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Modulation of T-cell function and survival by the tumor microenvironment

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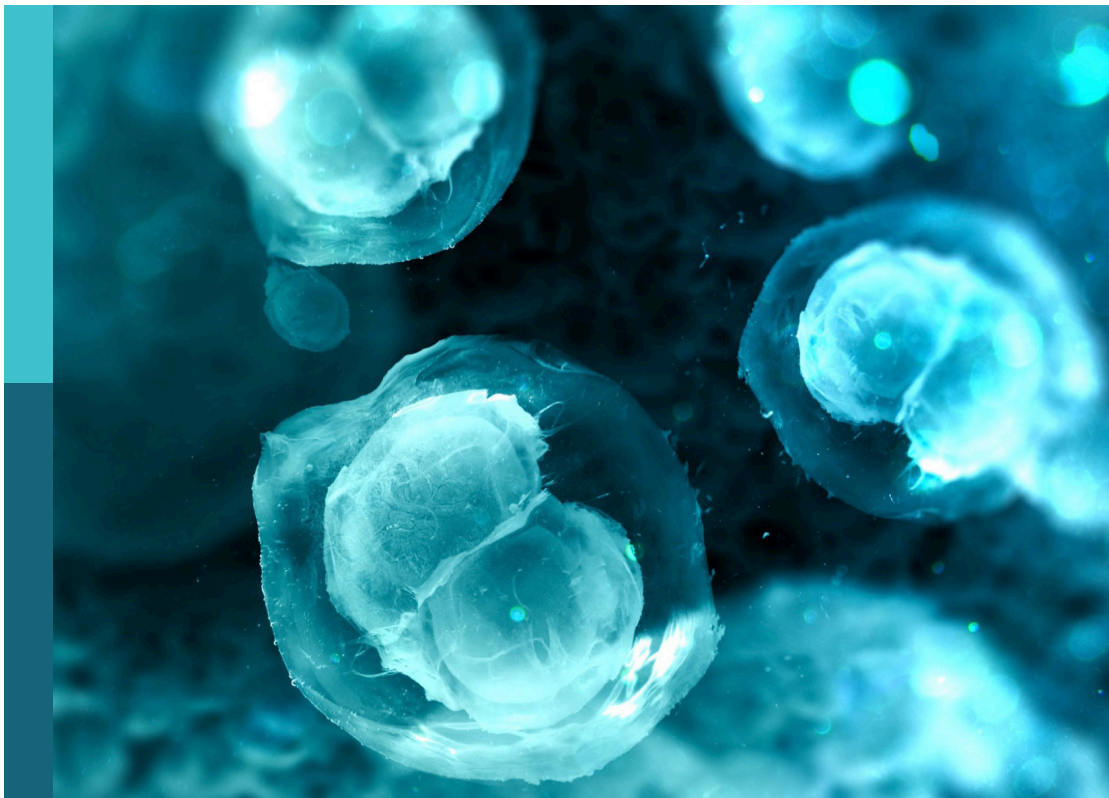

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Modulation of T-cell function and survival by the tumor microenvironment

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Editorial: Modulation of T-cell function and survival by the tumor microenvironment

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cancer, tumor microenvironment, T cell, immune regulation, tumor immunity

Editorial on the Research Topic
Modulation of T-cell function and survival by the tumor microenvironment

T lymphocytes are widely recognized as critical regulators of anti-tumor immune surveillance, and advances in our understanding of these cells in the context of cancer over the last two decades have sparked a rapid growth in T cell-based immunotherapies to treat the disease. From therapeutic vaccines that aim to elicit responses by otherwise dormant tumor-specific T lymphocytes, to adoptive cell transfer therapies that harness the anti-tumor potential of both natural and antigen-redirected T cells, to checkpoint blockade regimens that unleash the full power of these cells in their fight against cancer, the era of immuno-oncology has brought with it a dramatic change in the landscape of therapeutic options available for patients. At the same time, although these approaches have yielded response rates and clinical outcomes that are unprecedented in the history of cancer therapy, there remain significant challenges that often limit the reach and durability of T lymphocyte-based immunotherapies.

One challenge that has emerged as a particularly significant barrier to immunotherapy is the immunologically hostile nature of the tumor microenvironment (TME), a complex ecosystem comprised of diverse cell types (tumor cells, stromal cells, immune cells, fibroblasts, adipocytes, vascular endothelial cells, and microbes) and non-cellular components (metabolites, cytokines, extracellular matrix, exosomes, and other cell-derived factors) that evolves to support the dynamic needs of cancer cells over the course of tumor progression. The adaptation of cancer cells to not only survive in, but also thrive in, an ecological niche that at the same time promotes anti-tumor T cell dysfunction is indeed a driving force behind tumor immune escape.

In this Research Topic, we present a collection of original research and review articles from leading experts that highlight many of the complex interactions between T lymphocytes and the TME. These articles document the diverse ways in which the TME is now known to modulate T cell function and survival, and they highlight how interventions that aim to disrupt the immunosuppressive networks within this internal ecosystem can support more favorable outcomes of anti-tumor immune responses. Importantly, this collection also brings focus to important issues that remain to be addressed in this field as we seek to further improve the efficacy of immune-based interventions for cancer.

Our Research Topic opens with a comprehensive review by Mani et al., who describe various immune-modulating factors within the TME that compromise T cell functionality. Particular attention is given to the role of regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSC) in dampening T cell proliferation and effector function, oncometabolites and other

metabolic constraints that inhibit anti-tumor T cell reactivity, and immune checkpoint ligands that promote T cell exhaustion. Emphasis is also placed on inflammatory mediators and chemokines within the TME that act as key regulators of T lymphocyte-vascular endothelial cell interactions necessary for effector T cell entry and infiltration into the TME. A deeper discussion of T lymphocyte suppression by MDSC is then provided by Bhardwaj and Ansell, who focus on the influence of these immunoregulatory cells specifically within the context of hematologic malignancies, and Qi et al. bring focused attention to the immunosuppressive functions of Tregs, with particular emphasis on the role of the CBM complex/NF-κB, MAPK/CDK, and STAT3/P27 signaling pathways in these cells as contributing factors to TME formation and intratumoral Treg function.

We next present two review articles that highlight how non-immune cell populations and their products can influence the function of T lymphocytes within the TME. DiPalma and Blattman discuss the regulation of intratumoral T cell function by the tumor microbiome as well as distant microbiomes (such as the gut microbiome), highlighting the roles of specific microbial species, microbe-derived metabolites, and microbial dysbiosis in shaping both the TME and anti-tumor T cell reactivity at this site. Their article concludes with a discussion of recent insights from murine and clinical studies that have shed light on microbial dysbiosis and dietary influences on microbiome composition as determinants of therapeutic efficacy for adoptive cell transfer and checkpoint blockade regimens. Whiteside then reviews the mechanisms by which melanoma-derived exosomes released from the TME can suppress effector function and induce apoptosis of T cells even prior to tumor infiltration.

Among tumors that have been successfully infiltrated by T lymphocytes, a common phenomenon associated with loss of immunologic control and tumor progression is T cell exhaustion. Arising from chronic antigenic exposure and engagement of various immune checkpoints within the TME, T cell exhaustion is associated with reduced proliferative capacity, anti-tumor reactivity, and persistence of T cells within tumor tissue. Our Research Topic offers two excellent reviews on this Research Topic. First, Blake et al. describe epigenetic regulation of various T cell exhaustion phenotypes that have been reported in the TME, highlighting the role of both transcription factors and chromatin modifiers known to program the exhausted state. They also discuss epigenetic modulators that are currently being explored as a means of rewiring T cells for more robust anti-tumor reactivity. This review is followed by a more specific assessment of exhaustion in the context of CAR-T cell therapy. Zhu et al. summarize the various factors known to influence CAR-T cell exhaustion, from the design of CAR-T receptors, to *in vitro* expansion conditions during CAR-T cell preparation, to regulatory effects of the TME on CAR-T cell functionality. They conclude by discussing combinatorial strategies for targeting immunosuppressive factors within the TME as a means of preventing the exhaustion of CAR-T cells at this site.

Lastly, we close our Research Topic with two original research articles that have important implications for T lymphocyte-based cancer immunotherapy. Gamache et al. demonstrate in an orthotopic mouse model of pancreatic adenocarcinoma that CD40 stimulation remodels the TME to support more robust anti-tumor immunity. Notably, CD40 agonism in combination with checkpoint blockade led to a reduction in both conventional $CD4^+$ Tregs as well as $CD8^+$ Tregs within the TME, and this change was associated with enhanced T cell effector function, improved responses to checkpoint blockade therapy, and establishment of CD4-dependent immunologic memory. $CD4^+$ T cells within the TME are also the focus of a prognostic gene expression index for gastric cancer developed by Chen et al. Among $CD4^+$ T cell-related hub genes analyzed, *PROC* and *SERPINE1* were found to carry prognostic significance for gastric cancer and were used to construct a risk-score that correlated with patient outcome and tumor infiltration by $CD8^+$ T cells. As such, this risk-score model may serve as a useful predictor for gastric cancer responsiveness to immunotherapy.

As the era of immuno-oncology continues to unfold at a rapid pace, there is indeed a renewed optimism for the future of cancer care. Though the TME remains a daunting barrier to the success of many immunotherapies, advances in our understanding of this dynamic structure have reshaped the way we think about tumors and tumor immunity. Rather than a simple T cell versus tumor cell battleground, the TME is now appreciated to be a unique and complicated ecological niche, with a multitude of cellular and molecular interactions ultimately influencing the outcome of the anti-tumor immune response. Insights into these relationships within the TME are now opening up exciting opportunities to reshape this ecosystem in ways that support more robust tumor immune reactivity. With this goal in mind, it is our hope that the articles presented herein not only shed light on the complexities of the TME but also spark future research efforts to further our understanding of T cell regulation in the TME and improve treatment outcomes for cancer immunotherapy in the years ahead.

Author contributions

KH: Writing–original draft, Writing–review and editing. CL: Writing–review and editing. SJ: Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Crosstalk between the CBM complex/NF-κB and MAPK/P27 signaling pathways of regulatory T cells contributes to the tumor microenvironment

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Regulatory T cells (Tregs), which execute their immunosuppressive functions by multiple mechanisms, have been verified to contribute to the tumor microenvironment (TME). Numerous studies have shown that the activation of the CBM complex/NF-κB signaling pathway results in the expression of hypoxia-inducible factor-1 (HIF-1α) and interleukin-6 (IL-6), which initiate the TME formation. HIF-1α and IL-6 promote regulatory T cells (Tregs) proliferation and migration through the MAPK/CDK4/6/Rb and STAT3/SIAH2/P27 signaling pathways, respectively. IL-6 also promotes the production of HIF-1α and enhances the self-regulation of Tregs in the process of tumor microenvironment (TME) formation. In this review, we discuss how the crosstalk between the CARMA1–BCL10–MALT1 signalosome complex (CBM complex)/NF-κB and MAPK/P27 signaling pathways contributes to the formation of the TME, which may provide evidence for potential therapeutic targets in the treatment of solid tumors.

KEYWORDS

regulatory T cells, tumor microenvironment, CBM signaling pathway, MAPK/P27 signaling pathway, CARMA1

Highlights

1) The activation of the CBM complex/NF-κB signaling pathway results in TME formation.
2) HIF-1α and IL-6 promote Treg proliferation and migration via the MAPK/CDK4/6/Rb and STAT3/SIAH2/P27 signaling pathways.
3) The crosstalk between the CBM complex/NF-κB and MAPK/P27 signaling pathways appears in Tregs.

Introduction

The development of immune checkpoint therapy (ICT), which stimulates an immune response to cancer, has been one of the most rapid and important advances in cancer treatment over the past decade. Programmed cell death 1 (PD-1) is an immunosuppressive co-stimulatory signal receptor that belongs to the CD28 family. PD-1 and PD-L1 blockage at immune checkpoints can rejuvenate patients' T cells to achieve long-term remission (Qi et al., 2020). However, the clinical effect of programmed cell death 1(PD-1)/programmed cell death 1 ligand 1 (PD-L1) targeted therapy for solid malignant tumors is not ideal (Cai et al., 2019). Only 20% of patients achieve favorable long-term results after treatment, and most patients relapse after treatment (Yan et al., 2019). This phenomenon may be related to the tumor microenvironment (TME), which is characterized by nutrient competition, hypoxia, low pH, and metabolite accumulation. Such complex conditions accelerate exhaustion of T effector cells and promote differentiation and accumulation of regulatory T cells (Tregs), M2-like macrophages, and Myeloid-derived suppressor cells (MDSCs). The TME also produces unique subsets of myeloid cells known as tumor-associated dendritic cells (TADC) and tumor-associated neutrophils (TAN) (Bader et al., 2020; Wang et al., 2021).

In this complex microenvironment, T cells encounter many inhibitory cells and molecules that can disrupt the survival, activation, proliferation, and effector functions of T cells (Joyce and Fearon, 2015; Turley et al., 2015). Alongside the developments in antibody therapy, modulation of cell signaling pathways using small-molecule inhibitors has gained ground within the immunotherapy field. The functional profiles of immune cells are necessarily shaped in response to environmental cues, which are conveyed to the cellular machinery *via* a myriad of distinct but overlapping signaling cascades (Wicherska-Pawlowska et al., 2021).

Recent studies have shown that Tregs may be involved in PD-1/PD-L1 blockage treatment, and the PD-1/PD-L1 axis may affect the differentiation and function of Tregs (Cai et al., 2019). Tregs execute their immunosuppressive functions by multiple mechanisms, such as by consuming interleukin-2 (IL-2), expressing cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), secreting inhibitory cytokines (transforming growth factor-β, interleukin-10, interleukin-35) (Takeuchi and Nishikawa, 2016), and directly killing T cells or Antigen-presenting cells (APCs) by producing granular enzymes and perforin (Sakaguchi et al., 2010; Cai et al., 2019). These functions can be enhanced by classical interleukin-6 (IL-6) receptor (IL-6R) signaling (Hagenstein et al., 2019). These functions of Tregs may be related to hypoxia in the TME. The microenvironment of most tumors is usually hypoxic, and the expression level of hypoxia-inducible factor-1α (HIF-1α) is often increased in Treg (He et al., 2015). Hypoxia can also change the T cells CBM complex (CARMA1-BCL10-MALT1) activity, which is closely related to the development of solid tumor via NF-κB activation (Schaefer, 2020).

The effects of the hypoxic environment on the immunosuppressive function of Tregs are still inconclusive. Some studies have shown that HIF-1α positively affects the function of Tregs and plays a role in their suppressive function in tumors (Clambey et al., 2012; Westendorf et al., 2017). Other studies, however, have shown the opposite (Hsu and Lai, 2018). A recent study using a murine model of glioma has shown that ablation of HIF-1α leads to enhanced animal survival due to a decrease in the migratory abilities of HIF-1α Knockout Tregs (Miska et al., 2019). Here, we provide a brief review of signaling pathways in Tregs and the formation of the TME (Table 1).

Cyclin-dependent kinase signaling enhances treg proliferation and migration

Cyclin-dependent kinases (CDKs) are a class of serine/threonine kinases. As important signaling molecules that regulate transcription, CDK–cyclin complexes are involved in Treg growth, proliferation, dormancy, and apoptosis (Arnett et al., 2021). During the Treg cycle, cyclins are expressed and degraded periodically and are bound to the CDK activated by them. Through the activity of CDKs, phosphorylation of different substrates can be catalyzed to realize the promotion and transformation of the Treg cycle. Sequential phosphorylation of CDK4/6 and Rb proteins activates 1) downstream E2F and Stathmin, leading to the release of transcription factors such as E2F4 and E2F7 (Dyson, 1998; Pietrzak et al., 2018), and 2) some genes necessary for E2F4 and E2F7 activation and transcription, leading to progression into the S phase (Geng et al., 2020). As shown in Figure 1, when CDK4/6 phosphorylation is inhibited by P27 which is a CDK inhibitor (Scortegagna et al., 2020), some functions of Tregs such as gene transcription, cell proliferation, and migration, are affected.

Therefore, it is a good strategy to control the TME by inhibiting Treg proliferation by targeting CDKs with some target drugs (Johnson et al., 2010). Numerous clinical trials have been conducted with small molecules that target CDKs in patients with cancer (Albanese et al., 2010).

IL-6 enhances effects of the STAT3/SIAH2/CDK4/6 signaling pathway

The IL-6 signaling pathway is associated with tumor angiogenesis, and previous studies have found that the suppression of IL-6 signaling led to suppression of angiogenesis and migration of breast cancer (Luo et al., 2020). IL-6 is produced by fibroblasts, keratinocytes, and endothelial

TABLE 1 Points of concern between TME, Treg, CDKs, HIF-1α, CBM, MAPK, SIAH2 and STAT.

Correlation	Points of concern	References
TME and Treg	Treg is one of the important factors in the formation of tumor microenvironment	Sakaguchi et al. (2010)
CDKs and TME	TME was changed by drug targeting CDKs	Johnson et al. (2010)
HIF-1α and TME	Treg HIF-1α expression in TME is increased	He et al. (2015)
	SIAH2/PHD/HIF-1α pathway plays key role in the development of the TME	Nakayama et al. (2004)
		Albadari et al. (2019)
HIF-1α and Treg	Treg HIF-1α expression in TME is increased	He et al. (2015)
	HIF-1α positively affects Treg function	Westendorf et al. (2017)
	VEGF and VEGF receptor are closely related to HIF-1α in Tregs	Vaupel and Multhoff, (2018)
CBM complex and Treg	Partial disruption of CBM complex in Tregs improve immune checkpoint therapy	Di Pilato et al. (2019)
MAPK and Treg	MAPK regulates the Treg cell cycle and gene expression	Klomp et al. (2021)
	ERKs adjust Treg proliferation, differentiation, et al	Wang et al. (2019)
STAT and Treg	IL-6/STAT1/3 promote Treg proliferation	Yeh et al. (2013)
SIAH2 and Treg	immunosuppressive function of Siah2$^{-/-}$ Tregs was blunted	Nakayama et al. (2004)
CDKs and Treg	CDKs regulate Treg growth, proliferation, dormancy, and apoptosis	Arnett et al. (2021)
	CDK4/6 phosphorylation affects Tregs proliferation and migration	Scortegagna et al. (2020)

cells in response to injury, and corresponding receptors also exist on Tregs (Hagenstein et al., 2019). IL-6 transmits signals resulting in the activation of transcription factors, signal transducers and activators of transcription 1 (STAT1) and 3 (STAT3), through the association with gp130, and then promotes Foxp3 (+) Treg proliferation (Yeh et al., 2013).

STAT3 is an important member of the STAT family, which also includes STAT1, STAT2, STAT4, STAT5a, and STAT5b. The STAT6 and STAT3 signal transduction pathways are closely related to cell proliferation, differentiation, and apoptosis. The pathways control the production of growth factors and cytokines, and the extracellular signal stimulation, thereby regulating target gene transcription. The pathway activation can lead to abnormal cell proliferation and malignant transformation (Buettner et al., 2002). Jak–STAT3 can be activated by a variety of extracellular proteins, such as interleukins (Wang and Fuller, 1994). When activated, JAKs phosphorylate the tyrosine site on the receptor, causing the receptor to produce a region that binds to STAT3. At this point, the Src homology (SH2) domain on STAT3 binds to the phosphorylated tyrosine residues on the receptor, thereby forming homo- or heterodimers, which are transported to the nucleus and interact with other transduction factors to regulate gene transcription (Liu et al., 2017).

STAT3 activates the transcription and translation of the ubiquitin ligase, Seven In Absentia Homolog 2 (SIAH2). Then, P27, which inhibits CDK4/6 activation, is degraded by ubiquitination of SIAH2 (Figure 1). According to Hoshino et al., upregulation of P27 expression is necessary for specific blockage of tumor extracellular signal-regulated kinase pathways, which in turn leads to complete growth inhibition of tumor cells (Hoshino et al., 2001). A recent study has shown that the immunosuppressive function of Tregs in tumors of *Siah2*$^{-/-}$ mice was blunted owing to P27-dependent suppression of CDK4/6 signaling activation (Scortegagna et al., 2020).

STAT3 gene is highly expressed in hepatocellular carcinoma cells, and regorafenib (Stivarga), a drug targeting STAT2 for the treatment of hepatocellular carcinoma, has been identified as a second-line oral agent (Jindal et al., 2019).

SIAH2 enhances tumor HIF-1α expression

The SIAH2/PHD/HIF-1α pathway plays an important role in the development of the TME. The experiments by Nakayama et al. have confirmed that the E3 ubiquitin ligase SIAH2 shows significant ubiquitin-dependent degradation of prolyl-hydroxylase 1 (PHD1) and prolyl-hydroxylase 3 (PHD3), while its effect on PHD2 is not significant (Nakayama et al., 2004). There are two types of HIF-α, including HIF-1α and HIF-2α(Albadari et al., 2019). Among them, only the expression mechanism of HIF-1α has been well studied, and only HIF-1α has been found in a wide range of cells. As a substrate for PHD, HIF-1α can be hydroxylated in two forms, thereby undermining the stability of HIF-1α. When the intracellular oxygen concentration is below normal values (2–5%), a hypoxic environment is generated. Induction of SIAH2 expression by hypoxia serves to enhance the degradation of prolyl-hydroxylase 1/3(PHD1/3) and consequently increase the abundance of HIF-1α(Nakayama et al., 2004).

Upregulated HIF-1α expression in tumor cells and immune cells is characteristic of the TME. Some studies have shown that HIF-1α positively affects Treg function and plays a role in their

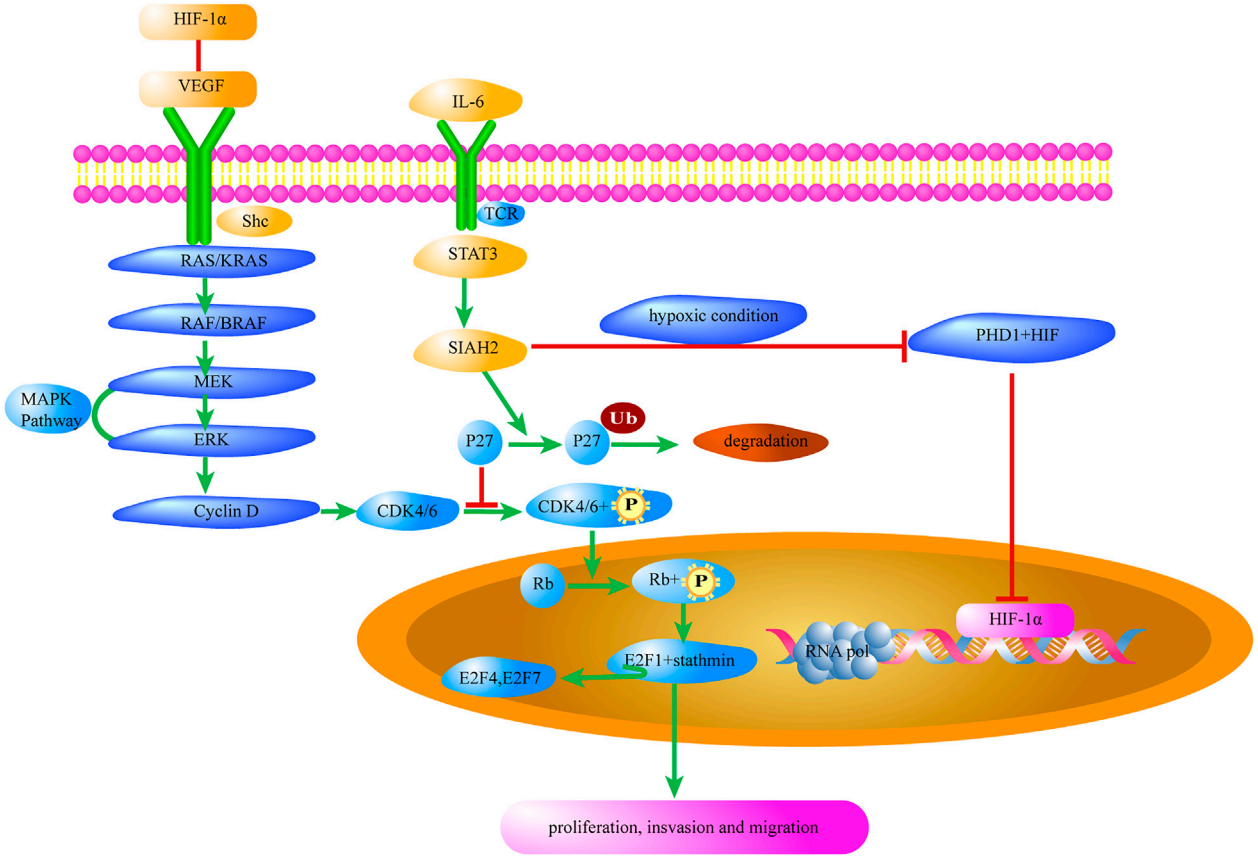

FIGURE 1
MAPK/P27 signaling of Tregs contributes to the tumor microenvironment. Il-6 in the tumor microenvironment promotes the expression of HIF-1α in Tregs and removes the inhibition of CDK4/6 by P27. Meanwhile, the interaction between HIF-1α and VEGF activates the MAPK/CDK4/6 signaling pathway to promote Treg cell proliferation. HIF-1α, Hypoxia-Inducible Factor-1α; VEGF, Vascular endothelial growth factor; shc, Src-homology collagen protein; RAS, small G-protein; KRAS, V-Ki-ras2 Kirsten ratsarcoma viral oncogene homolog; RAF, Raf kinase; BRAF, v-raf murine viral oncogene homolog B1; MEK, Mitogen-activated protein kinase; ERK, Extracellular-signal-regulated kinase; MAPK, mitogen-activated protein kinase; CDK, Cyclin-dependent kinase; Rb, Retinoblastoma protein; E2F, Transcription factor; P27, Potential tumor suppressor protein; Ub, Ubiquitin; SIAH2, Seven in absentia homolog 2; STAT3, Signal Transducer and Activator of Transcription 3; TCR, T cell receptor; PHD1, Hypoxia-inducible factor prolyl hydroxylase 1.

suppressive function in tumors (Clambey et al., 2012; Westendorf et al., 2017). An immune escape mechanism involves Treg-mediated immunosuppression, which is used by tumors to overcome the antitumor activity of $CD8^+$ cytotoxic T cells, dendritic cells, and natural killer cells (Bader, Voss, and Rathmell, 2020).

When the expression level of HIF-1α in Tregs is upregulated, hypoxia response element (HRE) binds to HIF-1α, resulting in the production of a variety of products, such as vascular endothelial growth factor (VEGF), which is associated with angiogenesis, and CXCR4, which is associated with cell migration (Forsythe et al., 1996). Overexpression of VEGF and activation of VEGF receptor are closely related to HIF-1α in Tregs (Vaupel and Multhoff, 2018).

VEGF and HIF-1α are also closely related to the development of blood vessels. Currently, inhibitor drugs targeting these two proteins are approved by the FDA for the treatment of some tumors (Jindal, Thadi, and Shailubhai, 2019). Given that VEGF and HIF-1α genes are also implicated in Treg reproduction, immunosuppressive drugs can be used to destroy the TME in Tregs.

Mitogen-activated protein kinase signaling enhances CDK4/6 activation

The mitogen-activated protein kinase (MAPK) signaling pathway is a signal transduction pathway that is widely found in animal cells. This pathway plays an important role in regulating the Treg cell cycle and gene expression (Liu et al., 2013; Klomp et al., 2021). The MAPK signaling pathway consists

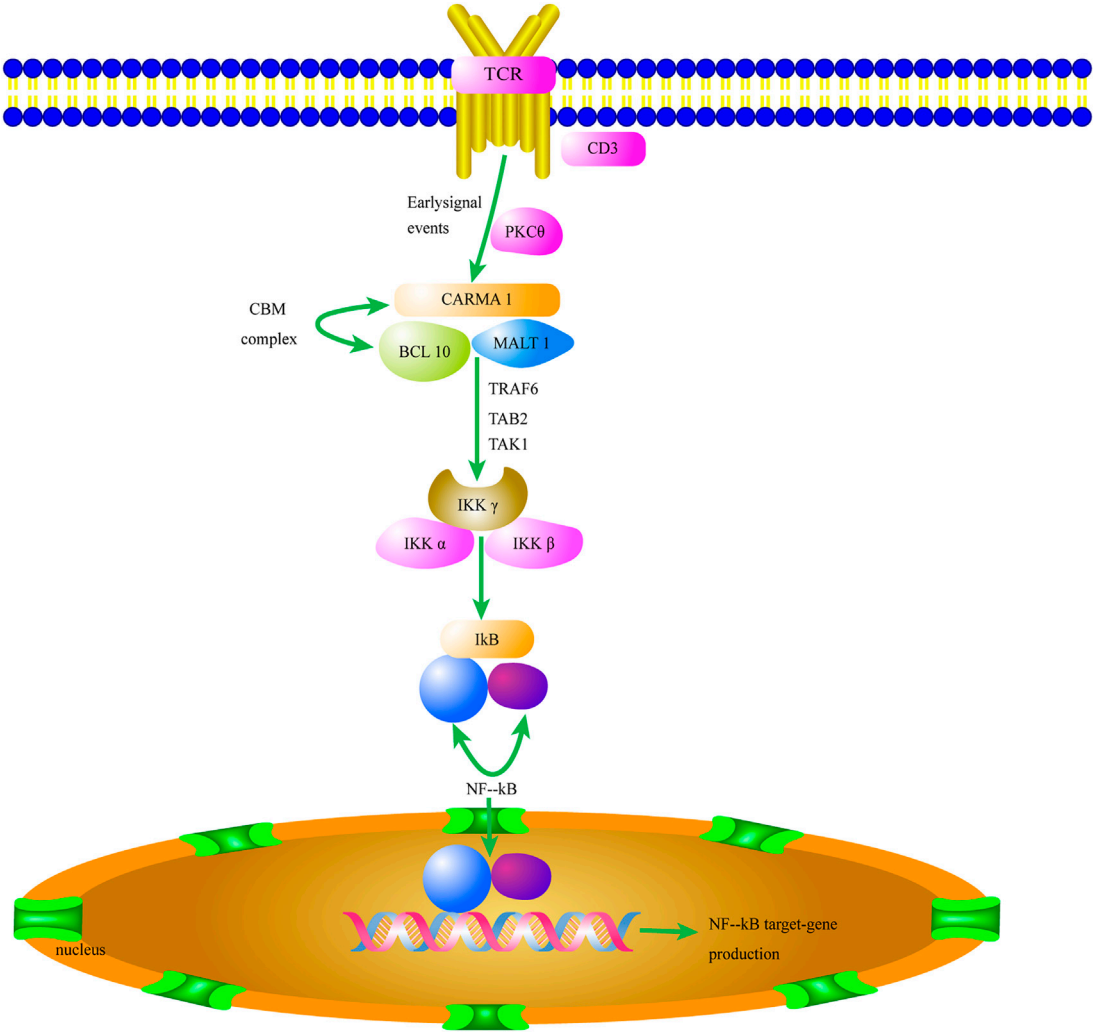

FIGURE 2
The CBM complex/NF-κB signaling pathway of Tregs contributes to the tumor microenvironment. Activation of the CBM complex/NF-κB signaling pathway in Tregs increases HIF-1α and IL-6 expression and initiates the TME. The secretion of HIF-1α and IL-6 in the TME promotes Treg proliferation and migration. TCR, T cell receptor; PKC, Protein kinase C; CARM1, Caspase recruitment domain-containing membrane-associated guanylate kinase protein-1; BCL10, B-cell lymphoma/leukemia 10; MALT 1, Mucosa-associated lymphoid tissue 1; TRAF6, TNF receptor associated factor 6; TAK1, TGFβ-activated kinase 1; TAB2, TAK1-binding protein 2; Iκκ, inhibitor of kappa kinase; NF-kB, nuclear factor kappa B.

of a cascade of successively activated serine/threonine protein kinases that amplify and transmit extracellular signals step by step to the cell and even to the nucleus, connecting membrane receptor-bound extracellular stimuli to effector molecules in the cytoplasm and nucleus (Tetu et al., 2021).

