

REVIEW

Cancer Focus

CD4⁺ T cell dysfunction in cancer

Hrishi Venkatesh¹  and Lawrence Fong¹ 

While the importance of CD8⁺ T cells in successful cancer immunotherapy is well-established, CD4⁺ T cells are increasingly recognized as key mediators of effective anti-tumor immunity. However, the mechanisms underlying the functional impairment of CD4⁺ T cells in tumors are not as well characterized as in CD8⁺ T cells. In this review, we will explore how CD4⁺ T cells are altered in tumor-bearing hosts, compare these changes to those observed in CD8⁺ T cells, and discuss how these changes impact tumor control. Approaches that counteract functional impairment in tumor-reactive CD4⁺ T cells may further enhance the efficacy of cancer immunotherapy.

Introduction

Accumulation of mutations due to DNA damage is one of the key hallmarks of cancer. These mutations allow cancer cells to continuously proliferate, evade growth suppressors/cell death pathways, and invade different tissues (Hanahan and Weinberg, 2011). However, these mutations also allow immune cells, in particular T cells, to identify and destroy cancerous cells. A plethora of studies have demonstrated a critical role for cytotoxic cluster of differentiation (CD)8⁺ T cells in effective anti-tumor immunity (Raskov et al., 2021; Giles et al., 2023). Over the past decade, pioneering immunotherapy approaches, like checkpoint blockade and adoptive T cell therapies, have aimed to harness tumor-reactive CD8⁺ T cells to treat cancer (Krishna et al., 2020; Chen et al., 2024b). However, many cancer patients fail to respond to these immunotherapies, necessitating a closer look at other immune cells that could modify tumor control (Morotti et al., 2021; Huang and Zappasodi, 2022).

CD4⁺ T cells have been shown to be indispensable for effective tumor control following immunotherapy (Tay et al., 2021; Montauti et al., 2024). In tumor-draining lymph nodes, T follicular helper (Tfh) CD4⁺ T cells drive the activation and maturation of B cells, while other helper CD4⁺ T cells promote CD8⁺ T cell priming by licensing cross-presenting dendritic cells (DCs) (Fig. 1) (Zander et al., 2019; Cui et al., 2021; Montauti et al., 2024). In tumors, CD4⁺ T cells activate anti-tumor immune effectors—CD8⁺ T cells (via IL2/IL21), natural killer cells (via IL2), B cells (via CD40L), macrophages (via IFN γ and TNF), and eosinophils (via IL5)—and can also directly kill off tumor cells (Fig. 1) (Speiser et al., 2023; Guo et al., 2024; Montauti et al., 2024). Effective anti-tumor CD4⁺ T cells have even been shown to lower

the likelihood of CD8⁺ T cell dysfunction in the tumor microenvironment (TME) (Arina et al., 2017; Khan et al., 2022; Espinosa-Carrasco et al., 2024). Given the pivotal role of CD4⁺ T cells in facilitating tumor control, understanding how CD4⁺ T cells are functionally impaired in the TME is essential to improving the efficacy of anti-tumor immune therapies. Although dysfunction in tumor-reactive CD4⁺ T cells has not been explored to the same detail as in CD8⁺ T cells, limited studies over the past decade have provided key insights into functional impairment of anti-tumor CD4⁺ T cells.

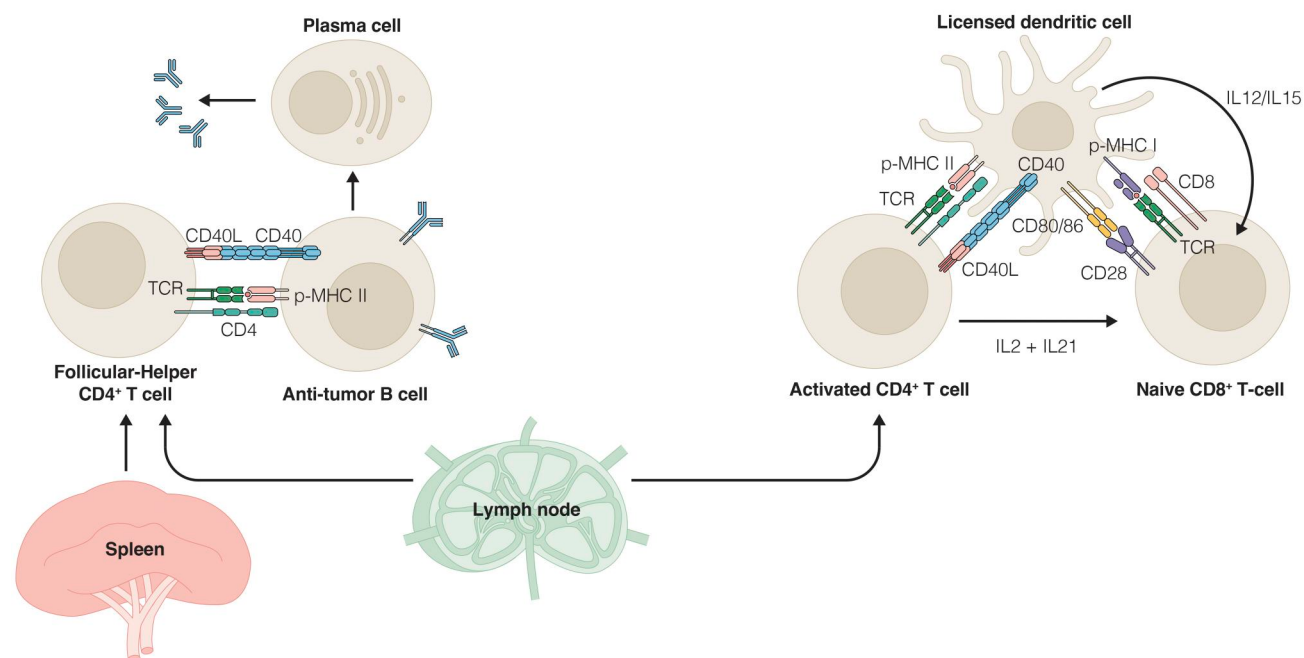
Many mechanisms constraining T cell function in tumors are consequences of immune adaptation to stress or chronic infections, with the aim of balancing threat removal with limiting damage to healthy cells. For example, T cell exhaustion—first described as an adaptation by effector T cells to persistent stimulation during chronic viral infections—is also seen in T cells in tumors (Saeidi et al., 2018; Baessler and Vignali, 2024). CD4⁺ and CD8⁺ T cells presumably evolved to play different roles in tumor control. CD8⁺ T cells are the frontline soldiers critical for directly killing tumor cells (Raskov et al., 2021; Giles et al., 2023). CD4⁺ T cells, though also capable of tumor killing, also serve as “generals” whose key role is to recruit and activate other anti-tumor immune cells (Speiser et al., 2023; Espinosa-Carrasco et al., 2024) (Fig. 1). The differences in function result in differences in the ability of anti-tumor CD4⁺ and CD8⁺ T cells to adapt to stressors. Indeed, CD4⁺ T cells have unique functional plasticity that enables them to adapt to environmental changes and reprogram other immune cells to restore homeostasis. In this review, we will first summarize the well-established processes by which tumor-reactive CD8⁺ T cells lose effector function. We will then describe analogous pathway of

¹Immunotherapy Integrated Research Center, Fred Hutchinson Cancer Center, Seattle, WA, USA.

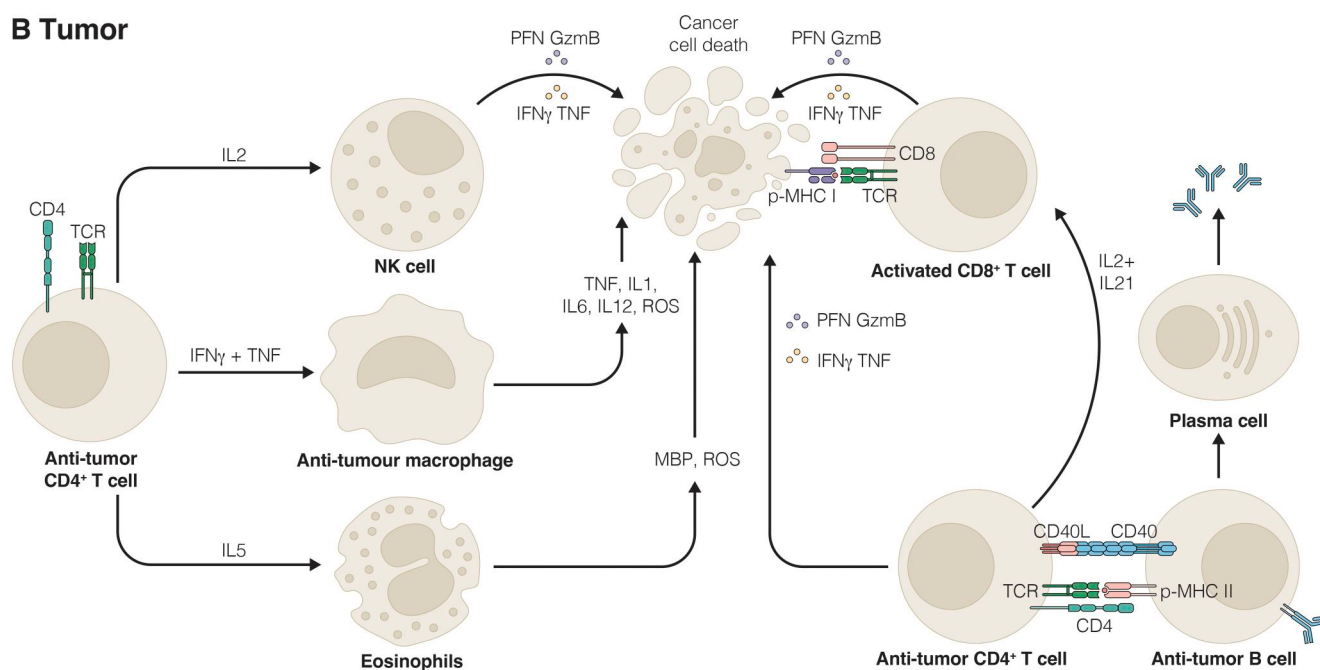
Correspondence to Lawrence Fong: lawrence.fong@fredhutch.org.

© 2025 Venkatesh and Fong. This article is distributed under the terms as described at <https://rupress.org/pages/terms102024/>.

A Tumor-draining lymph node/spleen



B Tumor



JEM | Journal of Experimental Medicine

Figure 1. Context-dependent anti-tumor functions of CD4⁺ T cells. (A) Within lymphoid tissues, CD4⁺ T cells are critical for licensing cross-presenting DCs, which is via CD40L in tumor-draining lymph nodes. CD4⁺ T cells can also directly activate memory CD8⁺ T cells via CD40 and IL2/IL21. Tfh CD4⁺ T cells activate anti-tumor B cells in draining lymph nodes and in the spleen. (B) Within tumors, CD4⁺ T cells in tumors enhance tumoricidal functions of various innate and adaptive immune effectors. These include macrophages, eosinophils, NK cells, and CD8⁺ T cells. CD4⁺ T cells can also directly kill tumor cells. CD4⁺ T cells are also known to activate B cells in tumors, which have been correlated with favorable responses after checkpoint blockade immunotherapy. Created in BioRender. <https://BioRender.com/p44y970>. NK, natural killer; PFN, perforin; Gzm, granzyme; p-MHC, peptide-MHC.

functional impairment in tumor-reactive CD4⁺ T cells, with focus on how functional plasticity allows tumor-reactive CD4⁺ T cells to adopt distinct functional states to CD8⁺ T cells in response to similar impediments.

CD8 T cell dysfunction in the TME

CD8⁺ T cell dysfunction, a hallmark of cancer, has been extensively studied over the past two decades. Exhaustion, a key mechanism of T cell dysfunction during chronic antigen

exposure, was first identified in CD8⁺ T cells during chronic lymphocytic choriomeningitis virus (LCMV) infection (Gallimore et al., 1998; Zajac et al., 1998; Wherry et al., 2003; Wherry et al., 2007) and later recognized as a key feature of impaired antigen-specific CD8⁺ T cells in tumors (Baitsch et al., 2011; Schietinger et al., 2016; Thommen and Schumacher, 2018; Rudloff et al., 2023).

Activated CD8⁺ T cells upregulate the expression of multiple inhibitory receptors (IRs), known as immune checkpoints, which act as a “brake” to limit T cells after activation (Guo et al., 2023). In acute infections, these checkpoints are downregulated after pathogen clearance as CD8⁺ T cells transition from effector to memory states. Chronic TCR stimulation due to continuous antigen exposure results in sustained upregulation of these checkpoints on exhausted CD8⁺ T cells, leading to a progressive loss of effector function (Wherry et al., 2007). Indeed, exhausted CD8⁺ T cells exist across a spectrum of states between progenitor-exhausted (Tpex) and terminally exhausted CD8⁺ T cells—with varying phenotypic and functional characteristics (Miller et al., 2019; Philip and Schietinger, 2019; Beltra et al., 2020). Tpex CD8⁺ T cells have self-renewal and proliferative capacity with limited effector potential (Fig. 2). Although they express the stemness-associated transcription factor 7 (*TCF7*), they also upregulate the IR programmed death-1 (PD-1) and the exhaustion-associated transcription factor thymocyte selection-associated high mobility group box (TOX)—indicating that an exhaustion program is induced early after activation (Blackburn et al., 2008; Paley et al., 2012; Utzschneider et al., 2016; Wu et al., 2016b; Miller et al., 2019; Beltra et al., 2020; Utzschneider et al., 2020). These cells typically reside in tumor-draining lymph nodes and give rise to intermediate-exhausted CD8⁺ T cells upon migration into tumors. Intermediate-exhausted CD8⁺ T cells lose self-renewal capacity, acquire T-box expressed in T cells (T-bet) expression and limited effector potential, and moderately upregulate PD-1 (Beltra et al., 2020) (Fig. 2). These cells eventually give rise to terminally exhausted CD8⁺ T cells that completely lose effector function and significantly upregulate the transcription factor TOX as well as multiple immune checkpoints—including PD-1, T cell Ig and mucin-domain containing-3 (TIM-3), lymphocyte-activation gene 3 (LAG-3), B and T lymphocyte attenuator, and T cell immunoreceptor with Ig and ITIM domains (TIGIT) (Miller et al., 2019; Beltra et al., 2020) (Fig. 2). Immune checkpoint blockade can expand CD8⁺ T cells with stronger effector functions that resemble intermediate-exhausted CD8⁺ T cells—including expression of multiple IRs (Zander and Cui, 2023) (Fig. 2). The “effector-exhausted” CD8⁺ T cells significantly express markers associated with terminal-effector CD8⁺ T cells, including the chemokine receptor CX3CR1, killer cell lectin receptors such as killer cell lectin-like receptor subfamily G member 1 (KLRG1), and the transcription factor zinc finger E-box-binding homeobox 2 (ZEB2) (Yan et al., 2018; Zander et al., 2019; Yamauchi et al., 2021). The induction of effector-exhausted CD8⁺ T cells relies on CD4⁺ T cell help during CD8⁺ T cell priming (Zander et al., 2019). Indeed, CD4⁺ T cell-mediated DC licensing significantly mitigates CD8⁺ T cell exhaustion, suggesting that the quality of CD8⁺ T cell priming

influences their subsequent differentiation into effector/exhausted fates (Zander et al., 2019) (Fig. 2).

