. Second primary malignancies after commercial CAR T-cell therapy: analysis of the FDA Adverse Events Reporting System. Blood 2024;143:2099–105.
143. Flugel CL, Majzner RG, Krenciute G, Dotti G, Riddell SR, Wagner DL, et al. Overcoming on-target, off-tumour toxicity of CAR T cell therapy for solid tumours. Nat Rev Clin Oncol 2023;20:49–62.
144. Goyco Vera D, Waghela H, Nuh M, Pan J, Lulla P. Approved CAR-T therapies have reproducible efficacy and safety in clinical practice. Hum Vaccin Immunother 2024;20:2378543.
145. Thirumalaisamy R, Vasuki S, Sindhu SM, Mothilal TM, Srimathi V, Poornima B, et al. FDA-approved chimeric antigen receptor (CAR)-T cell therapy for different cancers-A recent perspective. Mol Biotechnol 2025;67:469–83.
146. Baulu E, Gardet C, Chuvin N, Depil S. TCR-engineered T cell therapy in solid tumors: state of the art and perspectives. Sci Adv 2023;9:eadf3700.
147. Keam SJ. Afamitresgene autoleucel: first approval. Mol Diagn Ther 2024;28:861–6.