Vascular endothelial growth factors (VEGFs) constitute a subfamily of growth factors that stimulate the growth of new blood vessels. VEGFs are important signaling proteins involved in both vasculogenesis (*de novo* formation of the embryonic circulatory system) and angiogenesis (the growth of blood vessels from preexisting vasculature) (Negri et al., 2019). VEGFs can bind the Treg cell membrane receptor and increase Treg cell proliferation (Vasilev et al., 2019). NRP-1, a semaphorin III receptor involved in the activation of T cells, is constitutively expressed on the surface of Foxp3+ Tregs independently of their activation status. NRP-1 has been found to interact with VEGFs and interfere with Treg-mediated immunosuppression (Pucino et al., 2014).

RAS is a small GTP-binding protein, with the GTPase domain binding GDP in the inactive state and GTP in the active state; therefore, RAS plays the role of molecular switch. RAS adjusts T cell development, differentiation, and proliferation by inducing downstream signal transduction pathways. Inhibition of RAS has been found to be associated with an increased number and boosted function of Foxp3+ Tregs (Aizman et al., 2010).

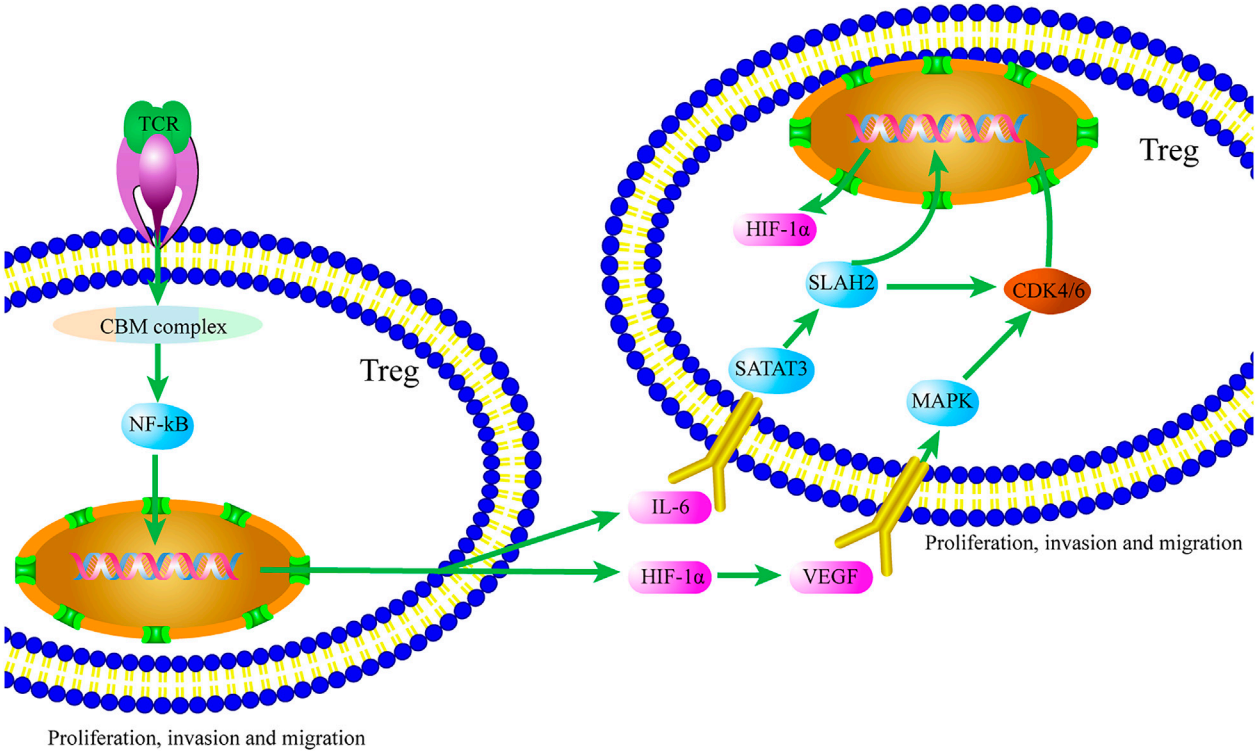

FIGURE 3
Crosstalk of the CBM complex/NF-κB and MAPK/P27 signaling pathways Tregs contributes to the TME. Ligand binding to TCR of Tregs, HIF-1α and IL-6 are produced via activating the CBM complex/NF-κB signaling pathway. In turn, Treg proliferation and migration are promoted by HIF-1α and IL-6 through the MAPK/CDK4/6/Rb and STAT3/SIAH2/P27 signaling pathways, respectively.

ERKs adjust Treg proliferation, differentiation, and survival, and regulate the production of a variety of downstream growth factors (EGF, model NGF, and PDGF) (Liu et al., 2013; Wang et al., 2019). The RAS/Raf/MEK/ERK axis is the main axis of the ERK pathway (Akula et al., 2019). Activation of ERK can promote the phosphorylation of cytoplasmic target proteins or regulate the activity of other protein kinases; the activated ERK is translocated into the nucleus, where it promotes the phosphorylation of a variety of transcription factors. The MAPK pathway transfers extracellular stimulatory signals to cells and their nuclei to regulate cell growth, differentiation, proliferation, apoptosis, and migration.

As shown in Figure 1, VEGF-R2 phosphorylates and activates SHC, which binds to spline protein, which binds to guanylate exchange protein via the SH2 domain to approach the RAS, thereby further activating the MAPK cascade (Raf1→MEK1/2→ERK1/2). It can also induce the activation of P38 MAPK, which in turn activates MAPKK-2/3 and phosphorylates both the polymerized regulatory molecule of filamin actin (F-actin) and heat shock protein 27 (HSP27), causing the recombination of the actin cytoskeleton and stimulating endothelial cell migration (Wu et al., 2016).

Effects of *CARMA1* on the NF-κB signaling pathway in tregs

CARMA1 (CARD11) proteins are composed of 1,147 amino acid residues. Their N-termini consist of a CARD domain and a coiled-coil structure, and the C-termini contain a PDZ domain, an SH3 domain, and a GUK domain. Activated downstream of protein kinase C (PKC), CARMA1 is coupled to lipid rafts on cell membranes (Figure 2). When MHC binds to molecules on the cell surface, TCR activates tyrosine phosphoric acid, which leads to activation of PKC. PKC is then phosphorylated and couples CARMA1 to the membrane, where BCL10 is connected to the Ig region of MALT1 and to CARMA1 to form a CBM complex. The CBM complex of all the T cells—which can respond to specific antigen receptor stimulation and includes the invariant components BCL10 and MALT1, assembled with CARD9, CARD10, CARD11, or CARD14—is an important mediator of NF-κB activation (Schaefer, 2020).

In addition, MALT1 interacts with CARMA1's coiled spiral region. This complex has enzymatic activity (Oruganti et al., 2017). NF-κB usually forms homo-/heterodimers with P65 and P50 and is inactivated in the cytoplasm by binding to the inhibitory protein IκB to form a trimer complex. CARMA1 binds to lipid rafts on the cell membrane. It also acts as a signal transmitter and ultimately

activates NF-κB when Tregs are stimulated by antigens (Di Pilato et al., 2019). CARMA1 plays an important role in the activation of the NF-κB signaling pathway as a junction between membrane-bound and cytoplasmic proteins (Ma et al., 2014).

The functional changes of the CBM complex in Tregs have an important relationship with the formation of TME. The CBM complex mediates TCR-induced NF-κB activation in Tregs and controls the conversion of resting Tregs to effector Tregs when needed. Partial disruption of the CBM complex in Tregs, such as due to a knockdown of *CARMA1* gene, can weaken the formed TME and improve sensitivity to PD-1/PD-L1 immune checkpoint therapy (Di Pilato et al., 2019). The interaction among CARMA1, BCL10, and MALT1 also affects the activity of downstream NK-KB signaling pathway, and the control of BCL10 on MALT1 paracaspase activity affects the formation of malignant melanoma TME (Rosenbaum et al., 2019). The role of Bcl10 in the development of Tregs and formation of TME is essential (Yang et al., 2021). Based on the results of a series of studies on the CBM complex, we can predict that drugs targeting CARMA1, BCL10 and MALT1 inhibitors will be able to effectively break the TME formed by Tregs (Keller et al., 2021).

The continuously activated NF-κB in Tregs or other cells can enhance the transcription of VEGF genes and also increase the levels of some tumor promoting cytokines, such as IL-1 (acute leukemia growth factor), TNF (malignant lymphogranuloma, T cell lymphoma, glioma growth factor), and IL-6 (growth factor for multiple myeloma), thereby promoting the above signaling pathways regulated by VEGF and IL-6. Two small-molecule inhibitors regulate cell signaling pathways in a synergistic manner to inhibit the proliferation and migration of Tregs, thereby blocking the secretion of inhibitory cytokines by Tregs to aid in the formation of the TME. Currently, four clinical trials have been recruited on the https://www.clinicaltrials.gov/, and they involve solid tumors and leukemia.

Conclusion

In conclusion, Tregs are involved in the regulation of autoimmune diseases, allergic diseases, and graft rejection, and Treg-mediated immunosuppression has become a major obstacle to effective treatment of tumors. Tregs play an immunosuppressive role in the TME through various mechanisms. Based on our analyses (Figure 3), we think that after specific ligand binding to TCR of Tregs, the CBM complex/NF-κB signaling pathway is activated, and factors such as HIF-1α and IL-6 are produced, thereby initiating the TME formation. HIF-1α and IL-6 promote Treg proliferation and migration through the MAPK/CDK4/6/Rb and STAT3/SIAH2/P27 signaling pathways, respectively. IL-6 also promotes the production of HIF-1α and enhances the self-regulation of Tregs in the process of TME formation.

In this review, we discussed the crosstalk between the CBM and MAPK/P27 signaling pathways, in order to gain a better understanding of the complexity of the role of Tregs in the process of TME formation. However, the complex role of Tregs in the formation of the TME *via* either CBM or the MAPK/P27 signaling pathway and its underlying mechanisms need further exploration. Therefore, further exploration of the complex role of CBM and MAPK/P27 signaling pathway in the formation of TME will provide stronger evidence for potential therapeutic targets in solid tumor therapy.

Author contributions

Conceptualization: QW and XT. Bibliographic retrieval: TQ, YL, YZ, and XM. Writing-original draft: TQ, WC, and DW. Writing-review and editing: YL, TQ, and WC. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Aizman, E., Mor, A., George, J., and Kloog, Y. (2010). Ras inhibition attenuates pancreatic cell death and experimental type 1 diabetes: possible role of regulatory T cells. *Eur. J. Pharmacol.* 643, 139–144. doi:10.1016/j.ejphar.2010.06.029

Akula, S. M., Abrams, S. L., Steelman, L. S., Emma, M. R., Augello, G., Cusimano, A., et al. (2019). RAS/RAF/MEK/ERK, PI3K/PTEN/AKT/mTORC1 and TP53 pathways and regulatory miRs as therapeutic targets in hepatocellular carcinoma. *Expert Opin. Ther. Targets* 23, 915–929. doi:10.1080/14728222.2019.1685501

Albadari, N., Deng, S., and Li, W. (2019). The transcriptional factors HIF-1 and HIF-2 and their novel inhibitors in cancer therapy. *Expert Opin. Drug Discov.* 14, 667–682. doi:10.1080/17460441.2019.1613370

Albanese, C., Alzani, R., Amboldi, N., Avanzi, N., Ballinari, D., Brasca, M. G., et al. (2010). Dual targeting of CDK and tropomyosin receptor kinase families by the oral inhibitor PHA-848125, an agent with broad-spectrum antitumor efficacy. *Mol. Cancer Ther.* 9, 2243–2254. doi:10.1158/1535-7163.MCT-10-0190

Arnett, A., Moo, K. G., Flynn, K. J., Sundberg, T. B., Johannessen, L., Shamji, A. F., et al. (2021). The cyclin-dependent kinase 8 (CDK8) inhibitor DCA promotes a tolerogenic chemical immunophenotype in $CD4^+$ T cells via a novel CDK8-GATA3-FOXP3 pathway. *Mol. Cell. Biol.* 41 (9), e0008521. doi:10.1128/MCB.00085-21

Bader, J. E., Voss, K., and Rathmell, J. C. (2020). Targeting metabolism to improve the tumor microenvironment for cancer immunotherapy. *Mol. Cell.* 78, 1019–1033. doi:10.1016/j.molcel.2020.05.034

Buettner, R., Mora, L. B., and Jove, R. (2002). Activated STAT signaling in human tumors provides novel molecular targets for therapeutic intervention. *Clin. Cancer Res.* 8, 945–954.

Cai, J., Wang, D., Zhang, G., and Guo, X. (2019). The role of PD-1/PD-L1 Axis in Treg development and function: Implications for cancer immunotherapy. *Onco. Targets. Ther.* 12, 8437–8445. doi:10.2147/OTT.S221340

Clambey, Eric T., McNamee, Eóin N., Westrich, Joseph A., Glover, Louise E., Campbell, Eric L., Jedlicka, Paul, et al. (2012). Hypoxia-inducible factor-1 alpha–dependent induction of FoxP3 drives regulatory T-cell abundance and function during inflammatory hypoxia of the mucosa. *Proc. Natl. Acad. Sci. U. S. A.* 109, E2784–E2793. doi:10.1073/pnas.1202366109

Di Pilato, M., Kim, E. Y., Cadilha, B. L., Prussmann, J. N., Nasrallah, M. N., Seruggia, D., et al. (2019). Targeting the CBM complex causes Treg cells to prime tumours for immune checkpoint therapy. *Nature* 570, 112–116. doi:10.1038/s41586-019-1215-2

Dyson, N. (1998). The regulation of E2F by pRB-family proteins. *Genes. Dev.* 12, 2245–2262. doi:10.1101/gad.12.15.2245

Forsythe, J. A., Jiang, B. H., Iyer, N. V., Agani, F., Leung, S. W., Koos, R. D., et al. (1996). Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol. Cell. Biol.* 16, 4604–4613. doi:10.1128/mcb.16.9.4604

Geng, R., Zheng, Y., Zhou, D., Li, Q., Li, R., Guo, X., et al. (2020). ZBTB7A, a potential biomarker for prognosis and immune infiltrates, inhibits progression of endometrial cancer based on bioinformatics analysis and experiments. *Cancer Cell. Int.* 20, 542. doi:10.1186/s12935-020-01600-5

Hagenstein, J., Melderis, S., Nosko, A., Warkotsch, M. T., Richter, J. V., Ramcke, T., et al. (2019). A novel role for IL-6 receptor classic signaling: Induction of RORγt+Foxp3+ Tregs with enhanced suppressive capacity. *J. Am. Soc. Nephrol.* 30, 1439–1453. doi:10.1681/ASN.2019020118

He, L. Y., Li, L., Guo, M. L., Zhang, Y., and Zhang, H. Z. (2015). Relationship between CD4+CD25+ Treg and expression of HIF-1α and ki-67 in NSCLC patients. *Eur. Rev. Med. Pharmacol. Sci.* 19, 1351–1355.

Hoshino, R., Tanimura, S., Watanabe, K., Kataoka, T., and Kohno, M. (2001). Blockade of the extracellular signal-regulated kinase pathway induces marked G1 cell cycle arrest and apoptosis in tumor cells in which the pathway is constitutively activated: Up-regulation of p27(kip1). *J. Biol. Chem.* 276, 2686–2692. doi:10.1074/jbc.M006132200

Hsu, T. S., and Lai, M. Z. (2018). Hypoxia-inducible factor 1α plays a predominantly negative role in regulatory T cell functions. *J. Leukoc. Biol.* 104, 911–918. doi:10.1002/JLB.MR1217-481R

Jindal, A., Thadi, A., and Shailubhai, K. (2019). Hepatocellular carcinoma: Etiology and current and future drugs. *J. Clin. Exp. Hepatol.* 9, 221–232. doi:10.1016/j.jceh.2019.01.004

Johnson, N., Bentley, J., Wang, L. Z., Newell, D. R., Robson, C. N., Shapiro, G. I., et al. (2010). Pre-clinical evaluation of cyclin-dependent kinase 2 and 1 inhibition in anti-estrogen-sensitive and resistant breast cancer cells. *Br. J. Cancer* 102, 342–350. doi:10.1038/sj.bjc.6605479

Joyce, J. A., and Fearon, D. T. (2015). T cell exclusion, immune privilege, and the tumor microenvironment. *Science* 348, 74–80. doi:10.1126/science.aaa6204

Keller, Peter, Mazo, Irina, Gao, Yun, Reddy, Vijayapal, Caballero, Francisco, Kazer, Sam, et al. (2021). 861 Reprogramming regulatory T cells (Treg) using a MALT1 inhibitor for cancer therapy. *J. Immunother. Cancer* 9, A902. doi:10.1136/jitc-2021-SITC2021.861

Klomp, J. E., Klomp, J. A., and Der, C. J. (2021). The ERK mitogen-activated protein kinase signaling network: The final frontier in RAS signal transduction. *Biochem. Soc. Trans.* 49, 253–267. doi:10.1042/BST20200507

Liu, H., Yao, S., Dann, S. M., Qin, H., Elson, C. O., Cong, Y., et al. (2013). ERK differentially regulates Th17- and Treg-cell development and contributes to the pathogenesis of colitis. *Eur. J. Immunol.* 43, 1716–1726. doi:10.1002/eji.201242889

Liu, X., Hu, H., Fan, H., Zuo, D., Shou, Z., Liao, Y., et al. (2017). The role of STAT3 and AhR in the differentiation of CD4+ T cells into Th17 and Treg cells. *Med. Baltim.* 96 (17), e6615. doi:10.1097/MD.0000000000006615

Luo, L. H., Rao, L., Luo, L. F., Chen, K., Ran, R. Z., Liu, X. L., et al. (2020). Long non-coding RNA NKILA inhibited angiogenesis of breast cancer through NF-κB/IL-6 signaling pathway. *Microvasc. Res.* 129, 103968. doi:10.1016/j.mvr.2019.103968

Ma, Y., Liao, Z., Xu, Y., Zhong, Z., Wang, X., Zhang, F., et al. (2014). Characteristics of CARMA1-BCL10-MALT1-A20-NF-κB expression in T cell-acute lymphocytic leukemia. *Eur. J. Med. Res.* 19, 62. doi:10.1186/s40001-014-0062-8

Miska, J., Lee-Chang, C., Rashidi, A., Muroski, M. E., Chang, A. L., Lopez-Rosas, A., et al. (2019). HIF-1α is a metabolic switch between glycolytic-driven migration and oxidative phosphorylation-driven immunosuppression of Tregs in glioblastoma. *Cell. Rep.* 27, 226–237. e4. doi:10.1016/j.celrep.2019.03.029

Nakayama, K., Frew, I. J., Hagensen, M., Skals, M., Habelhah, H., Bhoumik, A., et al. (2004). Siah2 regulates stability of prolyl-hydroxylases, controls HIF1alpha abundance, and modulates physiological responses to hypoxia. *Cell.* 117, 941–952. doi:10.1016/j.cell.2004.06.001

Negri, S., Faris, P., Berra-Romani, R., Guerra, G., and Moccia, F. (2019). Endothelial transient receptor potential channels and vascular remodeling: Extracellular Ca(2 +) entry for angiogenesis, arteriogenesis and vasculogenesis. *Front. Physiol.* 10, 1618. doi:10.3389/fphys.2019.01618

Oruganti, S. R., Torres, D. J., Krebsbach, S., Asperti-Boursin, F., Winters, J., Matlawska-Wasowska, K., et al. (2017). CARMA1 is a novel regulator of T-ALL disease and leukemic cell migration to the CNS. *Leukemia* 31, 255–258. doi:10.1038/leu.2016.272

Pietrzak, J., Spickett, C. M., Ploszaj, T., Virag, L., and Robaszkiewicz, A. (2018). PARP1 promoter links cell cycle progression with adaptation to oxidative environment. *Redox Biol.* 18, 1–5. doi:10.1016/j.redox.2018.05.017

Pucino, V., De Rosa, V., Procaccini, C., and Matarese, G. (2014). Regulatory T cells, leptin and angiogenesis. *Chem. Immunol. Allergy* 99, 155–169. doi:10.1159/000353557

Qi, Tongbing, Fu, Juan, Zhang, Wen, Cui, Weitong, Xu, Xiaosong, Yue, Jianmei, et al. (2020). Mutation of PD-1 immune receptor tyrosine-based switch motif (ITSM) enhances the antitumor activity of cytotoxic T cells. *Transl. Cancer Res.* 9, 6811–6819. doi:10.21037/tcr-20-2118

Rosenbaum, M., Gewies, A., Pechloff, K., Heuser, C., Engleitner, T., Gehring, T., et al. (2019). Bcl10-controlled Malt1 paracaspase activity is key for the immune suppressive function of regulatory T cells. *Nat. Commun.* 10, 2352. doi:10.1038/s41467-019-10203-2

Sakaguchi, S., Miyara, M., Costantino, C. M., and Hafler, D. A. (2010). FOXP3+ regulatory T cells in the human immune system. *Nat. Rev. Immunol.* 10, 490–500. doi:10.1038/nri2785

Schaefer, B. C. (2020). The CBM complex: A growing multiplicity of cellular functions, regulatory mechanisms and connections to human disease. *Cell. Immunol.* 356, 104189. doi:10.1016/j.cellimm.2020.104189

Scortegagna, M., Hockemeyer, K., Dolgalev, I., Pozniak, J., Rambow, F., Li, Y., et al. (2020). Siah2 control of T-regulatory cells limits anti-tumor immunity. *Nat. Commun.* 11, 99. doi:10.1038/s41467-019-13826-7

Takeuchi, Y., and Nishikawa, H. (2016). Roles of regulatory T cells in cancer immunity. *Int. Immunol.* 28, 401–409. doi:10.1093/intimm/dxw025

Tetu, P., Vercellino, L., Reger de Moura, C., Baroudjian, B., Dumaz, N., Mourah, S., et al. (2021). Mitogen-activated protein kinase blockade in melanoma: Intermittent versus continuous therapy, from preclinical to clinical data. *Curr. Opin. Oncol.* 33, 127–132. doi:10.1097/CCO.0000000000000706

Turley, S. J., Cremasco, V., and Astarita, J. L. (2015). Immunological hallmarks of stromal cells in the tumour microenvironment. *Nat. Rev. Immunol.* 15, 669–682. doi:10.1038/nri3902

Vasilev, G., Ivanova, M., Ivanova-Todorova, E., Tumangelova-Yuzeir, K., Krasimirova, E., Stoilov, R., et al. (2019). Secretory factors produced by adipose mesenchymal stem cells downregulate Th17 and increase Treg cells in peripheral blood mononuclear cells from rheumatoid arthritis patients. *Rheumatol. Int.* 39, 819–826. doi:10.1007/s00296-019-04296-7

Vaupel, P., and Multhoff, G. (2018). Hypoxia-/HIF-1α-Driven factors of the tumor microenvironment impeding antitumor immune responses and promoting malignant progression. *Adv. Exp. Med. Biol.* 1072, 171–175. doi:10.1007/978-3-319-91287-5_27

Wang, J., Shi, W., Miao, Y., Gan, J., Guan, Q., Ran, J., et al. (2021). Evaluation of tumor microenvironmental immune regulation and prognostic in lung adenocarcinoma from the perspective of purinergic receptor P2Y13. *Bioengineered* 12, 6286–6304. doi:10.1080/21655979.2021.1971029

Wang, W., Liu, Q. B., and Jing, W. (2019). Astragalus membranaceus improves therapeutic efficacy of asthmatic children by regulating the balance of Treg/Th17 cells. *Chin. J. Nat. Med.* 17, 252–263. doi:10.1016/S1875-5364(19)30029-9

Wang, Y., and Fuller, G. M. (1994). Phosphorylation and internalization of gp130 occur after IL-6 activation of Jak2 kinase in hepatocytes. *Mol. Biol. Cell.* 5, 819–828. doi:10.1091/mbc.5.7.819

Westendorf, A., Skibbe, K., Adamczyk, A., Buer, J., Geffers, R., Hansen, W., et al. (2017). Hypoxia enhances immunosuppression by inhibiting CD4+ effector T cell function and promoting Treg activity. *Cell. Physiol. Biochem.* 41, 1271–1284. doi:10.1159/000464429

Wicherska-Pawlowska, K., Wrobel, T., and Rybka, J. (2021). Toll-like receptors (TLRs), NOD-like receptors (NLRs), and RIG-I-like receptors (RLRs) in innate immunity. TLRs, NLRs, and RLRs ligands as immunotherapeutic agents for hematopoietic diseases. *Int. J. Mol. Sci.* 22, 13397. doi:10.3390/ijms222413397

Wu, M., Chen, X., Lou, J., Zhang, S., Zhang, X., Huang, L., et al. (2016). TGF-β1 contributes to CD8+ Treg induction through p38 MAPK signaling in ovarian cancer microenvironment. *Oncotarget* 7, 44534–44544. doi:10.18632/oncotarget.10003

Yan, Shijia, Sun, Lei, and Wan, Guohui (2019). The mechanism and research progress of drug resistance of PD-1/PD-L1 immunotherapy in tumors. *Acta Pharm. Sin.* 54, 1728–1734.

Yang, D., Zhao, X., and Lin, X. (2021). Bcl10 is required for the development and suppressive function of Foxp3(+) regulatory T cells. *Cell. Mol. Immunol.* 18, 206–218. doi:10.1038/s41423-019-0297-y

Yeh, H., Moore, D. J., Markmann, J. F., and Kim, J. I. (2013). Mechanisms of regulatory T cell counter-regulation by innate immunity. *Transpl. Rev.* 27, 61–64. doi:10.1016/j.trre.2013.02.001

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Mechanisms of CAR T cell exhaustion and current counteraction strategies

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The functional state of chimeric antigen receptor T (CAR T) cells determines their efficacy *in vivo*. Exhausted CAR T cells exhibit decreased proliferative capacity, impaired anti-tumor activity, and attenuated persistence. CAR T cell exhaustion has been recognized as a vital cause of nonresponse and relapse after CAR T cell therapy. However, the triggers and mechanisms leading to CAR T cell exhaustion remain blurry and complicated. Therefore, it is essential to clear the regulation network of CAR T cell exhaustion and explore potent solutions. Here, we review the diverse inducers of CAR T cell exhaustion in terms of manufacture process and immunosuppressive tumor microenvironment. In addition to the admitted immune checkpoint blockade, we also describe promising strategies that may reverse CAR T cell exhaustion including targeting the tumor microenvironment, epigenetics and transcriptomics.

KEYWORDS

CAR T cell exhaustion, tonic signaling, cytokines, tumor microenvironment, immune checkpoint blockade, epigenetics, transcriptomics

Introduction

Despite the tremendous efficacy of chimeric antigen receptor T (CAR T) cells in hematological malignancies such as B-cell acute lymphoblastic leukemia (B-ALL), B-cell lymphoma, and multiple myeloma, as well as in solid tumors, CAR T cell exhaustion remained a main obstacle to achieve remission (Fraietta et al., 2018; Holstein and Lunning, 2019; Marofi et al., 2021). T cell exhaustion was a dysfunctional state of T cells that usually observed in chronic infections and tumors (Hashimoto et al., 2018). It was first identified as a population of $CD8^+$ T cells without effector function which appeared during chronic lymphocytic choriomeningitis virus infection in mice, and the persistence of this cell population led to viral immune escape (Zajac et al., 1998). At the same time, Gallimore and colleagues also observed a similar phenomenon in the chronic lymphocytic choriomeningitis virus infection, which they defined as T cell exhaustion (Gallimore et al., 1998). T cell exhaustion was characterized by progressive loss of effector function, co-expression of multiple inhibitory receptors, and exaggerated effector cell differentiation. Besides, the exhausted T cells had distinct transcriptional, epigenetic, and metabolic signatures (Doering et al., 2012; Schietinger and Greenberg, 2014; Philip et al., 2017).

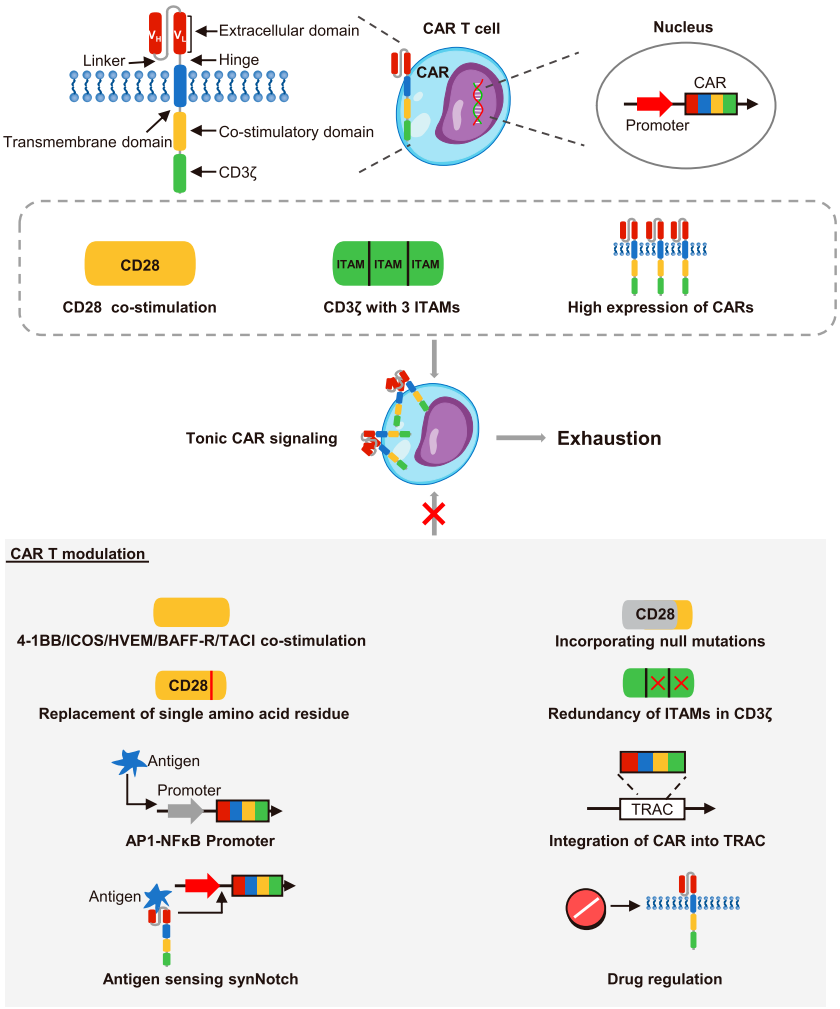

FIGURE 1
The effects of CARs structure on CAR T cell exhaustion. The CARs contained an extracellular domain, a hinge region, a transmembrane domain, a co-stimulatory domain and a CD3ζ stimulatory domain. The extracellular domain was composed of the variable heavy chain (V_H) and the variable light chain (V_L) connected by a linker. The upstream promoter initiated the strong expression of CARs on the cell surface. However, the CD3ζ with 3 immunoreceptor tyrosine-based activation motifs (ITAMs), CD28 co-stimulation and constitutively high expression of CARs could cause tonic CAR signaling and promote cell exhaustion. However, the 4-1BB, ICOS, HVEM, BAFF-R, and TACI co-stimulation reduced the exhaustion of CAR T cells. Both incorporation of null mutations and replacement of single amino acid residue in CD28 co-stimulation could inhibit exhaustion. Redundancy of ITAMs could also inhibit exhaustion. Besides, the synthetic activator protein 1-nuclear factor κB (AP1-NF-κB) promoter, the T cell receptor alpha constant locus (TRAC) that contained CAR structure, the synNotch-CAR circuits, and drug regulation could all restrain the CARs expression. Then the ligand-independent tonic signaling and exhaustion were thus reduced.