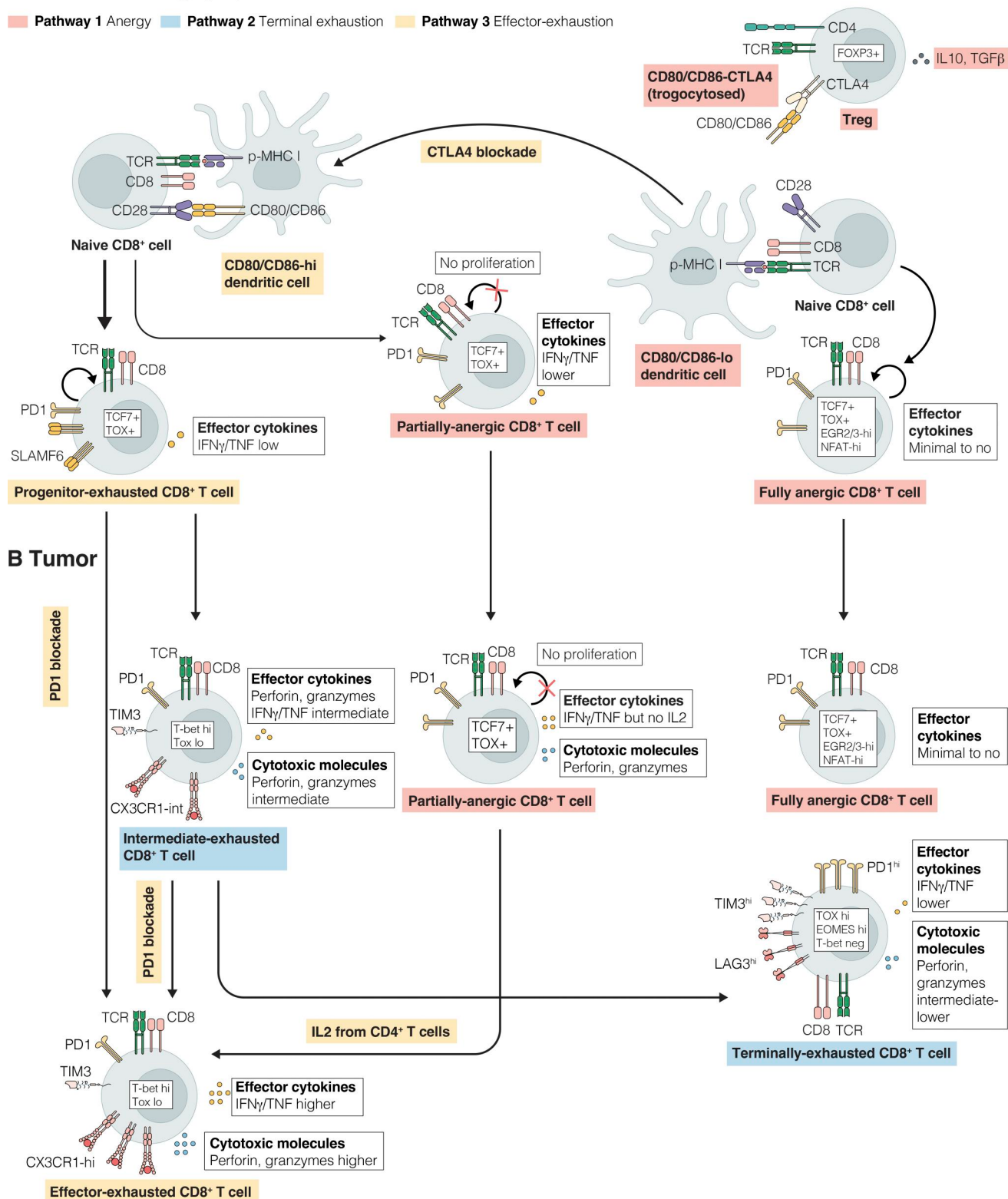
As cancer cells arise from healthy cells that T cells are normally tolerized to, the mechanisms that prevent self-reactive T cell responses also constrain anti-tumor T cell responses (Nüssing et al., 2020). Indeed, T cell anergy, a key mechanism that mitigates the activation of self-reactive T cells, has also been implicated in impaired priming of anti-tumor CD4⁺ and CD8⁺ T cells (Mescher et al., 2007; Crespo et al., 2013; Nüssing et al., 2020) (Figs. 2 and 3). T cells normally require three signals to be optimally activated: signal 1 via the TCR bound to a peptide-MHC complex on APCs, signal 2 via costimulatory molecules on APCs, and signal 3 via cytokines from APCs that dictates the functional properties of the activated T cell (Deeths and Mescher, 1997; Curtsinger et al., 1999). Anergic T cells are those that do not receive sufficient costimulatory signals (“signal 2”) (Schwartz, 1990; Greenwald et al., 2001; Schwartz, 2003). The transcription factor NFAT is a central regulator of anergy in CD4⁺ T cells. NFAT is a transcription factor induced downstream of TCR signaling. Strong TCR signaling with insufficient costimulatory signals leads to robust activation of NFAT, but weak activation of the transcription factor activator protein-1 (AP1) (Macián et al., 2002; Abe et al., 2012). This results in significant upregulation of transcription factors early growth response protein 2 (*Egr2*) and *Egr3*, which inhibit the expression of effector programs in T cells via induction of transcriptional repressors such as inhibitor of DNA binding 2 (*Id2*) (Li et al., 2012; Zheng et al., 2012; Omodho et al., 2018) (Figs. 2 and 3). Regulatory T cells (Tregs) constrain self-reactive CD8⁺ T cells via CTL-associated protein 4 (CTLA4)-mediated trogocytosis of costimulatory ligands CD80 and CD86 on APCs (Maeda et al., 2014), suggesting that a similar mechanism induces anergy in tumor-specific CD8⁺ T cells. Indeed, CTLA4 blockade is known to rescue CD8 T cell priming in tumor-draining lymph nodes (van Pul et al., 2022; Lax et al., 2023). Interestingly even with sufficient costimulatory signals via CD80/86, tumor-specific CD8⁺ T cells can adopt a “partial-anergic” phenotype characterized by impaired proliferation and IL2 production and normal effector/cytotoxic functions (Deeths et al., 1999; Mescher et al., 2007). Furthermore, exogenous IL2 from helper CD4⁺ T cells allows the partially anergic CD8⁺ T cells to overcome their impaired proliferation (Mescher et al., 2007). Thus, tumor-specific CD8⁺ T cells can adopt unique anergic states that differ from those seen in self-reactive CD8⁺ T cells.

Pathways to function and dysfunction in tumor-reactive CD4⁺ T cells

While anticancer immune therapies have traditionally targeted CD8⁺ T cells, CD4⁺ T cell help has been shown to be critical for effective tumor control in multiple therapeutic contexts (Arina et al., 2017; Alspach et al., 2019; Ferris et al., 2020; Tracy et al., 2022). Indeed, IR-expressing CD4⁺ T cells with functional deficits have also been observed in multiple tumor types and correlate with poor outcomes (Hohtari et al., 2019; Fu et al., 2020; Miggelbrink et al., 2021). These findings have prompted efforts to characterize mechanisms that functionally perturb tumor-reactive CD4⁺ T cells. In this section, we will describe the

A Tumor-draining lymph node

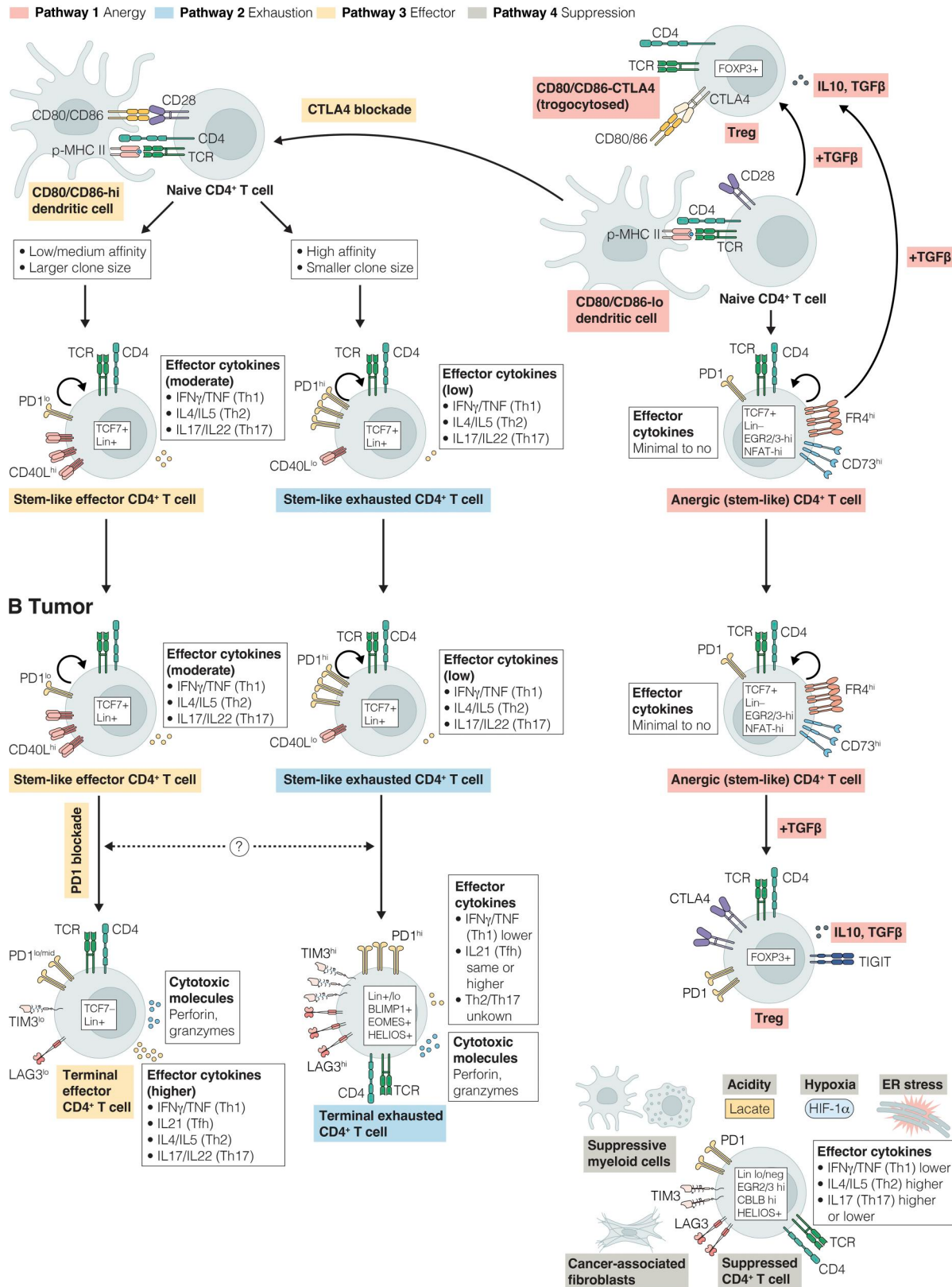
Pathway 1 Anergy Pathway 2 Terminal exhaustion Pathway 3 Effector-exhaustion



JEM | Journal of Experimental Medicine

Figure 2. Differentiation pathways of tumor-reactive CD8⁺ T cells. (A) In draining lymph nodes, a T_{pe} phenotype is induced in tumor-reactive CD8⁺ T cells early after priming. However, Tregs in draining lymph nodes from cold tumors can inhibit effective CD8⁺ T cell priming and induce a state of anergy comparable with what is seen in CD4⁺ T cells. CD8⁺ T cells can also adopt a unique “partial-anergic” phenotype that can be reversed by exogenous IL2 from CD4⁺ T cells. **(B)** In tumors, T_{pe} CD8⁺ T cells give rise to an intermediate-exhausted state and eventually a terminally exhausted T cells with impaired effector functions. Checkpoint blockade can expand effector-exhausted CD8⁺ T cells with significant effector function, but they still retain features of exhausted T cells. Created with BioRender. <https://BioRender.com/j7c8w6c>. p-MHC, peptide-MHC; EOMES, eomesodermin.

A Tumor-draining lymph node



JEM | Journal of Experimental Medicine

Figure 3. **Differentiation pathways of tumor-reactive CD4⁺ T cells.** (A) Within tumor-draining lymph nodes, Tregs can inhibit effective CD4⁺ T cell priming, leading to the induction of anergic CD4⁺ T cells. TGF β produced by Tregs and other immunosuppressive cells can also promote the differentiation of naive and

anergic CD4⁺ T cells into induced Tregs. In contrast, efficient priming generates stem-like CD4⁺ T cells that are imprinted with features of either effector or exhausted T cells, depending on the strength of priming. The strength of this priming is influenced by variables such as clone size and TCR affinity. **(B)** Within tumors, TGFβ can induce anergic CD4⁺ T cells and drive the differentiation into Tregs. The propensity of stem-like CD4⁺ T cells to differentiate into either effector or exhausted CD4⁺ T cells is likely shaped by their initial imprinting in the lymph node. Whether these differentiation pathways are mutually exclusive—i.e., whether stem-like exhausted CD4⁺ T cells can become effector CD4⁺ T cells, or whether exhausted CD4⁺ T cells can re-differentiate into effectors—remains unknown. Additionally, factors within the tumor microenvironment (TME) can suppress CD4⁺ T cell effector function through mechanisms that are independent of classical T cell exhaustion. Created with BioRender. <https://BioRender.com/ekcyog8>. p-MHC, peptide-MHC; EOMES, eomesodermin.

environmental factors and cell-intrinsic pathways that influence the functional state of tumor-reactive CD4⁺ T cells and highlight key similarities/differences with how these influence CD4⁺ and CD8⁺ T cells.

Impaired CD4⁺ T cell priming in tumor-draining lymph nodes

Similar to tumor-specific CD8⁺ T cells, impaired priming in tumor-draining lymph nodes induces anergic CD4⁺ T cells in many murine tumor models. Tregs in tumor-draining lymph nodes have been implicated in inducing anergic CD4⁺ T cells in murine models of lung adenocarcinoma (Alonso et al., 2018). As with self-reactive CD8⁺ T cells, this occurs via CTLA4-dependent trogocytosis of CD80 and CD86 on APCs by the Tregs (Tekguc et al., 2021). The anergic CD73^{hi} FR4^{hi} CD4⁺ T cells exhibited lower proliferative potential and impaired effector cytokine production after ex vivo stimulation, compared with CD73^{lo} FR4^{lo} effectors (Alonso et al., 2018). Similar findings have also been observed in other murine tumor models, where anergic CD4⁺ T cells have been shown to be ineffective at facilitating anti-tumor CD8⁺ T cell activation, resulting in cancer escape (Staveley-O'Carroll et al., 1998; Cuenca et al., 2003; Abe et al., 2012). The deletion of the key anergy regulator NFAT1 or vaccination with the TLR9 agonist CpG-ODN was sufficient to prevent CD4⁺ T cell anergy and delay tumor growth (Abe et al., 2012; Alonso et al., 2018). Additionally, CTLA-4 blockade has also been shown to mitigate T cell anergy by interfering with Treg-mediated trogocytosis and promotes the proliferation of effector CD4⁺ T cells (Peggs et al., 2009; Chan et al., 2014). While anergy impairs anti-tumor CD4⁺ T cells early on in tumor-draining lymph nodes, other mechanisms subsequently constrain their function in tumors and contribute to tumor growth (Abe et al., 2012). While anergic CD4⁺ T cells have been typically identified by high expression of CD73 and folate receptor 4 (FR4) in murine models (Kalekar et al., 2016; Trefzer et al., 2021), the utility of those markers to distinguish anergic CD4⁺ T cells from other dysfunctional CD4⁺ T cells in human/murine tumors remains controversial. A recent study by Haydn Kissick's group has described PD-1⁺TCF7⁺ cells as a significant CD4⁺ T cell subset in human and murine tumors (Cardenas et al., 2024). These stem-like progenitors had robust self-renewal abilities but were inhibited from T helper (Th)1 effector differentiation by tumor-induced Tregs (Cardenas et al., 2024). While the study did not explicitly describe these cells as "anergic," they had multiple traits associated with anergic CD4⁺ T cells—including upregulation of CD73/FR4, lack of expression of lineage-associated transcription factors (Lin: BCL6, T-BET, GATA3, and RAR-related orphan receptor gamma T), and reduced expression of both effector molecules as well as IRs (Cardenas et al., 2024). Thus, anergic tumor-reactive CD4⁺ T cells in murine and human

tumors can be potentially defined as TCF7⁺ PD-1⁺ Egr2/3^{hi} Lin[−] (Fig. 3). Indeed, the upregulation of TCF7 and downregulation of multiple IRs (apart from PD-1) is a key distinguishing feature between anergic and terminally exhausted CD4⁺ T cells (Fig. 3).

Unlike anergic CD8⁺ T cells, anergic CD4⁺ T cells can also differentiate into peripherally induced Tregs (pTregs) (Kalekar and Mueller, 2017; Alonso et al., 2018; Kuczma et al., 2021). The differentiation of anergic CD4⁺ T cells into pTregs is mainly driven by TGFβ (Fig. 3) (Kalekar and Mueller, 2017; Yamagiwa et al., 2024). pTregs have been suggested to play critical non-redundant roles in suppressing anti-tumor immune responses vis-à-vis thymically derived Tregs (Yadav et al., 2013; Hossain et al., 2024). Apart from pTregs, anergic cells can also give rise to FOXP3[−]-suppressive Tr1-CD4⁺ T cells, which have been linked to poor tumor control across different cancers (Bonnal et al., 2021; Thomann et al., 2021; Sultan et al., 2024). Thus, anergic CD4⁺ T cells have a unique ability to give rise to different suppressive CD4⁺ T cell subsets that inhibit anti-tumor immunity.

Exhaustion in chronically stimulated CD4⁺ T cells in tumors

While approaches that prevented CD4⁺ T cell anergy in draining lymph nodes transiently delayed tumor growth, the tumors eventually escaped and progressed (Abe et al., 2012). This suggests that distinct nonredundant mechanisms govern CD4⁺ T cell dysfunction in tumors, with implications for durable anti-tumor immune responses and tumor control. IR-expressing CD4⁺ T cells have been observed in multiple tumor types, hinting at a phenotype analogous to CD8⁺ T cell exhaustion (Crespo et al., 2013; Chen et al., 2020; Yang et al., 2020; Liu et al., 2022) (Table 1). While research remains limited, it has identified key traits of these cells—some shared with exhausted CD8⁺ T cells and others unique to CD4⁺ T cells. The sections below will compare the unique and shared traits of chronically stimulated CD4⁺ T cells with exhausted CD8⁺ T cells, first in chronic viral infections and then in tumors.

Evidence of exhaustion in chronically stimulated CD4⁺ T cells in chronic viral infections. As exhausted CD8⁺ T cells were first described in the context of chronic viral infections, we will first discuss features of exhausted CD4⁺ T cells in chronic viral infections. Chronic LCMV infections are known to induce a higher proportion of IR-expressing CD4⁺ T cells compared with acute LCMV infections (Brooks et al., 2005; Aubert et al., 2011; Fahey et al., 2011; Dow et al., 2013). Similar populations of IR-expressing CD4⁺ T cells are also seen in other chronic infections—including hepatitis (Aubert et al., 2011), HIV (Brunet-Ratnasingham et al., 2022), CMV (Parry et al., 2021), and EBV (Chatterjee et al., 2019). In these contexts, the kinetics of IR upregulation correlate with disease severity. Furthermore, the upregulation of IRs is greater on antigen-specific CD4⁺

Table 1. Comparison of key attributes of chronically stimulated CD4⁺ vs. CD8⁺ T cells

Attribute	Chronically stimulated CD8 ⁺ T cells	Chronically stimulated CD4 ⁺ T cells	References
IR expression	↑ PD-1, CTLA4, TIM-3 and LAG-3. ↑ 2B4, LILRB4	↑ PD-1, CTLA4, TIM-3 and LAG-3 ↑ CD200 and BTLA4	Dow et al. (2013), Goding et al. (2013), Crawford et al. (2014), Pour et al. (2019), Tracy et al. (2022)
Transcriptional regulation	TOX, NFAT, PRDM1, BACH2, BATF, and EOMES	TOX, NFAT, PRDM1, BACH2, BATF, and EOMES Uniquely upregulates <i>IKZF2</i> (Helios)	Crawford et al. (2014), Trefzer et al. (2021), Sumida et al. (2022)
Functional profile	↓ Self-renew	↓ Self-renew	Beltra et al. (2020), Fu et al. (2020)
	↓ IFN γ , TNF α , and IL2	↓ TNF α and IL2 but retains IFN γ , ↑ Tfh attributes (IL21 and IL10)	Crawford et al. (2014), Catakovic et al. (2017), Balançã et al. (2021)
Cytotoxicity attributes	++ to +++	0 to ++	Oh et al. (2020), Liu et al. (2022), Sultan et al. (2024)
Epigenetic traits	Characteristic epigenetic remodeling in IR, cytokine, and cytotoxicity genes	?	Pauken et al. (2016), Schietinger et al. (2016), Stephen et al. (2017), Satpathy et al. (2019)
Ontogeny	Terminally exhausted arise from a self-renewing TCF7 ⁺ Tpex subset	Arise from a self-renewing TCF7 ⁺ progenitor	Beltra et al. (2020), Xia et al. (2022), Zhou et al. (2024)

BATF, basic leucine zipper ATF-like transcription factor; EOMES, eomesodermin; BACH2, BTB domain and CNC homolog 2.

T cells compared with bystander CD4⁺ T cells (Raziorrouh et al., 2011; Jacobi et al., 2019). This suggests that chronic TCR signaling might drive an exhaustion phenotype analogous to CD8⁺ T cells. However, only a few studies have compared antigen-specific CD4⁺ and CD8⁺ T cells under conditions of persistent antigen that lead to exhaustion. Pioneering work by Wherry and colleagues addressed this by comparing the phenotype and functional profile of LCMV-specific CD4⁺ and CD8⁺ T cells from mice with chronic LCMV Cl13 infection (Crawford et al., 2014). Although IR upregulation was a common feature in chronically stimulated CD4⁺ and CD8⁺ T cells, there were some differences in the type of IRs expressed by each subset. The IRs 2B4, TIM-3,

and LILRB4 were mainly expressed by exhausted CD8⁺ T cells, while CTLA4, CD220, and B and T lymphocyte attenuator were mainly expressed by chronically stimulated CD4⁺ T cells (Crawford et al., 2014). Indeed, the increased expression of CTLA4 in CD4⁺ T cells results in CTLA4 blockade having a stronger impact on proliferation in CD4⁺ vs. CD8⁺ T cells (Chan et al., 2014). Both chronically stimulated CD4⁺ T cells and CD8⁺ T cells had an enhanced type-I IFN gene signature, consistent with the role of persistent IFN signaling in facilitating exhaustion (Benci et al., 2016; Sumida et al., 2022), and also upregulated the exhaustion-associated transcription factors BATF (Zhang et al., 2022), PRDM1 (Shin et al., 2009), EOMES (McLane et al., 2021), and TOX (Khan et al., 2019). The link between TOX and dysfunction in CD4⁺ T cells is harder to discern given its crucial role in the CD4⁺ T cell development (Aliahmad et al., 2011). While chronically stimulated CD4⁺ T cells and CD8⁺ T cells upregulate many of the same transcription factors, the transcription factor IKZF2 (Helios) has been implicated as a unique regulator in chronically stimulated CD4⁺ T cells, suggesting some differences in how CD4⁺ and CD8⁺ T cells are regulated during chronic stimulation (Crawford et al., 2014) (Table 1).

Chronically stimulated CD4⁺ and CD8⁺ T cells from LCMV-Cl13-infected mice had some common functional limitations—including reduced proliferation and motility (Table 1). However, they also had critical differences. Unlike chronically stimulated CD8⁺ T cells that were functionally impaired, chronically stimulated LCMV-specific CD4⁺ T cells appeared to be functionally remodeled (Table 1). Chronically stimulated LCMV-specific CD8⁺ T cells lost the ability to produce both IFN γ and TNF. In contrast, chronically stimulated LCMV-specific CD4⁺ T cells preferentially upregulated IL21 and IL10, retained IFN γ synthetic capacity, and lost TNF synthetic capacity—indicating acquisition of Tfh-like attributes (Fahey et al., 2011; Crawford et al., 2014). Indeed, chronically stimulated LCMV-specific CD4⁺ T cells upregulate costimulatory molecules associated with Tfh-CD4⁺ T cell development—such as inducible T cell costimulator and OX40 (Fahey et al., 2011; Crawford et al., 2014). This Th1-to-Tfh transition has been described in chronically stimulated CD4⁺ T cells (Fahey et al., 2011). Mechanistically, this could be driven by inhibition of TCR signaling, given that TCR signal strength governs Th1 vs. Tfh polarization in naïve CD4⁺ T cells (Kotov et al., 2019; Künzli et al., 2021). We now recognize that functional impairment in exhausted CD8⁺ T cells results from epigenetic remodeling that limits transcription of effector genes (Pauken et al., 2016; Schietinger et al., 2016; Sen et al., 2016; Khan et al., 2019). However, whether the functional changes in chronically stimulated CD4⁺ T cells are driven by epigenetic remodeling is still unclear. Future work is needed to determine whether CD4⁺ T cells have similar or distinct epigenetic programs compared with CD8⁺ T cells.

Unlike in CD8⁺ T cells, a detailed developmental trajectory to chronically stimulated CD4⁺ T cells has not been well-described. However, one common feature between the two is the existence of a stem-like progenitor population with self-renewal potential that eventually gives rise to a terminally differentiated “exhausted” population (Table 1). Indeed, a population of TCF7⁺ PD-1⁺ CD4⁺ T cells with features resembling TCF7⁺ PD-1⁺ Tpex CD8⁺

T cells has been observed in mice with chronic infections (Xia et al., 2022; Swaminathan et al., 2024). This “stem-like” CD4⁺ T cell population had significant self-renewal potential and gave rise to different effector CD4⁺ T cell subsets in LCMV Cl13-infected mice (Xia et al., 2022; Swaminathan et al., 2024). Thus, in chronic infections, terminally exhausted CD4⁺ and CD8⁺ T cells are continuously replenished from analogous pools of self-renewing TCF7⁺ stem-like T cell populations.

Evidence of exhaustion in tumor-reactive CD4⁺ T cells in cancer. IR-expressing CD4⁺ T cells have been described in various murine tumor models, which have a loss of proliferative capacity, impaired cytokine production, and consequently defective tumor control (Goding et al., 2013; Rausch and Hastings, 2015; Goding et al., 2018; Qin et al., 2018; Fu et al., 2020) (Fig. 3). PD-1 blockade expands CD4⁺ T cells with lower IR expression and enhanced effector function, leading to improved tumor control and survival (Goding et al., 2013; Rausch and Hastings, 2015; Fu et al., 2020). Similarly, the magnitude of IR upregulation cells strongly correlates with functional impairment of the CD4⁺ T cells and also lower progression-free survival in cancer patients (Yuan et al., 2017; Nakano et al., 2018; Huang et al., 2023; Ledergor et al., 2024). Indeed, tumor-specific PD-1⁺ CD39⁺ CD4⁺ T cells from patients with head and neck, ovarian, and cervical tumors exhibit impaired production of Th1-cytokines IFN γ and TNF (Balança et al., 2021). PD-1 blockade restores the helper function of these tumor-specific CD4⁺ T cells, which correlated with improved CD8⁺ T cell priming by DCs (Balança et al., 2021). These findings suggest that tumor-specific CD4⁺ T cells exhibit features of exhaustion, which contribute to impaired tumor control.

As mentioned earlier, a significant proportion of CD4⁺ T cells in tumors had a TCF7⁺PD-1⁺ stem-like phenotype and can give rise to Th1 effector CD4⁺ T cells (Fig. 3) (Cardenas et al., 2024). Treatment with PD-1 blockade induced the proliferation of stem-like CD4⁺ T cells, induced their differentiation into effector CD4⁺ T cells, and reduced the proportion of IR-expressing CD4⁺ T cells (Fig. 3) (Fu et al., 2020; Balança et al., 2021). These data suggest that the TCF7⁺PD-1⁺ stem-like CD4⁺ T cells can give rise to terminally differentiated effector or exhausted CD4⁺ T cells in tumors (Cardenas et al., 2024; Zhou et al., 2024) (Fig. 3). Indeed, stem-like and terminally differentiated CD4⁺ T cells share a gene expression program with progenitor- and terminally differentiated CD8⁺ T cells, respectively (Miller et al., 2019; Siddiqui et al., 2019; Zhou et al., 2024). While this suggests that tumor-reactive CD4⁺ T cells might be imprinted with features of exhaustion early on, this may not be uniform to all tumor-specific CD4⁺ T cell clones. Indeed, studies have shown that CD4⁺ T cell clones with a lower precursor frequency are more likely to become exhausted, while clones with a higher precursor frequency are more likely to acquire effector-memory fates (Malandro et al., 2016). Mechanistically, the low-precursor clones experience less clonal competition during priming and thus have a greater initial expansion. However, this stronger priming signal also hastens their terminal differentiation and predisposes them toward phenotypic exhaustion, including upregulation of multiple IRs and irreversible loss of Th1 effector function (Malandro et al., 2016). In contrast, high-precursor

clones experience significant clonal competition that blunts their expansion after priming, resulting in a bias toward functional effector-memory CD4⁺ T cells (Malandro et al., 2016). Interestingly, these exhaustion features can be imprinted on the low-precursor clones in the tumor-draining lymph node itself, indicating a T_{pex} state as seen in CD8⁺ T cells. These results are consistent with observations that high-affinity T cell clones have a greater propensity toward exhaustion, while low- to intermediate-affinity clones have a propensity toward effector/memory differentiation (Hoffmann and Slansky, 2020; Künzli et al., 2021; Shakiba et al., 2022). Nevertheless, at a certain threshold, T cell clones with the lowest affinity develop a functionally inert phenotype analogous to T cell anergy (Shakiba et al., 2022; Hay et al., 2023). Thus, the strength of priming represents a determinant of CD4⁺ T cell fate (Fig. 3).

As with chronic viral infections, persistent TCR stimulation in tumor-specific CD4⁺ T cells can result in a functional reprogramming as opposed to functional impairment. Indeed, scRNA-seq studies from patients with basal cell carcinoma showed that exhausted CD8⁺ T cells and T_{fh} CD4⁺ T cells in tumors are driven by a common regulatory program (Satpathy et al., 2019). Exhausted CD4⁺ T cells preferentially upregulate the T_{fh} cytokine IL21 (Crawford et al., 2014; Satpathy et al., 2019) (Fig. 3). Furthermore, chronic type-I IFN signaling, a pathway known to facilitate T cell exhaustion, preferentially induces T_{fh} polarization over Th1 CD4⁺ T cells (Osokine et al., 2014). Chronic TCR signaling is known to shift CD4⁺ T cells from a Th1 state to a T_{fh} state (Crawford et al., 2014). However, exhausted CD8⁺ T cells in tumors are also known to preferentially recruit CXCR5⁺ T_{fh} CD4⁺ T cells via CXCL13 production (Zhou et al., 2024). Thus, the shift of tumor-reactive CD4⁺ T cells from a Th1 to a T_{fh}-like state could be driven by preferential recruitment of T_{fh}-like precursors into tumors as well as induction of a T_{fh} program in chronically stimulated Th1 CD4⁺ T cells. Although exhausted CD4⁺ T cells do not lose all effector function, the effector functions they retain might not be significant to tumor control. Indeed, although TIGIT-expressing CD4⁺ T cells in chronic lymphocytic leukemia retain IFN γ synthetic capacity, their presence correlates with more advanced disease (Osokine et al., 2014). Furthermore, TIGIT blockade improved tumor control despite decreasing IFN γ production by the CD4⁺ T cells (Catakovic et al., 2017). Most studies have linked Th1 or T_{fh} CD4⁺ T cells with key roles in anti-tumor immune responses and tumor control and have thus studied CD4⁺ T cell exhaustion in the context of those effector programs. While Th2 and Th17 CD4⁺ T cells have been described in tumors, their role in tumor control remains controversial and context dependent (see section on TME below). Furthermore, chronic viral infections, where T cell exhaustion was first described, preferentially elicit Th1- and T_{fh}-polarized CD4⁺ T cells. For this reason, the impact of chronic TCR stimulation on effector functions of Th2/Th17-polarized CD4⁺ T cells and its impact on tumor control are still not well understood.

Interestingly, CD4⁺ T cells with cytotoxic attributes (cytotoxic CD4⁺ T cells) within tumors can co-express multiple IRs associated with exhausted T cells (Oh et al., 2020; Lin et al., 2023; Sultan et al., 2024; Zhou et al., 2024) (Fig. 3). Cytotoxic CD4⁺

T cells have context-dependent pro-tumor or anti-tumor roles. Many studies have described key roles for cytotoxic CD4⁺ T cells in facilitating tumor control by targeting MHCII⁺ cancer cells (Oh et al., 2020; Lin et al., 2023; Zhou et al., 2024). However, a recent study by Bob Schreiber's group described an IL10⁺ FOXP3⁺ "regulatory-cytotoxic" CD4⁺ T cell subset that preferentially kills anti-tumor MHCII⁺ myeloid cells, resulting in cancer immune evasion (Sultan et al., 2024). Although IR^{hi} cytotoxic CD4⁺ T cells are distinct from other helper CD4⁺ T cell subsets (Cachot et al., 2021), whether acquisition of cytotoxic function is a part of an "exhaustion" program in chronically stimulated CD4⁺ T cells in tumors or whether terminal effector CD4⁺ T cells acquire cytotoxic function via a pathway distinct from exhaustion is unknown.

In addition to effector CD4⁺ T cells, tumor-infiltrating Tregs also express various IRs (Yano et al., 2019). The role of these receptors on Tregs in tumors is complex. While significant up-regulation of PD-1 can reduce Treg proliferation and function (Lowther et al., 2016; Kamada et al., 2019), other IRs like Tim-3 (Sakuishi et al., 2013; Banerjee et al., 2021), LAG-3 (Huang et al., 2004; Camisaschi et al., 2010), and TIGIT (Chen et al., 2020; Yang et al., 2020) limit Treg proliferation while also enhancing their suppressive abilities—indicative of a transition to a terminal-effector phenotype. It is likely that the overall effect of IRs on Tregs depends on their expression level. While chronic TCR stimulation does impact Treg proliferation, whether it eventually impairs their suppressive function is not known. Thus, whether chronic TCR stimulation impacts Tregs in the same way as it impacts effector CD4⁺ or CD8⁺ T cells is still unclear.