Abundant clinical practice and research revealed that CAR T cell exhaustion ultimately contributed to the failure of CAR T cell therapy. For instance, Fraietta et al. performed transcriptomic sequencing and functional assessment of CAR T cells from 41 patients with chronic lymphocytic leukemia (CLL). They identified that CAR T cells in responders possessed memory-like characteristics, while CAR T cells in non-responders were in a highly exhaustion state (Fraietta et al., 2018). Consistent with this, other clinical studies on B-ALL and B-cell lymphoma also indicated that CAR T cell exhaustion was related to the treatment failure (Schuster et al., 2017; Finney et al., 2019; Deng et al., 2020). Therefore, the explorations focused on the mechanisms of CAR T cell exhaustion may provide potential insights on preventing CAR T cell exhaustion and improving CAR T cell efficacy.

In this article, we reviewed the underlying regulatory factors of CAR T cell exhaustion that existed in design, generation and infusion process. We also discussed the current strategies to combat CAR T cell exhaustion including blocking immune checkpoint, resisting the tumor microenvironment (TME), and regulating epigenetic and transcriptomic profiles.

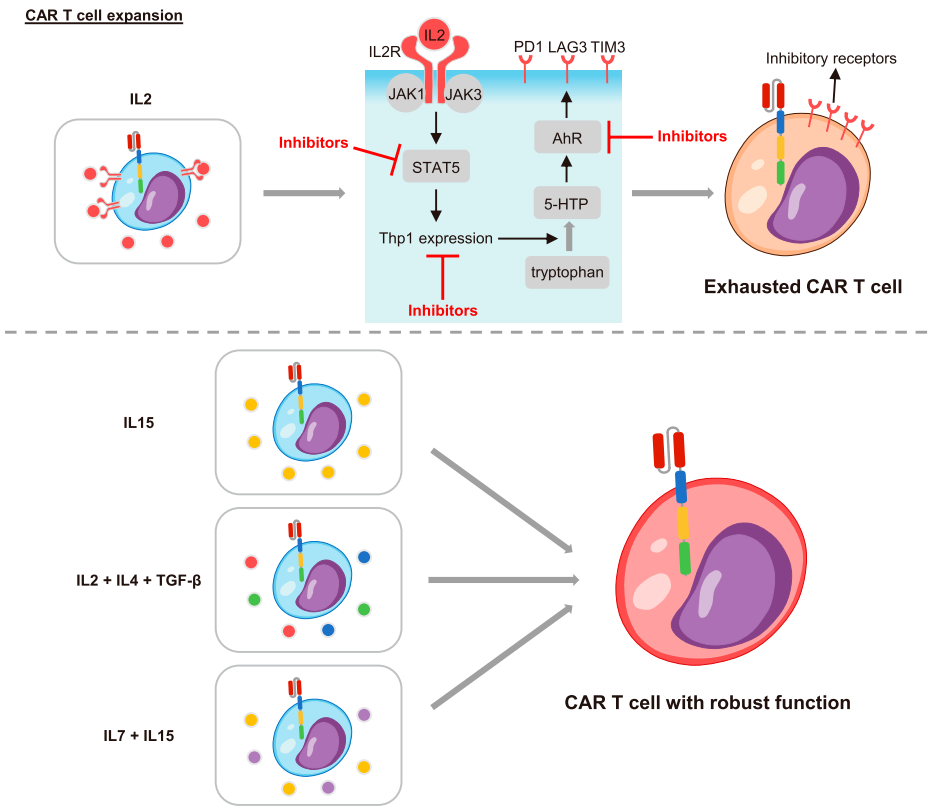

FIGURE 2
The effects of cytokines on CAR T cell exhaustion. The cytokine IL2 promoted CAR T cell exhaustion through the IL2 receptor (IL2R). The combination of IL2 and IL2R led to the recruitment and activation of the Janus family of tyrosine kinases (JAK1 and JAK3). Then the signal transducer and activator of transcription 5 (STAT5) was activated and further induced the expression of tryptophan hydroxylase 1 (Tph1). The Thp1 catalyzed tryptophan to produce 5-hydroxytryptophan (5-HTP), which activated the aryl hydrocarbon receptor (AhR) and led to T cell exhaustion. Inhibitors of STAT5, Thp1 or AhR could all restrain T cell exhaustion. Importantly, the cytokine IL15, IL2 supplemented with IL4/TGF-β, and IL15/IL7 improved the exhaustion. CAR T cells under these culture conditions manifested enhanced persistence and function.

CAR design affects CAR T cell exhaustion

The second generation of CARs approved by the US Food and Drug Administration contained an antigen binding domain, a hinge region, a transmembrane domain, an intracellular co-stimulatory domain and a signal transduction domain (Zhao et al., 2018). CAR molecules were activated upon stimulation by an antigen, which triggered the effector response (Salter et al., 2021). Nevertheless, undesirable CAR design would lead to suboptimal T cell activation and ligand-independent tonic signaling (Jayaraman et al., 2020). Long and colleagues described that GD2, CD22, or ErbB2 CAR T cells contained a CD28-CD3ζ intracellular domain tended to be exhausted during early expansion *in vitro*, but CD19.28z CAR T cells did not. This was due to the tonic CAR signaling induced by aggregation of single-chain variable fragments (scFv) on the surface (Figure 1). The co-stimulatory domain was also involved in tonic CAR signaling, with CD28 co-stimulation enhancing while 4-1BB co-stimulation reducing the tonic CAR signaling and exhaustion (Long et al., 2015). Intriguingly, it was proved that introduction of null mutations in the CD28 domain would down-regulate exhaustion-related genes and improve the anti-tumor function of CD19 CAR T cells *in vitro* and *in vivo* (Boucher et al., 2021). Similarly, ICOS co-stimulation also had an advantage over CD28 co-stimulation in averting exhaustion. Indeed, CD28 co-stimulation and ICOS co-stimulation had a shared motif but differed only in a single amino acid residue. Guedan et al. observed that this single amino acid residue asparagine in CD28 was related to the exhaustion of CAR T cells, and replacing asparagine with phenylalanine reduced CAR T cell differentiation and exhaustion in xenograft tumor models (Guedan et al., 2020). Moreover, Nunoya and colleagues evaluated the feature of CAR T cells with different co-stimulatory domain *in vitro*, and they identified that the co-stimulatory domain derived from herpes virus entry mediator (HVEM) was favorable for a low-level of exhaustion (Nunoya et al., 2019). More recently, a report using CAR pooling to evaluate

the propensity to resist exhaustion of CAR T cells during repetitive antigen stimulation. It was revealed that B cell-activating factor receptor (BAFF-R) and transmembrane activator and CAML interactor (TACI) delayed the exhaustion (Goodman et al., 2022). In addition to the co-stimulatory domain, signaling of the intracellular domain of CD3ζ also affected CAR T cell exhaustion. Using the xenograft model, Feucht et al. showed CD19 CAR T cells retaining only a single membrane-proximal immunoreceptor tyrosine-based activation motif (ITAM) had lower exhaustion levels and stronger persistence compared with conventional CAR T cells that had 3 ITAMs (Feucht et al., 2019).

Another important factor to consider when designing CAR T cells was the expression level of the CARs. In fact, the expression of T cell receptors (TCRs) was strictly controlled by the CD45 molecules and co-inhibitory receptors after initiating activation, but CARs typically contained a strong constitutive promoter to ensure their long-term and stable expression in T cells (Harris and Kranz, 2016; Wu et al., 2020). However, it had been recently reported that high expression of CAR molecules acted as a critical inducer of tonic CAR signaling and exhaustion (Figure 1). To disrupt constitutively expressed CARs, Webster and colleagues constructed the synthetic activator protein 1 (AP1)-nuclear factor κB (NFκB) promoter, which reduced the CAR expression at rest and drove the CAR expression under antigen stimulation. Consequently, AP1-NFκB promoted CAR T cells showed limited ligand-independent tonic signaling and exhaustion in preclinical models (Webster et al., 2021). In another attempt, Eyquem et al. designed CAR T cells through integrating the CD19 CAR into the T cell receptor alpha constant locus. These CAR T cells enabled CAR molecules to be low expressed but dynamically regulated under tumor antigen stimulation, thereby they could control tonic CAR signaling in the absence of antigen and avoid exhaustion *in vitro* (Eyquem et al., 2017). Recently, T cells with synthetic Notch (synNotch) receptors were developed to improve the specificity of CAR T cells. These CAR T cells with synNotch-CAR circuits activated the CAR expression only when they recognized the synNotch antigen (Srivastava et al., 2019). Impressively, this structure was showed to effectively prevent tonic signaling and enable CAR T cells to maintain memory phenotype and resist exhaustion in mouse models (Hyrenius-Wittsten et al., 2021). Besides, Weber et al. demonstrated that incorporating an FK506 binding protein 12 destabilizing domain into GD2 CAR structure would make the expression of CAR subject to the drug shield-1, this drug-regulatable system reversed the exhausted state of CAR T cells *in vitro* and in preclinical models. They also described that dasatinib which had been reported to reversibly inhibit TCR and CAR signaling had similar effects in reversing CAR T cells exhaustion (Weber et al., 2021). This study provided an extremely promising example for modulating the activation of CAR T cells to control their efficacy and exhaustion in clinical practice. The pharmacologic on/off switch may become a viable approach to steer the functional state of CAR T cells *in vivo*. Of note, Huang et al. initiated a clinical research (NCT04603872) to investigate the combination effects of dasatinib on *in vitro* manufacturing and *in vivo* infusion of CAR T cells.

Overall, the structure of CAR T cells was closely related to their functional characteristics. The component elements including scFv molecules, costimulatory domains and intracellular signaling domains all had complex regulatory effects on the activation and exhaustion. Optimizing the structure and regulating the expression pattern of CARs were feasible to weaken the tonic CAR signaling and prevent the exhaustion.

The *in vitro* expansion condition affects CAR T cell exhaustion

In order to obtain sufficient CAR T cell numbers, CAR T cells needed to be expanded *in vitro* before infused into the patient. There was a growing body of evidence that the culture system also affected the exhaustion of CAR T cells (Figure 2). The cytokine IL2, which was commonly added to the expansion system would cause CAR T cell exhaustion and reduce their *in vivo* persistence. However, CAR T cells expanded under cytokine IL15 or IL2 plus IL4 and TGF-β maintained a less differentiated phenotype. They exhibited reduced expression of inhibitory receptors and strengthened antitumor efficacy *in vitro* and *in vivo* (Gattinoni et al., 2005; Alizadeh et al., 2019; Liu et al., 2020). Consistently, Giuffrida et al. described that IL15 promoted the production of central memory T cells and up-regulated memory-related genes including TCF7. The persistence and response to adjuvant anti-PD1 therapy were thus significantly enhanced in preclinical models (Giuffrida et al., 2020). Similarly, addition of other cytokines such as IL4, IL7 and IL21 also supported the stemness of CAR T cells and restrained the exhaustion (Ptáčková et al., 2018). Based on these, the clinical research to ascertain the efficacy of IL15 plus IL7 and IL15 plus IL21 were initiated (NCT04186520, NCT04715191). Moreover, it was recently reported that the exhaustion level of CAR T cells increased with the prolongation of expansion time *in vitro*. The "younger" cells with shorter culture time manifested lowered exhaustion markers and enhanced long-term killing function *in vivo* (Caruso et al., 2019). In summary, the *in vitro* expansion condition greatly impacted the differentiation and exhaustion transition of CAR T cells. It was extremely important to modulate the cytokines and expansion time to obtain CAR T cells with optimal phenotypes and healthy states.

The TME promotes CAR T cell exhaustion

Recently, it was reported that the TME promoted by malignant cells played an important role in tumor development and immune regulation (DeBerardinis, 2020). The hallmarks of the TME including diverse cells such as tumor cells, immune cells and stromal cells, as

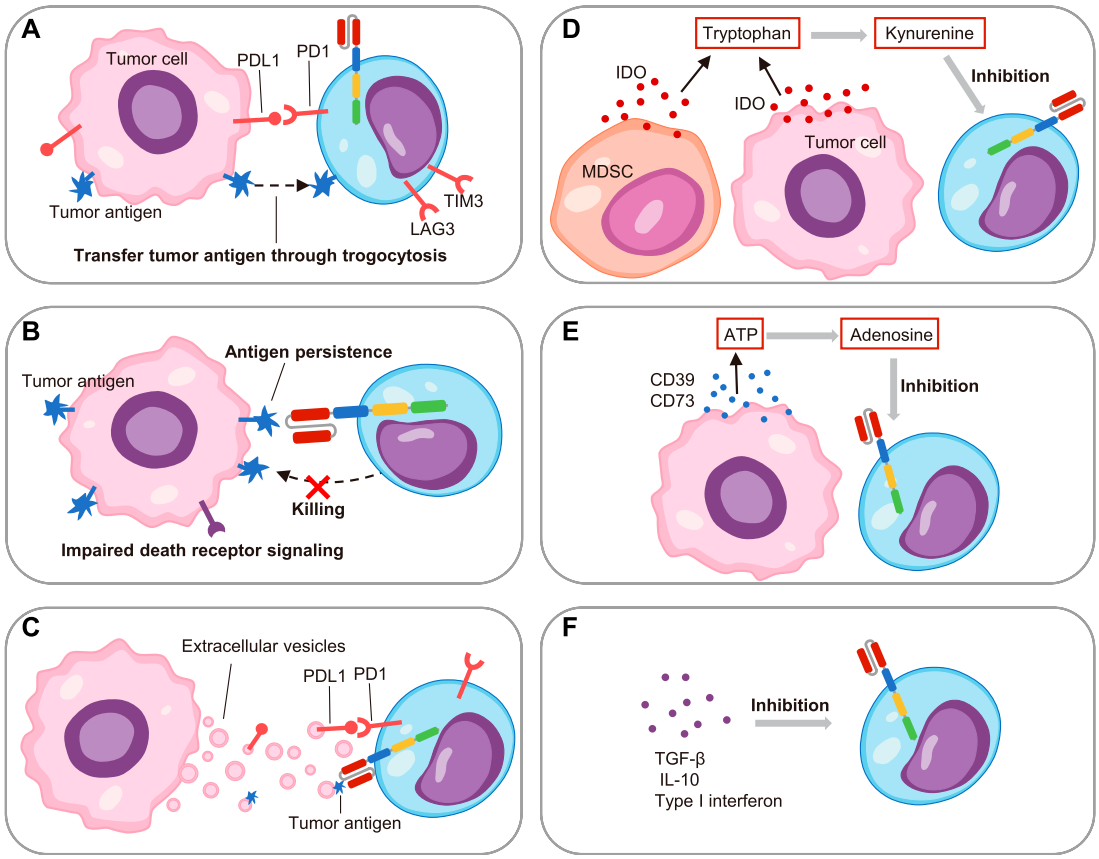

FIGURE 3
The factors promoting CAR T cell exhaustion in the tumor microenvironment.**(A)** On one hand, the PDL1 on tumor cells induced CAR T cell exhaustion through PD1/PDL1 pathway; on the other hand, the tumor antigen transferred to CAR T cells through trogocytosis and caused exhaustion. **(B)** The impaired death receptor signaling of leukemia cells led to their resistance to CAR T cells, and the persistent antigen in turn led to CAR T cell exhaustion. **(C)** The extracellular vesicles secreted by leukemia cells induced CAR T cell exhaustion through PDL1 and tumor antigen. **(D)** The myeloid-derived suppressor cells (MDSC) and leukemia cells secreted indoleamine 2,3-dioxygenase (IDO), which catalyzed tryptophan to kynurenine to suppress CAR T cell function. **(E)** The CD39 and CD73 released by tumor cells hydrolyzed ATP to adenosine, which inhibited CAR T cell activity. **(F)** Soluble factors including TGF-β, IL-10 and type I interferon restrained CAR T cell activity.

well as soluble factors such as cytokines, metabolites and extracellular vesicles (EVs) (Anderson and Simon, 2020). These complex components exerted intricate regulatory effects on CAR T cells (Figure 3).

As the pivot, tumor cells induced CAR T cell exhaustion versatilely. For example, the multiple immune checkpoint ligands such as PDL1 on tumor cells interacted with their corresponding receptors on CAR T cells and resulted in cell exhaustion (Wang et al., 2019). Additionally, it was reported that the targeted antigen on the ALL cells would be transferred to CAR T cells through trogocytosis in xenograft mouse models, which promoted the expression of PD1, LAG3 and TIM3. Intriguingly, this phenomenon also caused the fratricide of CAR T cells (Hamieh et al., 2019). More recently, Singh et al. showed the impaired death receptor signaling in ALL cells caused the resistance to cytotoxicity of T cells, and thus the persistence of antigens induced functional impairment of CAR T cells in turn. Impressively, they observed that the inherent dysregulation of death receptor signaling in ALL cells directly contributed to CAR T cell exhaustion and correlated with clinical outcomes (Singh et al., 2020). Although CD19 CAR T cells were applied to treat CLL, B-ALL, and B-cell lymphoma, they were less effective in CLL. An explanation for this discrepancy was that the imbalance between the suppressive TME and immune cells (Yoon et al., 2018). Jitschin and colleagues observed that the myeloid-derived suppressor cells (MDSC) were significantly increased in the peripheral blood of CLL patients. On one hand, this MDSC subset promoted the regulatory T cells (Treg). On the other hand, MDSC secreted high levels of indoleamine 2, 3-dioxygenase (IDO) to metabolize tryptophan to limit T cell proliferation and function (Jitschin et al., 2014). All in one, these findings highlighted the significance of the immunosuppressive cell components for inducing CAR T cell exhaustion.

As for immunosuppressive soluble factors in the TME, EVs have attracted the most attention. Cox et al. identified that the

number of EVs in patients with CLL was significantly higher than the number in normal individuals, and the same was true for PDL1 positive EVs. They further verified that PDL1 positive EVs induced CD19 CAR T cell exhaustion and attenuated effector function *in vitro* and *in vivo* (Cox et al., 2021). Our recent studies also demonstrated that EVs secreted by tumor cells such as B-ALL could induce CD19 CAR T cell exhaustion. Mechanistically, we found that B-ALL cells secreted EVs specifically carrying CD19 antigen, and thus their persistent antigenic stimulation led to exhaustion of CD19 CAR T cells (Zhu et al., 2022). In addition to EVs, tumor cells also secreted masses of metabolites to tame the TME, and consequently to regulate immunity. For instance, the AML cells in bone marrow and B-cell lymphoma cells released IDO to degrade tryptophan, resulting in local nutrient depletion while producing metabolites such as kynurenine to inhibit CAR T cell activity (Teague and Kline, 2013; Ninomiya et al., 2015). Recently, Mussai and colleagues described the AML blast in patients generated Arginase II, which consumed the arginine and led to low-arginine microenvironment. It was proved that the low-arginine conditions would cause the exhaustion and defective proliferation of CAR T cells *in vitro* (Mussai et al., 2019). Similarly, tumor cells produced ectoenzymes CD39 and CD73, which would hydrolyze ATP to generate adenosine. The adenosine further bound to adenosine 2A receptors on T cells to inhibit their function (Leone et al., 2015; Beavis et al., 2017). In addition, other soluble factors such as transforming growth factor-β (TGF-β), IL-10 and type I interferon were also important regulators of CAR T cell function in the TME (Chang et al., 2018; Stüber et al., 2019). Overall, a great deal of efforts had been devoted to the immune regulation of TME. However, the TME was extremely complex, new drivers of CAR T cell exhaustion were constantly emerging.

Strategies for combating CAR T cell exhaustion

Immune checkpoint blockade

An important hallmark of CAR T cell exhaustion was the up-regulation of multiple inhibitory receptors such as PD1, CTLA4, and LAG3. Their binding to corresponding ligands on tumor cells promoted the dysfunction of exhausted T cells and led to tumor immune escape. Cherkassky et al. proved that PD1-mediated cell exhaustion significantly inhibited CAR T cell function. Blocking the PD1-PDL1 pathway by PD1 inhibitors or endogenous blockade of PD1 expression could resist exhaustion and enhance function *in vitro* and in preclinical animal models (Cherkassky et al., 2016). Therefore, PD1 and CTLA4 inhibitors aimed at disrupting the inhibitory receptor pathway had gradually been applied for improving tumor immunity in clinical practice (Kon and Benhar, 2019; Liu, 2019). Cao et al. evaluated the efficacy of combination of CD19 CAR T cells with anti-PD1 antibody (nivolumab) in patients with refractory/relapsed B-cell non-Hodgkin lymphoma (Table 1). Encouragingly, they observed that complete response could be achieved in 45.45% of patients (Cao et al., 2019). Similarly, one recent study reported that the PD1 inhibitor, pembrolizumab, achieved complete remission or partial remission in 25% of patients with B-cell lymphoma refractory to and/or relapsed after CD19 CAR T cells treatment. Immune analysis revealed the patients who achieved remission had mild CAR T cell exhaustion, which indicating that PD1 inhibitors had certain benefits in improving exhaustion and enhancing the efficacy of CD19 CAR T cells (Chong et al., 2022). Meanwhile, administration of CAR T cells with nivolumab and ipilimumab (CTLA-4 inhibitor) was also being tested in clinical trials (NCT04003649).

In addition to PD1 and CTLA4 inhibitors, modifying CAR T cells to block immune checkpoint pathways had also been investigated intensively. For example, Pan and his colleagues introduced a fusion protein consisting of the extracellular domain of PD1 and CH3 from IgG4 into GPC3 CAR T cells to construct CAR T cells secreting a soluble PD1 protein. They found these CAR T cells were protected from exhaustion when stimulated by target cells *in vitro* and in murine xenogeneic models (Pan et al., 2018). Furthermore, Zou et al. demonstrated simultaneously downregulating three checkpoint receptors PD1, TIM3, and LAG3 could improve the exhaustion of CAR T cells through upregulation of CD56 in xenogenic mouse models (Zou et al., 2019). In line with this, a recent study described that simultaneous down-regulation of PD1 and TIGIT exerted a synergistic anti-tumor effect, the down-regulation of TIGIT was mainly responsible for maintaining the low-differentiation and low-exhaustion state of CAR T cells, while the down-regulation of PD1 enhanced the short-term cytotoxicity of CAR T cells *in vitro* functional assays (Lee et al., 2022). In summary, immune checkpoint blockade had achieved certain successes in preclinical and clinical practice, which was also the mainstream method for resisting CAR T cell exhaustion currently.

Combating the TME

With the clarification of the complex role of TME, new strategies targeting TME to improve the efficacy of CAR T cells had proliferated recently (Table 1). In terms of the excessive adenosine in the TME, the adenosine 2A receptor on CAR T cells was responsible for mediating its immunosuppressive effects. Accordingly, it was reported that using the adenosine 2A receptor antagonists and knockout of receptors in CAR T cells improved exhaustion and function in preclinical mouse models (Beavis et al., 2017; Li et al., 2020). Besides, to confront adenosine, immune cells could also catabolize it into inosine through adenosine deaminase 1 (ADA). Therefore, Qu et al.

TABLE 1 Strategies for combating CAR T cell exhaustion.

Strategies	Targets	References
Anti-PD1 antibody, CTLA-4 inhibitor	PD1-PDL1,CTLA-4	(Cao et al., 2019; Chong et al., 2022),NCT04003649
Soluble PD1-CH3 fusion protein	PD1-PDL1	Pan et al. (2018)
Downregulating immune checkpoint	TIGIT, PD1,TIM3, LAG3	Zou et al. (2019); Lee et al. (2022)
A2AR knockout, A2AR antagonists,ADA-overexpression	Adenosine	Beavis et al. (2017); Li et al. (2020); Qu et al. (2022)
ASS/OTC expression, Inhibiting arginine metabolism	Low-arginine	Mussai et al. (2019); Fultang et al. (2020)
DnTGF-βRII co-expression,TGF-βRII knockout, Bispecific trap protein	TGF-β	Kloss et al. (2018); Tang et al. (2020); Chen et al. (2021)
CD4 CAR T cell, IL7 co-expression		Agarwal et al. (2020); He et al. (2020)
DNMT3A knockout, Decitabine	Remodeled methylation programs	You et al. (2020); Wang et al. (2021b); Prinzing et al. (2021)
		NCT04697940
		NCT04850560
		NCT04553393
BET protein blockade	CAR silencing	Kong et al. (2021)
NR4A triple knockout	NR4A TFs	Chen et al. (2019)
TOX and TOX2 knockout	TOX and TOX2	Seo et al. (2019)
c-Jun-overexpression	AP1 TFs	Lynn et al. (2019)
Inhibiting HPK1	HPK1	Si et al. (2020)
TLE2 and IKZF2 knockout	TLE2 and IKZF2	Wang et al. (2021a)

engineered ADA-overexpressing CAR T cells and proved that they had an enhanced ability to resist exhaustion *in vitro* and *in vivo* (Qu et al., 2022).

As for the arginine, it was recognized that endogenous production of arginine was mainly dependent on argininosuccinate synthetase (ASS) enzymes and ornithine transcarbamylase (OTC). However, the expression of ASS and OTC in T cells was low, so they were dependent on exogenous arginine and were sensitive to low-arginine microenvironment. Encouragingly, inhibiting the arginine metabolism or engineering CAR T cells to express functional ASS and OTC could both significantly enhance the function (Mussai et al., 2019; Fultang et al., 2020).

To overcome the excess TGF-β in the TME, Kloss et al. blocked TGF-β signaling by co-expressing a dominant-negative TGF-β receptor II (TGF-βRII) in PSMA CAR T cells targeting prostate cancer. Tang et al. used CRISPR/Cas9 technology to knockout endogenous TGF-βRII in CAR T cells. Both these modifications prevented CAR T cell exhaustion and enhanced anti-tumor effects in tumor models (Kloss et al., 2018; Tang et al., 2020). And there were abundant agents that were being tested in clinical trials including TGF-β targeted neutralizing antibodies, vaccines, antisense oligonucleotides, and small molecule inhibitors. Of particular interest, a mass of research devoted to developing agents that dual-targeting TGF-β and PDL1 molecules. Especially the bintrafusp alfa, a bifunctional fusion protein targeting TGF-β and PD-L1 (Gulley et al., 2022). More recently, Chen and his team designed CAR T cells with a bispecific trap protein structure that secreted trap proteins, which could simultaneously target TGF-β and PD1. Such CAR T cells significantly attenuated suppressive T cell signaling and resisted exhaustion *in vitro* and *in vivo* (Chen et al., 2021).

Furthermore, in order to clear the ability of different types of CAR T cells to resist exhaustion in the TME, one study used CD4 or CD8-targeted lentivirus to generate CD4 CAR T cells or CD8 CAR T cells *in vivo*, respectively. It was showed that the anti-tumor efficacy of CD4 CAR T cells alone was better than that of CD8 CAR T cells alone or mixture of CD4 and CD8 CAR T cells, because CD8 CAR T cells were more prone to exhaustion under high tumor burden. This report suggested that the phenotype could be adjusted to obtain CAR T cells that were resistant to exhaustion *in vivo* (Agarwal et al., 2020). In addition, promoting the secretion of cytokines could also resist CAR T cell exhaustion caused by the TME. For example, co-expression of IL7 gene enabled CAR T cells to produce IL7 under antigen stimulation, which promoted CAR T cell proliferation and inhibited CAR T cell exhaustion and apoptosis. These CAR T cells exhibited a less differentiated phenotype with a higher proportion of central memory T cells in preclinical models (He et al., 2020). Therefore, it was feasible to engineer CAR T cells that directly counter the immune suppressors in the TME to resist exhaustion. However, the clinical efficacy and safety of these CAR T cells remained to be further observed.

Epigenetic regulation of CAR T cell exhaustion

It had been established that exhausted T cells had completely different epigenetic characteristics from effector T cells and memory T cells. For instance, exhausted T cells lacked several open chromatin regions presented in the IFNG locus in effector T cells and memory T cells. Because blocking PD1 was unable to fully remodel the epigenetics of exhausted T cells, T cells would be

re-exhausted when the antigen persisted (Pauken et al., 2016). Subsequently, Ghoneim et al. performed whole-genome sequencing of effector T cells and exhausted T cells, they identified that the acquired *de novo* methylation programs limited T cell proliferation and clonal diversity during transition to exhaustion (Ghoneim et al., 2017). In line with this, Zebley and colleagues observed CAR T cells acquired exhaustion-related methylation programs after infusion in patients with relapsed/refractory B-ALL, the genes associated with T cell memory maintenance including TCF and LEF1 were suppressed (Zebley et al., 2021). Consequently, restraining these epigenetic modifications had the potential to reverse exhaustion transition (Table 1). In a recent work, it was described that blocking *de novo* methylation programs through *de novo* DNA methyltransferase 3α (DNMT3A) knockout could overcome CAR T cell exhaustion *in vitro* and in preclinical solid tumor models (Prinzing et al., 2021). Similarly, DNA methyltransferase inhibitor, decitabine, had also been proved to reverse exhaustion-associated DNA-methylation programs. Two research teams successively demonstrated that decitabine enhanced the antitumor activity of CAR T cells both *in vitro* and *in vivo*. Through transcriptome and epigenetic sequencing, it was revealed that memory-related genes and immune synapse genes were promoted while exhaustion-related genes were suppressed (You et al., 2020; Wang Y. et al., 2021b). In addition, clinical trials of CD19 CAR T cells in relapsed/refractory CLL (NCT01029366 and NCT01747486) showed the CAR silencing was associated with CAR T treatment failure, this was caused by abnormal methylation programs of the CAR promoter. Kong and colleagues presented that epigenetic modulation by bromodomain and extra-terminal (BET) family protein blockade could reverse CAR silencing and improve CAR T cell exhaustion *in vitro* (Kong et al., 2021). Overall, these clinical and preclinical data provided evidence for the epigenetic involvement in the regulation of CAR T cell exhaustion and efficacy. In particular, the combination of decitabine with CAR T cell therapy was currently being tested in clinical trials (NCT04697940, NCT04850560 and NCT04553393). Thus, epigenetic regulation had the potential to become an important approach to reverse CAR T cell exhaustion in the future.

Transcriptomic regulation of CAR T cell exhaustion

Multiple genes and transcription factors were involved in the regulatory network of CAR T cell exhaustion. One of the most important was the transcription factor TCF1, it was a key regulator of T cell differentiation. TCF1-positive CAR T cells exhibited a less exhaustion level and enhanced persistence *in vitro* and *in vivo* (Wu et al., 2016; Zheng et al., 2021). The other key transcription factors were the nuclear factor of activated T cells (NFAT) family and NR4A nuclear receptor family (NR4A1, NR4A2 and NR4A3). They played an important role in the immune homeostasis, including maintaining the development of regulatory T cells, regulating the activation of T cells, controlling the survival of B cells and T cells when encountered antigen (Macian, 2005; Sekiya et al., 2013; Hiwa et al., 2022). However, their strong activity and redundant effects were thought to be related to T cell exhaustion. It was reported that the NFAT/NR4A axis cooperatively controlled the expression of inhibitory receptors including PD1, TIM3 and LAG3. Deleting all three NR4A transcription factors could enhance the anti-tumor effect in solid tumor models (Table 1) (Chen et al., 2019). And meanwhile, Seo and colleagues observed that the transcription factors TOX and TOX2 were also downstream targets of NFAT to prompt CAR T cell exhaustion. Disruption the expression and activity of TOX and TOX2 in CAR T cells promoted tumor regression in tumor-bearing mouse models (Seo et al., 2019). Another NFAT partner, transcription factor AP1, was also involved in the regulation of CAR T cell exhaustion. Lynn et al. described that there was an epigenetic dysregulation of AP1 in exhausted CAR T cells using an exhaustion cell model with tonic CAR signaling, they proposed that this may be associated with the immunoregulatory AP-1/IRF transcriptional complexes (Lynn et al., 2019). In addition, some genes that may regulate CAR T cell exhaustion had been gradually uncovered through genetic screening. For example, one study identified that MAP4K1 gene (encoding HPK1) was highly correlated with the expression of inhibitory receptors in a variety of cancer through database analysis, the HPK1-NFκB-Blimp1 axis may mediate CAR T cell exhaustion. (Si et al., 2020). More recently, Wang et al. screened the whole genome of glioblastoma stem cells and CAR T cells to obtain the key molecules that determine the cytotoxicity of CAR T cells. They found that genes TLE2 and IKZF2 had a suppressive effect on CAR T cells. Intervention to down-regulate the expression of these two genes could inhibit CAR T cell exhaustion and enhance function in glioblastoma mouse models (Wang D. et al., 2021a). Therefore, with the clarity of genetic changes, the intricate regulation network behind CAR T cell exhaustion gradually emerged. Although the intervention of these transcription factors and genes was still confined to *in vitro* and preclinical stage currently, these regulators provided attractive drug targets for inhibiting CAR T cell exhaustion and improving immunotherapy response. Interventions against these key factors were expected to enter clinical applications soon.