Factors in the TME that regulate suppressed CD4⁺ T cells

Cancer cells do not exist in isolation: indeed, they recruit a diverse cast of cell types to form an ecosystem favorable to their survival and growth. The TME presents various obstacles that impede T cell function—such as physical barriers, metabolic stressors, and immunosuppressive cell populations (Schreiber et al., 2011; Chen and Mellman, 2013). These features of the TME can significantly suppress effector functions in CD4⁺ T cells via mechanisms distinct from anergy or exhaustion (Fig. 3). Indeed, as these factors can affect CD4⁺ T cells at different stages of the aforementioned differentiation pathways, identifying suppressed CD4⁺ T cells *ex vivo* using surface markers and functional profiling is challenging. Rather, these TME effects can be discerned by identifying the suppressive mediators and elucidating pathways associated with the different suppressive mechanisms. This will also inform immunotherapies that rescue CD4⁺ T cell function by blocking these pathways. (Borst et al., 2018; Renaude et al., 2021). In the section below, we will describe how key players/factors in the TME impact the functional state of tumor-reactive CD4⁺ T cells (summarized in Table 2).

Metabolic stressors. A key feature of the TME, particularly in large solid tumors, is a paucity of oxygen and nutrients due to rapid tumor growth (Visser and Joyce, 2023). Rapidly dividing cancer cells outcompete T cells for critical metabolic resources. Furthermore, during tumor growth, cancer cells adapt their metabolism from energy production toward biosynthetic

Table 2. Summary of how the factors in the TME impact CD4⁺ T cell functions

TME factor/Cell type	Impact on CD4 ⁺ T cell functions	References
Acidity/lactate	↑ Treg differentiation	Comito et al. (2019)
	↓ Th1/Th17 effector programs	Comito et al. (2019), Krol et al. (2022)
	↓ TCR signaling via induction of Cbl-b	Bosticardo et al. (2001), Pilon-Thomas et al. (2016), Huber et al. (2017), Tsai et al. (2023)
Hypoxia	↑ Treg differentiation	Ben-Shoshan et al. (2008), Neildez-Nguyen et al. (2015)
	↑ Suppressive IL10 ⁺ Th17 CD4s	Volchenkov et al. (2017)
	↓ Th1 effector program via IL10 signaling	Shehade et al. (2015)
ER stress	↓ Activation and effector functions in CD4 ⁺ T cells	Song et al. (2018), Cao et al. (2019)
	↓ Th1 polarization	
CAFs	↓ Effector function in CD4 ⁺ T cells	Erez et al. (2010), Fiori et al. (2019), Butti et al. (2021), Chen et al. (2024a)
	↑ Suppressive Tregs	Liao et al. (2009), Monte et al. (2011), Huang et al. (2022)
	↑ Th2-CD4 ⁺ T cells	Barnas et al. (2010), Keridani et al. (2022)
	Prime anti-tumor Th1/Th17 CD4 ⁺ T cells	
Tumor-associated suppressive myeloid cells	Produce suppressive factors that inhibit Th1 effector programs in CD4 ⁺ T cells	Beltra et al. (2020), Xia et al. (2022), Zhou et al. (2024)
	↓ Amino acids essential for T cell function	
	Prime CD4 ⁺ T cells to become anergic/Tregs	
	Induce tumor-promoting Th17 CD4 ⁺ T cells	
cDC2s, cDC1s, and M1-like macrophages	Prime anti-tumor CD4 ⁺ T cells	Ferris et al. (2020), Iwanowycz et al. (2021), Patterson et al. (2023)

cDC2, type-2 conventional DCs; cDC1, type-1 conventional DCs.

pathways to produce molecules required for rapid cell division. This "aerobic" glycolysis consumes oxygen and releases substantial lactate, resulting in a hypoxic and acidic TME that becomes progressively hostile with tumor growth (Zhou et al., 2022; Visser and Joyce, 2023). This hostile environment has profound impact on the functional state of tumor-specific CD4⁺ T cells.

Tumors have a high amount of lactate, which creates an acidic environment (Zhou et al., 2022; Visser and Joyce, 2023). The high lactate can induce effector CD4⁺ T cells in tumors to

upregulate FOXP3 and thus become Tregs (Comito et al., 2019; Krol et al., 2022). Lactate signaling also impairs effector function in preexisting helper CD4⁺ T cells: lactate mediates degradation of the Th1-associated transcription factor T-bet and also results in inhibition of IL17 expression in Th17 CD4⁺ T cells (Comito et al., 2019; Krol et al., 2022). Low pH impairs the effector function of CD4⁺ T cells even in the absence of lactate, mainly via casitas B lymphoma-b (Cbl-b)-mediated inhibition of TCR signaling, suggesting that lactate inhibits effector functions in tumor-CD4⁺ T cells through both direct and indirect mechanisms (Bosticardo et al., 2001; Pilon-Thomas et al., 2016; Huber et al., 2017; Tsai et al., 2023).

Hypoxia has a similar inhibitory impact on CD4⁺ T cell function, which is mediated by the activation of the transcription factor HIF1 α . Similar to lactate, HIF1 α is also known to facilitate iTreg differentiation via induction and stabilization of FOXP3 in Tregs (Ben-Shoshan et al., 2008; Neildez-Nguyen et al., 2015). HIF1 α signaling is also known to inhibit effector functions of Th1 cells and induce immune-suppressive IL10⁺ Th17 CD4⁺ T cells (Shehade et al., 2015; Volchenkov et al., 2017).

ER stress is a common phenomenon in immune cells in tumors, which is driven by a plethora of stressors, including hypoxia, acidity, and nutrient deprivation. ER stress is known to inhibit the activation and effector function of both CD4⁺ and CD8⁺ T cells, particularly under glucose starvation condition in tumors where IRE1-XBP1 signaling induce mitochondrial dysfunction by inhibiting glutamine influx (Song et al., 2018; Cao et al., 2019). ER stress also uniquely inhibits Th1-polarized CD4⁺ T cells by repressing T-bet (Song et al., 2018; Cao et al., 2019).

Cancer-associated fibroblasts (CAFs). One of the first responders to these inflammatory signals is fibroblasts. During wound healing, fibroblasts respond to persistent inflammatory signals by recruiting suppressive immune cells and producing a fibrous “clot” that closes wounds (Dvorak, 2015). Similarly in tumors, cancer cells reprogram fibroblasts toward an anti-inflammatory state, which is a key event that facilitates tumor progression (Erez et al., 2010; Fiori et al., 2019; Butti et al., 2021). CAFs have direct suppressive effects on T cell function via production of soluble mediators such as IL10, TGF β , and adenosine (from ATP via CD39 and CD73), as well as via direct contact through PDL1/PDL2 and FasL (Chen et al., 2024a). CAFs induce naïve T cells to become Tregs via direct antigen presentation and TGF β production and induce effector CD4⁺ T cells to acquire an anti-inflammatory Th2 phenotype via thymic stromal lymphopoietin—both of which are associated with impaired anti-tumor immunity and tumor progression (Liao et al., 2009; Monte et al., 2011; Huang et al., 2022). However, CAFs are not necessarily pro-tumor and have in some cases been associated with anti-tumor CD4⁺ T cell responses. Antigen presentation, as well as complement protein-1q (C1q) production, by CAFs was shown to be critical for optimal expansion and survival of anti-tumor CD4⁺ T cells in lung cancer (Keridani et al., 2022). In skin cancer, inflammatory CAFs induce IFN γ - and IL17A-producing effector CD4⁺ T cells (Barnas et al., 2010). The anti-tumor function of the CAFs required autocrine IL6 signaling, which was enhanced by soluble factors produced by T cells (Barnas et al., 2010). Anti-tumor CAFs and Th1/Th17 CD4⁺ T cells

thus co-regulate each other and can facilitate tumor control in some solid tumors. Thus, CAFs can polarize CD4⁺ T cells toward either pro-tumor or anti-tumor states, depending on the context (Fig. 3 and Table 2).

Tumor-associated myeloid cells. Tumors recruit and foster a variety of suppressive myeloid cells, which play critical roles in suppressing anti-tumor T cell responses (Cassetta and Pollard, 2018; Li et al., 2021). These include myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages, and tolerogenic DC (toDCs). These myeloid subsets produce a plethora of immunosuppressive factors, such as IL10 and TGF β , that inhibit effector Th1 CD4⁺ T cells and impair Th1 CD4⁺ T cell polarization (Tsukamoto et al., 2013). Suppressive myeloid cells also produce enzymes that deplete amino acids essential for T cell function, such as IDO (depletes tryptophan) (Munn and Mellor, 2016) and arginase (depletes arginine) (Grzywa et al., 2020). Direct interaction with MDSCs and toDCs can cause naïve CD4⁺ T cells to either become anergic or differentiate into Tregs (FOXP3⁺ Tregs or FOXP3⁻ Tr1s) (Steinbrink et al., 1997; Zhen et al., 2004; Raker et al., 2015), with Treg/Tr1 differentiation mediated by costimulatory signals such as CD40 on MDSCs (Pan et al., 2010) and ILT family proteins on toDCs (Comi et al., 2018). Interestingly in lupus, MDSCs are known to induce CD4⁺ T cells to acquire a Th17 phenotype (Wu et al., 2016a; Hu et al., 2023). Th17 polarization is driven by high systemic levels of the inflammation-associated cytokines IL6 and TGF β , as well as arginase-mediated arginine depletion (Wu et al., 2016a; Hu et al., 2023). The observations in lupus patients suggest that MDSCs induce Th17 CD4⁺ T cells in a chronic inflammatory environment—which could likely extend to inflammation-driven tumors. Indeed, MDSCs in colorectal, oral cell squamous, and pancreatic tumors are known to induce Th17 CD4⁺ T cells (Chatterjee et al., 2013; Blair et al., 2019; Dar et al., 2020; Wang et al., 2020). In those tumors, the Th17 CD4⁺ T cells facilitate tumor progression by recruiting more MDSCs, promoting angiogenesis, and promoting tumor survival and metastasis, as well as inducing fibrosis (Du et al., 2012; Wu et al., 2014; Lücke et al., 2021; Fesneau et al., 2024). In contrast, type-2 conventional DCs, as well as type-1 conventional DCs and “M1-like” MHCII⁺ macrophages, prime and expand effector anti-tumor CD4⁺ T cells (Ferris et al., 2020; Iwanowycz et al., 2021; Patterson et al., 2023). The relative composition of pro-tumor and anti-tumor myeloid cell subsets in tumors thus plays a key role in regulating the functional state of anti-tumor CD4⁺ T cells.

Summary

CD8⁺ T cells have been at the forefront of immunotherapy approaches to fight cancer. However, recent studies have illuminated key roles for CD4⁺ T cells in initiating and directing anti-tumor immune responses that curtail tumor progression. There has thus been an interest in better defining mechanisms of dysfunction in tumor-associated CD4⁺ T cells. In this review, we integrated insights from studies on tumor-reactive CD4⁺ T cells with cutting-edge studies on chronically stimulated CD4⁺ T cells in viral infections to define three main differentiation trajectories for CD4⁺ T cells in tumor-draining lymph nodes and tumors (Fig. 3): (1) Impaired priming in tumor-draining lymph nodes resulting in CD4⁺ T cell anergy, (2) strong priming in tumor-

draining lymph nodes predisposes stem-like CD4⁺ T cells toward phenotypic exhaustion in the TME due to chronic TCR signaling, and (3) moderate/weak priming in tumor-draining lymph nodes, as well as treatment with immune-checkpoint inhibitors, predisposes stem-like CD4⁺ T cells toward effector differentiation. Finally, effector CD4⁺ T cells can also be functionally suppressed by factors in the TME.

The quality of priming in draining lymph nodes plays a key role in determining the eventual functional state of tumor-reactive CD4⁺ T cells. In many tumors, Tregs in the draining lymph node impair costimulatory signals that CD4⁺ T cells receive during priming—resulting in CD4⁺ T cell anergy. On the flip side, excessively strong activation signals during priming can predispose CD4⁺ T cells toward terminal exhaustion. As in CD8⁺ T cells, this exhaustion phenotype is imprinted early on in stem-like CD4⁺ T cells in draining lymph nodes. Although exhausted CD4⁺ T cells in tumors have functional deficits, studies have described exhausted CD4⁺ T cells with varying degrees of functional impairment (Malandro et al., 2016; Catakovic et al., 2017; Balanço et al., 2021; Tracy et al., 2022). Whether the severity of the functional deficits correlates with TCR affinity or clone size, and thus the strength of the initial priming signals, remains to be determined. Still, these studies suggest that effective anti-tumor CD4⁺ T cell priming requires activation signals of an intermediate strength.

PD-1 and CTLA4 blockade impact CD4⁺ T cells via distinct mechanisms. CTLA-4 blockade mitigates T cell anergy by interfering with Treg-mediated trogocytosis, and promotes the proliferation of effector CD4⁺ T cells (Peggs et al., 2009; Chan et al., 2014). In contrast, based on observation in CD8⁺ T cells, PD-1 blockade likely promotes the differentiation of stem-like CD4⁺ T cell to effector CD4⁺ T cells within tumors (Siddiqui et al., 2019; Connolly et al., 2021). However, some studies suggest that PD-1 blockade can restore of effector function in exhausted CD4⁺ T cells (Balanço et al., 2021). As the studies describing effector vs. exhaustion pathways in tumor-reactive CD4⁺ T cells focused on Th1/Tfh effector states, whether this model can be applied to tumor-reactive Th2 and Th17 CD4⁺ T cells is not yet known.

Lastly, immunosuppressive factors in the TME play a key role in attenuating CD4⁺ T cell function via pathways that are distinct from anergy or exhaustion (Table 2). As the TME can impact different effector CD4⁺ T cell states, understanding how factors in the TME influence the functional attributes of effector and regulatory CD4⁺ T cell subsets is crucial to elucidating their impact on CD4⁺ T cell-mediated tumor control. While the studies highlighted in this review have described the functional impact of individual TME factors on tumor-reactive CD4⁺ T cells, integrating these insights to understand the net impact of the TME on CD4⁺ T cell function is a crucial next step. One approach, pioneered by Max Krummel and others, is to classify tumors based on “immune archetypes:” groups of tumors with characteristic microenvironments and immune cell profiles (Combes et al., 2022; Xiao et al., 2022; Anderson et al., 2023; Hamidi et al., 2024). Future studies could build on this work by characterizing how the different TME factors in distinct tumor “niches” influence CD4⁺ T cell function in each archetype. Recent advances in

interaction analysis leveraging single-cell RNA sequencing and spatial transcriptomics/proteomics have made it possible to answer such questions (Dimitrov et al., 2022; Jing et al., 2025).

In conclusion, CD4⁺ and CD8⁺ T cells experience distinct functional perturbations in tumors. Effective use of immunotherapy for cancer treatment requires understanding how tumors and anti-tumor therapies affect different CD4⁺ T cell subsets and how these changes influence the anti-tumor immune response and tumor growth.

Acknowledgments

L. Fong is supported by National Institutes of Health grant R35CA253175.

Author contributions: H. Venkatesh: conceptualization, investigation, visualization, and writing—original draft, review, and editing. L. Fong: conceptualization, data curation, funding acquisition, supervision, and writing—original draft, review, and editing.

Disclosures: L. Fong reported personal fees from AbbVie, Bio-Atla, BMS, Daiichi Sankyo, Dendreon, ImmunoGenesis, Inovvent, Merck, Nutcracker, Sutro, and Roche/Genentech during the conduct of the study. No other disclosures were reported.

Submitted: 6 January 2025

Revised: 25 March 2025

Accepted: 24 July 2025

References

- Abe, B.T., D.S. Shin, E. Mocholi, and F. Macian. 2012. NFAT1 supports tumor-induced anergy of CD4(+) T cells. *Cancer Res.* 72:4642–4651. <https://doi.org/10.1158/0008-5472.can-11-3775>
- Aliaahmad, P., A. Kadavallor, B. de la Torre, D. Kappes, and J. Kaye. 2011. TOX is required for development of the CD4 T cell lineage gene program. *J. Immunol.* 187:5931–5940. <https://doi.org/10.4049/jimmunol.1101474>
- Alonso, R., H. Flament, S. Lemoine, C. Sedlik, E. Bottasso, I. Péguillet, V. Prémel, J. Denizeau, M. Salou, A. Darbois, et al. 2018. Induction of anergic or regulatory tumor-specific CD4⁺ T cells in the tumor-draining lymph node. *Nat. Commun.* 9:2113. <https://doi.org/10.1038/s41467-018-04524-x>
- Alspach, E., D.M. Lussier, A.P. Miceli, I. Kizhvatov, M. DuPage, A.M. Luoma, W. Meng, C.F. Licht, E. Esaulova, A.N. Vomund, et al. 2019. MHC-II neoantigens shape tumour immunity and response to immunotherapy. *Nature.* 574:696–701. <https://doi.org/10.1038/s41586-019-1671-8>
- Anderson, K.G., D.A. Braun, A. Buqué, S.B. Gitto, J.L. Guerriero, B. Horton, B.P. Keenan, T.S. Kim, A. Overacre-Delgoffe, M. Ruella, et al. 2023. Leveraging immune resistance archetypes in solid cancer to inform next-generation anticancer therapies. *J. Immunother. Cancer.* 11: e006533. <https://doi.org/10.1136/jitc-2022-006533>
- Arina, A., T. Karrison, E. Galka, K. Schreiber, R.R. Weichselbaum, and H. Schreiber. 2017. Transfer of allogeneic CD4⁺ T cells rescues CD8⁺ T cells in anti-PD-L1-resistant tumors leading to tumor eradication. *Cancer Immunol. Res.* 5:127–136. <https://doi.org/10.1158/2326-6066.CIR-16-0293>
- Aubert, R.D., A.O. Kamphorst, S. Sarkar, V. Vezys, S.J. Ha, D.L. Barber, L. Ye, A.H. Sharpe, G.J. Freeman, and R. Ahmed. 2011. Antigen-specific CD4 T-cell help rescues exhausted CD8 T cells during chronic viral infection. *Proc. Natl. Acad. Sci. USA.* 108:21182–21187. <https://doi.org/10.1073/pnas.1118450109>
- Baessler, A., and D.A.A. Vignali. 2024. T cell exhaustion. *Annu. Rev. Immunol.* 42:179–206. <https://doi.org/10.1146/annurev-immunol-090222-110914>
- Baitsch, L., P. Baumgaertner, E. Devèvre, S.K. Raghav, A. Legat, L. Barba, S. Wiekowski, H. Bouzourene, B. Deplancke, P. Romero, et al. 2011.