Conclusion

The mechanisms of CAR T cell exhaustion are extraordinarily complex and need to be explored in depth. First, inappropriate CAR T cell structure could induce ligand-independent tonic signaling and thus leading to CAR T cell exhaustion. Then, the cytokines and the duration of *in vitro* expansion also affect CAR T cell exhaustion. More importantly, there are abundant immunosuppressive factors in the TME. Hence, these multifaceted attacks make it difficult to reverse CAR T cell exhaustion. At present, PD1 and

CTLA4 inhibitors are the dominant agents to combat CAR T cell exhaustion in clinical practice. However, the experience using immune checkpoint inhibitors with CAR T cells is limited. Besides, exhausted CAR T cells have epigenetic remodeling as well as transcriptomic abnormalities that increase the difficulty to intervene. Therefore, designing CAR T cells that can resist exhaustion or targeting these exhaustion-inducing factors may offer the potential to improve the efficacy of CAR T cells.

Author contributions

XyZ wrote the first draft of this manuscript. QL and XjZ revised the manuscript. All authors have read and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Agarwal, S., Hanauer, J. D. S., Frank, A. M., Riechert, V., Thalheimer, F. B., and Buchholz, C. J. (2020). *In vivo* generation of CAR T cells selectively in human CD4+ lymphocytes. *Mol. Ther.* 28, 1783–1794. doi:10.1016/j.ymthe.2020.05.005

Alizadeh, D., Wong, R. A., Yang, X., Wang, D., Pecoraro, J. R., Kuo, C.-F., et al. (2019). IL15 enhances CAR-T cell antitumor activity by reducing mTORC1 activity and preserving their stem cell memory phenotype. *Cancer Immunol. Res.* 7, 759–772. doi:10.1158/2326-6066.CIR-18-0466

Anderson, N. M., and Simon, M. C. (2020). The tumor microenvironment. *Curr. Biol.* 30, R921–R925. doi:10.1016/j.cub.2020.06.081

Beavis, P. A., Henderson, M. A., Giuffrida, L., Mills, J. K., Sek, K., Cross, R. S., et al. (2017). Targeting the adenosine 2A receptor enhances chimeric antigen receptor T cell efficacy. *J. Clin. Invest.* 127, 929–941. doi:10.1172/JCI89455

Boucher, J. C., Li, G., Kotani, H., Cabral, M. L., Morrissey, D., Lee, S. B., et al. (2021). CD28 costimulatory domain-targeted mutations enhance chimeric antigen receptor T-cell function. *Cancer Immunol. Res.* 9, 62–74. doi:10.1158/2326-6066.CIR-20-0253

Cao, Y., Lu, W., Sun, R., Jin, X., Cheng, L., He, X., et al. (2019). Anti-CD19 chimeric antigen receptor T cells in combination with nivolumab are safe and effective against relapsed/refractory B-cell non-hodgkin lymphoma. *Front. Oncol.* 9, 767. doi:10.3389/fonc.2019.00767

Caruso, H. G., Tanaka, R., Liang, J., Ling, X., Sabbagh, A., Henry, V. K., et al. (2019). Shortened *ex vivo* manufacturing time of EGFRvIII-specific chimeric antigen receptor (CAR) T cells reduces immune exhaustion and enhances antiglioma therapeutic function. *J. Neurooncol.* 145, 429–439. doi:10.1007/s11060-019-03311-y

Chang, Z. L., Lorenzini, M. H., Chen, X., Tran, U., Bangayan, N. J., and Chen, Y. Y. (2018). Rewiring T-cell responses to soluble factors with chimeric antigen receptors. *Nat. Chem. Biol.* 14, 317–324. doi:10.1038/nchembio.2565

Chen, J., López-Moyado, I. F., Seo, H., Lio, C. J., Hempleman, L. J., Sekiya, T., et al. (2019). NR4A transcription factors limit CAR T cell function in solid tumours. *Nature* 567, 530–534. doi:10.1038/s41586-019-0985-x

Chen, X., Yang, S., Li, S., Qu, Y., Wang, H.-Y., Liu, J., et al. (2021). Secretion of bispecific protein of anti-PD-1 fused with TGF-β trap enhances antitumor efficacy of CAR-T cell therapy. *Mol. Ther. Oncolytics* 21, 144–157. doi:10.1016/j.omto.2021.03.014

Cherkassky, L., Morello, A., Villena-Vargas, J., Feng, Y., Dimitrov, D. S., Jones, D. R., et al. (2016). Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. *J. Clin. Invest.* 126, 3130–3144. doi:10.1172/JCI83092

Chong, E. A., Alanio, C., Svoboda, J., Nasta, S. D., Landsburg, D. J., Lacey, S. F., et al. (2022). Pembrolizumab for B-cell lymphomas relapsing after or refractory to CD19-directed CAR T-cell therapy. *Blood* 139, 1026–1038. doi:10.1182/blood.2021012634

Cox, M. J., Lucien, F., Sakemura, R., Boysen, J. C., Kim, Y., Horvei, P., et al. (2021). Leukemic extracellular vesicles induce chimeric antigen receptor T cell dysfunction in chronic lymphocytic leukemia. *Mol. Ther.* 29, 1529–1540. doi:10.1016/j.ymthe.2020.12.033

DeBerardinis, R. J. (2020). Tumor microenvironment, metabolism, and immunotherapy. *N. Engl. J. Med.* 382, 869–871. doi:10.1056/NEJMcibr1914890

Deng, Q., Han, G., Puebla-Osorio, N., Ma, M. C. J., Strati, P., Chasen, B., et al. (2020). Characteristics of anti-CD19 CAR T cell infusion products associated with efficacy and toxicity in patients with large B cell lymphomas. *Nat. Med.* 26, 1878–1887. doi:10.1038/s41591-020-1061-7

Doering, T. A., Crawford, A., Angelosanto, J. M., Paley, M. A., Ziegler, C. G., and Wherry, E. J. (2012). Network analysis reveals centrally connected genes and pathways involved in CD8+ T cell exhaustion versus memory. *Immunity* 37, 1130–1144. doi:10.1016/j.immuni.2012.08.021

Eyquem, J., Mansilla-Soto, J., Giavridis, T., van der Stegen, S. J., Hamieh, M., Cunanan, K. M., et al. (2017). Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. *Nature* 543, 113–117. doi:10.1038/nature21405

Feucht, J., Sun, J., Eyquem, J., Ho, Y.-J., Zhao, Z., Leibold, J., et al. (2019). Calibration of CAR activation potential directs alternative T cell fates and therapeutic potency. *Nat. Med.* 25, 82–88. doi:10.1038/s41591-018-0290-5

Finney, O. C., Brakke, H. M., Rawlings-Rhea, S., Hicks, R., Doolittle, D., Lopez, M., et al. (2019). CD19 CAR T cell product and disease attributes predict leukemia remission durability. *J. Clin. Invest.* 129, 2123–2132. doi:10.1172/JCI125423

Fraietta, J. A., Lacey, S. F., Orlando, E. J., Pruteanu-Malinici, I., Gohil, M., Lundh, S., et al. (2018). Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. *Nat. Med.* 24, 563–571. doi:10.1038/s41591-018-0010-1

Fultang, L., Booth, S., Yogev, O., Martins da Costa, B., Tubb, V., Panetti, S., et al. (2020). Metabolic engineering against the arginine microenvironment enhances CAR-T cell proliferation and therapeutic activity. *Blood* 136, 1155–1160. doi:10.1182/blood.2019004500

Gallimore, A., Glithero, A., Godkin, A., Tissot, A. C., Plückthun, A., Elliott, T., et al. (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. doi:10.1084/jem.187.9.1383

Gattinoni, L., Klebanoff, C. A., Palmer, D. C., Wrzesinski, C., Kerstann, K., Yu, Z., et al. (2005). Acquisition of full effector function *in vitro* paradoxically impairs the *in vivo* antitumor efficacy of adoptively transferred CD8+ T cells. *J. Clin. Invest.* 115, 1616–1626. doi:10.1172/JCI24480

Ghoneim, H. E., Fan, Y., Moustaki, A., Abdelsamed, H. A., Dash, P., Dogra, P., et al. (2017). De novo epigenetic programs inhibit PD-1 blockade-mediated T cell rejuvenation. *Cell* 170, 142–157. e19. doi:10.1016/j.cell.2017.06.007

Giuffrida, L., Sek, K., Henderson, M. A., House, I. G., Lai, J., Chen, A. X. Y., et al. (2020). IL-15 preconditioning augments CAR T cell responses to checkpoint blockade for improved treatment of solid tumors. *Mol. Ther.* 28, 2379–2393. doi:10.1016/j.ymthe.2020.07.018

Goodman, D. B., Azimi, C. S., Kearns, K., Talbot, A., Garakani, K., Garcia, J., et al. (2022). Pooled screening of CAR T cells identifies diverse immune signaling domains for next-generation immunotherapies. *Sci. Transl. Med.* 14, eabm1463. doi:10.1126/scitranslmed.abm1463

Guedan, S., Madar, A., Casado-Medrano, V., Shaw, C., Wing, A., Liu, F., et al. (2020). Single residue in CD28-costimulated CAR-T cells limits long-term persistence and antitumor durability. *J. Clin. Invest.* 130, 3087–3097. doi:10.1172/JCI133215

Gulley, J. L., Schlom, J., Barcellos-Hoff, M. H., Wang, X.-J., Seoane, J., Audhuy, F., et al. (2022). Dual inhibition of TGF-β and PD-L1: A novel approach to cancer treatment. *Mol. Oncol.* 16, 2117–2134. doi:10.1002/1878-0261.13146

Hamieh, M., Dobrin, A., Cabriolu, A., van der Stegen, S. J. C., Giavridis, T., Mansilla-Soto, J., et al. (2019). CAR T cell trogocytosis and cooperative killing regulate tumour antigen escape. *Nature* 568, 112–116. doi:10.1038/s41586-019-1054-1

Harris, D. T., and Kranz, D. M. (2016). Adoptive T cell therapies: A comparison of T cell receptors and chimeric antigen receptors. *Trends Pharmacol. Sci.* 37, 220–230. doi:10.1016/j.tips.2015.11.004

Hashimoto, M., Kamphorst, A. O., Im, S. J., Kissick, H. T., Pillai, R. N., Ramalingam, S. S., et al. (2018). CD8 T cell exhaustion in chronic infection and cancer: Opportunities for interventions. *Annu. Rev. Med.* 69, 301–318. doi:10.1146/annurev-med-012017-043208

He, C., Zhou, Y., Li, Z., Farooq, M. A., Ajmal, I., Zhang, H., et al. (2020). Co-expression of IL-7 improves nkg2d-based CAR T cell therapy on prostate cancer by enhancing the expansion and inhibiting the apoptosis and exhaustion. *Cancers* 12, 1969. doi:10.3390/cancers12071969

Hiwa, R., Brooks, J. F., Mueller, J. L., Nielsen, H. V., and Zikherman, J. (2022). NR4A nuclear receptors in T and B lymphocytes: Gatekeepers of immune tolerance. *Immunol. Rev.* 307, 116–133. doi:10.1111/imr.13072

Holstein, S. A., and Lunning, M. A. (2019). CAR T-cell therapy in hematologic malignancies: A voyage in progress. *Clin. Pharmacol. Ther.* 107, 112–122. doi:10.1002/cpt.1674

Hyrenius-Wittsten, A., Su, Y., Park, M., Garcia, J. M., Alavi, J., Perry, N., et al. (2021). SynNotch CAR circuits enhance solid tumor recognition and promote persistent antitumor activity in mouse models. *Sci. Transl. Med.* 13, eabd8836. doi:10.1126/scitranslmed.abd8836

Jayaraman, J., Mellody, M. P., Hou, A. J., Desai, R. P., Fung, A. W., Pham, A. H. T., et al. (2020). CAR-T design: Elements and their synergistic function. *EBioMedicine* 58, 102931. doi:10.1016/j.ebiom.2020.102931

Jitschin, R., Braun, M., Büttner, M., Dettmer-Wilde, K., Bricks, J., Berger, J., et al. (2014). CLL-cells induce IDO^{hi} $CD14^{+}HLA\text{-}DR^{lo}$ myeloid-derived suppressor cells that inhibit T-cell responses and promote T_{Regs}. *Blood* 124, 750–760. doi:10.1182/blood-2013-12-546416

Kloss, C. C., Lee, J., Zhang, A., Chen, F., Melenhorst, J. J., Lacey, S. F., et al. (2018). Dominant-negative TGF-β receptor enhances PSMA-targeted human CAR T cell proliferation and augments prostate cancer eradication. *Mol. Ther.* 26, 1855–1866. doi:10.1016/j.ymthe.2018.05.003

Kon, E., and Benhar, I. (2019). Immune checkpoint inhibitor combinations: Current efforts and important aspects for success. *Drug resist. updat.* 45, 13–29. doi:10.1016/j.drup.2019.07.004

Kong, W., Dimitri, A., Wang, W., Jung, I.-Y., Ott, C. J., Fasolino, M., et al. (2021). BET bromodomain protein inhibition reverses chimeric antigen receptor extinction and reinvigorates exhausted T cells in chronic lymphocytic leukemia. *J. Clin. Invest.* 131, e145459. doi:10.1172/JCI145459

Lee, Y.-H., Lee, H. J., Kim, H. C., Lee, Y., Nam, S. K., Hupperetz, C., et al. (2022). PD-1 and TIGIT downregulation distinctly affect the effector and early memory phenotypes of CD19-targeting CAR T cells. *Mol. Ther.* 30, 579–592. doi:10.1016/j.ymthe.2021.10.004

Leone, R. D., Lo, Y.-C., and Powell, J. D. (2015). A2aR antagonists: Next generation checkpoint blockade for cancer immunotherapy. *Comput. Struct. Biotechnol. J.* 13, 265–272. doi:10.1016/j.csbj.2015.03.008

Li, N., Tang, N., Cheng, C., Hu, T., Wei, X., Han, W., et al. (2020). Improving the anti-solid tumor efficacy of CAR-T cells by inhibiting adenosine signaling pathway. *Oncoimmunology* 9, 1824643. doi:10.1080/2162402X.2020.1824643

Liu, D. (2019). CAR-T "the living drugs", immune checkpoint inhibitors, and precision medicine: A new era of cancer therapy. *J. Hematol. Oncol.* 12, 113. doi:10.1186/s13045-019-0819-1

Liu, L., Bi, E., Ma, X., Xiong, W., Qian, J., Ye, L., et al. (2020). Enhanced CAR-T activity against established tumors by polarizing human T cells to secrete interleukin-9. *Nat. Commun.* 11, 5902. doi:10.1038/s41467-020-19672-2

Long, A. H., Haso, W. M., Shern, J. F., Wanhainen, K. M., Murgai, M., Ingaramo, M., et al. (2015). 4-1BB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors. *Nat. Med.* 21, 581–590. doi:10.1038/nm.3838

Lynn, R. C., Weber, E. W., Sotillo, E., Gennert, D., Xu, P., Good, Z., et al. (2019). c-Jun overexpression in CAR T cells induces exhaustion resistance. *Nature* 576, 293–300. doi:10.1038/s41586-019-1805-z

Macian, F. (2005). NFAT proteins: Key regulators of T-cell development and function. *Nat. Rev. Immunol.* 5, 472–484. doi:10.1038/nri1632

Marofi, F., Motavalli, R., Safonov, V. A., Thangavelu, L., Yumashev, A. V., Alexander, M., et al. (2021). CAR T cells in solid tumors: Challenges and opportunities. *Stem Cell Res. Ther.* 12, 81. doi:10.1186/s13287-020-02128-1

Mussai, F., Wheat, R., Sarrou, E., Booth, S., Stavrou, V., Fultang, L., et al. (2019). Targeting the arginine metabolic brake enhances immunotherapy for leukaemia. *Int. J. Cancer* 145, 2201–2208. doi:10.1002/ijc.32028

Ninomiya, S., Narala, N., Huye, L., Yagyu, S., Savoldo, B., Dotti, G., et al. (2015). Tumor indoleamine 2, 3-dioxygenase (Ido) inhibits CD19-CAR T cells and is downregulated by lymphodepleting drugs. *Blood* 125, 3905–3916. doi:10.1182/blood-2015-01-621474

Nunoya, J.-I., Masuda, M., Ye, C., and Su, L. (2019). Chimeric antigen receptor T cell bearing herpes virus entry mediator Co-stimulatory signal domain exhibits high functional potency. *Mol. Ther. Oncolytics* 14, 27–37. doi:10.1016/j.omto.2019.03.002

Pan, Z., Di, S., Shi, B., Jiang, H., Shi, Z., Liu, Y., et al. (2018). Increased antitumor activities of glypican-3-specific chimeric antigen receptor-modified T cells by coexpression of a soluble PD1–CH3 fusion protein. *Cancer Immunol. Immunother.* 67, 1621–1634. doi:10.1007/s00262-018-2221-1

Pauken, K. E., Sammons, M. A., Odorizzi, P. M., Manne, S., Godec, J., Khan, O., et al. (2016). Epigenetic stability of exhausted T cells limits durability of reinvigoration by PD-1 blockade. *Science* 354, 1160–1165. doi:10.1126/science.aaf2807

Philip, M., Fairchild, L., Sun, L., Horste, E. L., Camara, S., Shakiba, M., et al. (2017). Chromatin states define tumour-specific T cell dysfunction and reprogramming. *Nature* 545, 452–456. doi:10.1038/nature22367

Prinzing, B., Zebley, C. C., Petersen, C. T., Fan, Y., Anido, A. A., Yi, Z., et al. (2021). Deleting DNMT3A in CAR T cells prevents exhaustion and enhances antitumor activity. *Sci. Transl. Med.* 13, eabh0272. doi:10.1126/scitranslmed.abh0272

Ptáčková, P., Musil, J., Štach, M., Lesný, P., Němečková, Š., Král, V., et al. (2018). A new approach to CAR T-cell gene engineering and cultivation using piggyBac transposon in the presence of IL-4, IL-7 and IL-21. *Cytotherapy* 20, 507–520. doi:10.1016/j.jcyt.2017.10.001

Qu, Y., Dunn, Z. S., Chen, X., MacMullan, M., Cinay, G., Wang, H.-Y., et al. (2022). Adenosine deaminase 1 overexpression enhances the antitumor efficacy of chimeric antigen receptor-engineered T cells. *Hum. Gene Ther.* 33, 223–236. doi:10.1089/hum.2021.050

Salter, A. I., Rajan, A., Kennedy, J. J., Ivey, R. G., Shelby, S. A., Leung, I., et al. (2021). Phosphoproteomic analysis of chimeric antigen receptor signaling reveals kinetic and quantitative differences that affect cell function. *Sci. Signal.* 14, eaat6753. doi:10.1126/scisignal.aat6753

Schietinger, A., and Greenberg, P. D. (2014). Tolerance and exhaustion: Defining mechanisms of T cell dysfunction. *Trends Immunol.* 35, 51–60. doi:10.1016/j.it.2013.10.001

Schuster, S. J., Svoboda, J., Chong, E. A., Nasta, S. D., Mato, A. R., Anak, Ö., et al. (2017). Chimeric antigen receptor T cells in refractory B-cell lymphomas. *N. Engl. J. Med.* 377, 2545–2554. doi:10.1056/NEJMoa1708566

Sekiya, T., Kashiwagi, I., Yoshida, R., Fukaya, T., Morita, R., Kimura, A., et al. (2013). Nr4a receptors are essential for thymic regulatory T cell development and immune homeostasis. *Nat. Immunol.* 14, 230–237. doi:10.1038/ni.2520

Seo, H., Chen, J., González-Avalos, E., Samaniego-Castruita, D., Das, A., Wang, Y. H., et al. (2019). TOX and TOX2 transcription factors cooperate with NR4A transcription factors to impose CD8+ T cell exhaustion. *Proc. Natl. Acad. Sci. U. S. A.* 116, 12410–12415. doi:10.1073/pnas.1905675116

Si, J., Shi, X., Sun, S., Zou, B., Li, Y., An, D., et al. (2020). Hematopoietic progenitor Kinase1 (HPK1) mediates T cell dysfunction and is a druggable target for T cell-based immunotherapies. *Cancer Cell* 38, 551–566. e11. doi:10.1016/j.ccell.2020.08.001

Singh, N., Lee, Y. G., Shestova, O., Ravikumar, P., Hayer, K. E., Hong, S. J., et al. (2020). Impaired death receptor signaling in leukemia causes antigen-independent resistance by inducing CAR T-cell dysfunction. *Cancer Discov.* 10, 552–567. doi:10.1158/2159-8290.CD-19-0813

Srivastava, S., Salter, A. I., Liggitt, D., Yechan-Gunja, S., Sarvothama, M., Cooper, K., et al. (2019). Logic-gated ROR1 chimeric antigen receptor expression rescues T cell-mediated toxicity to normal tissues and enables selective tumor targeting. *Cancer Cell* 35, 489–503. doi:10.1016/j.ccell.2019.02.003

Stüber, T., Monjezi, R., Wallstabe, L., Kühnemundt, J., Nietzer, S. L., Dandekar, G., et al. (2019). Inhibition of TGF-β-receptor signaling augments the antitumor function of ROR1-specific CAR T-cells against triple-negative breast cancer. *J. Immunother. Cancer* 8, e000676. doi:10.1136/jitc-2020-000676

Tang, N., Cheng, C., Zhang, X., Qiao, M., Li, N., Mu, W., et al. (2020). TGF-β inhibition via CRISPR promotes the long-term efficacy of CAR T cells against solid tumors. *JCI Insight* 5, e133977. doi:10.1172/jci.insight.133977

Teague, R. M., and Kline, J. (2013). Immune evasion in acute myeloid leukemia: Current concepts and future directions. *J. Immunother. Cancer* 1, 1. doi:10.1186/2051-1426-1-13

Wang, D., Prager, B. C., Gimple, R. C., Aguilar, B., Alizadeh, D., Tang, H., et al. (2021a). CRISPR screening of CAR T cells and cancer stem cells reveals critical dependencies for cell-based therapies. *Cancer Discov.* 11, 1192–1211. doi:10.1158/2159-8290.Cd-20-1243

Wang, H., Kaur, G., Sankin, A. I., Chen, F., Guan, F., and Zang, X. (2019). Immune checkpoint blockade and CAR-T cell therapy in hematologic malignancies. *J. Hematol. Oncol.* 12, 59. doi:10.1186/s13045-019-0746-1

Wang, Y., Tong, C., Dai, H., Wu, Z., Han, X., Guo, Y., et al. (2021b). Low-dose decitabine priming endows CAR T cells with enhanced and persistent antitumour potential via epigenetic reprogramming. *Nat. Commun.* 12, 409. doi:10.1038/s41467-020-20696-x

Weber, E. W., Parker, K. R., Sotillo, E., Lynn, R. C., Anbunathan, H., Lattin, J., et al. (2021). Transient rest restores functionality in exhausted CAR-T cells through epigenetic remodeling. *Science* 372, eaba1786. doi:10.1126/science.aba1786

Webster, B., Xiong, Y., Hu, P., Wu, D., Alabanza, L., Orentas, R. J., et al. (2021). Self-driving armored CAR-T cells overcome a suppressive milieu and eradicate CD19+ Raji lymphoma in preclinical models. *Mol. Ther.* 29, 2691–2706. doi:10.1016/j.ymthe.2021.05.006

Wu, L., Wei, Q., Brzostek, J., and Gascoigne, N. R. J. (2020). Signaling from T cell receptors (TCRs) and chimeric antigen receptors (CARs) on T cells. *Cell. Mol. Immunol.* 17, 600–612. doi:10.1038/s41423-020-0470-3

Wu, T., Ji, Y., Moseman, E. A., Xu, H. C., Manglani, M., Kirby, M., et al. (2016). The TCF1-Bcl6 axis counteracts type I interferon to repress exhaustion and maintain T cell stemness. *Sci. Immunol.* 1, eaai8593. doi:10.1126/sciimmunol.aai8593

Yoon, D., Osborn, M., Tolar, J., and Kim, C. (2018). Incorporation of immune checkpoint blockade into chimeric antigen receptor T cells (CAR-Ts): Combination or built-in CAR-T. *Int. J. Mol. Sci.* 19, 340. doi:10.3390/ijms19020340

You, L., Han, Q., Zhu, L., Zhu, Y., Bao, C., Yang, C., et al. (2020). Decitabine-mediated epigenetic reprograming enhances anti-leukemia efficacy of cd123-targeted chimeric antigen receptor T-cells. *Front. Immunol.* 11, 1787. doi:10.3389/fimmu.2020.01787

Zajac, A. J., Blattman, J. N., Murali-Krishna, K., Sourdive, D. J. D., Suresh, M., Altman, J. D., et al. (1998). Viral immune evasion due to persistence of activated T cells without effector function. *J. Exp. Med.* 188, 2205–2213. doi:10.1084/jem.188.12.2205

Zebley, C. C., Brown, C., Mi, T., Fan, Y., Alli, S., Boi, S., et al. (2021). CD19-CAR T cells undergo exhaustion DNA methylation programming in patients with acute lymphoblastic leukemia. *Cell Rep.* 37, 110079. doi:10.1016/j.celrep.2021.110079

Zhao, J., Lin, Q., Song, Y., and Liu, D. (2018). Universal CARs, universal T cells, and universal CAR T cells. *J. Hematol. Oncol.* 11, 132. doi:10.1186/s13045-018-0677-2

Zheng, W., Wei, J., Zebley, C. C., Jones, L. L., Dhungana, Y., Wang, Y. D., et al. (2021). Regnase-1 suppresses TCF-1+ precursor exhausted T-cell formation to limit CAR-T-cell responses against ALL. *Blood* 138, 122–135. doi:10.1182/blood.2020009309

Zhu, X., Hu, H., Xiao, Y., Li, Q., Zhong, Z., Yang, J., et al. (2022). Tumor-derived extracellular vesicles induce invalid cytokine release and exhaustion of CD19 CAR-T Cells. *Cancer Lett.* 536, 215668. doi:10.1016/j.canlet.2022.215668

Zou, F., Lu, L., Liu, J., Xia, B., Zhang, W., Hu, Q., et al. (2019). Engineered triple inhibitory receptor resistance improves anti-tumor CAR-T cell performance via CD56. *Nat. Commun.* 10, 4109. doi:10.1038/s41467-019-11893-4

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Fundamentals to therapeutics: Epigenetic modulation of CD8$^+$ T Cell exhaustion in the tumor microenvironment

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In the setting of chronic antigen exposure in the tumor microenvironment (TME), cytotoxic CD8$^+$ T cells (CTLs) lose their immune surveillance capabilities and ability to clear tumor cells as a result of their differentiation into terminally exhausted CD8$^+$ T cells. Immune checkpoint blockade (ICB) therapies reinvigorate exhausted CD8$^+$ T cells by targeting specific inhibitory receptors, thus promoting their cytolytic activity towards tumor cells. Despite exciting results with ICB therapies, many patients with solid tumors still fail to respond to such therapies and patients who initially respond can develop resistance. Recently, through new sequencing technologies such as the assay for transposase-accessible chromatin with sequencing (ATAC-seq), epigenetics has been appreciated as a contributing factor that enforces T cell differentiation toward exhaustion in the TME. Importantly, specific epigenetic alterations and epigenetic factors have been found to control CD8$^+$ T cell exhaustion phenotypes. In this review, we will explain the background of T cell differentiation and various exhaustion states and discuss how epigenetics play an important role in these processes. Then we will outline specific epigenetic changes and certain epigenetic and transcription factors that are known to contribute to CD8$^+$ T cell exhaustion. We will also discuss the most recent methodologies that are used to study and discover such epigenetic modulations. Finally, we will explain how epigenetic reprogramming is a promising approach that might facilitate the development of novel exhausted T cell-targeting immunotherapies.

KEYWORDS

CD8$^+$ T cell, epigenetics, tumor microenvironment, TOX, SLAMF7, PD-1, HDAC, ATAC-seq

Introduction

Immunotherapy is a promising new treatment for cancer, one which harnesses a patient's own immune system to target tumor cells. These therapies aim to boost anti-tumor activity of cytotoxic immune cells, such as cytotoxic CD8$^+$ T cells and Natural Killer (NK) cells. During acute infections, naïve CD8$^+$ T cells undergo robust proliferation and clonal expansion to differentiate into effector and memory CD8$^+$ T cells. In contrast, in cancer and during chronic infections persistent antigen stimulation abrogates the development of memory T cells and T cells become exhausted. In cancer, immunotherapies attempt to enhance CD8$^+$ T cell effector function by targeting various inhibitory receptors on the cells' surfaces. For example, anti-programmed death protein 1 (PD-1) monoclonal antibodies are exceptional for PD-1 receptor inhibition, thereby promoting anti-tumor CD8$^+$ T cell activity (Hellmann et al., 2019; Hellmann and Ramalingam, 2020). Anti-PD-1 therapies such as pembrolizumab and nivolumab were first

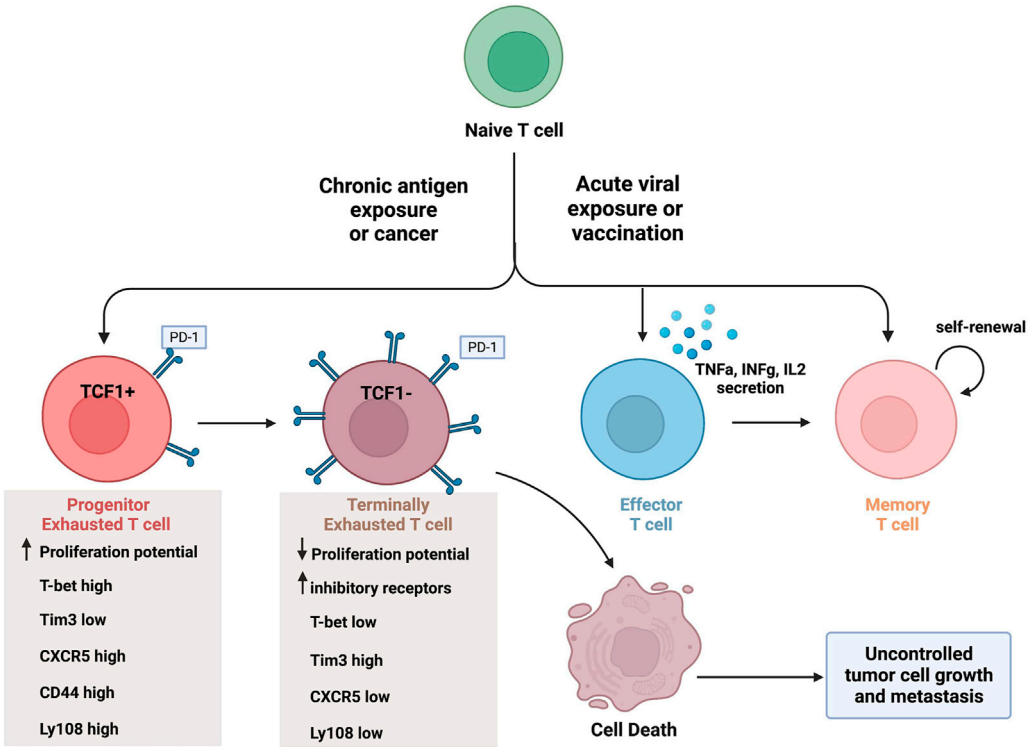

FIGURE 1
CD8⁺ T cell differentiation in the tumor microenvironment and during acute infection. Naïve T cells can differentiate into various CD8⁺ T cell subsets upon acute antigen exposure, and this varies in comparison to chronic antigen exposure. Effector T cells are capable of secreting cytokines and have cytolytic activity. A portion of effector T cells can differentiate into memory T cells, capable of self-renewal, or undergo cell death. In contrast, exhausted T cell populations lack effector activity and undergo cell death. Progenitor T_{ex} cells express TCF1 (as well as other markers) and have enhanced proliferation potential. Terminal T_{ex} cells do not express TCF1 (and increased inhibitory receptor expression) and have reduced proliferation potential. Created with BioRender.com.

approved for melanoma (Wolchok et al., 2013) and have since shown exceptional results in other cancers as well (Tawbi et al., 2018; Doki et al., 2022). Similarly, chimeric antigen receptor-T (CAR-T) cell therapy harnesses T cell-mediated anti-tumoral immune activity, an immune therapy approach that optimizes T cell activity against tumor cells. As promising as this therapy is for specific types of hematological malignancies, CAR-T therapy are less effective against solid tumors, and are also associated with some significant safety concerns (Gust et al., 2020; Rubin and Vaitkevicius, 2021). Specifically, in both CAR-T and immune checkpoint blockade (ICB) such as anti-PD-1 therapy, resistance to treatment remains a significant issue that limits their efficacy and utilization for a large subset of cancer patients (Gust et al., 2020; Rubin and Vaitkevicius, 2021). Therefore, defining the mechanisms that mediate resistance to CTL-based immunotherapies is of upmost importance (Sade-Feldman et al., 2018; Klemen et al., 2019; Lu et al., 2019).