- Exhaustion of tumor-specific CD8⁺ T cells in metastases from melanoma patients. *J. Clin. Invest.* 121:2350–2360. <https://doi.org/10.1172/JCI46102>
- Balanca, C.-C., A. Salvioni, C.-M. Scarlata, M. Michelas, C. Martinez-Gomez, C. Gomez-Roca, V. Sarradin, M. Tosolini, C. Valle, F. Pont, et al. 2021. PD-1 blockade restores helper activity of tumor-infiltrating, exhausted PD-1hiCD39⁺ CD4⁺ T cells. *JCI Insight.* 6:e142513. <https://doi.org/10.1172/jci.insight.142513>
- Banerjee, H., H. Nieves-Rosado, A. Kulkarni, B. Murter, K.V. McGrath, U.R. Chandran, A. Chang, A.L. Szymczak-Workman, L. Vujanovic, G.M. Delgoffe, et al. 2021. Expression of Tim-3 drives phenotypic and functional changes in Treg cells in secondary lymphoid organs and the tumor microenvironment. *Cell Rep.* 36:109699. <https://doi.org/10.1016/j.celrep.2021.109699>
- Barnas, J.L., M.R. Simpson-Abelson, S.P. Brooks, R.J. Kelleher Jr., and R.B. Bankert. 2010. Reciprocal functional modulation of the activation of T lymphocytes and fibroblasts derived from human solid tumors. *J. Immunol.* 185:2681–2692. <https://doi.org/10.4049/jimmunol.1000896>
- Beltra, J.-C., S. Manne, M.S. Abdel-Hakeem, M. Kurachi, J.R. Giles, Z. Chen, V. Casella, S.F. Ngiew, O. Khan, Y.J. Huang, et al. 2020. Developmental relationships of four exhausted CD8⁺ T cell subsets reveals underlying transcriptional and epigenetic landscape control mechanisms. *Immunity.* 52:825–841.e8. <https://doi.org/10.1016/j.immuni.2020.04.014>
- Ben-Shoshan, J., S. Maysel-Auslender, A. Mor, G. Keren, and J. George. 2008. Hypoxia controls CD4⁺CD25⁺ regulatory T-cell homeostasis via hypoxia-inducible factor-1alpha. *Eur. J. Immunol.* 38:2412–2418. <https://doi.org/10.1002/eji.200838318>
- Benci, J.L., B. Xu, Y. Qiu, T.J. Wu, H. Dada, C.T.-S. Victor, L. Cucolo, D.S.M. Lee, K.E. Pauken, A.C. Huang, et al. 2016. Tumor interferon signaling regulates a multigenic resistance program to immune checkpoint blockade. *Cell.* 167:1540–1554.e12. <https://doi.org/10.1016/j.cell.2016.11.022>
- Blackburn, S.D., H. Shin, G.J. Freeman, and E.J. Wherry. 2008. Selective expansion of a subset of exhausted CD8 T cells by alphaPD-L1 blockade. *Proc. Natl. Acad. Sci. USA.* 105:15016–15021. <https://doi.org/10.1073/pnas.0801497105>
- Blair, A.B., J. Kleponis, D.L. Thomas, S.T. Muth, A.G. Murphy, V. Kim, and L. Zheng. 2019. Idol inhibition potentiates vaccine-induced immunity against pancreatic adenocarcinoma. *J. Clin. Invest.* 129:1742–1755. <https://doi.org/10.1172/JCI124077>
- Bonnal, R.J.P., G. Rossetti, E. Lugli, M. De Simone, P. Gruarin, J. Brummelman, L. Druifuca, M. Passaro, R. Bason, F. Gervasoni, et al. 2021. Clonally expanded EOMES⁺ Tr1-like cells in primary and metastatic tumors are associated with disease progression. *Nat. Immunol.* 22:735–745. <https://doi.org/10.1038/s41590-021-00930-4>
- Borst, J., T. Ahrends, N. Băbala, C.J.M. Melief, and W. Kastenmüller. 2018. CD4⁺ T cell help in cancer immunology and immunotherapy. *Nat. Rev. Immunol.* 18:635–647. <https://doi.org/10.1038/s41577-018-0044-0>
- Bosticardo, M., S. Ariotti, G. Losana, P. Bernabei, G. Forni, and F. Novelli. 2001. Biased activation of human T lymphocytes due to low extracellular pH is antagonized by B7/CD28 costimulation. *Eur. J. Immunol.* 31:2829–2838. [https://doi.org/10.1002/1521-4141\(200109\)31:9<2829::aid-immu2829>3.0.co;2-u](https://doi.org/10.1002/1521-4141(200109)31:9<2829::aid-immu2829>3.0.co;2-u)
- Brooks, D.G., L. Teyton, M.B.A. Oldstone, and D.B. McGavern. 2005. Intrinsic functional dysregulation of CD4 T cells occurs rapidly following persistent viral infection. *J. Virol.* 79:10514–10527. <https://doi.org/10.1128/JVI.79.16.10514-10527.2005>
- Brunet-Ratnasingham, E., A. Morou, M. Dubé, J. Niessl, A.E. Baxter, O. Tastet, N. Brassard, G. Ortega-Delgado, R. Charlebois, G.J. Freeman, et al. 2022. Immune checkpoint expression on HIV-specific CD4⁺ T cells and response to their blockade are dependent on lineage and function. *EBio-Medicine.* 84:104254. <https://doi.org/10.1016/j.ebiom.2022.104254>
- Butti, R., R. Nimma, G. Kundu, A. Bulbule, T.V.S. Kumar, V.P. Gunasekaran, D. Tomar, D. Kumar, A. Mane, S.S. Gill, et al. 2021. Tumor-derived osteopontin drives the resident fibroblast to myofibroblast differentiation through Twist1 to promote breast cancer progression. *Oncogene.* 40:2002–2017. <https://doi.org/10.1038/s41388-021-01663-2>
- Cachot, A., M. Bilous, Y.-C. Liu, X. Li, M. Saillard, M. Cenerenti, G.A. Rockinger, T. Wyss, P. Guillaume, J. Schmidt, et al. 2021. Tumor-specific cytolytic CD4 T cells mediate immunity against human cancer. *Sci. Adv.* 7:eabe3348. <https://doi.org/10.1126/sciadv.abe3348>
- Camisaschi, C., C. Casati, F. Rini, M. Perego, A. De Filippo, Triebel F., G. Parmiani, F. Belli, L. Rivoltini, and C. Castelli. 2010. LAG-3 expression defines a subset of CD4(+)CD25(high)Foxp3(+) regulatory T cells that are expanded at tumor sites. *J. Immunol.* 184:6545–6551. <https://doi.org/10.4049/jimmunol.0903879>
- Cao, Y., J. Trillo-Tinoco, R.A. Sierra, C. Anadon, W. Dai, E. Mohamed, L. Cen, T.L. Costich, A. Magliocco, D. Marchion, et al. 2019. ER stress-induced mediator C/EBP homologous protein thwarts effector T cell activity in tumors through T-bet repression. *Nat. Commun.* 10:1280. <https://doi.org/10.1038/s41467-019-09263-1>
- Cardenas, M.A., N. Prokhnevskaya, E. Sobierajska, P. Gregorova, C.B. Medina, R.M. Valanparambil, R. Greenwald, L. DelBalzo, M.A. Bilen, S.S. Joshi, et al. 2024. Differentiation fate of a stem-like CD4 T cell controls immunity to cancer. *Nature.* 636:224–232. <https://doi.org/10.1038/s41586-024-08076-7>
- Cassetta, L., and J.W. Pollard. 2018. Targeting macrophages: Therapeutic approaches in cancer. *Nat. Rev. Drug Discov.* 17:887–904. <https://doi.org/10.1038/nrd.2018.169>
- Catakovic, K., F.J. Gassner, C. Ratswohl, N. Zaborsky, S. Rebhandl, M. Schubert, M. Steiner, J.C. Gutjahr, L. Pleyer, A. Egle, et al. 2017. TIGIT expressing CD4⁺T cells represent a tumor-supportive T cell subset in chronic lymphocytic leukemia. *Oncotarget.* 7:e1371399. <https://doi.org/10.1080/2162402X.2017.1371399>
- Chan, D.V., H.M. Gibson, B.M. Aufiero, A.J. Wilson, M.S. Hafner, Q.-S. Mi, and H.K. Wong. 2014. Differential CTLA-4 expression in human CD4⁺ versus CD8⁺ T cells is associated with increased NFAT1 and inhibition of CD4⁺ proliferation. *Genes Immun.* 15:25–32. <https://doi.org/10.1038/gene.2013.57>
- Chatterjee, B., Y. Deng, A. Holler, N. Nunez, T. Azzi, L.D. Vanoaica, A. Müller, H. Zdimerova, O. Antsiferova, A. Zbinden, et al. 2019. CD8⁺ T cells retain protective functions despite sustained inhibitory receptor expression during Epstein-Barr virus infection in vivo. *PLoS Pathog.* 15:e1007748. <https://doi.org/10.1371/journal.ppat.1007748>
- Chatterjee, S., S. Das, P. Chakraborty, A. Manna, M. Chatterjee, and S.K. Choudhuri. 2013. Myeloid derived suppressor cells (MDSCs) can induce the generation of Th17 response from naïve CD4⁺ T cells. *Immunobiology.* 218:718–724. <https://doi.org/10.1016/j.imbio.2012.08.271>
- Chen, D.S., and I. Mellman. 2013. Oncology meets immunology: The cancer-immunity cycle. *Immunity.* 39:1–10. <https://doi.org/10.1016/j.immuni.2013.07.012>
- Chen, F., Y. Xu, Y. Chen, and S. Shan. 2020. TIGIT enhances CD4⁺ regulatory T-cell response and mediates immune suppression in a murine ovarian cancer model. *Cancer Med.* 9:3584–3591. <https://doi.org/10.1002/cam4.2976>
- Chen, M., F. Chen, Z. Gao, X. Li, L. Hu, S. Yang, S. Zhao, and Z. Song. 2024a. CAFs and T cells interplay: The emergence of a new arena in cancer combat. *Biomed. Pharmacother.* 177:117045. <https://doi.org/10.1016/j.biopha.2024.117045>
- Chen, Y., D. Yu, H. Qian, Y. Shi, and Z. Tao. 2024b. CD8⁺ T cell-based cancer immunotherapy. *J. Transl. Med.* 22:1–17. <https://doi.org/10.1186/s12967-024-0134-6>
- Combes, A.J., S. Bushra, J. Tsui, N.W. Chew, P. Yan, G.C. Reeder, D. Kushnoor, A. Shen, B. Davidson, A.J. Barczak, et al. 2022. Discovering dominant tumor immune archetypes in a pan-cancer census. *Cell.* 185:184–203.e19. <https://doi.org/10.1016/j.cell.2021.12.004>
- Comi, M., G. Amodio, and S. Gregori. 2018. Interleukin-10-Producing DC-10 is a unique tool to promote tolerance via antigen-specific T regulatory type 1 cells. *Front. Immunol.* 9:682. <https://doi.org/10.3389/fimmu.2018.00682>
- Comito, G., A. Iscaro, M. Bacci, A. Morandi, L. Ippolito, M. Parri, I. Montagnani, M.R. Raspollini, S. Serni, L. Simeoni, et al. 2019. Lactate modulates CD4⁺ T-cell polarization and induces an immunosuppressive environment, which sustains prostate carcinoma progression via TLR8/miR21 axis. *Oncogene.* 38:3681–3695. <https://doi.org/10.1038/s41388-019-0688-7>
- Connolly, K.A., M. Kuchroo, A. Venkat, A. Khatun, J. Wang, I. William, N.I. Hornick, B.L. Fitzgerald, M. Damo, M.Y. Kasmani, et al. 2021. A reservoir of stem-like CD8⁺ T cells in the tumor-draining lymph node preserves the ongoing antitumor immune response. *Sci. Immunol.* 6:eabg7836. <https://doi.org/10.1126/sciimmunol.abg7836>
- Crawford, A., J.M. Angelosanto, C. Kao, T.A. Doering, P.M. Odorizzi, B.E. Barnett, and E.J. Wherry. 2014. Molecular and transcriptional basis of CD4⁺ T cell dysfunction during chronic infection. *Immunity.* 40:289–302. <https://doi.org/10.1016/j.immuni.2014.01.005>
- Crespo, J., H. Sun, T.H. Welling, Z. Tian, and W. Zou. 2013. T cell anergy, exhaustion, senescence, and stemness in the tumor microenvironment. *Curr. Opin. Immunol.* 25:214–221. <https://doi.org/10.1016/j.coi.2012.12.003>
- Cuenca, A., F. Cheng, H. Wang, J. Brayer, P. Horna, L. Gu, H. Bien, I.M. Borrello, H.I. Levitsky, and E.M. Sotomayor. 2003. Extra-lymphatic solid

- tumor growth is not immunologically ignored and results in early induction of antigen-specific T-cell anergy: Dominant role of cross-tolerance to tumor antigens. *Cancer Res.* 63:9007–9015.
- Cui, C., J. Wang, E. Fagerberg, P.M. Chen, K.A. Connolly, M. Damo, J.F. Cheung, T. Mao, A.S. Askari, S. Chen, et al. 2021. Neoantigen-driven B cell and CD4 T follicular helper cell collaboration promotes anti-tumor CD8 T cell responses. *Cell.* 184:6101–6118.e13. <https://doi.org/10.1016/j.cell.2021.11.007>
- Curtsinger, J.M., C.S. Schmidt, A. Mondino, D.C. Lins, R.M. Kedl, M.K. Jenkins, and M.F. Mescher. 1999. Inflammatory cytokines provide a third signal for activation of naive CD4⁺ and CD8⁺ T cells. *J. Immunol.* 162: 3256–3262.
- Dar, A.A., R.S. Patil, T.N. Pradhan, D.A. Chaukar, A.K. D'Cruz, and S.V. Chiplunkar. 2020. Myeloid-derived suppressor cells impede T cell functionality and promote Th17 differentiation in oral squamous cell carcinoma. *Cancer Immunol. Immunother.* 69:1071–1086. <https://doi.org/10.1007/s00262-020-02523-w>
- Deeths, M.J., and M.F. Mescher. 1997. B7-1-dependent co-stimulation results in qualitatively and quantitatively different responses by CD4⁺ and CD8⁺ T cells. *Eur. J. Immunol.* 27:598–608. <https://doi.org/10.1002/eji.1830270305>
- Deeths, M.J., R.M. Kedl, and M.F. Mescher. 1999. CD8⁺ T cells become non-responsive (anergic) following activation in the presence of co-stimulation. *J. Immunol.* 163:102–110. <https://doi.org/10.4049/jimmunol.163.1.102>
- Dimitrov, D., D. Türe, M. Garrido-Rodriguez, P.L. Burmedi, J.S. Nagai, C. Boys, R.O. Ramirez Flores, H. Kim, B. Szalai, I.G. Costa, et al. 2022. Comparison of methods and resources for cell-cell communication inference from single-cell RNA-Seq data. *Nat. Commun.* 13:3224. <https://doi.org/10.1038/s41467-022-30755-0>
- Dow, C., R. Henderson, A. Sette, and B.R. Mothé. 2013. CD4(+) T-cell inhibitory ligands: A tool for characterizing dysfunctional CD4(+) T cells during chronic infection. *Immunology.* 140:61–69. <https://doi.org/10.1111/imm.12109>
- Du, J.W., K.Y. Xu, L.Y. Fang, and X.L. Qi. 2012. Interleukin-17, produced by lymphocytes, promotes tumor growth and angiogenesis in a mouse model of breast cancer. *Mol. Med. Rep.* 6:1099–1102. <https://doi.org/10.3892/mmr.2012.1036>
- Dvorak, H.F. 2015. Tumors: Wounds that do not heal-redux. *Cancer Immunol. Res.* 3:1–11. <https://doi.org/10.1158/2326-6066.CIR-14-0209>
- Erez, N., M. Truitt, P. Olson, S.T. Arron, and D. Hanahan. 2010. Cancer-Associated fibroblasts are activated in incipient neoplasia to orchestrate tumor-promoting inflammation in an NF-kappaB-Dependent manner. *Cancer Cell.* 17:135–147. <https://doi.org/10.1016/j.ccr.2009.12.041>
- Espinosa-Carrasco, G., E. Chiu, A. Scrivo, P. Zumbo, A. Dave, D. Betel, S.W. Kang, H.-J. Jang, M.D. Hellmann, B.M. Burt, et al. 2024. Intratumoral immune triads are required for immunotherapy-mediated elimination of solid tumors. *Cancer Cell.* 42:1202–1216.e8. <https://doi.org/10.1016/j.ccell.2024.05.025>
- Fahey, L.M., E.B. Wilson, H. Elsaesser, C.D. Fistonich, D.B. McGavern, and D.G. Brooks. 2011. Viral persistence redirects CD4 T cell differentiation toward T follicular helper cells. *J. Exp. Med.* 208:987–999. <https://doi.org/10.1084/jem.20101773>
- Ferris, S.T., V. Durai, R. Wu, D.J. Theisen, J.P. Ward, M.D. Bern, J.T. Davidson 4th, P. Bagadia, T. Liu, C.G. Briseño, et al. 2020. cDC1 prime and are licensed by CD4⁺ T cells to induce anti-tumour immunity. *Nature.* 584: 624–629. <https://doi.org/10.1038/s41586-020-2611-3>
- Fesneau, O., V. Thevin, V. Pinet, C. Goldsmith, B. Vieille, S. M'Homa Soudja, R. Lattanzio, M. Hahne, V. Dardalhon, H. Hernandez-Vargas, et al. 2024. An intestinal TH17 cell-derived subset can initiate cancer. *Nat. Immunol.* 25:1637–1649. <https://doi.org/10.1038/s41590-024-01909-7>
- Fiori, M.E., S. Di Franco, L. Villanova, P. Bianca, G. Stassi, and R. De Maria. 2019. Cancer-associated fibroblasts as abettors of tumor progression at the crossroads of EMT and therapy resistance. *Mol. Cancer.* 18:70. <https://doi.org/10.1186/s12943-019-0994-2>
- Fu, J., A. Yu, X. Xiao, J. Tang, X. Zu, W. Chen, and B. He. 2020. CD4⁺ T cell exhaustion leads to adoptive transfer therapy failure which can be prevented by immune checkpoint blockade. *Am. J. Cancer Res.* 10: 4234–4250.
- Gallimore, A., A. Glithero, A. Godkin, A.C. Tissot, A. Plückthun, T. Elliott, H. Hengartner, and R. Zinkernagel. 1998. Induction and exhaustion of lymphocytic choriomeningitis virus-specific cytotoxic T lymphocytes visualized using soluble tetrameric major histocompatibility complex class I-peptide complexes. *J. Exp. Med.* 187:1383–1393. <https://doi.org/10.1084/jem.187.9.1383>
- Giles, J.R., A.M. Globig, S.M. Kaech, and E.J. Wherry. 2023. CD8⁺ T cells in the cancer-immunity cycle. *Immunity.* 56:2231–2253. <https://doi.org/10.1016/j.immuni.2023.09.005>
- Goding, S.R., K.A. Wilson, C. Rosinsky, and P.A. Antony. 2018. PD-L1-Independent mechanisms control the resistance of melanoma to CD4⁺ T cell adoptive immunotherapy. *J. Immunol.* 200:3304–3311. <https://doi.org/10.4049/jimmunol.1701617>
- Goding, S.R., K.A. Wilson, Y. Xie, K.M. Harris, A. Baxi, A. Akpınarlı, A. Fulton, K. Tamada, S.E. Strome, and P.A. Antony. 2013. Restoring immune function of tumor-specific CD4⁺ T cells during recurrence of melanoma. *J. Immunol.* 190:4899–4909. <https://doi.org/10.4049/jimmunol.1300271>
- Greenwald, R.J., V.A. Boussiotis, R.B. Lorschach, A.K. Abbas, and A.H. Sharpe. 2001. CTLA-4 regulates induction of energy in vivo. *Immunity.* 14: 145–155. [https://doi.org/10.1016/s1074-7613\(01\)00097-8](https://doi.org/10.1016/s1074-7613(01)00097-8)
- Grzywa, T.M., A. Sosnowska, P. Matryba, Z. Rydzynska, M. Jasinski, D. Nowis, and J. Golab. 2020. Myeloid cell-derived arginase in cancer immune response. *Front. Immunol.* 11:938. <https://doi.org/10.3389/fimmu.2020.00938>
- Guo, M., M.Y.R. Liu, and D.G. Brooks. 2024. Regulation and impact of tumor-specific CD4⁺ T cells in cancer and immunotherapy. *Trends Immunol.* 45: 303–313. <https://doi.org/10.1016/j.it.2024.02.005>
- Guo, Z., R. Zhang, A.G. Yang, and G. Zheng. 2023. Diversity of immune checkpoints in cancer immunotherapy. *Front. Immunol.* 14:1121285. <https://doi.org/10.3389/fimmu.2023.1121285>
- Hamidi, H., Y. Senbabaoglu, N. Beig, J. Roels, C. Manuel, X. Guan, H. Koeppen, Z.J. Assaf, B.Y. Nabet, A. Waddell, et al. 2024. Molecular heterogeneity in urothelial carcinoma and determinants of clinical benefit to PD-L1 blockade. *Cancer Cell.* 42:2098–2112.e4. <https://doi.org/10.1016/j.ccell.2024.10.016>
- Hanahan, D., and R.A. Weinberg. 2011. Hallmarks of cancer: The next generation. *Cell.* 144:646–674. <https://doi.org/10.1016/j.ccell.2011.02.013>
- Hay, Z.L.Z., J.R. Knapp, R.E. Magallon, B.P. O'Connor, and J.E. Slansky. 2023. Low TCR binding strength results in increased progenitor-like CD8⁺ tumor-infiltrating lymphocytes. *Cancer Immunol. Res.* 11:570–582. <https://doi.org/10.1158/2326-6066.CIR-22-0761>
- Hoffmann, M.M., and J.E. Slansky. 2020. T-cell receptor affinity in the age of cancer immunotherapy. *Mol. Carcinog.* 59:862–870. <https://doi.org/10.1002/mc.23212>
- Hohtari, H., O. Brück, S. Blom, R. Turkki, M. Sinisalo, P.E. Kovanen, O. Kallioniemi, T. Pellinen, K. Porkka, and S. Mustjoki. 2019. Immune cell constitution in bone marrow microenvironment predicts outcome in adult ALL. *Leukemia.* 33:1570–1582. <https://doi.org/10.1038/s41375-018-0360-1>
- Hossain, M.M., P. King, J. Hackett, H.C. Gerard, R. Niwinski, L. Wu, L. Van Kaer, G. Dyson, H. Gibson, A.D. Borowsky, and E. Sebzda. 2024. Peripheral-derived regulatory T cells contribute to tumor-mediated immune suppression in a nonredundant manner. *Proc. Natl. Acad. Sci. USA.* 121:e2404916121. <https://doi.org/10.1073/pnas.2404916121>
- Hu, C., Y. Zhen, Z. Ma, L. Zhao, H. Wu, C. Shu, B. Pang, J. Yu, Y. Xu, X. Zhang, et al. 2023. Polyamines from myeloid-derived suppressor cells promote Th17 polarization and disease progression. *Mol. Ther.* 31:569–584. <https://doi.org/10.1016/j.ymthe.2022.10.013>
- Huang, A.C., and R. Zappasodi. 2022. A decade of checkpoint blockade immunotherapy in melanoma: Understanding the molecular basis for immune sensitivity and resistance. *Nat. Immunol.* 23:660–670. <https://doi.org/10.1038/s41590-022-01141-1>
- Huang, C.-T., C.J. Workman, D. Flies, X. Pan, A.L. Marson, G. Zhou, E.L. Hipkiss, S. Ravi, J. Kowalski, H.I. Levitsky, et al. 2004. Role of LAG-3 in regulatory T cells. *Immunity.* 21:503–513. <https://doi.org/10.1016/j.immuni.2004.08.010>
- Huang, H., Z. Wang, Y. Zhang, R.N. Pradhan, D. Ganguly, R. Chandra, G. Murimwa, S. Wright, X. Gu, R. Maddipati, et al. 2022. Mesothelial cell-derived antigen-presenting cancer-associated fibroblasts induce expansion of regulatory T cells in pancreatic cancer. *Cancer Cell.* 40: 656–673.e7. <https://doi.org/10.1016/j.ccell.2022.04.011>
- Huang, S., Y. Zhao, W. Lai, J. Tan, X. Zheng, X. Zha, Y. Li, and S. Chen. 2023. Higher PD-1/Tim-3 expression on IFN-γ⁺ T cells is associated with poor prognosis in patients with acute myeloid leukemia. *Cancer Biol. Ther.* 24: 2278229. <https://doi.org/10.1080/15384047.2023.2278229>
- Huber, V., C. Camisaschi, A. Berzi, S. Ferro, L. Lugini, T. Triulzi, A. Tuccitto, E. Tagliabue, C. Castelli, and L. Rivoltini. 2017. Cancer acidity: An ultimate frontier of tumor immune escape and a novel target of

- immunomodulation. *Semin. Cancer Biol.* 43:74–89. <https://doi.org/10.1016/j.semcancer.2017.03.001>
- Iwanowycz, S., S. Ngoi, Y. Li, M. Hill, C. Koivisto, M. Parrish, B. Guo, Z. Li, and B. Liu. 2021. Type 2 dendritic cells mediate control of cytotoxic T cell resistant tumors. *JCI Insight.* 6:e145885. <https://doi.org/10.1172/jci.insight.145885>
- Jacobi, F.J., K. Wild, M. Smits, K. Zoldan, B. Csernalabics, T. Flecken, J. Lang, P. Ehrenmann, F. Emmerich, M. Hofmann, et al. 2019. OX40 stimulation and PD-L1 blockade synergistically augment HBV-specific CD4 T cells in patients with HBeAg-negative infection. *J. Hepatol.* 70:1103–1113. <https://doi.org/10.1016/j.jhep.2019.02.016>
- Jing, S.-y., H.-q. Wang, P. Lin, J. Yuan, Z.-x. Tang, and H. Li. 2025. Quantifying and interpreting biologically meaningful spatial signatures within tumor microenvironments. *NPJ Precis. Oncol.* 9:68. <https://doi.org/10.1038/s41698-025-00857-1>
- Kalekar, L.A., and D.L. Mueller. 2017. Relationship between CD4 regulatory T cells and anergy in vivo. *J. Immunol.* 198:2527–2533. <https://doi.org/10.4049/jimmunol.1602031>
- Kalekar, L.A., S.E. Schmiel, S.L. Nandiwada, W.Y. Lam, L.O. Barsness, N. Zhang, G.L. Stritesky, D. Malhotra, K.E. Pauken, J.L. Linehan, et al. 2016. CD4⁺ T cell anergy prevents autoimmunity and generates regulatory T cell precursors. *Nat. Immunol.* 17:304–314. <https://doi.org/10.1038/ni.3331>
- Kamada, T., Y. Togashi, C. Tay, D. Ha, A. Sasaki, Y. Nakamura, E. Sato, S. Fukuoka, Y. Tada, A. Tanaka, et al. 2019. PD-1⁺ regulatory T cells amplified by PD-1 blockade promote hyperprogression of cancer. *Proc. Natl. Acad. Sci. USA.* 116:9999–10008. <https://doi.org/10.1073/pnas.1822001116>
- Kerididani, D., E. Aerakis, K.-M. Verrou, I. Angelidis, K. Douka, M.-A. Maniou, P. Stamoulis, K. Goudevenou, A. Prados, C. Tzaferis, et al. 2022. Lung tumor MHCII immunity depends on in situ antigen presentation by fibroblasts. *J. Exp. Med.* 219:e20210815. <https://doi.org/10.1084/jem.20210815>
- Khan, O., J.R. Giles, S. McDonald, S. Manne, S.F. Ngiew, K.P. Patel, M.T. Werner, A.C. Huang, K.A. Alexander, J.E. Wu, et al. 2019. TOX transcriptionally and epigenetically programs CD8⁺ T cell exhaustion. *Nature.* 571:211–218. <https://doi.org/10.1038/s41586-019-1325-x>
- Khan, S.M., R. Desai, A. Coxon, A. Livingstone, G.P. Dunn, A. Petti, and T.M. Johans. 2022. Impact of CD4 T cells on intratumoral CD8 T-cell exhaustion and responsiveness to PD-1 blockade therapy in mouse brain tumors. *J. Immunother. Cancer.* 10:e005293. <https://doi.org/10.1136/jitc-2022-005293>
- Kotov, D.I., J.S. Mitchell, T. Pengo, C. Ruedl, S.S. Way, R.A. Langlois, B.T. Fife, and M.K. Jenkins. 2019. TCR Affinity Biases Th Cell Differentiation by Regulating CD25, Eef1e1, and Gbp2. *J. Immunol.* 202:2535–2545. <https://doi.org/10.4049/jimmunol.1801609>
- Krishna, S., F.J. Lowery, A.R. Copeland, E. Bahadiroglu, R. Mukherjee, L. Jia, J.T. Anibal, A. Sachs, S.O. Adebola, D. Gurusamy, et al. 2020. Stem-like CD8 T cells mediate response of adoptive cell immunotherapy against human cancer. *Science.* 370:1328–1334. <https://doi.org/10.1126/science.abb9847>
- Krol, A.L., H.P. Nehring, F.F. Krause, A. Wempe, H. Raifer, A. Nist, T. Stiewe, W. Bertrams, B. Schmeck, M. Luu, et al. 2022. Lactate induces metabolic and epigenetic reprogramming of pro-inflammatory Th17 cells. *EMBO Rep.* 23:e54685. <https://doi.org/10.15252/embr.202254685>
- Kuczma, M.P., E.A. Szurek, A. Cebula, V.L. Ngo, M. Pietrzak, P. Kraj, T.L. Denning, and L. Ignatowicz. 2021. Self and microbiota-derived epitopes induce CD4⁺ T cell anergy and conversion into CD4⁺Foxp3⁺ regulatory cells. *Mucosal Immunol.* 14:443–454. <https://doi.org/10.1038/s41385-020-00349-4>
- Künzli, M., P. Reuther, D.D. Pinschewer, and C.G. King. 2021. Opposing effects of T cell receptor signal strength on CD4 T cells responding to acute versus chronic viral infection. *Elife.* 10:e61869. <https://doi.org/10.7554/eLife.61869>
- Lax, B.M., J.R. Palmeri, E.A. Lutz, A. Sheen, J.A. Stinson, L. Duhamel, L. Santollani, A. Kennedy, A.M. Rothschilds, S. Spranger, et al. 2023. Both intratumoral regulatory T cell depletion and CTLA-4 antagonism are required for maximum efficacy of anti-CTLA-4 antibodies. *Proc. Natl. Acad. Sci. USA.* 120:e2300895120. <https://doi.org/10.1073/pnas.2300895120>
- Ledergor, G., Z. Fan, K. Wu, E. McCarthy, A. Hyrenius-Wittsten, A. Starzinski, H. Chang, M. Bridge, S. Kwek, A. Cheung, et al. 2024. CD4⁺ CAR T-cell exhaustion associated with early relapse of multiple myeloma after BCMA CAR T-cell therapy. *Blood Adv.* 8:3562–3575. <https://doi.org/10.1182/bloodadvances.2023012416>
- Li, K., H. Shi, B. Zhang, X. Ou, Q. Ma, Y. Chen, P. Shu, D. Li, and Y. Wang. 2021. Myeloid-derived suppressor cells as immunosuppressive regulators and therapeutic targets in cancer. *Signal Transduct. Target. Ther.* 6:362. <https://doi.org/10.1038/s41392-021-00670-9>
- Li, S., T. Miao, M. Sebastian, P. Bhullar, E. Ghaffari, M. Liu, A.L.J. Symonds, and P. Wang. 2012. The transcription factors Egr2 and Egr3 are essential for the control of inflammation and antigen-induced proliferation of B and T cells. *Immunity.* 37:685–696. <https://doi.org/10.1016/j.immuni.2012.08.001>
- Liao, D., Y. Luo, D. Markowitz, R. Xiang, and R.A. Reisfeld. 2009. Cancer associated fibroblasts promote tumor growth and metastasis by modulating the tumor immune microenvironment in a 4T1 murine breast cancer model. *PloS One.* 4:e7965. <https://doi.org/10.1371/journal.pone.0007965>
- Lin, W., V. Singh, R. Springer, G. Choonoo, N. Gupta, A. Patel, D. Frleta, J. Zhong, T. Owczarek, C. Decker, et al. 2023. Human CD4 cytotoxic T lymphocytes mediate potent tumor control in humanized immune system mice. *Commun. Biol.* 6:447. <https://doi.org/10.1038/s42003-023-04812-3>
- Liu, Q., L. Wang, H. Lin, Z. Wang, J. Wu, J. Guo, S. Wen, L. Ran, Z. Yue, X. Su, et al. 2022. Tumor-Specific CD4⁺ T cells restrain established metastatic melanoma by developing into cytotoxic CD4- T cells. *Front. Immunol.* 13: 875718. <https://doi.org/10.3389/fimmu.2022.875718>
- Lowther, D.E., B.A. Goods, L.E. Luca, B.A. Lerner, K. Raddassi, D. van Dijk, A.L. Hernandez, X. Duan, M. Gunel, V. Coric, et al. 2016. PD-1 marks dysfunctional regulatory T cells in malignant gliomas. *JCI Insight.* 1: e85935. <https://doi.org/10.1172/jci.insight.85935>
- Lücke, J., A.M. Shiri, T. Zhang, J. Kempster, A.D. Giannou, and S. Huber. 2021. Rationalizing heptadecaphobia: TH 17 cells and associated cytokines in cancer and metastasis. *FEBS J.* 288:6942–6971. <https://doi.org/10.1111/febs.15711>
- Macián, F., F. García-Cózar, S.-H. Im, H.F. Horton, M.C. Byrne, and A. Rao. 2002. Transcriptional mechanisms underlying lymphocyte tolerance. *Cell.* 109:719–731. [https://doi.org/10.1016/s0092-8674\(02\)00767-5](https://doi.org/10.1016/s0092-8674(02)00767-5)
- Maeda, Y., H. Nishikawa, D. Sugiyama, D. Ha, M. Hamaguchi, T. Saito, M. Nishioka, J.B. Wing, D. Adeegbe, I. Katayama, and S. Sakaguchi. 2014. Detection of self-reactive CD8⁺ T cells with an anergic phenotype in healthy individuals. *Science.* 346:1536–1540. <https://doi.org/10.1126/science.aaa1292>
- Malandro, N., S. Budhu, N.F. Kuhn, C. Liu, J.T. Murphy, C. Cortez, H. Zhong, X. Yang, G. Rizzuto, G. Altan-Bonnet, et al. 2016. Clonal abundance of tumor-specific CD4(+) T cells potentiates efficacy and alters susceptibility to exhaustion. *Immunity.* 44:179–193. <https://doi.org/10.1016/j.immuni.2015.12.018>
- McLane, L.M., S.F. Ngiew, Z. Chen, J. Attanasio, S. Manne, G. Ruthel, J.E. Wu, R.P. Staupé, W. Xu, R.K. Amaravadi, et al. 2021. Role of nuclear localization in the regulation and function of T-bet and Eomes in exhausted CD8 T cells. *Cell Rep.* 35:109120. <https://doi.org/10.1016/j.celrep.2021.109120>
- Mescher, M.F., F.E. Popescu, M. Gerner, C.D. Hammerbeck, and J.M. Custers. 2007. Activation-induced non-responsiveness (anergy) limits CD8 T cell responses to tumors. *Semin. Cancer Biol.* 17:299–308. <https://doi.org/10.1016/j.semcancer.2007.06.008>
- Miggelbrink, A.M., J.D. Jackson, S.J. Lorrey, E.S. Srinivasan, J. Waibl-Polania, D.S. Wilkinson, and P.E. Fecci. 2021. CD4 T-cell exhaustion: Does it exist and what are its roles in cancer? *Clin. Cancer Res.* 27:5742–5752. <https://doi.org/10.1158/1078-0432.CCR-21-0206>
- Miller, B.C., D.R. Sen, R. Al Abosy, K. Bi, Y.V. Virkud, M.W. LaFleur, K.B. Yates, A. Lako, K. Felt, G.S. Naik, et al. 2019. Subsets of exhausted CD8⁺ T cells differentially mediate tumor control and respond to checkpoint blockade. *Nat. Immunol.* 20:326–336. <https://doi.org/10.1038/s41590-019-0312-6>
- Montauti, E., D.Y. Oh, and L. Fong. 2024. CD4⁺ T cells in antitumor immunity. *Trends Cancer.* 10:969–985. <https://doi.org/10.1016/j.trecan.2024.07.009>
- Monte, L.D., M. Reni, E. Tassi, D. Clavenna, I. Papa, H. Recalde, M. Braga, V. Di Carlo, C. Doglioni, and M.P. Protti. 2011. Intratumor T helper type 2 cell infiltrate correlates with cancer-associated fibroblast thymic stromal lymphopoietin production and reduced survival in pancreatic cancer. *J. Exp. Med.* 208:469–478. <https://doi.org/10.1084/jem.20101876>
- Morotti, M., A. Albukhari, A. Alsaadi, M. Artibani, J.D. Brenton, S.M. Curbishley, T. Dong, M.L. Dustin, Z. Hu, N. McGranahan, et al. 2021. Promises and challenges of adoptive T-cell therapies for solid tumours. *Br. J. Cancer.* 124:1759–1776. <https://doi.org/10.1038/s41416-021-01353-6>