One mechanism of resistance to T cell-targeting immunotherapies is loss of T cell effector activity (Pauken et al., 2016; Balança et al., 2021), whereby dysfunctional/exhausted CD8⁺ T cells fail to adequately clear cancer cells. Recently, epigenetic alterations were found to reinforce the differentiation of naive T cells to the exhausted state (Yang and Wang, 2021; Belk et al., 2022; Chen et al., 2022). Therefore, we and others believe that CD8⁺ T cells can be re-sensitized to ICB therapy by modulating the activity of specific epigenetic factors that mediate CD8⁺ T cell exhaustion. Here, we will describe effector and exhausted CD8⁺ T cell differentiation in the tumor microenvironment, explore transcription factors and genomic alterations reinvigorating such differentiation, and examine therapies which target these epigenetic pathways as a means to enhance the efficacy of ICB therapy.

T cell development in the TME

CTLs are very effective at removing foreign invaders in the body, such as during a viral infection. To do so, CTLs differentiate into their antigen-specific subtypes. Upon antigen recognition, naïve CD8⁺ T cells undergo clonal expansion and differentiate into effector CD8⁺ T cells, as shown in Figure 1. These immune cells are capable of cytokine secretion, have cytolytic activity, and function to target and clear virally infected cells. Once an infection is cleared from the body, the majority of these effector cells die leaving a small percentage (about 5%) of them to differentiate into long-lasting memory T cells (Tonnerre et al., 2021). This process in comparison with chronic antigen exposure is illustrated in Figure 1.

In the setting of a chronic antigen exposure, such as in cancer or chronic viral infections, the effector activity of CD8⁺ T cells is hindered. Even in tumors with a high mutational burden and the existence of a large amount of neoantigens, the immune system fails to completely clear all cancerous cells (Caushi et al., 2021; Belk et al.,

2022). Thus, CD8$^+$ T cells in the TME are thought of as being dysfunctional throughout tumorigenesis. This is mainly due to persistent antigenic stimulation during chronic infections or in the TME where CD8$^+$ T cells lose their effector functions and are skewed towards an exhausted phenotype. There is now a wide belief that terminally exhausted CD8$^+$ T cells are attributed to immune checkpoint blockade failures in the clinic, a point which will be discussed later in this review.

An overview of CD8$^+$ T cell exhaustion

T cell differentiation into an exhausted phenotype is a dynamic process, moving through a series of cellular states. Upon consistent antigenic stimulation either during chronic viral infections or cancer, effector CD8$^+$ T cells begin to lose their proliferative capacity, cytokine production, and effector potential. They upregulate various inhibitory receptors (such as PD-1, CTLA4, Tim3, LAG3, *etc.*), alter known transcription factors (such as TCF1, T-bet, NFAT, TOX, Blimp-1, *etc.*), and incur a unique epigenetic state, all of which hinder their effector and cytolytic abilities (Tang et al., 2021). The upregulation of inhibitory receptors on exhausted CD8$^+$ T cells, especially on progenitor exhausted CD8$^+$ T cells (Tang et al., 2021), are targeted with ICBs and as a means of stimulating CTL effector functions of exhausted T cells. It is important to note that although chronic viral infection and cancer can both cause similar T cell differentiation into exhausted phenotypes as mentioned here, exhaustion in the TME is a subtly but importantly unique form of exhaustion which will be focused on throughout this review.

The different stages of exhausted CD8$^+$ T cells differ greatly based on the expression levels of specific transcription factors and T cell inhibitory receptors. The progenitor exhausted T cells (T_{ex}^{prog1}) have a high proliferative potential with stem-like properties but low cytolytic ability (Jiang et al., 2021). In contrast, terminally exhausted CD8$^+$ T cells (T_{ex}^{term}) have a low proliferative potential but high cytotoxicity (Jiang et al., 2021). The terminally exhausted CD8$^+$ T cell subset is characterized by higher PD-1, Tim-3 and CD38 expression, and lower CXCR5, CD44, and Ly108 expression than progenitor CD8$^+$ T_{ex} cells (Jiang et al., 2021). Furthermore, terminally exhausted CD8$^+$ T cells overexpress the transcription factor TOX and do not express the transcription factors TCF1 and T-bet (Beltra et al., 2020). The differentiation into terminally exhausted CD8+T cells varies as well. For example, progenitor exhausted CD8$^+$ T cells do not always turn into terminally exhausted subtype. Some reports suggest the existence of multiple intermediate cell subsets during this progression, if the progression occurs at all (Yang and Wang, 2021; Chen et al., 2022). To further outline the transcriptional heterogeneity of exhausted T cells, a new exhausted CD8$^+$ T cell subset was identified recently which expresses unique natural killer (NK) cell related genes (i.e. *Klr*, *Fcg2rb*) and surface proteins (i.e. NK1.1, Ly49I, NKG2D) (Giles et al., 2022b).

The distinction between exhausted T cell subtypes is critical because although progenitor exhausted CD8$^+$ T cells are responsive to ICBs, terminally exhausted cells are not (Kallies et al., 2020; Jiang et al., 2021). This distinction in part explains why such a large proportion of people fail to reach a complete response to ICB therapy, and why many people eventually progress with such treatments (Kallies et al., 2020; Jiang et al., 2021). One specific reason for this is that when antigen concentration remains high, exhausted CD8$^+$ T cells which initially responded to checkpoint inhibitor therapy can become "re-exhausted" (Pauken et al., 2016). Pauken et al. demonstrated this finding in a mouse model of lymphocytic choriomeningitis virus (LCMV) with anti-PD-1 therapy. They found that CD8$^+$ T cells failed to acquire memory after immune checkpoint therapy, even if exhausted T cells were reinvigorated (Pauken et al., 2016). This represents an important truism in cancer immunotherapy: once cells reach terminal exhaustion, it cannot be undone and likelihood of ICB resistance increases (Pauken et al., 2016).

Some immune receptors on T cells have been shown to modulate the expression levels of the inhibitory receptors that ICB therapy is trying to target, unveiling potential new approaches to immunotherapy. For example, our group demonstrated that the self-ligand immune receptor SLAMF7 was co-expressed with multiple inhibitory receptors (i.e. PD-1, Tim-3, LAG3) and when SLAMF7 signaling was induced in CD8$^+$ T cells, the expression of PD-1, Tim-3, and LAG3 inhibitory receptors were upregulated (O'Connell et al., 2021). Also, SLAMF7 activation resulted in enhanced expression of several T cell exhaustion-promoting transcription factors and epigenetic regulators (O'Connell et al., 2021). We further demonstrated that presence of SLAMF7 on tumor-associated macrophages (TAMs) was necessary for TAMs to induce CD8$^+$ T cell exhaustion during their cross-talk with CD8$^+$ T cells (O'Connell et al., 2021). Accordingly, SLAMF7 expression on certain TAMs may be a prognostic factor in clear cell renal cell carcinoma (ccRCC) (O'Connell et al., 2021).

Key transcription factors and chromatin modifiers regulating T cell exhaustion

It is now widely recognized that exhausted CD8$^+$ T cells represent a distinct state in CD8$^+$ T cell differentiation and, as such, have a unique profile of transcription factors (TFs) and epigenetic factors that drive and enforce this state *via* epigenetic remodeling and other mechanisms (Sen et al., 2016; Yao et al., 2019; Ranzoni et al., 2021). Understanding the mechanisms that regulate the expression of specific TFs, their temporal dynamics during the process of T cell exhaustion, and the cellular processes regulated by each TF has critical implications. These details help us not only understand the complex process of exhaustion, but also unveil how to pharmacologically alter this process for clinical benefit. A myriad of TFs have been implicated in regulating T cell exhaustion, some better defined than others, and a complete in-depth review of each can be found elsewhere (Wherry and Kurachi, 2015). There are also a number of TFs suspected to regulate exhaustion which have not yet been formally investigated, including E2F2 (Giles et al., 2022a), and likely others. Our group recently found that the immune cell surface receptor SLAMF7 is capable of regulating some of the critical exhaustion-defining TFs and epigenetic modifiers that regulate different aspects of T cell exhaustion (O'Connell et al., 2021). Below we detail what is currently known about each of these TFs and chromatin modifiers and how they regulate CD8$^+$ T cell exhaustion programs.

TOX

TOX has been described as the "master regulator" of T cell exhaustion (Alfei et al., 2019; Khan et al., 2019; Scott et al., 2019; Yao et al., 2019). However, the precise role TOX plays in T cell exhaustion is more nuanced and involves interplay with other TFs and temporal expression dynamics throughout T cell differentiation. As a brief review, $CD8^+$ T cell exhaustion is not a binary state cells enter, but rather a continuous developmental trajectory which was definitively separated into four cell states by Beltra et al. (2020). Two of these cell states, T_{ex}^{prog1} and T_{ex}^{prog2}, are progenitor states with stem-like capabilities. On the opposing end of the exhaustion developmental spectrum are the T_{ex}^{int} and T_{ex}^{term} states, which represent progressively more dysfunctional $CD8^+$ T cells with T_{ex}^{term} having a terminal and irreversible phenotype as reviewed here and in the literature (Beltra et al., 2020).

During the progression of $CD8^+$ T cells towards terminal exhaustion, expression of TOX gradually increases along with a concomitant decrease in the transcription factor TCF1 (Scott et al., 2019; Beltra et al., 2020). This transition from TCF1 expression to TOX expression is critical in defining these divergent $CD8^+$ T cell states. TCF1 is a well-established regulator of stem cell capabilities in $CD8^+$ T cells (Siddiqui et al., 2019) and TOX functions to enforce the terminal exhaustion phenotype, while repressing the terminal effector state (Khan et al., 2019). Specifically, TOX epigenetically reinforces exhaustion programs *via* chromatin remodeling at promoters and enhancers of various genes driving T cell exhaustion. One way it does this is by inducing histone H3 and H4 acetylation and DNA methylation (Zeng et al., 2020).

There are a number of signals which induce TOX expression and this transition from stem-like $CD8^+$ T cells to exhausted cells, including: repetitive TCR stimulation (Blank et al., 2019; Philip and Schietinger, 2019), IL-12 (Maurice et al., 2021), calcineurin (Khan et al., 2019), NFAT2 (Khan et al., 2019), STAT3 (Yao et al., 2019), SLAMF7 signaling (O'Connell et al., 2021), and likely others yet to be discovered. A number of these signals (calcineurin, NFAT2, and STAT3) are secondary to primary stimuli such as TCR activation (calcineurin and NFAT2) and SLAMF7 activation (STAT3 (O'Connell et al., 2021)). TOX expression in T cells does not itself induce exhaustion, nor is it a marker of T cell exhaustion. Indeed, increased TOX expression has been noted in PD-1+$CD39^+CD8^+$ T cells and EMRA $CD8^+$ T cells from healthy control subjects (Balança et al., 2021; Maurice et al., 2021; Tallón de Lara et al., 2021). Furthermore, we and others have noted that TOX is transiently upregulated in T cells upon TCR stimulation (Khan et al., 2019; Maurice et al., 2021). Together, these findings suggest that TOX can be transiently expressed in CD8+T cells without committing them towards exhaustion, in a manner analogous to FOXP3 which is also transiently upregulated during $CD4^+$ T cell TCR stimulation (Wang et al., 2007). Additionally, the expression levels of TOX vary significantly between terminally exhausted $CD8^+$ T cells and any other T cell subset expressing TOX (Alfei et al., 2019; Khan et al., 2019; Scott et al., 2019; Yao et al., 2019). TOX expression also increases in $CD8^+$ T cells along the exhaustion developmental trajectory defined by Beltra et al. (Beltra et al., 2020), with T_{ex}^{term} having a TOX^{high} phenotype. This data further confirms that high TOX expression is indicative of true $CD8^+$ T cell exhaustion, as can be appreciated in the difference in TOX expression noted between tumor infiltrating lymphocytes (TILs) and *in vitro* stimulated T cells (Khan et al., 2019; Giles et al., 2022a).

T-bet

The T-box family member transcription factor T-box-expressed-in-T-cells (T-bet) has been implicated in playing an important role in the regulation of $CD8^+$ T cell exhaustion, and it's mechanism has recently been described. T-bet has well established roles in Th1 $CD4^+$ T cell polarization (Szabo et al., 2000) and promoting T cell effector functions (Sullivan et al., 2003). It accomplishes such roles through binding T-box DNA elements leading to control of gene expression programs promoting these activities. More recently, roles for T-bet in the regulation of other cellular phenotypes in non-T immune cells have been described, such as its role in isotype switching and propagation of an antigen-experienced subset of B cells (Knox et al., 2019). Work by McLane et al. identified that T-bet antagonizes terminal exhaustion in $CD8^+$ T cells *via* competition with another TF linked to T cell exhaustion, called Eomes (McLane et al., 2021). While both TF's can bind to T-box sites in the promoter region of *Pdcd1* and block its expression, Eomes does so very weakly while T-bet strongly prevents PD-1 expression (McLane et al., 2021). McLane et al. identified that T-bet and Eomes compete for the same T-box binding domain in the promoter of *Pdcd1* and other exhaustion-linked genes, resulting in competition between the two. Therefore, the fraction of nuclear-localized T-bet *versus* Eomes plays a strong role in regulating $CD8^+$ T cell exhaustion (McLane et al., 2021). Another group has made similar findings in humans with chronic HIV infection (Buggert et al., 2014). Work from Beltra et al. further supports the hypothesis that high nuclear T-bet expression antagonizes terminal exhaustion. They discovered that Eomes expression is highest in terminally exhausted (T_{ex}^{term}) cells and that these same cells have lower T-bet (Beltra et al., 2020). Accordingly, the $T\text{-bet}^{high}$ $PD\text{-}1^{mid}$ subset of $CD8^+$ TILs is the subset capable of reinvigoration by checkpoint blockage, while the $Eomes^{high}$ subset is not (Wherry and Kurachi, 2015). These studies on the role of T-bet in CTL exhaustion highlight the need to measure sub-cellular localization of many of these various proteins and TF's linked to exhaustion. Thus, identifying appropriate roles for each in the context of exhaustion cell biology and revealing that merely measuring expression levels is likely not enough.

Blimp-1

Blimp-1 (which is encoded by *prdm1)* was one of the first TF's to be linked to T cell exhaustion (Wherry et al., 2007; Shin et al., 2009) and is thus one of the best characterized. Blimp-1 was first identified as a TF critical in the development of plasma cells (Shaffer et al., 2002) and has since been identified as a regulator of various functions in additional immune cell subsets (Nadeau and Martins, 2022). Blimp-1 primarily functions as a transcriptional repressor by either competing with other TFs for binding to specific DNA regulatory regions or by recruitment of other chromatin-modifying factors to specific genes (Nadeau and Martins, 2022). However, there are occasional instances where Blimp-1 can function as a transcriptional activator, as in the case of its regulation of *SLAMF7* (Kim et al., 2016).

The association of Blimp-1 with T cell exhaustion occurred after it was discovered that it was upregulated in T cells expressing multiple inhibitory receptors (Shin et al., 2009) and, more recently, in terminally exhausted CD8$^+$ T cells (Yao et al., 2019). Conditional deletion of *prdm1* in activated CD8$^+$ T cells *via* a granzymeB-Cre driver, so as to remove the possibility of *prdm1* deletion interfering with T cell development, revealed lower levels of multiple inhibitory receptors in *prdm1* cKO CD8$^+$ T cells (Shin et al., 2009). It is important to note that while the *prdm1* cKO CD8$^+$ T cells displayed decreased levels of multiple inhibitory receptors such as PD-1, 2B4, LAG3, TIGIT, and CD160, changes in PD-1 and LAG3 were minimal and 2B4 expression changes were the most pronounced (Shin et al., 2009; Zhu et al., 2017). 2B4 (*SLAMF4*) is a member of the SLAM family of receptors. Considering Blimp-1 has been shown to regulate the expression of another SLAM family member (*SLAMF7*) (Kim et al., 2016), and SLAMF7 has been shown to regulate Blimp-1 (O'Connell et al., 2021), this suggests Blimp-1 may preferentially regulate T cell exhaustion *via* SLAM signaling networks over that of other T cell inhibitory receptors.

It was also found that mice with conditional deletion of *prdm1* in CD8$^+$ T cells controlled LCMV infection more effectively than WT mice (Shin et al., 2009), and that anti-CD19 CAR-T cells lacking Blimp-1 have improved *in vivo* tumor control (Yoshikawa et al., 2022). This data confirms the role for Blimp-1 in CD8$^+$ T cell responses *in vivo*. These findings further support the theory that Blimp-1 regulates T cell exhaustion and effector functions, in conjunction with findings that high Blimp-1 expression in T cells inhibits IL-2 production (Gong and Malek, 2007).

Mechanistically, Blimp-1 drives T cell exhaustion in much the same way that it regulates cell states in other cell types, *via* regulation of chromatin accessibility to key genes important in driving functional T cell dysfunction (Yoshikawa et al., 2022). As a testament to how critical high Blimp-1 expression is in enforcing T cell exhaustion, Wu et al. demonstrated that Blimp-1 expression must be repressed by TCF1, the master TF regulating CD8$^+$ T cell stemness (Wu et al., 2016). As discussed above, TCF1 functions in an inverse manner to TOX (the master positive regulator of exhaustion). Thus, the finding that TCF1 must directly antagonize both TOX and Blimp-1 demonstrates the importance of Blimp-1-controlled transcriptional programs in CD8$^+$ T cell exhaustion.

EZH2

The histone methyltransferase enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) is a critical member of the polycomb repressive complex and has well-studied roles across developmental biology. Its importance in organismal development is exemplified by the fact that homozygous deletion of *EZH2* is embryonic lethal (O'Carroll et al., 2001), and necessitates conditional knockout models for its *in vivo* study. More recently, the contribution of EZH2 to immune cell biology has been investigated with a number of studies showing complex and important roles for this chromatin modifier in T cell biology (He et al., 2017; Goswami et al., 2018; Wang et al., 2018; Li et al., 2020; Stairiker et al., 2020). EZH2 functions as a methyltransferase which is capable of mediating di- and tri-methylation of histone H3 at lysine 27 (H3K27) on the promoter of target genes resulting in heterochromatin formation and repression of gene expression (Stairiker et al., 2020). In addition to its methyltransferase activity, EZH2 has been detected in the cytosol and shown to regulate actin polymerization and cell signaling (such as TCR signaling) (Su et al., 2005)].

Currently, EZH2 has been linked to memory T cell responses (He et al., 2017; Li et al., 2020), regulatory T cell responses in the TME (Goswami et al., 2018), altered T cell inhibitory receptor expression (He et al., 2017), and T cell differentiation towards an exhaustion phenotype (O'Connell et al., 2021). EZH2 has pleotropic effects in T cells as evidenced by the numerous above-mentioned roles of this TF and the variety of pathways it is known to cooperate in. For example, one mechanism by which EZH2 may be able to induce T cell exhaustion is *via* STAT3 phosphorylation, as demonstrated in part by our group and others (Kim et al., 2013; O'Connell et al., 2021). STAT3 has previously been shown to induce PD-1 expression, limit T cell effector functions, and promote formation of regulatory T cells, which together can drive T cell exhaustion (O'Connell et al., 2021). On the contrary, active EZH2 has also been shown to alter chromatin at the *prdm1* locus resulting in decreased Blimp-1 levels which may temper T cell exhaustion (He et al., 2017). To further complicate matters, EZH2 is able to regulate CD8$^+$ T cells *via* activation of *Id3* and inhibition of *Id2* and *Eomes* (a TF which also plays an important role in T cell exhaustion and has been reviewed elsewhere (Li et al., 2018)) which together promote memory T cell formation and tumor control (He et al., 2017).

The key to EZH2's diverse T cell regulatory abilities is its phosphorylation status, which determines if this chromatin modifier is active or not. When EZH2 is phosphorylated (*via* AKT), it is inhibited and unable to bind to and remodel chromatin and induce memory T cell formation (He et al., 2017). Additionally, signaling through the SLAMF7 cell surface receptor is capable of robustly inducing EZH2 levels in CD8$^+$ T cells (O'Connell et al., 2021), but if this also alters EZH2 phosphorylation remains to be seen. Countering the induction of EZH2 expression in T cells by SLAMF7 signaling (which can be prevalent in the TME of certain cancers (O'Connell et al., 2021)) are unknown factors in the TME which negatively regulate EZH2 levels (Stairiker et al., 2020). Dissecting the dominant role of EZH2 in T cells in the TME is complicated by the above-mentioned pleotropic effects of this chromatin modifier, and also the fact that most studies on EZH2 have been performed in mice. As efforts move forward to modulate EZH2 for anti-tumor benefit with small molecule inhibitors (Kim and Roberts, 2016; Adema and Colla, 2022), there will need to be human-centric studies on the role of EZH2 in human TILs. The need to perform intranuclear staining for EZH2 has likely been a major factor contributing to the lack of human TIL EZH2 research, but as our group has shown (O'Connell et al., 2021), it is possible to optimize staining for this chromatin modifier.

YY1

YY1 (Yin Yang 1) is a ubiquitously expressed TF harboring a large array of diverse and often opposing regulatory abilities (Gordon et al., 2006). YY1's ability to regulate various cellular processes comes from its DNA binding function, where it serves as a TF capable of direct or indirect regulation of gene expression. Most early studies on YY1 focused on its function in cancer cells, however, more recent studies have highlighted additional roles as a TF capable of

intrinsically modulating immune cell function (Gordon et al., 2006; Balkhi et al., 2018).

With regard to T cell exhaustion, YY1 has been found to control multiple processes during this phenomenon including modulation of inhibitory receptors and cytokine production (Balkhi et al., 2018). Specifically, it was found to drive transcription of PD-1, LAG3, and Tim3 in chronically stimulated $CD8^+$ T cells *in vitro* (Balkhi et al., 2018). Additionally, YY1 was noted to induce T cell dysfunction *via* repression of T cell-intrinsic IL-2 production in a mechanism involving recruitment of EZH2 to the IL-2 promoter (Balkhi et al., 2018). As lack of IL-2 during active T cell proliferation is known to cause T cell dysfunction (Ross and Cantrell, 2018), this was thought to be a major mechanism whereby YY1 controls T cell exhaustion. Similar to Blimp-1, YY1 is also known to regulate SLAMF7 expression, although YYA does this *via* direct transcriptional repression (Dongre et al., 2013). Likewise, SLAMF7 can induce YY1 expression (O'Connell et al., 2021). This suggests the presence of a negative feedback loop involving YY1 and SLAMF7, although each likely only contributes to a fraction of the many regulatory signals each protein receives during normal immune cell function (O'Connell et al., 2021). Since YY1 has a well described role in regulating NFκB function (Gordon et al., 2006; Balkhi et al., 2018), its immune cell-modifying functions can be partially attributed to this mechanism as well. However, like many of YY1's functions, its regulation of NFκB is complex (Gordon et al., 2006; Balkhi et al., 2018) and dissecting these mechanisms may be difficult.

Epigenetic regulation of T cell exhaustion

Recent studies have demonstrated that T cell exhaustion is commonly associated with significant and unique genome-wide epigenetic remodeling programs which differentiate T_{ex} cells from naïve, effector, and memory T cells (Pauken et al., 2016; Sen et al., 2016; Philip et al., 2017). These epigenetic programs are associated with changes in expression of specific TFs, histone modifications, DNA methylation, and chromatin accessibility. For example, multiple epigenetic profiling studies in tumor infiltrating T_{ex} cells demonstrated altered chromatin accessibility in thousands of differentially accessible regions, including decreases in chromatin accessibility of genes associated with effector T cell differentiation, such as IFNγ, TNFα, IL-2, KLRG1, and others, and increases in chromatin accessibility of genes associated with T cell exhaustion, such as PD-1, Tim3, LAG3, TIGIT, TOX, Tcf7, NFAT, and others (Sen et al., 2016; Abdel-Hakeem et al., 2021; Giles et al., 2022a; Belk et al., 2022). These changes in chromatin accessibility were also associated with extensive transcriptional alteration in T_{ex} cells. Importantly, the transcriptional programs and chromatin profiles of T_{ex} cells were largely conserved between murine T_{ex} cells of LCMV-infected mice and human T_{ex} cells derived from cancer and chronically-infected patients (Bengsch et al., 2018; Pritykin et al., 2021), revealing common differentiation programs and regulatory mechanisms. These results also demonstrate that T cell alteration in tumors and chronic infections follows a shared epigenetic differentiation trajectory. In addition to having a similar transcriptional and chromatin profile, studies using murine tumor models identified CD38 and CD101 as differentiating markers for T_{ex} reprogrammability in PD-1 high $CD8^+$ T cells. Specifically, $PD\text{-}1^{Hi}CD101^{Low}CD38^{Low}$ was a plastic therapeutically reprogrammable chromatin state and $PD\text{-}1^{High}CD101^{High}CD38^{High}$ was a fixed dysfunctional state of exhausted CD8+T cells (Philip et al., 2017). These findings demonstrate that the epigenetic state of T_{ex} cells is highly regulated and heterogenous, and this state governs the phenotype and function of T_{ex} cells, as well as their response to ICB therapy (Gyorki et al., 2009).

It is now well appreciated that the terminal epigenetic state of T_{ex}^{term} is fixed and irreversible, even after reactivation, curative therapy, or following ICB therapies (Pauken et al., 2016; Hensel et al., 2021; Tonnerre et al., 2021; Yates et al., 2021). For example, unlike memory $CD8^+$ T cells which robustly expanded upon restimulation, $CD8^+$ T_{ex}^{term} cells from anti-PD-1-treated tumor-bearing mice failed to respond to secondary stimuli (Pauken et al., 2016). These results were associated with persistent PD-1 upregulation and a specific DNA methylation signature at the *Pdcd1* and other gene loci (Pauken et al., 2016). Also, anti-PD-1 therapy was able to only change 10% of the epigenetic landscape to T_{ex}^{term} cells, implicating that the fixed chromatin state of T_{ex}^{term} cells is a major limitation for anti-PD-1 therapy (Pauken et al., 2016). Similarly, the chromatin accessibility of T_{ex}^{term} cells derived from HIV- and HCV-infected patients remained unchanged before and after resolution of the infection, and in particular at the *Tox* and *Pdcd1* loci, confirming a fixed epigenetic state in T_{ex} cells after they reach terminal exhaustion (Abdel-Hakeem et al., 2021; Yates et al., 2021). Additionally, and due to the nature of the fixed chromatin state of T_{ex}^{term} cells, when T_{ex} cells from cured HCV patients were re-challenged with HCV virus, robust upregulation of T cell exhaustion-related genes like PD-1 occurred (Abdel-Hakeem et al., 2021). This phenotype was also associated with reduced expression of genes that regulate effector T cell activity, which further impaired their effector functions. These results necessitate the need to develop novel therapeutic approaches that increase the epigenetic plasticity of T_{ex} cells. It is important to note that the T_{ex} cell differentiation state, which is enforced at the epigenetic level, defines specific subsets of T_{ex} cells. For example, T_{ex}^{prog1} ($Ly108+CD69^+$) and T_{ex}^{int} ($Ly108+CD69^-$), which can proliferate and self-renew with ICB therapy (Gyorki et al., 2009) have accessible chromatin in various gene loci that is associated with T cell stemness, such as the Ly108 (*SLAMF6*) and TCF1 (*Tcf7)* gene loci (Zehn et al., 2022). In contrast, the terminally exhausted (($Ly108^-CD69^+$); T_{ex}^{term}) T cell subset, which does not respond to ICB therapy, has accessible chromatin at the gene loci of NR4A, EOMES, and TOX TFs and PD-1, TIM-3, LAG3, CD101, and CD38 T cell inhibitory receptors (Hudson et al., 2019). These data reveal intricacies within the exhausted T cell population, and their implications in cancer progression and tumor evasion of ICB therapy.