- Munn, D.H., and A.L. Mellor. 2016. Ido in the tumor microenvironment: Inflammation, counter-regulation, and tolerance. *Trends Immunol.* 37: 193–207. <https://doi.org/10.1016/j.it.2016.01.002>
- Nakano, M., M. Ito, R. Tanaka, K. Yamaguchi, H. Ariyama, K. Mitsugi, T. Yoshihiro, H. Ohmura, N. Tsuruta, F. Hanamura, et al. 2018. PD-1+ TIM-3+ T cells in malignant ascites predict prognosis of gastrointestinal cancer. *Cancer Sci.* 109:2986–2992. <https://doi.org/10.1111/cas.13723>
- Neildez-Nguyen, T.M.A., J. Bigot, S. Da Rocha, G. Corre, F. Boisgerault, A. Paldi, and A. Galy. 2015. Hypoxic culture conditions enhance the generation of regulatory T cells. *Immunology.* 144:431–443. <https://doi.org/10.1111/imm.12388>
- Nüssing, S., J.A. Trapani, and I.A. Parish. 2020. Revisiting T cell tolerance as a checkpoint target for cancer immunotherapy. *Front. Immunol.* 11: 589641. <https://doi.org/10.3389/fimmu.2020.589641>
- Oh, D.Y., S.S. Kwek, S.S. Raju, T. Li, E. McCarthy, E. Chow, D. Aran, A. Ilano, C.-C.S. Pai, C. Rancan, et al. 2020. Intratumoral CD4⁺ T cells mediate anti-tumor cytotoxicity in human bladder cancer. *Cell.* 181: 1612–1625.e13. <https://doi.org/10.1016/j.cell.2020.05.017>
- Omodho, B., T. Miao, A.L.J. Symonds, R. Singh, S. Li, and P. Wang. 2018. Transcription factors early growth response gene (Egr) 2 and 3 control inflammatory responses of tolerant T cells. *Immun. Inflamm. Dis.* 6: 221–233. <https://doi.org/10.1002/iid3.210>
- Osokine, I., L.M. Snell, C.R. Cunningham, D.H. Yamada, E.B. Wilson, H.J. Elsaesser, J.C. de la Torre, and D. Brooks. 2014. Type I interferon suppresses de novo virus-specific CD4 Th1 immunity during an established persistent viral infection. *Proc. Natl. Acad. Sci. USA.* 111:7409–7414. <https://doi.org/10.1073/pnas.1401662111>
- Paley, M.A., D.C. Kroy, P.M. Odorizzi, J.B. Johnnidis, D.V. Dolfi, B.E. Barnett, E.K. Bikoff, E.J. Robertson, G.M. Lauer, S.L. Reiner, and E.J. Wherry. 2012. Progenitor and terminal subsets of CD8⁺ T cells cooperate to contain chronic viral infection. *Science.* 338:1220–1225. <https://doi.org/10.1126/science.1229620>
- Pan, P.Y., G. Ma, K.J. Weber, J. Ozao-Choy, G. Wang, B. Yin, C.M. Divino, and S.H. Chen. 2010. Immune stimulatory receptor CD40 is required for T-cell suppression and T regulatory cell activation mediated by myeloid-derived suppressor cells in cancer. *Cancer Res.* 70:99–108. <https://doi.org/10.1158/0008-5472.CAN-09-1882>
- Parry, H.M., A.C. Dowell, J. Zuo, K. Verma, F.A.M. Kinsella, J. Begum, W. Croft, A. Sharma-Oates, G. Pratt, and P. Moss. 2021. PD-1 is imprinted on cytomegalovirus-specific CD4⁺ T cells and attenuates Th1 cytokine production whilst maintaining cytotoxicity. *PLoS Pathog.* 17:e1009349. <https://doi.org/10.1371/journal.ppat.1009349>
- Patterson, M.T., A.L. Burrack, Y. Xu, G.H. Hickok, Z.C. Schmichen, S. Becker, E. Cruz-Hinojosa, P.R. Schrank, A.E. Kennedy, M.M. Firulyova, et al. 2023. Tumor-specific CD4 T cells instruct monocyte fate in pancreatic ductal adenocarcinoma. *Cell Rep.* 42:112732. <https://doi.org/10.1016/j.celrep.2023.112732>
- Pauken, K.E., M.A. Sammons, P.M. Odorizzi, S. Manne, J. Godec, O. Khan, A.M. Drake, Z. Chen, D.R. Sen, M. Kurachi, et al. 2016. Epigenetic stability of exhausted T cells limits durability of reinvigoration by PD-1 blockade. *Science.* 354:1160–1165. <https://doi.org/10.1126/science.aaf2807>
- Peggs, K.S., S.A. Quezada, C.A. Chambers, A.J. Korman, and J.P. Allison. 2009. Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies. *J. Exp. Med.* 206:1717–1725. <https://doi.org/10.1084/jem.20082492>
- Philip, M., and A. Schietinger. 2019. Heterogeneity and fate choice: T cell exhaustion in cancer and chronic infections. *Curr. Opin. Immunol.* 58: 98–103. <https://doi.org/10.1016/j.coi.2019.04.014>
- Pilon-Thomas, S., K.N. Kodumudi, A.E. El-Kenawi, S. Russell, A.M. Weber, K. Luddy, M. Damaghi, J.W. Wojtkowiak, J.J. Mulé, A. Ibrahim-Hashim, and R.J. Gillies. 2016. Neutralization of tumor acidity improves antitumor responses to immunotherapy. *Cancer Res.* 76:1381–1390. <https://doi.org/10.1158/0008-5472.CAN-15-1743>
- Pour, S.R., H. Morikawa, N.A. Kiani, M. Yang, A. Azimi, G. Shafi, M. Shang, R. Baumgartner, D.F.J. Ketelhuth, M.A. Kamleh, et al. 2019. Exhaustion of CD4⁺ T-cells mediated by the kynurenine pathway in melanoma. *Sci. Rep.* 9:12150. <https://doi.org/10.1038/s41598-019-48635-x>
- Qin, H., K. Ishii, S. Nguyen, P.P. Su, C.R. Burk, B.-H. Kim, B.B. Duncan, S. Tarun, N.N. Shah, M.E. Kohler, and T.J. Fry. 2018. Murine pre-B-cell ALL induces T-cell dysfunction not fully reversed by introduction of a chimeric antigen receptor. *Blood.* 132:1899–1910. <https://doi.org/10.1182/blood-2017-12-815548>
- Raker, V.K., M.P. Domogalla, and K. Steinbrink. 2015. Tolerogenic dendritic cells for regulatory T cell induction in man. *Front. Immunol.* 6:569. <https://doi.org/10.3389/fimmu.2015.00569>
- Raskov, H., A. Orhan, J.P. Christensen, and I. Gögenur. 2021. Cytotoxic CD8⁺ T cells in cancer and cancer immunotherapy. *Br. J. Cancer.* 124:359–367. <https://doi.org/10.1038/s41416-020-01048-4>
- Rausch, M.P., and K.T. Hastings. 2015. An exhaustion-like phenotype constrains the activity of CD4⁺ T cells specific for a self and melanoma antigen. *PLoS One.* 10:e0123332. <https://doi.org/10.1371/journal.pone.0123332>
- Raziorrouh, B., A. Ulsenheimer, W. Schraut, M. Heeg, P. Kurtschiev, R. Zachoval, M.-C. Jung, R. Thimme, C. Neumann-Haefelin, S. Horster, et al. 2011. Inhibitory molecules that regulate expansion and restoration of HCV-specific CD4⁺ T cells in patients with chronic infection. *Gastroenterology.* 141:1422–1431.e1-6. <https://doi.org/10.1053/j.gastro.2011.07.004>
- Renaude, E., M. Kroemer, C. Borg, P. Peixoto, E. Hervouet, R. Loyon, and O. Adotévi. 2021. Epigenetic reprogramming of CD4⁺ helper T cells as a strategy to improve anticancer immunotherapy. *Front. Immunol.* 12: 669992. <https://doi.org/10.3389/fimmu.2021.669992>
- Rudloff, M.W., P. Zumbo, N.R. Favret, J.J. Roetman, C.R. Detrés Román, M.M. Erwin, K.A. Murray, S.T. Jonnakuti, F. Dünder, D. Betel, and M. Philip. 2023. Hallmarks of CD8⁺ T cell dysfunction are established within hours of tumor antigen encounter before cell division. *Nat. Immunol.* 24: 1527–1539. <https://doi.org/10.1038/s41590-023-01578-y>
- Saeidi, A., K. Zandi, Y.Y. Cheok, H. Saeidi, W.F. Wong, C.Y.Q. Lee, H.C. Cheong, Y.K. Yong, M. Larsson, and E.M. Shankar. 2018. T-Cell exhaustion in chronic infections: Reversing the state of exhaustion and reinvigorating optimal protective immune responses. *Front. Immunol.* 9: 2569. <https://doi.org/10.3389/fimmu.2018.02569>
- Sakuishi, K., S.F. Ngiew, J.M. Sullivan, M.W.L. Teng, V.K. Kuchroo, M.J. Smyth, and A.C. Anderson. 2013. TIM3+FOXP3⁺ regulatory T cells are tissue-specific promoters of T-cell dysfunction in cancer. *Oncotarget.* 2:e23849. <https://doi.org/10.4161/onc.23849>
- Satpathy, A.T., J.M. Granja, K.E. Yost, Y. Qi, F. Meschi, G.P. McDermott, B.N. Olsen, M.R. Mumbach, S.E. Pierce, M.R. Corces, et al. 2019. Massively parallel single-cell chromatin landscapes of human immune cell development and intratumoral T cell exhaustion. *Nat. Biotechnol.* 37: 925–936. <https://doi.org/10.1038/s41587-019-0206-z>
- Schietinger, A., M. Philip, V.E. Krisnawan, E.Y. Chiu, J.J. Delrow, R.S. Basom, P. Lauer, D.G. Brockstedt, S.E. Knoblaugh, G.J. Hammerling, et al. 2016. Tumor-Specific T cell dysfunction is a dynamic antigen-driven differentiation program initiated early during tumorigenesis. *Immunity.* 45: 389–401. <https://doi.org/10.1016/j.immuni.2016.07.011>
- Schreiber, R.D., L.J. Old, and M.J. Smyth. 2011. Cancer immunoediting: Integrating immunity's roles in cancer suppression and promotion. *Science.* 331:1565–1570. <https://doi.org/10.1126/science.1203486>
- Schwartz, R.H. 1990. A cell culture model for T lymphocyte clonal anergy. *Science.* 248:1349–1356. <https://doi.org/10.1126/science.2113314>
- Schwartz, R.H. 2003. T cell anergy. *Annu. Rev. Immunol.* 21:305–334. <https://doi.org/10.1146/annurev.immunol.21.120601.141110>
- Sen, D.R., J. Kaminski, R.A. Barnitz, M. Kurachi, U. Gerdemann, K.B. Yates, H.-W. Tsao, J. Godec, M.W. LaFleur, F.D. Brown, et al. 2016. The epigenetic landscape of T cell exhaustion. *Science.* 354:1165–1169. <https://doi.org/10.1126/science.aae0491>
- Shakiba, M., P. Zumbo, G. Espinosa-Carrasco, L. Menocal, F. Dünder, S.E. Carson, E.M. Bruno, F.J. Sanchez-Rivera, S.W. Lowe, S. Camara, et al. 2022. TCR signal strength defines distinct mechanisms of T cell dysfunction and cancer evasion. *J. Exp. Med.* 219:e20201966. <https://doi.org/10.1084/jem.20201966>
- Shehade, H., V. Acotly, M. Moser, and G. Oldenhove. 2015. Cutting edge: Hypoxia-inducible factor 1 negatively regulates Th1 function. *J. Immunol.* 195:1372–1376. <https://doi.org/10.4049/jimmunol.1402552>
- Shin, H., S.D. Blackburn, A.M. Intlekofer, C. Kao, J.M. Angelosanto, S.L. Reiner, and E.J. Wherry. 2009. A role for the transcriptional repressor Blimp-1 in CD8(+) T cell exhaustion during chronic viral infection. *Immunity.* 31:309–320. <https://doi.org/10.1016/j.immuni.2009.06.019>
- Siddiqui, I., K. Schaeuble, V. Chennupati, S.A. Fuertes Marraco, S. Calderon-Copete, D. Pais Ferreira, S.J. Carmona, L. Scarpellino, D. Gfeller, S. Pradervand, et al. 2019. Intratumoral Tcf1+PD-1+CD8⁺ T cells with stem-like properties promote tumor control in response to vaccination and checkpoint blockade immunotherapy. *Immunity.* 50:195–211.e10. <https://doi.org/10.1016/j.immuni.2018.12.021>
- Song, M., T.A. Sandoval, C.S. Chae, S. Chopra, C. Tan, M.R. Rutkowski, M. Raundhal, R.A. Chaurio, K.K. Payne, C. Konrad, et al. 2018. IRE1α-XBP1 controls T cell function in ovarian cancer by regulating mitochondrial activity. *Nature.* 562:423–428. <https://doi.org/10.1038/s41586-018-0597-x>