Technologies for studying epigenetics

ATAC-seq

Various unique techniques have been developed and optimized to study the epigenetic profile of exhausted T cells, with one of the most prominent being the assay for transposase-accessible chromatin with sequencing, or ATAC-seq. Figure 2 illustrates ATAC-seq and the other methods that are discussed in this review. This specific technique involves the cleavage of chromatin by Tn5, which directly cuts only accessible chromatin (Belk et al., 2022). ATAC-seq therefore profiles

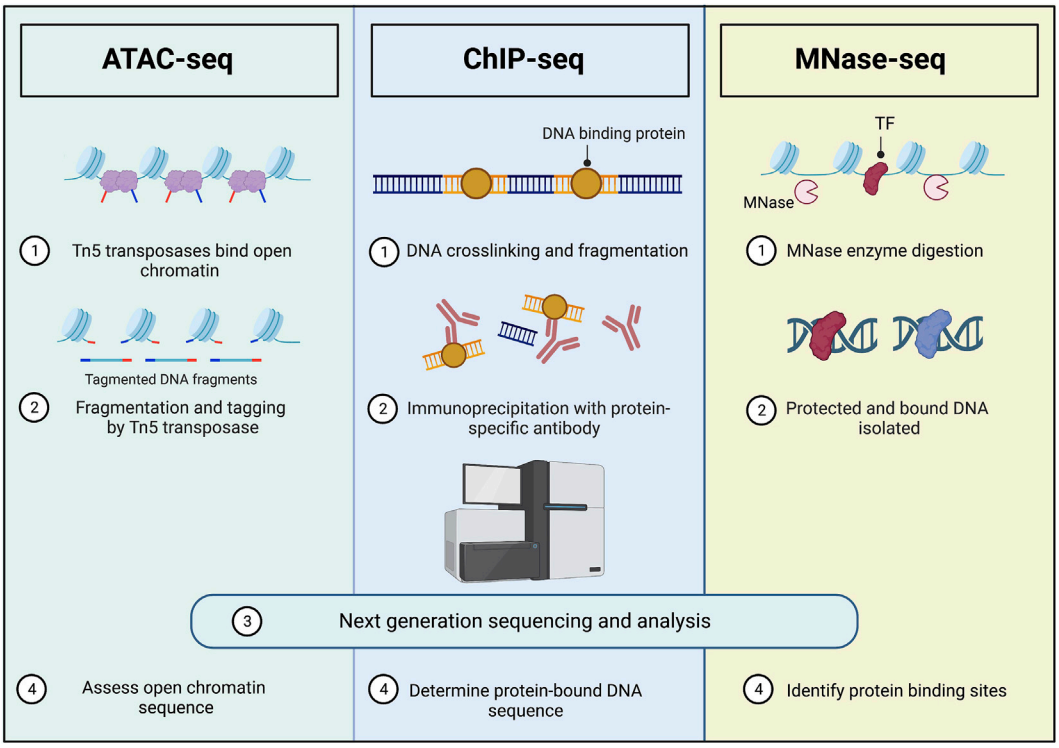

FIGURE 2
Common methods for studying epigenetic modifications of chromatin. ATAC-seq, ChIP-seq, and MNase-seq have different mechanisms for DNA isolation, but all result in sequencing of specific DNA structures to identify epigenetic modulations. ATAC-seq involves the use of Tn5 to assess the location and sequence of open chromatin, ChIP-seq assesses the sequence of protein-bound DNA through crosslinking and fragmentation, and MNase identifies protein binding sites on DNA using an MNase enzyme digestion which leaves bound DNA to be isolated and sequenced. These techniques can be completed on bulk cells or single cells as described in this review. Created with Biorender.com and adapted from Chen et al., 2022.

only DNA fragments of genomic locations which are accessible for transcription. This technology allows one to identify the genomic accessibility at a precise location. Indeed, ATAC-seq transposes native chromatin for epigenomic profiling in a fast and sensitive way, and is thus a powerful technique for profiling the epigenetic regulation of T cell exhaustion. Recently, advances have made it possible to conduct ATAC-seq on single cells (Fang et al., 2021; Ranzoni et al., 2021)and in a spatial context (Thornton et al., 2021), making the unique cell populations and architecture of TME possible to profile.

Specific to T cell exhaustion, studies found that exhausted T cells had similar chromatin accessibility in different tumor models (Pritykin et al., 2021). This work was done by analyzing over 300 assays of ATAC-seq and RNA-seq. Authors found, through ATAC-seq, that functional and dysfunctional T cells diverged from a progenitor-like population of T cells (Pritykin et al., 2021). Multiple other studies have been done using the ATAC-seq technique, revealing different and specific chromatin accessibility changes in effector T cells, exhausted T cells, and memory T cells (Sen et al., 2016). Studies also have shown significant differences in regulatory region patterns between these different T cell populations with ATAC-seq, overall supporting its clear importance. Thorough reviews have been done on the application of ATAC-seq in the tumor microenvironment and we recommend their review for more information (Sen et al., 2016; Chen et al., 2022).

ChIP-seq and MNase-seq

Other techniques can be used to locate specific histone modifications or DNA-binding protein locations. Chromatin immunoprecipitation with sequencing (ChIP-seq) (Figure 2) is a technology for identifying genome-wide DNA binding of a protein of interest (or chromatin modification) and involves crosslinking DNA with all bound proteins, fragmenting the DNA, immunoprecipitation, followed with sequencing of the pulled-down DNA (Mikkelsen et al., 2007; Belk et al., 2022). Antibody-tethered micrococcal nuclease (MNase-seq) (Figure 2) is an advancement of ChIP-seq, also known as the CUT&RUN method, where the chromatin is cut directly next to the bound protein, allowing the DNA fragments to move out of the nucleus where they can then be isolated and sequenced (Skene and Henikoff, 2017; Belk et al., 2022). The main advantage of this technique is that is does not require fixation and can be done with fewer cells than ChIP-seq, allowing for sequencing of rare cells. Similarly to ATAC-seq, ChIP-seq can now be done on single cells, advancing its ability to detect changes in small immune populations (Grosselin et al., 2019). Furthermore, HI-ChIP is a new "protein-centric" chromatin conformation technique which improves readability and requires a lower sample size than ChIP-seq (Mumbach et al., 2016).

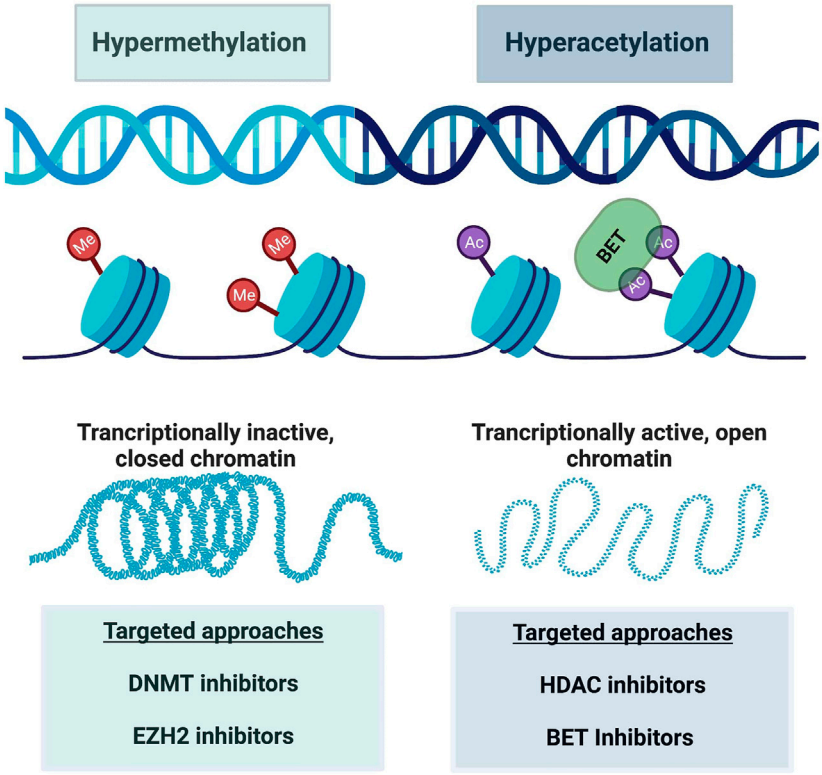

FIGURE 3
A schematic of major epigenetic alterations reinforcing CD8⁺ T cell exhaustion. Hypermethylation is characterized by increased methyl group attachment to DNA on chromatin, making the DNA transcriptionally inactive and closed. Hyperacetylation on the other hand acts in an opposite manner, where acetyl group binding causes chromatin to open and enhanced transcription of DNA. BET targeting to acetyl groups can also modify this transcription. These alterations are manipulated by various targeted drug approaches as listed in this figure. Created with BioRender.com.

Multiomic approach

The above mentioned epigenetic techniques are able to be performed with other transcription- and proteome-wide profiling techniques as well, constituting a multiomic method. This allows for profiling multiple layers of cell processes at the same time in a single cell. A couple examples include nucleosome occupancy methylome sequencing (NOME-seq), which measures DNA methylation and chromatin accessibility at the same time, showing the nucleosome position along with DNA methylation in the chromatin (Mehrmohamadi et al., 2021). However, these assays are limited to the CpG frequency in the genome. To account for this limitation, one can alternatively combine ATAC-seq with bisulfite conversion in a technique known as EpiMethylTag to study DNA methylation and chromatin accessibility on the same DNA molecule (Mehrmohamadi et al., 2021). Although most of these approaches are applicable to single-cell sequencing, they are not as optimal for single cells due to low throughput (Mehrmohamadi et al., 2021). Nonetheless, the technology is improving quickly and will likely be applicable to single cells in a robust manner soon.

T cell reprogramming

As mentioned throughout this review, resistance to ICB and CAR-T cell therapy is a critical clinical problem requiring further research. Scientists have discovered that this resistance, at least in part, occurs due to epigenetic changes in T cell differentiation and in particular in exhausted CD8⁺ T cells. Thereby, it is hypothesized that certain epigenetic-targeting drugs could be applied to T_{ex} cells as a means of restoring stemness. Indeed, there are various classes of epigenetic modifiers, both in development and FDA approved, which will be discussed here along with their current utility. It is important to note that most of these epigenetic modifiers do so on a global scale, so specificity to a subset of immune cells in the tumor microenvironment is currently limited. Figure 3 demonstrates how these therapies can be conceptualized in addition to the altered epigenetic modifiers discussed here.

Broad-spectrum epigenetic inhibitors target genome-wide cancer specific gene expression and include inhibition of various targets. Specifically, inhibition of DNA methyltransferase (DNMT) (Chiappinelli et al., 2015; Giri and Aittokallio, 2019), histone deacetylase (HDAC) (Eckschlager et al., 2017; Lue et al., 2019), and bromodomain and extra-terminal motif proteins (BET) (Kong et al., 2021; Shorstova et al., 2021) are the most common. DNMT inhibitors target hypermethylated DNA regions, and thus transcriptionally inactive chromatin. This reversal of silenced gene transcription by loss of DNA methylation allows the genes to become active again, which is desirable in the setting of tumor suppressor gene transcriptional silencing. DNMT inhibitors have been used in treating blood malignancies for years (Gore, 2005). Specifically, decitabine and azacytidine, two DNMTi drugs, are approved for

treating myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). The drugs' activity towards malignant cells supports the importance of epigenetic regulation in tumors. More recently, DNMTi use has been found to affect T cell exhaustion (Wang et al., 2021). Wang *et al.* found that that decitabine epigenetically reprogrammed CAR-T cells, enhancing and prolongating their antitumor activity (Wang et al., 2021). Furthermore, other groups showed that DNMTi therapy reversed exhausted $CD8^+$ T cells and enhanced their effector function in different cancer models (Chiappinelli et al., 2015; Roulois et al., 2015). DNMT inhibitor therapy with various different drugs are currently being tested in clinical trials for multiple hematologic and solid malignancies, most in combination with another agent such as chemotherapy, immunotherapy, or second epigenetic modifying drug (Gore, 2005; Hu et al., 2021; Baer et al., 2022).

HDAC inhibitors on the other hand, preserve acetyl groups on lysine residues in histone tails, allowing open chromatin configurations and thus favoring gene transcription (Laino et al., 2019). Indeed, HDAC inhibitors cause apoptosis and arrest tumor cell growth and potential angiogenesis. There are multiple HDAC isoforms, and some inhibitors target specific HDAC isoforms while others are global inhibitors (Laino et al., 2019). For example, selective HDAC6 inhibition with ACY-1215 and ACY-241 was efficacious towards melanoma patients' T cells *ex-vivo*, harvested from either peripheral blood or tumor biopsies (Laino et al., 2019). Specifically, HDAC6 inhibitor increased T cell effector functions, reduced exhaustive markers, and in turn enhanced the killing of malignant cells (Laino et al., 2019). Importantly, higher frequencies of T effector memory cells remained after treatment with ACY-1215 as well (Laino et al., 2019). Chromatin accessibility was increased in genetic regions associated with such effector and memory T cell functions. Furthermore, McCaw et al. found that an HDAC inhibitor, specifically the HDAC1 inhibitor etinostat (ENT), promoted immune-modulatory molecule expression which in turn re-sensitized tumors to ICB (McCaw et al., 2019). These findings support that epigenetic targeting can reprogram exhausted T cells and enhance effector function of T cells once resistant to ICB.

Another global inhibitor of epigenetic processes are bromodomain and extra-terminal domain (BET) inhibitors. BET overexpression can contribute to carcinogenesis, such as in hyper-acetylation of proliferation-promoting genes or hyper-acetylation of oncogene enhances (Jin et al., 2021). BET inhibitors recognize and bind to acetylated lysine residues, altering transcriptional activation and chromatin remodeling (Shorstova et al., 2021). Indeed, mouse studies revealed that suppression of BET proteins reduced AML burden (Zhong et al., 2022). Moreso, the specific BET inhibitor JQ1 reversed T cell exhaustion from ICB therapy in AML T cells *in vitro* and *in vivo* (Zhong et al., 2022). This BET inhibitor also reduced PD-1 and Tim-3 expression and increased cytokine secretion by AML patient-derived T cells (Zhong et al., 2022). As exciting as this data is, whether BET inhibition can reverse exhaustion in CML *in vivo* models which are resistant to anti-PD-1 therapy remains to be seen, and would be an important next step. Using a chronic lymphocytic leukemia (CLL) model, Kong et al. did demonstrate that BET inhibition reversed CAR-T cell exhaustion as measured by multiple factors including reduced inhibitory receptor expression and increasing their proliferation abilities (Kong et al., 2021).

Other methods of epigenetic modulation include narrow spectrum inhibitors. One of these methods focuses on EZH2 inhibition, due to its methyltransferase activity. Wang *et al.* found that EZH2 depletion reduced MYC expression and hindered neuroblastoma and small cell lung carcinoma tumor growth (Wang et al., 2022). However, they found that this EZH2 inhibition was independent of its methyltransferase activity (Wang et al., 2022), revealing that EZH2 may play a role in cancer beyond its enzymatic epigenetic reprogramming abilities. In 2020, the first EZH2 inhibitor was FDA approved for patients with relapsed or refractory follicular lymphoma. To receive this treatment, patients require a confirmed EZH2 mutation, as the studies determined a higher response rate in patients with confirmed EZH2 mutation in comparison to WT (69% vs 35%) (Morschhauser et al., 2020). However, the therapy was found to be tolerable and produce reasonably efficacy independent of EZH2 mutation (Morschhauser et al., 2020). Thus, it could be argued that this could be an option for refractory lymphoma patients (or other B cell malignancies) without EZH2 mutation, given its reasonable efficacy and safety profile.

Different EZH2 inhibitors exist, most targeting the methyltransferase abilities of EZH2. For this reason, it has been proposed as a promising combination with cytotoxic chemotherapy, as it makes DNA more accessible for DNA-damaging agents (Adema and Colla, 2022). In theory, one could use a lower dose of cytotoxic drug with a largely not toxic EZH2 inhibitor. This would improve safety profiles of chemotherapy, especially in older individuals (Adema and Colla, 2022). Furthermore, it can be combined with other epigenetic modifiers for better results. For example, the combination of EZH2 inhibitor with HDAC inhibitor increased lymphoma cell line apoptosis (Lue et al., 2019). The impact of combination epigenetic reprogramming on $CD8^+$ T cell exhaustion and possible synergistic effects *in vivo* are unclear and warrant further investigations.

Outside of targeting epigenetic enzymes, research has supported targeting important exhaustion-driving transcription factors, and preclinical results have been promising. For example, CAR-T cells with Nrf4A family proteins knocked out had improved tumor-directed efficacy in mouse models, and knockout of TOX improved CAR-T cell efficacy as well (Tang et al., 2021). Although there are no current TOX inhibitors, this would seem to be a viable option for reversing ICB resistance. Another option could be to use an upstream regulator of TOX. Specifically, a regulator such as SLAMF7 which regulates TOX expression as discussed above. Thus, we propose that epigenetic directed therapy in combination with a SLAMF7 inhibitor may be a way to reduce CD8+T cell terminal exhaustion and warrants future investigations.

Discussion

It is no question that the discovery of checkpoint inhibitors and development of CAR-T cell-based therapy have drastically changed the way clinicians approach cancer treatment and management. However, despite great success, many tumors unfortunately remain insensitive to such therapies, with solid tumors remaining particularly challenging to treat *via* immune-based mechanisms. Recently, the discovery of epigenetic changes driving T cell differentiation toward exhaustion has unveiled that epigenetic factors reinforce $CD8^+$ T cell exhaustion as well. These studies, together with known transcription factors driving $CD8^+$ T cell exhaustion, support that the exhausted $CD8^+$ T cell phenotype is at least in part due to epigenetic modulations.

Thus, the utilization of epigenetic modifying drugs either as single agents or in combination with potent therapies might improve the effector and memory responses of $CD8^+$ T cells.

As important as the understanding of epigenetic remodeling of T_{ex} cells is for the tumor immunology field, how these drugs will translate *in vivo* remains to be seen. For example, many of these drugs are global modifiers of specific epigenetic processes. Thus, they would need to be directed towards exhausted $CD8^+$ T cells specifically to confirm these theories. Utilization of a drug-antibody conjugate for an inhibitory receptor highly expressed on exhausted $CD8^+$ T cells, such as PD-1, may be one way to localize epigenetic modifying therapies to the T_{ex} immune cell subset in the TME. In practice, it may be more beneficial to epigenetically modify both cancer cells and T_{ex} cells, in which case a purely T cell-targeted approach would not be as desirable, but this remains to be seen. Not only may the inhibitors have effects on tumor cells, but they also can have off target effects on healthy cells and tissues unrelated to cancer. In addition, it is well-known that potent immune activation in the setting of cancer can trigger autoimmune-like reactions (Young et al., 2018). Thus, ensuring the therapy is selective for exhausted $CD8^+$ T cells is an important limitation of current studies and should be further addressed.

This work leads one to wonder whether the combination of broad-spectrum epigenetic regulators in combination with ICB or CAR-T cells therapy would be optimal in cancer patients who do not respond to ICB alone, and some studies already support this hypothesis (Eckschlager et al., 2017; Li et al., 2018; McCaw et al., 2019; Wang et al., 2021; Zhong et al., 2022). Whether it is due to reversing $CD8^+$ T cell exhaustion or some other factor remains to be seen. The lack of specificity with global epigenetic regulators may limit their efficacy and makes mechanistic understanding of the inhibitors in relation to exhaustion difficult to discern. This muddies identifying patients who will respond to the therapy *versus* those who will not. Currently, genetic testing of specific epigenetic regulators is being done on patients to predict their response to such inhibition. However, as we have learned from targeted therapy approaches, because a gene is altered does not mean that targeted inhibition will reverse the phenotype. Indeed, it is unclear whether patients without such mutations could also respond to therapy, which would support that these epigenetic therapies could be efficacious for a larger population. Given patients with reduced options, we propose that epigenetic approaches should be considered. The authors here believe that this is a field of study with great promise and may be a possible solution to ICB or CAR-T cell resistance. Further research is necessary to unweave the intricate epigenetic mechanisms in exhausted $CD8^+$ T cells, improve specificity of epigenetic modifying therapy, and discover optimal approaches for reversing terminal $CD8^+$ T cell exhaustion in the TME.

Author contributions

This review was written and edited by MKB, PC, and YAA.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Abdel-Hakeem, M. S., Manne, S., Beltra, J.-C., Stelekati, E., Chen, Z., Nzingha, K., et al. (2021). Epigenetic scarring of exhausted T cells hinders memory differentiation upon eliminating chronic antigenic stimulation. *Nat. Immunol.* 22, 1008–1019. doi:10.1038/s41590-021-00975-5

Adema, V., and Colla, S. (2022). EZH2 inhibitors: The unpacking revolution. *Cancer Res.* 82, 359–361. doi:10.1158/0008-5472.CAN-21-4311

Alfei, F., Kanev, K., Hofmann, M., Wu, M., Ghoneim, H. E., Roelli, P., et al. (2019). TOX reinforces the phenotype and longevity of exhausted T cells in chronic viral infection. *Nature* 571, 265–269. doi:10.1038/s41586-019-1326-9

Baer, M. R., Kogan, A. A., Bentzen, S. M., Mi, T., Lapidus, R. G., Duong, V. H., et al. (2022). Phase I clinical trial of DNA methyltransferase inhibitor decitabine and PARP inhibitor talazoparib combination therapy in relapsed/refractory acute myeloid leukemia. *Clin. Cancer Res.* 28, 1313–1322. doi:10.1158/1078-0432.CCR-21-3729

Balança, C.-C., Salvioni, A., Scarlata, C.-M., Michelas, M., Martinez-Gomez, C., Gomez-Roca, C., et al. (2021). PD-1 blockade restores helper activity of tumor-infiltrating, exhausted PD-1hiCD39+ CD4 T cells. *JCI Insight* 6, e142513. doi:10.1172/jci.insight.142513

Balkhi, M. Y., Wittmann, G., Xiong, F., and Junghans, R. P. (2018). YY1 upregulates checkpoint receptors and downregulates type I cytokines in exhausted, chronically stimulated human T cells. *iScience* 2, 105–122. doi:10.1016/j.isci.2018.03.009

Belk, J. A., Daniel, B., and Satpathy, A. T. (2022). Epigenetic regulation of T cell exhaustion. *Nat. Immunol.* 23, 848–860. doi:10.1038/s41590-022-01224-z

Beltra, J.-C., Manne, S., Abdel-Hakeem, M. S., Kurachi, M., Giles, J. R., Chen, Z., 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. doi:10.1016/j.immuni.2020.04.014

Bengsch, B., Ohtani, T., Khan, O., Setty, M., Manne, S., O'Brien, S., et al. (2018). Epigenomic-guided mass cytometry profiling reveals disease-specific features of exhausted CD8 T cells. *Immunity* 48, 1029–1045. e5. doi:10.1016/j.immuni.2018.04.026

Blank, C. U., Haining, W. N., Held, W., Hogan, P. G., Kallies, A., Lugli, E., et al. (2019). Defining "T cell exhaustion". *Nat. Rev. Immunol.* 19, 665–674. doi:10.1038/s41577-019-0221-9

Buggert, M., Tauriainen, J., Yamamoto, T., Frederiksen, J., Ivarsson, M. A., Michaëlsson, J., et al. (2014). T-bet and Eomes are differentially linked to the exhausted phenotype of CD8+ T cells in HIV infection. *PLoS Pathog.* 10, e1004251. doi:10.1371/journal.ppat.1004251

Caushi, J. X., Zhang, J., Ji, Z., Vaghasia, A., Zhang, B., Hsiue, E. H.-C., et al. (2021). Transcriptional programs of neoantigen-specific TIL in anti-PD-1-treated lung cancers. *Nature* 596, 126–132. doi:10.1038/s41586-021-03752-4

Chen, C., Liu, J., Chen, Y., Lin, A., Mou, W., Zhu, L., et al. (2022). Application of ATAC-seq in tumor-specific T cell exhaustion. *Cancer Gene Ther.* doi:10.1038/s41417-022-00495-w

Chiappinelli, K. B., Strissel, P. L., Desrichard, A., Li, H., Henke, C., Akman, B., et al. (2015). Inhibiting DNA methylation causes an interferon response in cancer via dsRNA including endogenous retroviruses. *Cell* 162, 974–986. doi:10.1016/j.cell.2015.07.011

Doki, Y., Ajani, J. A., Kato, K., Xu, J., Wyrwicz, L., Motoyama, S., et al. (2022). Nivolumab combination therapy in advanced esophageal squamous-cell carcinoma. *N. Engl. J. Med.* 386, 449–462. doi:10.1056/NEJMoa2111380

Dongre, P., Mathew, S., Akopova, I., Gryczynski, I., and Mathew, P. (2013). YY1 and a unique DNA repeat element regulates the transcription of mouse CS1 (CD319, SLAMF7) gene. *Mol. Immunol.* 54, 254–263. doi:10.1016/j.molimm.2012.12.017

Eckschlager, T., Plch, J., Stiborova, M., and Hrabeta, J. (2017). Histone deacetylase inhibitors as anticancer drugs. *Int. J. Mol. Sci.* 18, 1414. doi:10.3390/ijms18071414

Fang, R., Preissl, S., Li, Y., Hou, X., Lucero, J., Wang, X., et al. (2021). Comprehensive analysis of single cell ATAC-seq data with SnapATAC. *Nat. Commun.* 12, 1337. doi:10.1038/s41467-021-21583-9

Giles, J. R., Manne, S., Freilich, E., Oldridge, D. A., Baxter, A. E., George, S., et al. (2022a). Human epigenetic and transcriptional T cell differentiation atlas for identifying functional T cell-specific enhancers. *Immunity* 55, 557–574.e7. e7. doi:10.1016/j.immuni.2022.02.004

Giles, J. R., Ngiow, S. F., Manne, S., Baxter, A. E., Khan, O., Wang, P., et al. (2022b). Shared and distinct biological circuits in effector, memory and exhausted CD8+ T cells revealed by temporal single-cell transcriptomics and epigenetics. *Nat. Immunol.* 23, 1600–1613. doi:10.1038/s41590-022-01338-4

Giri, A. K., and Aittokallio, T. (2019). DNMT inhibitors increase methylation in the cancer genome. *Front. Pharmacol.* 10, 385. doi:10.3389/fphar.2019.00385

Gong, D., and Malek, T. R. (2007). Cytokine-dependent Blimp-1 expression in activated T cells inhibits IL-2 production. *J. Immunol.* 178, 242–252. doi:10.4049/jimmunol.178.1.242

Gordon, S., Akopyan, G., Garban, H., and Bonavida, B. (2006). Transcription factor YY1: Structure, function, and therapeutic implications in cancer biology. *Oncogene* 25, 1125–1142. doi:10.1038/sj.onc.1209080

Gore, S. D. (2005). Combination therapy with DNA methyltransferase inhibitors in hematologic malignancies. *Nat. Clin. Pract. Oncol.* 2, S30–S35. doi:10.1038/ncponc0346

Goswami, S., Apostolou, I., Zhang, J., Skepner, J., Anandhan, S., Zhang, X., et al. (2018). Modulation of EZH2 expression in T cells improves efficacy of anti-CTLA-4 therapy. *J. Clin. Invest.* 128, 3813–3818. doi:10.1172/JCI99760

Grosselin, K., Durand, A., Marsolier, J., Poitou, A., Marangoni, E., Nemati, F., et al. (2019). High-throughput single-cell ChIP-seq identifies heterogeneity of chromatin states in breast cancer. *Nat. Genet.* 51, 1060–1066. doi:10.1038/s41588-019-0424-9

Gust, J., Ponce, R., Liles, W. C., Garden, G. A., and Turtle, C. J. (2020). Cytokines in CAR T cell-associated neurotoxicity. *Front. Immunol.* 11, 577027. doi:10.3389/fimmu.2020.577027

Gyorki, D. E., Asselin-Labat, M.-L., van Rooijen, N., Lindeman, G. J., and Visvader, J. E. (2009). Resident macrophages influence stem cell activity in the mammary gland. *Breast Cancer Res.* 11, R62. doi:10.1186/bcr2353

He, S., Liu, Y., Meng, L., Sun, H., Wang, Y., Ji, Y., et al. (2017). Ezh2 phosphorylation state determines its capacity to maintain CD8(+) T memory precursors for antitumor immunity. *Nat. Commun.* 8, 2125. doi:10.1038/s41467-017-02187-8

Hellmann, M. D., Paz-Ares, L., Bernabe Caro, R., Zurawski, B., Kim, S.-W., Carcereny Costa, E., et al. (2019). Nivolumab plus ipilimumab in advanced non-small-cell lung cancer. *N. Engl. J. Med.* 381, 2020–2031. doi:10.1056/NEJMoa1910231

Hellmann, M. D., and Ramalingam, S. S. (2020). Nivolumab plus ipilimumab in non-small-cell lung cancer. Reply. *Reply. N. Engl. J. Med.* 382, 875. doi:10.1056/NEJMc1916859

Hensel, N., Gu, Z., SagarWieland, D., Jechow, K., Kemming, J., Llewellyn-Lacey, S., et al. (2021). Memory-like HCV-specific CD8(+) T cells retain a molecular scar after cure of chronic HCV infection. *Nat. Immunol.* 22, 229–239. doi:10.1038/s41590-020-00817-w

Hu, C., Liu, X., Zeng, Y., Liu, J., and Wu, F. (2021). DNA methyltransferase inhibitors combination therapy for the treatment of solid tumor: Mechanism and clinical application. *Clin. Epigenetics* 13, 166. doi:10.1186/s13148-021-01154-x

Hudson, W. H., Gensheimer, J., Hashimoto, M., Wieland, A., Valanparambil, R. M., Li, P., et al. (2019). Proliferating transitory T cells with an effector-like transcriptional signature emerge from PD-1(+) stem-like CD8(+) T cells during chronic infection. *Immunity* 51, 1043–1058. e4. doi:10.1016/j.immuni.2019.11.002

Jiang, W., He, Y., He, W., Wu, G., Zhou, X., Sheng, Q., et al. (2021). Exhausted CD8+T cells in the tumor immune microenvironment: New pathways to therapy. *Front. Immunol.* 11, 622509. doi:10.3389/fimmu.2020.622509

Jin, N., George, T. L., Otterson, G. A., Verschraegen, C., Wen, H., Carbone, D., et al. (2021). Advances in epigenetic therapeutics with focus on solid tumors. *Clin. Epigenetics* 13, 83. doi:10.1186/s13148-021-01069-7

Kallies, A., Zehn, D., and Utzschneider, D. T. (2020). Precursor exhausted T cells: Key to successful immunotherapy? *Nat. Rev. Immunol.* 20, 128–136. doi:10.1038/s41577-019-0223-7

Khan, O., Giles, J. R., McDonald, S., Manne, S., Ngiow, S. F., Patel, K. P., et al. (2019). TOX transcriptionally and epigenetically programs CD8(+) T cell exhaustion. *Nature* 571, 211–218. doi:10.1038/s41586-019-1325-x

Kim, E., Kim, M., Woo, D.-H., Shin, Y., Shin, J., Chang, N., et al. (2013). Phosphorylation of EZH2 activates STAT3 signaling via STAT3 methylation and promotes tumorigenicity of glioblastoma stem-like cells. *Cancer Cell* 23, 839–852. doi:10.1016/j.ccr.2013.04.008

Kim, J. R., Mathew, S. O., and Mathew, P. A. (2016). Blimp-1/PRDM1 regulates the transcription of human CS1 (SLAMF7) gene in NK and B cells. *Immunobiology* 221, 31–39. doi:10.1016/j.imbio.2015.08.005

Kim, K. H., and Roberts, C. W. M. (2016). Targeting EZH2 in cancer. *Nat. Med.* 22, 128–134. doi:10.1038/nm.4036

Klemen, N. D., Wang, M., Feingold, P. L., Cooper, K., Pavri, S. N., Han, D., et al. (2019). Patterns of failure after immunotherapy with checkpoint inhibitors predict durable progression-free survival after local therapy for metastatic melanoma. *J. Immunother. Cancer* 7, 196. doi:10.1186/s40425-019-0672-3

Knox, J. J., Myles, A., and Cancro, M. P. (2019). T-bet(+) memory B cells: Generation, function, and fate. *Immunol. Rev.* 288, 149–160. doi:10.1111/imr.12736

Kong, W., Dimitri, A., Wang, W., Jung, I.-Y., Ott, C. J., Fasolino, M., et al. (2021). BET bromodomain protein inhibition reverses chimeric antigen receptor extinction and reinvigorates exhausted T cells in chronic lymphocytic leukemia. *J. Clin. Invest.* 131, 1454599–e145516. doi:10.1172/JCI145459

Laino, A. S., Betts, B. C., Veerapathran, A., Dolgalev, I., Sarnaik, A., Quayle, S. N., et al. (2019). HDAC6 selective inhibition of melanoma patient T-cells augments anti-tumor characteristics. *J. Immunother. Cancer* 7, 33. doi:10.1186/s40425-019-0517-0

Li, J., He, Y., Hao, J., Ni, L., and Dong, C. (2018). High levels of Eomes promote exhaustion of anti-tumor CD8(+) T cells. *Front. Immunol.* 9, 2981. doi:10.3389/fimmu.2018.02981

Li, R., Pan, Z., Wu, J., Yue, S., Lin, Y., Yang, Y., et al. (2020). The epigenetic regulator EZH2 instructs CD4 T cell response to acute viral infection via coupling of cell expansion and metabolic fitness. *J. Virol.* 94, 016277-e1720. doi:10.1128/JVI.01627-20

Lu, S., Stein, J. E., Rimm, D. L., Wang, D. W., Bell, J. M., Johnson, D. B., et al. (2019). Comparison of biomarker modalities for predicting response to PD-1/PD-L1 checkpoint blockade: A systematic review and meta-analysis. *JAMA Oncol.* 5, 1195–1204. doi:10.1001/jamaoncol.2019.1549

Lue, J. K., Prabhu, S. A., Liu, Y., Gonzalez, Y., Verma, A., Mundi, P. S., et al. (2019). Precision targeting with EZH2 and HDAC inhibitors in epigenetically dysregulated lymphomas. *Clin. Cancer Res.* 25, 5271–5283. doi:10.1158/1078-0432.CCR-18-3989

Maurice, N. J., Berner, J., Taber, A. K., Zehn, D., and Prlic, M. (2021). Inflammatory signals are sufficient to elicit TOX expression in mouse and human CD8+ T cells. *JCI Insight* 6, e150744. doi:10.1172/jci.insight.150744

McCaw, T. R., Li, M., Starenki, D., Liu, M., Cooper, S. J., Arend, R. C., et al. (2019). Histone deacetylase inhibition promotes intratumoral CD8(+) T-cell responses, sensitizing murine breast tumors to anti-PD1. *Cancer Immunol. Immunother.* 68, 2081–2094. doi:10.1007/s00262-019-02430-9

McLane, L. M., Ngiow, S. F., Chen, Z., Attanasio, J., Manne, S., Ruthel, G., 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. doi:10.1016/j.celrep.2021.109120

Mehrmohamadi, M., Sepehri, M. H., Nazer, N., and Norouzi, M. R. (2021). A comparative overview of epigenomic profiling methods. *Front. Cell Dev. Biol.* 9, 714687. doi:10.3389/fcell.2021.714687

Mikkelsen, T. S., Ku, M., Jaffe, D. B., Issac, B., Lieberman, E., Giannoukos, G., et al. (2007). Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* 448, 553–560. doi:10.1038/nature06008

Morschhauser, F., Tilly, H., Chaidos, A., McKay, P., Phillips, T., Assouline, S., et al. (2020). Tazemetostat for patients with relapsed or refractory follicular lymphoma: An open-label, single-arm, multicentre, phase 2 trial. *Lancet Oncol.* 21, 1433–1442. doi:10.1016/S1470-2045(20)30441-1

Mumbach, M. R., Rubin, A. J., Flynn, R. A., Dai, C., Khavari, P. A., Greenleaf, W. J., et al. (2016). HiChIP: Efficient and sensitive analysis of protein-directed genome architecture. *Nat. Methods* 13, 919–922. doi:10.1038/nmeth.3999

Nadeau, S., and Martins, G. A. (2022). Conserved and unique functions of Blimp1 in immune cells. *Front. Immunol.* 12, 805260. doi:10.3389/fimmu.2021.805260

O'Carroll, D., Erhardt, S., Pagani, M., Barton, S. C., Surani, M. A., and Jenuwein, T. (2001). The polycomb-group gene Ezh2 is required for early mouse development. *Mol. Cell Biol.* 21, 4330–4336. doi:10.1128/MCB.21.13.4330-4336.2001

O'Connell, P., Hyslop, S., Blake, M. K., Godbehere, S., Amalfitano, A., and Aldhamen, Y. A. (2021). SLAMF7 signaling reprograms T cells toward exhaustion in the tumor microenvironment. *J. Immunol.* 206, 193–205. doi:10.4049/jimmunol.2000300

Pauken, K. E., Sammons, M. A., Odorizzi, P. M., Manne, S., Godec, J., Khan, O., et al. (2016). Epigenetic stability of exhausted T cells limits durability of reinvigoration by PD-1 blockade. *Science* 354, 1160–1165. doi:10.1126/science.aaf2807

Philip, M., Fairchild, L., Sun, L., Horste, E. L., Camara, S., Shakiba, M., et al. (2017). Chromatin states define tumour-specific T cell dysfunction and reprogramming. *Nature* 545, 452–456. doi:10.1038/nature22367

Philip, M., and Schietinger, A. (2019). Heterogeneity and fate choice: T cell exhaustion in cancer and chronic infections. *Curr. Opin. Immunol.* 58, 98–103. doi:10.1016/j.coi.2019.04.014

Pritykin, Y., van der Veeken, J., Pine, A. R., Zhong, Y., Sahin, M., Mazutis, L., et al. (2021). A unified atlas of CD8 T cell dysfunctional states in cancer and infection. *Mol. Cell* 81, 2477–2493.e10. e10. doi:10.1016/j.molcel.2021.03.045

Ranzoni, A. M., Tangherloni, A., Berest, I., Riva, S. G., Myers, B., Strzelecka, P. M., et al. (2021). Integrative single-cell RNA-seq and ATAC-seq analysis of human developmental hematopoiesis. *Cell Stem Cell* 28, 472–487.e7. e7. doi:10.1016/j.stem.2020.11.015

Ross, S. H., and Cantrell, D. A. (2018). Signaling and function of interleukin-2 in T lymphocytes. *Annu. Rev. Immunol.* 36, 411–433. doi:10.1146/annurev-immunol-042617-053352

Roulois, D., Loo Yau, H., Singhania, R., Wang, Y., Danesh, A., Shen, S. Y., et al. (2015). DNA-demethylating agents target colorectal cancer cells by inducing viral mimicry by endogenous transcripts. *Cell* 162, 961–973. doi:10.1016/j.cell.2015.07.056

Rubin, D. B., and Vaitkevicius, H. (2021). Neurological complications of cancer immunotherapy (CAR T cells). *J. Neurol. Sci.* 424, 117405. doi:10.1016/j.jns.2021.117405

Sade-Feldman, M., Yizhak, K., Bjorgaard, S. L., Ray, J. P., de Boer, C. G., Jenkins, R. W., et al. (2018). Defining T cell states associated with response to checkpoint immunotherapy in melanoma. *Cell* 175, 998–1013. e20. doi:10.1016/j.cell.2018.10.038

Scott, A. C., Dündar, F., Zumbo, P., Chandran, S. S., Klebanoff, C. A., Shakiba, M., et al. (2019). TOX is a critical regulator of tumour-specific T cell differentiation. *Nature* 571, 270–274. doi:10.1038/s41586-019-1324-y

Sen, D. R., Kaminski, J., Barnitz, R. A., Kurachi, M., Gerdemann, U., Yates, K. B., et al. (2016). The epigenetic landscape of T cell exhaustion. *Science* 354, 1165–1169. doi:10.1126/science.aae0491

Shaffer, A. L., Lin, K. I., Kuo, T. C., Yu, X., Hurt, E. M., Rosenwald, A., et al. (2002). Blimp-1 orchestrates plasma cell differentiation by extinguishing the mature B cell gene expression program. *Immunity* 17, 51–62. doi:10.1016/s1074-7613(02)00335-7

Shin, H., Blackburn, S. D., Intlekofer, A. M., Kao, C., Angelosanto, J. M., Reiner, S. L., et al. (2009). A role for the transcriptional repressor Blimp-1 in CD8(+) T cell exhaustion during chronic viral infection. *Immunity* 31, 309–320. doi:10.1016/j.immuni.2009.06.019

Shorstova, T., Foulkes, W. D., and Witcher, M. (2021). Achieving clinical success with BET inhibitors as anti-cancer agents. *Br. J. Cancer* 124, 1478–1490. doi:10.1038/s41416-021-01321-0

Siddiqui, I., Schaeuble, K., Chennupati, V., Fuertes Marraco, S. A., Calderon-Copete, S., Pais Ferreira, D., 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. doi:10.1016/j.immuni.2018.12.021

Skene, P. J., and Henikoff, S. (2017). An efficient targeted nuclease strategy for high-resolution mapping of DNA binding sites. *Elife* 6, e21856. doi:10.7554/eLife.21856

Stairiker, C. J., Thomas, G. D., and Salek-Ardakani, S. (2020). EZH2 as a regulator of CD8+ T cell fate and function. *Front. Immunol.* 11, 593203. doi:10.3389/fimmu.2020.593203

Su, I., Dobenecker, M.-W., Dickinson, E., Oser, M., Basavaraj, A., Marqueron, R., et al. (2005). Polycomb group protein ezh2 controls actin polymerization and cell signaling. *Cell* 121, 425–436. doi:10.1016/j.cell.2005.02.029

Sullivan, B. M., Juedes, A., Szabo, S. J., von Herrath, M., and Glimcher, L. H. (2003). Antigen-driven effector CD8 T cell function regulated by T-bet. *Proc. Natl. Acad. Sci. U. S. A.* 100, 15818–15823. doi:10.1073/pnas.2636938100

Szabo, S. J., Kim, S. T., Costa, G. L., Zhang, X., Fathman, C. G., and Glimcher, L. H. (2000). A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 100, 655–669. doi:10.1016/s0092-8674(00)80702-3

Tallón de Lara, P., Castañón, H., Vermeer, M., Núñez, N., Silina, K., Sobottka, B., et al. (2021). CD39(+)PD-1(+)CD8(+) T cells mediate metastatic dormancy in breast cancer. *Nat. Commun.* 12, 769. doi:10.1038/s41467-021-21045-2

Tang, L., Zhang, Y., Hu, Y., and Mei, H. (2021). T cell exhaustion and CAR-T immunotherapy in hematological malignancies. *Biomed. Res. Int.* 2021, 6616391. doi:10.1155/2021/6616391

Tawbi, H. A., Forsyth, P. A., Algazi, A., Hamid, O., Hodi, F. S., Moschos, S. J., et al. (2018). Combined nivolumab and ipilimumab in melanoma metastatic to the brain. *N. Engl. J. Med.* 379, 722–730. doi:10.1056/NEJMoa1805453

Thornton, C. A., Mulqueen, R. M., Torkenczy, K. A., Nishida, A., Lowenstein, E. G., Fields, A. J., et al. (2021). Spatially mapped single-cell chromatin accessibility. *Nat. Commun.* 12, 1274. doi:10.1038/s41467-021-21515-7

Tonnerre, P., Wolski, D., Subudhi, S., Aljabban, J., Hoogeveen, R. C., Damasio, M., et al. (2021). Differentiation of exhausted CD8(+) T cells after termination of chronic antigen stimulation stops short of achieving functional T cell memory. *Nat. Immunol.* 22, 1030–1041. doi:10.1038/s41590-021-00982-6

Wang, D., Quiros, J., Mahuron, K., Pai, C.-C., Ranzani, V., Young, A., et al. (2018). Targeting EZH2 reprograms intratumoral regulatory T cells to enhance cancer immunity. *Cell Rep.* 23, 3262–3274. doi:10.1016/j.celrep.2018.05.050

Wang, J., Ioan-Facsinay, A., van der Voort, E. I. H., Huizinga, T. W. J., and Toes, R. E. M. (2007). Transient expression of FOXP3 in human activated nonregulatory CD4+ T cells. *Eur. J. Immunol.* 37, 129–138. doi:10.1002/eji.200636435

Wang, L., Chen, C., Song, Z., Wang, H., Ye, M., Wang, D., et al. (2022). EZH2 depletion potentiates MYC degradation inhibiting neuroblastoma and small cell carcinoma tumor formation. *Nat. Commun.* 13, 12. doi:10.1038/s41467-021-27609-6

Wang, Y., Tong, C., Dai, H., Wu, Z., Han, X., Guo, Y., et al. (2021). Low-dose decitabine priming endows CAR T cells with enhanced and persistent antitumour potential via epigenetic reprogramming. *Nat. Commun.* 12, 409. doi:10.1038/s41467-020-20696-x

Wherry, E. J., Ha, S.-J., Kaech, S. M., Haining, W. N., Sarkar, S., Kalia, V., et al. (2007). Molecular signature of CD8+ T cell exhaustion during chronic viral infection. *Immunity* 27, 670–684. doi:10.1016/j.immuni.2007.09.006

Wherry, E. J., and Kurachi, M. (2015). Molecular and cellular insights into T cell exhaustion. *Nat. Rev. Immunol.* 15, 486–499. doi:10.1038/nri3862

Wolchok, J. D., Kluger, H., Callahan, M. K., Postow, M. A., Rizvi, N. A., Lesokhin, A. M., et al. (2013). Nivolumab plus ipilimumab in advanced melanoma. *N. Engl. J. Med.* 369, 122–133. doi:10.1056/NEJMoa1302369

Wu, T., Ji, Y., Moseman, E. A., Xu, H. C., Manglani, M., Kirby, M., et al. (2016). The TCF1-Bcl6 axis counteracts type I interferon to repress exhaustion and maintain T cell stemness. *Sci. Immunol.* 1, eaai8593. doi:10.1126/sciimmunol.aai8593

Yang, Y., and Wang, Y. (2021). Role of epigenetic regulation in plasticity of tumor immune microenvironment. *Front. Immunol.* 12, 640369. doi:10.3389/fimmu.2021.640369

Yao, C., Sun, H.-W., Lacey, N. E., Ji, Y., Moseman, E. A., Shih, H.-Y., et al. (2019). Single-cell RNA-seq reveals TOX as a key regulator of CD8(+) T cell persistence in chronic infection. *Nat. Immunol.* 20, 890–901. doi:10.1038/s41590-019-0403-4

Yates, K. B., Tonnerre, P., Martin, G. E., Gerdemann, U., Al Abosy, R., Comstock, D. E., et al. (2021). Epigenetic scars of CD8(+) T cell exhaustion persist after cure of chronic infection in humans. *Nat. Immunol.* 22, 1020–1029. doi:10.1038/s41590-021-00979-1

Yoshikawa, T., Wu, Z., Inoue, S., Kasuya, H., Matsushita, H., Takahashi, Y., et al. (2022). Genetic ablation of PRDM1 in antitumor T cells enhances therapeutic efficacy of adoptive immunotherapy. *Blood* 139, 2156–2172. doi:10.1182/blood.2021012714

Young, A., Quandt, Z., and Bluestone, J. A. (2018). The balancing act between cancer immunity and autoimmunity in response to immunotherapy. *Cancer Immunol. Res.* 6, 1445–1452. doi:10.1158/2326-6066.CIR-18-0487

Zehn, D., Thimme, R., Lugli, E., de Almeida, G. P., and Oxenius, A. (2022). Stem-like" precursors are the fount to sustain persistent CD8(+) T cell responses. *Nat. Immunol.* 23, 836–847. doi:10.1038/s41590-022-01219-w

Zeng, Z., Wei, F., and Ren, X. (2020). Exhausted T cells and epigenetic status. *Cancer Biol. Med.* 17, 923–936. doi:10.20892/j.issn.2095-3941.2020.0338

Zhong, M., Gao, R., Zhao, R., Huang, Y., Chen, C., Li, K., et al. (2022). BET bromodomain inhibition rescues PD-1-mediated T-cell exhaustion in acute myeloid leukemia. *Cell Death Dis.* 13, 671. doi:10.1038/s41419-022-05123-x

Zhu, L., Kong, Y., Zhang, J., Claxton, D. F., Ehmann, W. C., Rybka, W. B., et al. (2017). Blimp-1 impairs T cell function via upregulation of TIGIT and PD-1 in patients with acute myeloid leukemia. *J. Hematol. Oncol.* 10, 124. doi:10.1186/s13045-017-0486-z

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Immunosuppressive functions of melanoma cell-derived exosomes in plasma of melanoma patients

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Tumor-derived exosomes (TEX) are a subset of small extracellular vesicles (sEV) present in all body fluids of patients with cancer. In plasma of patients with metastatic melanoma, numbers of exosomes produced by melanoma cells called MTEX are elevated. To study the role of MTEX in melanoma progression, immunoaffinity-based separation of MTEX from total plasma exosomes was performed. The surface of MTEX was decorated by various checkpoint inhibitory proteins, and upon coincubation with immune recipient cells, MTEX suppressed anti-tumor functions of these cells. MTEX emerge as a major mechanism of immune suppression in melanoma and thus might play a role in promoting melanoma progression.

KEYWORDS

melanoma, small extracellular vesicles, exosomes, melanoma cell-derived exosomes (MTEX), immune capture, immune suppression

Introduction

Extracellular vesicles (EVs) are produced and released by all cells. However, stressed cells, including cancer cells, produce an excess of EVs, and plasma of cancer patients is enriched in circulating tumor cell-derived vesicles called TEX. The main function of EVs is intercellular communication, which involves the transfer of information between cells, especially cells distantly located from one another. EVs are heterogenous and vary broadly in size, cellular origin, biogenesis and molecular/genetic cargos they carry (Whiteside, 2017). TEX are a subset of circulating EVs with unique characteristics that set them apart from other vesicles (Czystowska-Kuzmicz and Whiteside, 2021). Specifically, TEX originate from the late endosomes/multivesicular bodies (MVBs) in tumor cells and are released into extracellular space upon fusion of MVBs with the cell membrane. They are small vesicles sized at 30–150nm, and their surface topography as well as the lumen content resemble those of parent tumor cells (Czystowska-Kuzmicz and Whiteside, 2021). The current EV nomenclature places TEX in the category of small EVs (sEV) or exosomes (Thery et al., 2018), and it emphasizes their distinction from larger microvesicles (MVs) ranging in size from 200 to 500 nm and from much larger apoptotic bodies based not only on the vesicle size but also distinct biogenesis. Melanoma cell-derived TEX

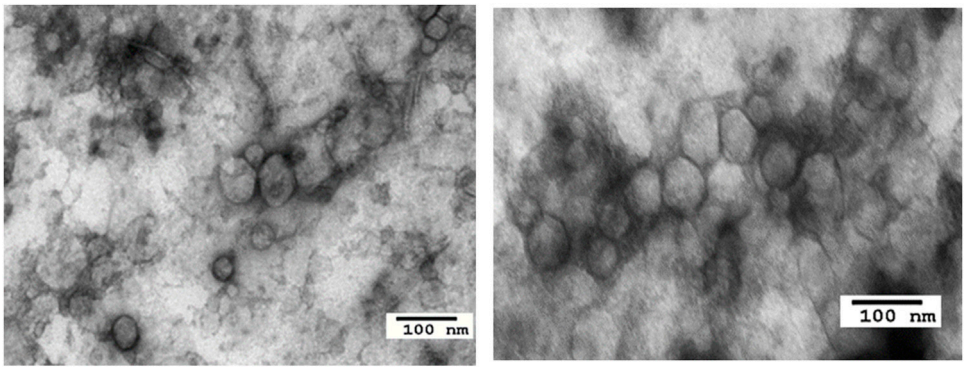

FIGURE 1
Transmission electron microscopy images of sEV from a healthy donor's plasma (left) and from a melanoma patient's plasma (right).

(MTEX) circulate freely and cross the BBB as well as all organ barriers (Banks et al., 2022). Upon contact with the cell membrane of recipient cells, MTEX enter into the cytosol, engaging various mechanisms that facilitate their entry, including receptor/ligand signaling, membrane fusion, integrin-mediated uptake, opsonization, endocytosis or phagocytosis (Mulcahy et al., 2014). The vesicle entry results in transcriptional and molecular changes in the recipient cell. These changes reflect the ability of MTEX to re-program functions of recipient cells (Czystowska-Kuzmicz and Whiteside, 2021).

This commentary discusses the interactions of TEX derived from melanoma cells (MTEX) with immune cells and describes the consequences of MTEX-mediated transfer of information from the tumor to immune cells. The impact of the MTEX-T cell crosstalk is emphasized as an example of cancer-driven functional reprogramming that promotes melanoma progression by inducing dysfunction of immune cells, especially $CD8^+$ effector T-cell.

MTEX isolation from melanoma patients' plasma

For the study described here (Sharma et al., 2020), the banked plasma specimens from patients with metastatic melanoma and healthy donors (HD) were obtained from the U. of Pittsburgh Melanoma SPORE Bank (IRB #991206). All study participants signed an informed consent form. The banked specimens were annotated and were randomly selected for the studies described here. Thawed plasma samples were pre-cleared by centrifugation and were ultrafiltered prior to size exclusion chromatography (SEC) as previously described (Hong et al., 2016). Small extracellular vesicles (sEV) were eluted in the void volume with PBS and harvested in fraction #4. Following concentration with 100,000 MWCO Vivaspin 500 centrifugal concentrators, protein levels of vesicles were measured using a BCA protein assay kit. The vesicle size and particle numbers were verified using qNano or NanoSite. Transmission electron microscopy (TEM) was used to visualize the vesicles. The sEV isolated from melanoma plasma had the vesicular morphology, a mean size of 90–110 nm and were positive for tetraspanins (CD63, CD81), TSG101 and ALIX, but were negative for the cytosolic proteins, calnexin and grp94 in western blots. TAM images of sEV obtained from a melanoma patient's plasma were similar in the morphology and size to sEV isolated from plasma of patients and HDs (Figure 1). Total sEV protein levels were higher in patients than in HDs: mean 76 μg/ml vs. 54 μg/ml (Figure 2A) and were not significantly different in melanoma patients with no evident disease (NED) or active metastatic disease at blood draw for this study (Sharma et al., 2020). Total plasma-derived sEV in the SEC fraction #4 were used for the separation of MTEX from non-malignant cell-derived vesicles, non-MTEX.

MTEX isolation from total plasma exosomes was performed by immune capture using the melanoma cell-specific monoclonal Abs provided by Dr. Soldano Ferrone (Harvard U.) Anti-CSPG4 mAbs (clones 763.64 and 225.28) recognize an epitope of CSPG4 (also known as the high molecular weight melanoma associated antigen) present on melanoma cells but not on any other non-malignant cells or tissues as determined by immunostaining and previously reported (Campoli et al., 2004). The capture mAb (763.64) was biotinylated, and MTEX captured by the biotinylated mAb harvested on streptavidin-charged magnetic beads as described (Sharma et al., 2018). The non-captured vesicles were recaptured using biotinylated anti-CD63 mAb and streptavidin beads. Both fractions, MTEX and non-MTEX, were studied by on-bead flow cytometry for their protein profiles (Theodoraki et al.,

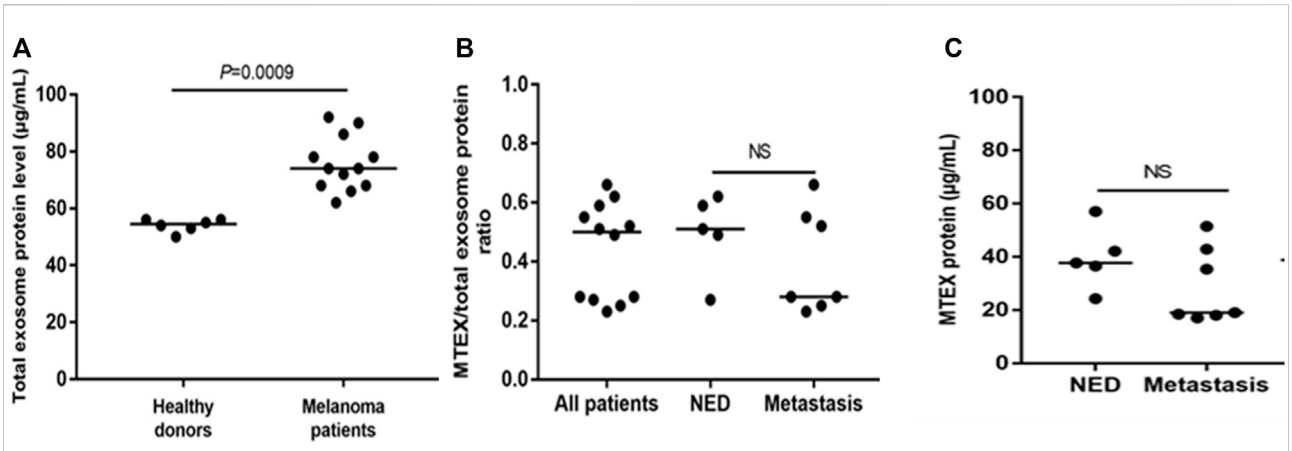

FIGURE 2
Protein levels in total plasma exosomes of melanoma patients or HDs and in isolated MTEX. In **(A)**, total exosome protein (TEP) levels in plasma of HDs or melanoma patients. In **(B)**, the total MTEX/TEP ratio for all patients vs. patients with NED or with progressive disease following oncological therapy. In **(C)**, protein levels in MTEX obtained from plasma of NED patients or melanoma patients with metastases (i.e., progressive disease following oncological.

2021), and the detected protein expression levels were calculated as Relative Fluorescence Intensity (RFI) values equal to the ratio of MFI detection mAb/MFI isotype control. TEM of isolated MTEX and non-MTEX showed vesicles with morphology similar to that of total plasma EVs, although the MTEX size was somewhat smaller than that of non-MTEX. The protein content of isolated MTEX ranged from 20 to 60µg/ml and was no different for melanoma patients with NED vs. patients with progressive disease (PD) after oncologic therapy (Figure 1B). The MTEX/total sEV protein (TEP) ratio was also not different for these two patient groups (Figure 1C). Among 12 melanoma patients, MTEX represented 23%–66% of total plasma sEV (Sharma et al., 2020). This may appear as a very high proportion of recovered MTEX, and it contrasts with the scarce data for the recovery of cancer TEX from plasma reported in the literature. In one study of patients with NSCLC, anti-EpCAM Abs were used to capture TEX, and their recovery ranged from 0.5%–11% of total plasma sEVs(Yoh et al., 2021). In other studies, TEX were identified based on the TEX associated gene expression profiles (Wu et al., 2021), with recoveries that were very low (Vitale et al., 2021) or by microfluidics based capture on chips, where co-expression levels of tumor-associated antigens (TAA) present on TEX were high, but TEX recovery was not evaluated (Zhao et al., 2016; Yu et al., 2021). The broad frequency range of MTEX recovered from plasma seen in our study suggests that the MTEX/non-MTEX ratios vary with disease activity, as two of the patients in our small cohort with the highest MTEX recovery had advanced ocular melanoma with pulmonary and liver metastases.

Immunocapture of MTEX with anti-CSPG4 mAb proved to be highly effective, largely due to its specificity for the melanoma cells/exosomes and its high binding avidity. As such, it has been repeatedly used in our studies of MTEX. Specificity of the immunocapture for MTEX was verified by demonstrating that MTEX isolated from various melanoma cell lines showed the presence of CSPPG4 on all sEVs albeit at various expression levels; only MTEX isolated from plasma were CSPG4+, while non-MTEX were CSPG4 (-); sEV obtained from plasma of HDs were CSPG4 (-) and only MTEX were highly enriched in melanoma-associated antigens (MAA), TYRP2, Melan A, Gp100, VLA4; only non-MTEX were $CD3^{+}$, while MTEX were CD3 (−). When MTEX were added to vesicles obtained from HDs plasma in spiking experiments, immune capture recovered all CSPG4+ vesicles, while the non-captured fraction was CSPG4 (−).

Protein profiles of MTEX and non-MTEX

On-bead flow cytometry of MTEX and non-MTEX was performed as previously described (Theodoraki et al., 2021) to evaluate their surface protein profiles, primarily looking at the expression levels of immunoregulatory proteins. Table 1 and Table 2 list RFI values for immunosuppressive and immunostimulatory surface proteins on MTEX isolated from 12 patients with melanoma. By adding individual RFI values for all surface proteins carried on MTEX, we calculated the suppressor and stimulatory RFI scores for each patient (Table 1). The sum of the suppressor or stimulatory scores gave the mean RFI scores for all 12 patients. The same set of data was obtained for all paired non-MTEX fractions (data not shown). Although the same immunoregulatory proteins were detectable in MTEX and non-MTEX, the mean RFI score for

TABLE 1 Immunosuppressive proteins in MTEX[a].

Patient	Supp RFI Score[b]	PD1	PDL-1	CD39	CD73	Fas	FasL	LAP-TGFβ	TRAIL	CTLA-4
1	16.8	3.3	4	1	1.2	4	1.8	4	3.8	1
2	13.4	1.6	1	1.8	2.4	2	2.2	1	3.8	1.2
3	13	1.1	2.5	3	1	1	2	1	1	2.5
4	20.3	8	1.6	1.1	1	4.8	7.2	1.8	6	1.6
5	15.2	2.8	1.8	2	1	2.3	2.8	3	3.6	1
6	10.8	5.9	1	1	2.9	2.3	2.3	1	1	1.6
7	19.9	2	1.2	1.5	1	2.8	4.8	4.8	4	2.6
8	16.7	6.8	3.9	1.4	1	2.6	4.4	3.7	1.3	1
9	17.1	6.2	4.2	1.4	1	1.3	2.4	3.8	2.5	1.8
10	9.8	5.8	1.3	1.3	1	5	2.4	1	1	1.8
11	15.5	6.5	1.4	2.7	2.2	2.5	2.8	1.8	3.6	1
12	15.2	6.1	1	3.4	1.4	2.4	2.3	1.3	3.2	2.6

[a]Proteins detected on the surface of MTEX by on-bead flow cytometry.
[b]Supp RFI Score = the sum of PD1, PD-L1, CD39, CD73, Fas, FasL, LAP-TGFβ, and CTLA4.
The mean RFI Score for immunosuppressive proteins in MTEX, 15.3.

TABLE 2 Immunostimulatory proteins in MTEX.[a].

Patient	Stim RFI score[a]	CD40	CD40L	CD80	OX40	OX40L
1	14.4	1	1	3	4	5.4
2	5.3	1	1.1	1	1.1	1.1
3	6.2	1	1.1	1	1.1	2
4	12	1	4.2	1	3.5	2.3
5	6.4	1	1	1	1	2.4
6	6	1	1.1	1	1.4	1.5
7	10.7	1	1	1	3.8	3.9
8	6.6	1	1.2	1.2	1.2	2
9	7.2	1	1	1.2	1.9	2.1
10	14.6	1	2.2	1.2	3	7.2
11	8.9	1	1.1	1	3.7	2.1
12	7.2	1	1	1	1.7	2.5

[a]Stim RFI score = the sum of CD40, CD40L, CD80, OX40 and OX40L.
The mean RFI Score for immunostimulatory proteins in MTEX, 8.8.

immunosuppressive proteins was significantly higher (p = 0.03) for MTEX (15.3) than for non-MTEX (11.7). The mean RFI score for *immunostimulatory* proteins was significantly lower (p = 0.002) for MTEX (8.8) than for non-MTEX (16.2). Both suppressive and stimulatory scores for non-MTEX were similar to those calculated for sEV of HDs. We also calculated the stimulatory/suppressor ratio for MTEX, which was significantly lower (p = 0.001) than the ratio for non-MTEX or for sEV of HDs at 0.6, 1.4 and 2.2, respectively. In aggregate, the quantitative flow cytometry data indicated that MTEX were significantly enriched in immunosuppressive surface proteins, while non-MTEX largely carried immunostimulatory surface proteins. This observation indicated that MTEX are more likely to mediate suppression of immune cells than non-MTEX or sEV from plasma of HDs.