- Speiser, D.E., O. Chijioke, K. Schaeuble, and C. Münz. 2023. CD4⁺ T cells in cancer. *Nat. Cancer*. 4:317–329. <https://doi.org/10.1038/s43018-023-00521-2>
- Staveley-O'Carroll, K., E. Sotomayor, J. Montgomery, I. Borrello, L. Hwang, S. Fein, D. Pardoll, and H. Levitsky. 1998. Induction of antigen-specific T cell anergy: An early event in the course of tumor progression. *Proc. Natl. Acad. Sci. USA*. 95:1178–1183. <https://doi.org/10.1073/pnas.95.3.1178>
- Steinbrink, K., M. Wölfl, H. Jonuleit, J. Knop, and A.H. Enk. 1997. Induction of tolerance by IL-10-treated dendritic cells. *J. Immunol.* 159:4772–4780.
- Stephen, T.L., K.K. Payne, R.A. Chaurio, M.J. Allegranza, H. Zhu, J. Perez-Sanz, A. Perales-Puchalt, J.M. Nguyen, A.E. Vara-Ailor, E.B. Eruslanov, et al. 2017. SATB1 expression governs epigenetic repression of PD-1 in tumor-reactive T cells. *Immunity*. 46:51–64. <https://doi.org/10.1016/j.immuni.2016.12.015>
- Sultan, H., Y. Takeuchi, J.P. Ward, N. Sharma, T.-T. Liu, V. Sukhov, M. Firulyova, Y. Song, S. Ameh, S. Briochi, et al. 2024. Neoantigen-specific cytotoxic Tr1 CD4 T cells suppress cancer immunotherapy. *Nature*. 632: 182–191. <https://doi.org/10.1038/s41586-024-07752-y>
- Sumida, T.S., S. Dulberg, J.C. Schupp, M.R. Lincoln, H.A. Stillwell, P.P. Axisa, M. Comi, A. Unterman, N. Kaminski, A. Madi, et al. 2022. Type I interferon transcriptional network regulates expression of coinhibitory receptors in human T cells. *Nat. Immunol.* 23:632–642. <https://doi.org/10.1038/s41590-022-01152-y>
- Swaminathan, S., L.T. Mai, A.P. Meli, L. Carmona-Pérez, T. Charpentier, A. Lamarre, I.L. King, and S. Stäger. 2024. LAG-3- and CXCR5-expressing CD4 T cells display progenitor-like properties during chronic visceral leishmaniasis. *Cell Rep.* 43:113879. <https://doi.org/10.1016/j.celrep.2024.113879>
- Tay, R.E., E.K. Richardson, and H.C. Toh. 2021. Revisiting the role of CD4⁺ T cells in cancer immunotherapy—new insights into old paradigms. *Cancer Gene Ther.* 28:5–17. <https://doi.org/10.1038/s41417-020-0183-x>
- Tekguc, M., J.B. Wing, M. Osaki, J. Long, and S. Sakaguchi. 2021. Treg-expressed CTLA-4 depletes CD80/CD86 by trogocytosis, releasing free PD-L1 on antigen-presenting cells. *Proc. Natl. Acad. Sci. USA*. 118: e2023739118. <https://doi.org/10.1073/pnas.2023739118>
- Thomann, A.S., T. Schneider, L. Cyran, I.N. Eckert, A. Kerstan, and M.B. Lutz. 2021. Conversion of anergic T cells into Foxp3- IL-10⁺ regulatory T cells by a second antigen stimulus in vivo. *Front. Immunol.* 12:704578. <https://doi.org/10.3389/fimmu.2021.704578>
- Thommen, D.S., and T.N. Schumacher. 2018. T cell dysfunction in cancer. *Cancer Cell*. 33:547–562. <https://doi.org/10.1016/j.ccell.2018.03.012>
- Tracy, S.I., H. Venkatesh, C. Hekim, L.M. Heltemes-Harris, T.P. Knutson, V. Bachanova, and M.A. Farrar. 2022. Combining nilotinib and PD-L1 blockade reverses CD4⁺ T-cell dysfunction and prevents relapse in acute B-cell leukemia. *Blood*. 140:335–348. <https://doi.org/10.1182/blood.2021015341>
- Trefzer, A., P. Kadam, S.-H. Wang, S. Pennavaria, B. Lober, B. Akçabozan, J. Kranich, T. Brocker, N. Nakano, M. Irrmler, et al. 2021. Dynamic adoption of anergy by antigen-exhausted CD4⁺ T cells. *Cell Rep.* 34:108748. <https://doi.org/10.1016/j.celrep.2021.108748>
- Tsai, Y.L., M. Arias-Badia, T.A. Kadlecsek, Y.M. Lwin, A. Srinath, N.H. Shah, Z.E. Wang, D. Barber, J. Kuriyan, L. Fong, and A. Weiss. 2023. TCR signaling promotes formation of an STS1-Cbl-b complex with pH-sensitive phosphatase activity that suppresses T cell function in acidic environments. *Immunity*. 56:2682–2698.e9. <https://doi.org/10.1016/j.immuni.2023.11.010>
- Tsukamoto, H., R. Nishikata, S. Senju, and Y. Nishimura. 2013. Myeloid-derived suppressor cells attenuate TH1 development through IL-6 production to promote tumor progression. *Cancer Immunol. Res.* 1:64–76. <https://doi.org/10.1158/2326-6066.CIR-13-0030>
- Utzschneider, D.T., M. Charmoy, V. Chennupati, L. Pousse, D.P. Ferreira, S. Calderon-Copete, M. Danilo, F. Alfei, M. Hofmann, D. Wieland, et al. 2016. T cell factor 1-expressing memory-like CD8(+) T cells sustain the immune response to chronic viral infections. *Immunity*. 45:415–427. <https://doi.org/10.1016/j.immuni.2016.07.021>
- Utzschneider, D.T., S.S. Gabriel, D. Chisanga, R. Gloury, P.M. Gubser, A. Vasanthakumar, W. Shi, and A. Kallies. 2020. Early precursor T cells establish and propagate T cell exhaustion in chronic infection. *Nat. Immunol.* 21:1256–1266. <https://doi.org/10.1038/s41590-020-0760-z>
- van Pul, K.M., J.C.L. Notohardjo, M.F. Franssen, B.D. Koster, A.G.M. Stam, D. Chondronasiou, S.M. Loughheed, J. Bakker, V. Kandiah, M.P. van den Tol, et al. 2022. Local delivery of low-dose anti-CTLA-4 to the melanoma lymphatic basin leads to systemic T_{reg} reduction and effector T cell activation. *Sci. Immunol.* 7:eabn8097. <https://doi.org/10.1126/sciimmunol.abn8097>
- Visser, K.d., and J.A. Joyce. 2023. The evolving tumor microenvironment: From cancer initiation to metastatic outgrowth. *Cancer Cell*. 41:374–403. <https://doi.org/10.1016/j.ccell.2023.02.016>
- Volchenkov, R., V. Nygaard, Z. Sener, and B.S. Skålhegg. 2017. Th17 polarization under hypoxia results in increased IL-10 production in a pathogen-independent manner. *Front. Immunol.* 8:698. <https://doi.org/10.3389/fimmu.2017.00698>
- Wang, Y., Y. Ding, Y. Deng, Y. Zheng, and S. Wang. 2020. Role of myeloid-derived suppressor cells in the promotion and immunotherapy of colitis-associated cancer. *J. Immunother. Cancer*. 8:e000609. <https://doi.org/10.1136/jitc-2020-000609>
- Wherry, E.J., J.N. Blattman, K. Murali-Krishna, R. van der Most, and R. Ahmed. 2003. Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. *J. Virol.* 77:4911–4927. <https://doi.org/10.1128/jvi.77.8.4911-4927.2003>
- Wherry, E.J., S.-J. Ha, S.M. Kaech, W.N. Haining, S. Sarkar, V. Kalia, S. Subramaniam, J.N. Blattman, D.L. Barber, and R. Ahmed. 2007. Molecular signature of CD8⁺ T cell exhaustion during chronic viral infection. *Immunity*. 27:670–684. <https://doi.org/10.1016/j.immuni.2007.09.006>
- Wu, H., Y. Zhen, Z. Ma, H. Li, J. Yu, Z.-G. Xu, X.-Y. Wang, H. Yi, and Y.-G. Yang. 2016a. Arginase-1-dependent promotion of TH17 differentiation and disease progression by MDSCs in systemic lupus erythematosus. *Sci. Transl. Med.* 8:331ra40. <https://doi.org/10.1126/scitranslmed.aae0482>
- Wu, P., D. Wu, C. Ni, J. Ye, W. Chen, G. Hu, Z. Wang, C. Wang, Z. Zhang, W. Xia, et al. 2014. γδT17 cells promote the accumulation and expansion of myeloid-derived suppressor cells in human colorectal cancer. *Immunity*. 40:785–800. <https://doi.org/10.1016/j.immuni.2014.03.013>
- Wu, T., Y. Ji, E.A. Moseman, H.C. Xu, M. Mangani, M. Kirby, S.M. Anderson, R. Handon, E. Kenyon, A. Elkahlon, et al. 2016b. The TCF1-Bcl6 axis counteracts type I interferon to repress exhaustion and maintain T cell stemness. *Sci. Immunol.* 1:eaai8593. <https://doi.org/10.1126/sciimmunol.aai8593>
- Xia, Y., K. Sandor, J.A. Pai, B. Daniel, S. Raju, R. Wu, S. Hsiung, Y. Qi, T. Yangdon, M. Okamoto, et al. 2022. BCL6-dependent TCF-1⁺ progenitor cells maintain effector and helper CD4⁺ T cell responses to persistent antigen. *Immunity*. 55:1200–1215.e6. <https://doi.org/10.1016/j.immuni.2022.05.003>
- Xiao, X., Q. Guo, C. Cui, Y. Lin, L. Zhang, X. Ding, Q. Li, M. Wang, W. Yang, Y. Kong, and R. Yu. 2022. Multiplexed imaging mass cytometry reveals distinct tumor-immune microenvironments linked to immunotherapy responses in melanoma. *Commun. Med.* 2:131. <https://doi.org/10.1038/s43856-022-00197-2>
- Yadav, M., S. Stephan, and J.A. Bluestone. 2013. Peripherally induced tregs - role in immune homeostasis and autoimmunity. *Front. Immunol.* 4:232. <https://doi.org/10.3389/fimmu.2013.00232>
- Yamaguchi, S., J.D. Gray, S. Hashimoto, and D.A. Horwitz. 2001. A role for TGF-β in the generation and expansion of CD4⁺CD25⁺ regulatory T cells from human peripheral blood. *J. Immunol.* 166:7282–7289. <https://doi.org/10.4049/jimmunol.166.12.7282>
- Yamauchi, T., T. Hoki, T. Oba, V. Jain, H. Chen, K. Attwood, S. Battaglia, S. George, G. Chatta, I. Puzanov, et al. 2021. T-cell CX3CR1 expression as a dynamic blood-based biomarker of response to immune checkpoint inhibitors. *Nat. Commun.* 12:1402. <https://doi.org/10.1038/s41467-021-21619-0>
- Yan, Y., S. Cao, X. Liu, S.M. Harrington, W.E. Bindeman, A.A. Adjei, J.S. Jang, J. Jen, Y. Li, P. Chanana, et al. 2018. CX3CR1 identifies PD-1 therapy-responsive CD8⁺ T cells that withstand chemotherapy during cancer chemioimmunotherapy. *JCI Insight*. 3:e97828. <https://doi.org/10.1172/jci.insight.97828>
- Yang, Z.Z., H.J. Kim, H. Wu, S. Jalali, X. Tang, J.E. Krull, W. Ding, A.J. Novak, and S.M. Ansell. 2020. TIGIT expression is associated with T-cell suppression and exhaustion and predicts clinical outcome and anti-PD-1 response in follicular lymphoma. *Clin. Cancer Res.* 26:5217–5231. <https://doi.org/10.1158/1078-0432.CCR-20-0558>
- Yano, H., L.P. Andrews, C.J. Workman, and D.A.A. Vignali. 2019. Intratumoral regulatory T cells: Markers, subsets and their impact on anti-tumor immunity. *Immunology*. 157:232–247. <https://doi.org/10.1111/imm.13067>
- Yuan, L., B. Xu, P. Yuan, J. Zhou, P. Qin, L. Han, G. Chen, Z. Wang, Z. Run, P. Zhao, and Q. Gao. 2017. Tumor-infiltrating CD4⁺ T cells in patients with gastric cancer. *Cancer Cell Int.* 17:114. <https://doi.org/10.1186/s12935-017-0489-4>
- Zajac, A.J., J.N. Blattman, K. Murali-Krishna, D.J. Sourdive, M. Suresh, J.D. Altman, and R. Ahmed. 1998. Viral immune evasion due to persistence

- of activated T cells without effector function. *J. Exp. Med.* 188:2205–2213. <https://doi.org/10.1084/jem.188.12.2205>
- Zander, R., and W. Cui. 2023. Exhausted CD8⁺ T cells face a developmental fork in the road. *Trends Immunol.* 44:276–286. <https://doi.org/10.1016/j.it.2023.02.006>
- Zander, R., D. Schauder, G. Xin, C. Nguyen, X. Wu, A. Zajac, and W. Cui. 2019. CD4⁺ T cell help is required for the formation of a cytolytic CD8⁺ T cell subset that protects against chronic infection and cancer. *Immunity*. 51: 1028–1042.e4. <https://doi.org/10.1016/j.immuni.2019.10.009>
- Zhang, X., C. Zhang, M. Qiao, C. Cheng, N. Tang, S. Lu, W. Sun, B. Xu, Y. Cao, X. Wei, et al. 2022. Depletion of BATF in CAR-T cells enhances antitumor activity by inducing resistance against exhaustion and formation of central memory cells. *Cancer Cell*. 40:1407–1422.e7. <https://doi.org/10.1016/j.ccell.2022.09.013>
- Zheng, Y., Y. Zha, G. Driessens, F. Locke, and T.F. Gajewski. 2012. Transcriptional regulator early growth response gene 2 (Egr2) is required for T cell anergy in vitro and in vivo. *J. Exp. Med.* 209:2157–2163. <https://doi.org/10.1084/jem.20120342>
- ZhenG, Z., M. Narita, M. Takahashi, A. Liu, T. Furukawa, K. Toba, and Y. Aizawa. 2004. Induction of T cell anergy by the treatment with IL-10-treated dendritic cells. *Comp. Immunol. Microbiol. Infect. Dis.* 27:93–103. [https://doi.org/10.1016/S0147-9571\(03\)00054-7](https://doi.org/10.1016/S0147-9571(03)00054-7)
- Zhou, D., Z. Duan, Z. Li, F. Ge, R. Wei, and L. Kong. 2022. The significance of glycolysis in tumor progression and its relationship with the tumor microenvironment. *Front. Pharmacol.* 13:1091779. <https://doi.org/10.3389/fphar.2022.1091779>
- Zhou, W., S. Kawashima, T. Ishino, K. Kawase, Y. Ueda, K. Yamashita, T. Watanabe, M. Kawazu, H. Dansako, Y. Suzuki, et al. 2024. Stem-like progenitor and terminally differentiated TFH-like CD4⁺ T cell exhaustion in the tumor microenvironment. *Cell Rep.* 43:113797. <https://doi.org/10.1016/j.celrep.2024.113797>