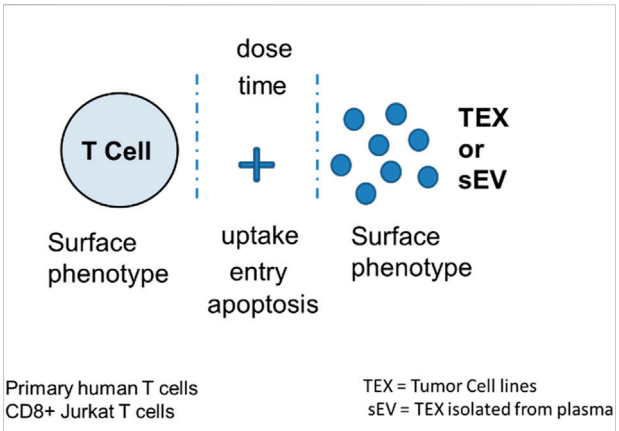

FIGURE 3
Measurement of TEX-induced phenotypic and functional changes in T cells following their co-incubation with TEX or sEV from cancer plasma. Following co-incubation at optimized doses for different time periods depending on the assay, T cells were tested for the vesicle uptake or entry into the cytosol or for changes in the surface protein profile, in growth and apoptosis levels or in mRNA transcription. Vertical stipled lines indicate that inhibitors of the T cell-TEX crosstalk can be used to block T cell-TEX interactions.

assays, while non-MTEX and sEV of HDs were in solution. The presence of beads alone or beads coated with biotinylated CSPG4 capture mAbs did not interfere with the functional assays (data not shown). Table 3 summarizes results of these assays.

Coincubation of the isolated vesicles with human primary T cells or NK cells led to significant inhibition of activation, proliferation and survival of the immune cells interacting with MTEX but not of cells coincubated with non-MTEX or sEV obtained from plasma of HDs. The data in Table 3 are median values for inhibitory functions of the vesicles from 12 patients with metastatic melanoma. The ranges of inhibitory activity of MTEX and non-MTEX varied broadly among the patients. While non-MTEX paralleled the functional behavior of sEV of HDs in all assays, in some patients, non-MTEX showed mild inhibitory activity (Sharma et al., 2020). This observation suggested that non-MTEX, derived from plasma of melanoma patients, were not functionally identical with sEV of HDs.

We previously reported that TEX co-incubated for 6 h with human primary T cells were not readily internalized but remained at the T-cell surface for 15–30min (Muller et al., 2017). This observation suggested that both CD69 (an

TABLE 3 Results of coincubation assays of the isolated vesicles with immune cells [a].

Assay	MTEX	Non-MTEX	HD sEV
% T-cell with CD69 expression (flow cytometry)	45% (p = 0.0005)	90%	90%
$CD8^+$T-cell NF-κB activation (p65 nuclear trans-location by confocal microscopy	Yes	No	No
%$CD8^+$T-cell Proliferation (CSFE)	25% (p = 0.0005)	85%	75%
%$CD8^+$T-cell Apoptosis (Annexin/PI binding)	83% (p = 0.0005)	20%	25%
%NK cells with surface NKG2D expression	70% (p = 0.001)	95%	98%
$CD8^+$T-cell Changes in CD69 transcripts (RT-PCR)	Decrease	No change	No change

[a]Primary human T cells and NK cells were co-incubated with paired MTEX, and non-MTEX, or with sEV from HDs (6 h for apoptosis, NKG2D assays or RT-PCR; 72 h for CSFE assays and 30min for NF-κB activation). The data are median values of results obtained in experiments performed in triplicates for 12 patients with metastatic melanoma (Sharma et al., 2020).

Immunosuppressive functions of MTEX and non-MTEX

Immunoregulatory activities of MTEX, non-MTEX and sEV of HDs were evaluated in co-incubation experiments with human primary immune cells (Figure 3). The following functional assays were performed: CD69 protein downregulation on the surface of T cells, changes in CD69 mRNA transcripts in T cells, NF-κB activation in $CD8^+$ T cells, CSFE-based proliferation of $CD8^+$ T cells, apoptosis of $CD8^+$ T cells and NKG2D down-regulation on the surface of NK cells. Utilizing primary *in vitro* activated CD8+T cells or natural killer (NK) cells as vesicle recipient cells in co-incubation experiments, we compared inhibitory effects of MTEX and non-MTEX on the above listed immune cell functions. MTEX captured on beads were used in these

activation antigen) downregulation in T cells and apoptosis of $CD8^+$ T cells measured at 6 h of coincubation with MTEX may be initiated by cell surface signaling events, which then translate into cellular alterations in T cells. Indeed, using RT-PCR we showed that MTEX, but not non-MTEX, induced downregulation of CD69 mRNA transcripts in $CD8^+$ T cells following 6 h coincubation. Also, a 30 min coincubation of $CD8^+$ T cells with MTEX induced translocation of the NF-κB subunit p65 to the nucleus of a T cells, confirming activation of the NF-κB pathway and surface signaling by MTEX, which leads to downregulation of the CD69 expression level. It has been reported that the normally pro-inflammatory NF-κB pathway leads to cellular apoptosis in cancer, where stress due to genetic, metabolic or environmental factors drives the cell damage responses (Janssens et al., 2014; Cao et al., 2016). Vesicles in

all three fractions, MTEX, non-MTEX and HD sEV, induced vesicle dose-dependent apoptosis in activated $CD8^+$T cells; however, MTEX induced significantly higher apoptosis than non-MTEX, which was only partially blocked by neutralizing anti-Fas (ZB4) mAbs. MTEX induced down-regulation of NKG2D (a cytolysis activating antigen) expression levels on the surface of NK cells, and thus inhibited NK cell activity. Preliminary experiments in which we attempted to interfere with the inhibitory signaling of MTEX by preincubation with neutralizing Abs to surface proteins on the MTEX surface showed only a partial and never complete inhibition of apoptosis. Also, the correlations linking the observed functional inhibition with expression of any single inhibitory protein on the MTEX surface were not significant, except for apoptosis, which was only partly blocked with anti-Fas mAb. The experiments with neutralizing mAbs suggested that not any one but rather several simultaneously delivered receptor-ligand signals might be responsible for MTEX mediated apoptosis in activated human primary $CD8^+$T cells (Sharma P, et al., 2020).

Interestingly, MTEX mediate suppression of immune functions that exclusively target anti-tumor immune responses and do not appear to interfere with responses to infections. Such selective suppression of anti-tumor immunity is seen in most patients with malignancies (Whiteside, 2006; Chen and Mellman, 2013), but its severity varies broadly among patients and may relate to disease progression. Thus, patients with melanoma have variously depressed anti-tumor immunity but appear to respond normally to viral or bacterial antigens, except for patients with advanced metastatic disease, whose immune system may be generally compromised.

MTEX and non-MTEX protein profiles *versus* their functional attributes

Functional changes induced in recipient immune cells by MTEX and non-MTEX were correlated with the protein profiles of these vesicles. Total exosome protein (TEP) levels in plasma correlated with the MTEX immunosuppressive score ($p = 0.002$, $r = 0.79$), linking the high TEP levels with the enrichment in suppressive MTEX. Thus, apoptosis correlated with the MTEX/TEP ratio ($p = 0.01$, $r = 0.68$). The RFI scores for FasL and TRAIL in MTEX were significantly elevated relative to non-MTEX, accounting for high MTEX-driven apoptosis. The stim/supp ratio correlated positively with the immuno-stimulatory score ($p = 0.006$, $r = 0.74$). Unexpectedly, apoptosis mediated by non-MTEX was inversely correlated with the stim/supp ratio ($p = 0.007$, $r = -0.75$) and with the immunostimulatory score ($p = 0.009$, $r = -0.72$). Non-MTEX -induced proliferation of T cells positively correlated with their immunostimulatory score ($p = 0.04$, $r = 0.59$). In aggregate, these and other correlations linking immune activities of MTEX and non-MTEX with their phenotypic characteristics showed that: i) MTEX had superior immune suppressor activity *vis a vis* non-MTEX; ii) the stimulatory/inhibitory vesicle activities were dependent on the surface profile of immunoregulatory proteins in MTEX and non-MTEX; and iii) MTEX and non-MTEX abilities to alter functions of immune receptor cells depended on the stim/suppr protein ratios in these vesicles.

MTEX and non-MTEX profiles and patients' clinicopathological data

The group of patients we evaluated consisted of 12 individuals (6 males and six females) aged 32–82 years, previously treated with oncological therapies. Five of these patients had no evident disease (NED) and seven had progressive disease at the time of blood draw for this study. We attempted to explore associations of the observed characteristics of MTEX and non-MTEX with disease status or activity in this small cohort of melanoma patients. While the study was not powered for a formal correlative assessment, it provided several potentially important insights. While MTEX-mediated apoptosis of $CD8^+$T cells did not correlate with disease status or stage at diagnosis, the non-MTEX ability to induce apoptosis associated with disease stage ($p = 0.04$, r = 0.61). This was an unexpected observation, which suggests that non-MTEX might potentially serve as a correlate of disease progression in future studies. We also observed a significant inverse correlation between of the stim/supp ratio with disease stage ($p = 0.0007$, r = -0.83). This suggests that the stim/supp ratio might be more informative about disease progression than individual regulatory protein expression in the vesicular profile. In this study, with the exception of an inverse correlation between disease status and PD-L1 expression levels in MTEX ($p = 0.03$, r = -0.62), no other regulatory proteins on the MTEX surface correlated with disease status or activity.

Concluding remarks

It has been reported that TEX isolated from supernatants of tumor cell lines carry on their surface immunosuppressive ligands, FasL, TRAIL and immunosuppressive proteins such as TGF-β or CD39/CD73, and suppress functions of immune cells *in vitro* and *in vivo* in tumor-bearing mice (Ludwig et al., 2018; Razzo et al., 2020). Recent studies confirm that the surface of sEV (exosomes) isolated from plasma of melanoma patients is decorated by immunosuppressive proteins, including PD-L1 (Chen et al., 2018; Cordonnier et al., 2020). The cellular source of these immunosuppressive vesicles in plasma has remained unknown, however. Here, we considered the evidence for a major role played by MTEX isolated from plasma of patients with melanoma in suppression of immune cell functions. The immunoaffinity-based separation of MTEX

from the non-malignant cell-derived exosomes (non-MTEX) in melanoma patients' plasma allowed for the analysis and comparison of molecular cargos of MTEX and non-MTEX and for establishing MTEX as the main source of immunosuppressive signals delivered to recipient immune cells. MTEX are abundant in plasma of melanoma patients, and in patients with metastatic melanoma, MTEX might represent a majority of circulating vesicles. In this context, MTEX emerge as a major immunosuppressive mechanism that promotes melanoma escape from the host immune system. Our data are in agreement with other reports on tumor-promoting attributes of melanoma-derived EVs (Peinado et al., 2012; Gyukity-Sebestyen et al., 2019; Bollard et al., 2020; Amor Lopez et al., 2021).

To support this conclusion, we showed that the short-term (6 h) coincubation of MTEX with activated effector ($CD8^+$T and NK cells) reduced CD69 expression levels and initiated apoptosis in $CD8^+$T-cell or reduced NKG2D expression in NK cells contributing to the attenuation of NK activity (Hong et al., 2014). These results suggest that signaling *via* MTEX-associated surface proteins is sufficient for eliciting changes in the phenotype or function of recipient T or NK cells. The downregulation of CD69 expression on the T-cell surface was followed by changes in CD69 mRNA transcripts in recipient T cells, indicating that signals delivered by MTEX resulted in transcriptional activation. Further, a 30min coincubation of MTEX with CD8+T cells induced activation of the NF-κB pathway, as evidenced by the translocation of p65 to the nucleus of the recipient cells. These results clearly implicate MTEX in functional reprogramming of normal human effector cells. Interestingly, non-MTEX isolated from peripheral blood of patients with melanoma, but not sEV from plasma of HDs, also downregulated functions of immune cells, albeit much less effectively than MTEX. This observation indicates that non-malignant cells in patients with melanoma may be subverted by the tumor to also produce immunosuppressive vesicles, contributing to melanoma progression. The presented evidence for MTEX as drivers of molecular and transcriptional changes in effector immune cells of patients with melanoma calls for additional studies of MTEX in larger cohorts of patients to better define their role in melanoma progression and confirm their clinical significance.

Author contributions

TW wrote this commentary based on the data reported in the paper by Sharma et al., 2020 which described MTEX isolation and characterization performed in TW's laboratory.

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Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Amor Lopez, A., Mazariegos, M. S., Capuano, A., Ximenez-Embun, P., Hergueta-Redondo, M., Recio, J. A., et al. (2021). Inactivation of EMILIN-1 by proteolysis and secretion in small extracellular vesicles favors melanoma progression and metastasis. *Int. J. Mol. Sci.* 22, 7406. doi:10.3390/ijms22147406

Banks, W. A. S., Hansen, K. M., Ludwig, N., and Whiteside, T. L. (2022). Characteristics of exosomes and the vascular landscape regulate exosome sequestration by peripheral tissues and brain. *Int. J. Mol. Sci.* 23, 12513. doi:10.3390/ijms232012513

Bollard, S. M., Casalou, C., Goh, C. Y., Tobin, D. J., Kelly, P., Mccann, A., et al. (2020). Circulating melanoma-derived extracellular vesicles: Impact on melanoma diagnosis, progression monitoring, and treatment response. *Pharm. (Basel)* 13, 475. doi:10.3390/ph13120475

Cao, S. S., Luo, K. L., and Shi, L. (2016). Endoplasmic reticulum stress interacts with inflammation in human diseases. *J. Cell Physiol.* 231, 288–294. doi:10.1002/jcp.25098

Chen, D. S., and Mellman, I. (2013). Oncology meets immunology: The cancer-immunity cycle. *Immunity* 39, 1–10. doi:10.1016/j.immuni.2013.07.012

Chen, G., Huang, A. C., Zhang, W., Zhang, G., Wu, M., Xu, W., et al. (2018). Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. *Nature* 560, 382–386. doi:10.1038/s41586-018-0392-8

Cordonnier, M., Nardin, C., Chanteloup, G., Derangere, V., Algros, M. P., Arnould, L., et al. (2020). Tracking the evolution of circulating exosomal-PD-L1 to monitor melanoma patients. *J. Extracell. Vesicles* 9, 1710899. doi:10.1080/20013078.2019.1710899

Czystowska-Kuzmicz, M., and Whiteside, T. L. (2021). The potential role of tumor-derived exosomes in diagnosis, prognosis, and response to therapy in cancer. *Expert Opin. Biol. Ther.* 21, 241–258. doi:10.1080/14712598.2020.1813276

Gyukity-Sebestyen, E., Harmati, M., Dobra, G., Nemeth, I. B., Mihaly, J., Zvara, A., et al. (2019). Melanoma-derived exosomes induce PD-1 overexpression and tumor progression via mesenchymal stem cell oncogenic reprogramming. *Front. Immunol.* 10, 2459. doi:10.3389/fimmu.2019.02459

Hong, C. S., Funk, S., Muller, L., Boyiadzis, M., and Whiteside, T. L. (2016). Isolation of biologically active and morphologically intact exosomes from plasma of patients with cancer. *J. Extracell. Vesicles* 5, 29289. doi:10.3402/jev.v5.29289

Hong, C. S., Muller, L., Whiteside, T. L., and Boyiadzis, M. (2014). Plasma exosomes as markers of therapeutic response in patients with acute myeloid leukemia. *Front. Immunol.* 5, 160. doi:10.3389/fimmu.2014.00160

Janssens, S., Pulendran, B., and Lambrecht, B. N. (2014). Emerging functions of the unfolded protein response in immunity. *Nat. Immunol.* 15, 910–919. doi:10.1038/ni.2991

Ludwig, N., Yerneni, S. S., Razzo, B. M., and Whiteside, T. L. (2018). Exosomes from HNSCC promote angiogenesis through reprogramming of endothelial cells. *Mol. Cancer Res.* 16, 1798–1808. doi:10.1158/1541-7786.MCR-18-0358

Mulcahy, L. A., Pink, R. C., and Carter, D. R. (2014). Routes and mechanisms of extracellular vesicle uptake. *J. Extracell. Vesicles* 3, 24641. doi:10.3402/jev.v3.24641

Muller, L., Simms, P., Hong, C. S., Nishimura, M. I., Jackson, E. K., Watkins, S. C., et al. (2017). Human tumor-derived exosomes (TEX) regulate Treg functions via cell surface signaling rather than uptake mechanisms. *Oncoimmunology* 6, e1261243. doi:10.1080/2162402X.2016.1261243

Peinado, H., Aleckovic, M., Lavotshkin, S., Matei, I., Costa-Silva, B., Moreno-Bueno, G., et al. (2012). Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat. Med.* 18, 883–891. doi:10.1038/nm.2753

Razzo, B. M., Ludwig, N., Hong, C. S., Sharma, P., Fabian, K. P., Fecek, R. J., et al. (2020). Tumor-derived exosomes promote carcinogenesis of murine oral squamous cell carcinoma. *Carcinogenesis* 41, 625–633. doi:10.1093/carcin/bgz124

Sharma, P., Diergaarde, B., Ferrone, S., Kirkwood, J. M., and Whiteside, T. L. (2020). Melanoma cell-derived exosomes in plasma of melanoma patients suppress functions of immune effector cells. *Sci. Rep.* 10, 92. doi:10.1038/s41598-019-56542-4

Theodoraki, M. N., Hong, C. S., Donnenberg, V. S., Donnenberg, A. D., and Whiteside, T. L. (2021). Evaluation of exosome proteins by on-bead flow cytometry. *Cytom. A* 99, 372–381. doi:10.1002/cyto.a.24193

Thery, C., Witwer, K. W., Aikawa, E., Alcaraz, M. J., Anderson, J. D., Andriantsitohaina, R., et al. (2018). Minimal information for studies of extracellular vesicles 2018 (MISEV2018): A position statement of the international society for extracellular vesicles and update of the MISEV2014 guidelines. *J. Extracell. Vesicles* 7, 1535750. doi:10.1080/20013078.2018.1535750

Vitale, S. R., Helmijr, J. A., Gerritsen, M., Coban, H., Van Dessel, L. F., Beije, N., et al. (2021). Detection of tumor-derived extracellular vesicles in plasma from patients with solid cancer. *BMC Cancer* 21, 315. doi:10.1186/s12885-021-08007-z

Whiteside, T. L. (2006). Immune suppression in cancer: Effects on immune cells, mechanisms and future therapeutic intervention. *Semin. Cancer Biol.* 16, 3–15. doi:10.1016/j.semcancer.2005.07.008

Whiteside, T. L. (2017). The effect of tumor-derived exosomes on immune regulation and cancer immunotherapy. *Future Oncol.* 13, 2583–2592. doi:10.2217/fon-2017-0343

Wu, J., Zeng, D., Zhi, S., Ye, Z., Qiu, W., Huang, N., et al. (2021). Single-cell analysis of a tumor-derived exosome signature correlates with prognosis and immunotherapy response. *J. Transl. Med.* 19, 381. doi:10.1186/s12967-021-03053-4

Yoh, K. E., Lowe, C. J., Mahajan, S., Suttmann, R., Nguy, T., Reichelt, M., et al. (2021). Enrichment of circulating tumor-derived extracellular vesicles from human plasma. *J. Immunol. Methods* 490, 112936. doi:10.1016/j.jim.2020.112936

Yu, W., Hurley, J., Roberts, D., Chakrabortty, S. K., Enderle, D., Noerholm, M., et al. (2021). Exosome-based liquid biopsies in cancer: Opportunities and challenges. *Ann. Oncol.* 32, 466–477. doi:10.1016/j.annonc.2021.01.074

Zhao, Z., Yang, Y., Zeng, Y., and He, M. (2016). A microfluidic ExoSearch chip for multiplexed exosome detection towards blood-based ovarian cancer diagnosis. *Lab. Chip* 16, 489–496. doi:10.1039/c5lc01117e

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The impact of microbiome dysbiosis on T cell function within the tumor microenvironment (TME)

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Insights into the effect of the microbiome's composition on immune cell function have recently been discerned and further characterized. Microbiome dysbiosis can result in functional alterations across immune cells, including those required for innate and adaptive immune responses to malignancies and immunotherapy treatment. Dysbiosis can yield changes in or elimination of metabolite secretions, such as short-chain fatty acids (SCFAs), from certain bacterial species that are believed to impact proper immune cell function. Such alterations within the tumor microenvironment (TME) can significantly affect T cell function and survival necessary for eliminating cancerous cells. Understanding these effects is essential to improve the immune system's ability to fight malignancies and the subsequent efficacy of immunotherapies that rely on T cells. In this review, we assess typical T cell response to malignancies, classify the known impact of the microbiome and particular metabolites on T cells, discuss how dysbiosis can affect their function in the TME then further describe the impact of the microbiome on T cell-based immunotherapy treatment, with an emphasis on recent developments in the field. Understanding the impact of dysbiosis on T cell function within the TME can carry substantial implications for the design of immunotherapy treatments and further our understanding of factors that could impact how the immune system combats malignancies.

KEYWORDS

microbiome, T cell, tumor microenvironment (TME), dysbiosis, metabolites, T cell signaling, short chain fatty acids (SCFAs), immunotherapy

Introduction

Microbial species inhabit nearly every organ of the human body; their significance has recently been established in proper health and immune cell function, with potential impacts in the tumor microenvironment (TME) through the presence or absence of microbial-derived metabolites such as short-chain fatty acids (SCFAs), that can impact T cell functioning. The human microbiome comprises a complex network of various organisms, including those of bacterial, archaeal, fungal, viral, and protozoan populations, many of which are capable of symbiotic or pathogenic manifestations on the host, particularly when oscillations in microbial composition occur (Riiser, 2015). In a healthy host, microbial populations typically outnumber human cells; current studies have estimated the number of bacteria alone in the human body is roughly the same as that of human cells (Sender et al., 2016). Technological advancements such as metagenomic sequencing and sophisticated data analysis have allowed scientists to characterize the

abundance and diversity of the human microbiome, classifications that have allowed researchers to elucidate the potential mechanisms by which these species impact health and disease (Freilich et al., 2009; Qin et al., 2010; Methé et al., 2012). Studies support the necessity of a diverse, stable, and balanced microbiome to maintain general health and proper immunity to disease, with negative impacts during microbiome dysbiosis (Tuddenham and Sears, 2015). Microbial dysbiosis is an imbalance in the composition of microbial communities within a host resulting in perturbations from normal cellular and organ function, illness, or reduced treatment efficacy for an infection or disease (Petersen and Round, 2014). The etiology of dysbiosis is diverse and includes pathologies resulting from inflammation, infection, diet, genetics, and antibiotic administration (Willing et al., 2010; Claesson et al., 2012; Pham and Lawley, 2014). Current studies have also demonstrated the impact of host-microbiome interactions during dysbiosis on cancer progression and treatment, including disease presence and chronic inflammation (Czesnikiewicz-Guzik and Müller, 2018). Dysbiosis is believed to impact the regular functioning of immune cells caused by modifications in microbial-produced metabolites needed for proper performance (Arpaia et al., 2013; Luu et al., 2019). Such alterations in immune cells can impact not only how we respond to pathogenic infections but also the immune response to neoplastic events.

The degree of T cell tumor site infiltration and proper function within tumor sites can significantly affect tumor progression or regression (Al-Shibli et al., 2008). Therefore, an alteration in T cell function can cause a massive change in the efficacy of how T cells respond within the TME and, ultimately, their ability to clear malignancies. Understanding the mechanistic impact of microbial composition on T cells and their function within the TME is essential if we hope to understand and improve treatment strategies for malignancies. Here, we review canonical T-cell responses to malignancies, the impacts of the microbiome in the context of its typical and dysbiotic state on T-cell signaling and further how this can change T-cell function within the TME, recent discoveries in the field, and the potential that research stemming from these investigations can have on how we design and administer cancer therapies.

Conventional anti-tumoral activity of T-cells

Adaptive immune cells, such as T cells, play a considerable role in the antitumor immune response, with the presence of tumor-infiltrating T lymphocytes exhibiting positive prognostic value across a wide range of cancers (Zhang et al., 2003; Taylor et al., 2007). To understand how microbiome dysbiosis can affect the ability of T cells to react to malignancies, we must first understand conventional anti-tumoral T-cell mechanisms.

Typical CD4 T cell signaling and function in response to malignancies

CD4 T cells are directly and peripherally involved in the antitumor immune response through effects on innate and adaptive immune cells such as macrophages and T cells. Individually, CD4 T cells can be cytotoxic to incipient and progressive malignancies, acting directly on unhealthy cells that present neoepitopes to eliminate them through differentiation into Th1 cells and ensuing perforin/granzyme B-dependent killing or target cell elimination *via* ligation of Fas/FasL (or other TNF/TNFR family) death receptors (Figure 1A) (Lundin et al., 2004). Peripherally, CD4 T cells can also secrete IL-2 and IFN-γ, which have been found to elicit M1 macrophages to inhibit tumor propagation *via* secretion of nitric oxide synthase (iNOS), reactive oxygen species (ROS), and IL-12, resulting in direct and indirect clearance of tumor cells. Additionally, M1 macrophages can engulf malignant cells to eliminate them (Figure 1B) (Zhou et al., 2021). These M1 mechanisms are productive during MHC class II negativity, which can result from the loss of MHC Class II trans-activator (CIITA) expression during carcinogenesis (Haabeth et al., 2014). Additionally, CD4 T cells are necessary to support continued CD8 memory T cell survival and function (Janssen et al., 2003). They secrete cytokines, such as INFγ and IL-2, that can act on CD8 T cells to improve the anticancer immune response (Figure 1C) (Ossendorp et al., 1998).

Typical CD8 T Cell signaling and function in response to malignancies

The presence of CD8 T cells within the TME has been associated with improved tumor clearance and overall prognosis, often characterized by the concomitant presence of pro-inflammatory cytokines such as Type I IFN (Trujillo et al., 2018). The mechanisms by which CD8 T cells within the TME are believed to eliminate cancerous cells are *via* ligation of the Fas ligand (Fas L) on T cells with the Fas receptor (FasR) on target cells or by the perforin/granzyme B pathway, with a preference for FasL/FasR pathway for cancerous cells (Figure 1D) (Chávez-Galán et al., 2009). Though these mechanisms can aid in the clearance of tumors, tumor-infiltrating lymphocytes often display upregulation of inhibitory markers, such as PD-1 and CTLA-4, which bind PD-L1 and CD80/CD86, respectively; these can halt anti-tumoral effector functions and result in reduced effector cytokine production (Ahmadzadeh et al., 2009).

Molecular impact of the microbiome on anti-tumoral activity of T-cells

There is evidence that microbial species within the mucosal tissue of a host and within the tumor microbiome contribute to patient tumor immune response variations, with correlations between metabolic functions of microbes present within the TME and clinical patient presentation (Nejman et al., 2020). These trends have been documented for several pancreatic, bone, and breast cancers (Riquelme et al., 2019; Nejman et al., 2020; Banerjee et al., 2021). Alteration of the host microbiome can change host cell function, including that of innate and adaptive immune cells (Russo et al., 2016; Thaiss et al., 2016). The mechanisms underlying these cellular changes are still being investigated but have been better characterized in recent studies and are believed to be primarily associated with alterations in metabolite secretions by microbial species that subsequently impact immune cell function (Luu et al., 2019; Yang et al., 2020). In particular, microbes produce

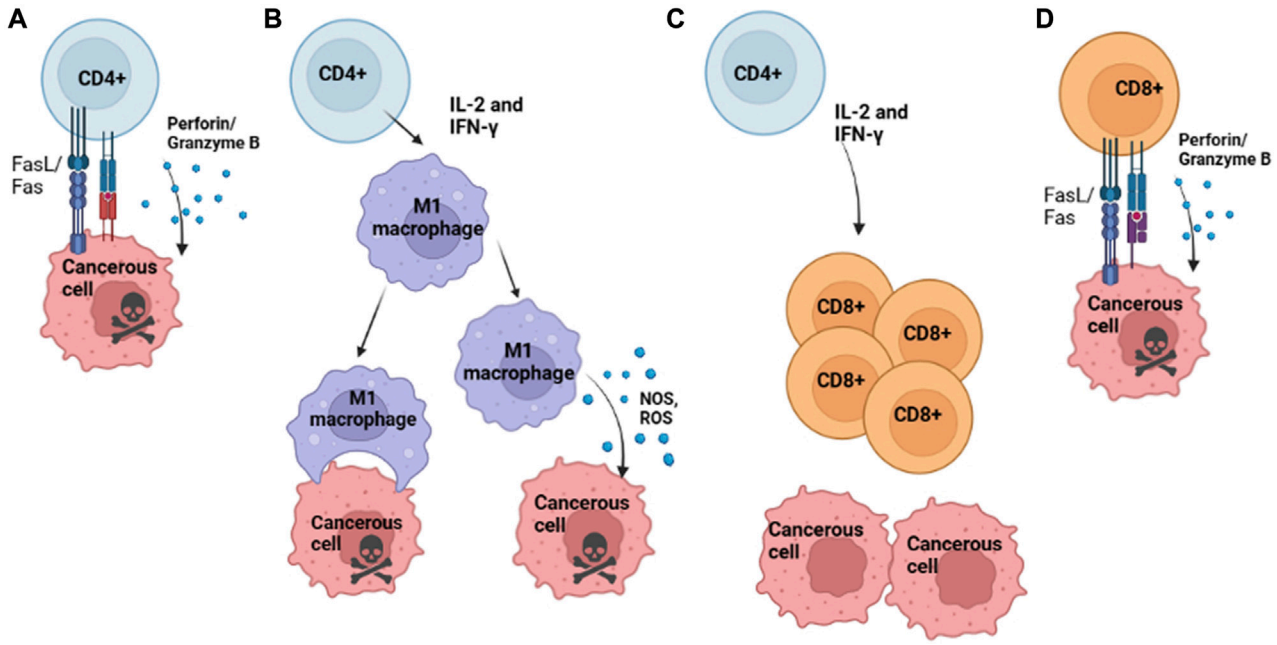

FIGURE 1
Conventional Mechanisms of T Cell Anti-Tumoral Activity. **(A)** Th1 differentiated CD4 T cell killing of cancerous cells that present neoepitopes on MHC II via secretion of perforin and granzyme B. **(B)** CD4 T cells release cytokines IL-2 and IFN-γ to recruit M1 macrophages; activated M1 Macrophages can kill cancerous cells either by direct phagocytosis or release of NOS or ROS, which results in tumor cell death and continue to secrete IL-12, among other cytokines which recruit T cells to the site of the tumor. **(C)** Activated CD4 T cell release of cytokines such as IL-2 and IFN-γ, which recruits CD8 T cells to the site of tumor growth. **(D)** CD8 T cells directly kill cancerous cells that present neoepitopes via MHC I via the Fas/FasL and the release of perforin/ granzyme B, resulting in tumor cell killing. Image Created with BioRender.com.

fermentation products known as short-chain fatty acids (SCFAs); these free fatty acids contain short aliphatic carbon chains and are composed of less than six carbons. Typically when referring to SCFAs, the following are included: formic acid (C1), acetic acid (C2), propionic acid (C3), butyric acid (C4), and valeric acid (C5) (Tan et al., 2014). SCFAs are predominantly water-soluble, and therefore easily transported throughout the body, and are believed to play a significant role in the differentiation, function, and regulation of T cells and other immune cells through the promotion of pro- or anti-inflammatory cytokines needed for particular effector functions (Arpaia et al., 2013; Ryu et al., 2022). Thus, alterations in microbiome composition and consequent changes in microbial metabolite secretion may disrupt T cells' conventional effector functions against malignancies.

Tumor-specific microbiome associations with malignancies

Microbial composition within the TME varies across cancers and further differs from adjacent healthy tissues, even in solid tumors with no direct contact with the external environment (Nejman et al., 2020). Several cancers, including breast, lung, ovarian, colorectal, melanoma, brain, prostate, and bone, have exhibited the presence of specific microbial species contributing to a dysbiotic state within tumor tissue (Sfanos et al., 2008; Apostolou et al., 2011; Castellarin et al., 2012; Thompson et al., 2017; Costantini et al., 2018; Greathouse et al., 2018; Nejman et al., 2020). These tumor-specific microbial populations can sometimes vary for different cancer types within the same organ systems (Banerjee et al., 2018). Considering the known impact of microbial changes on immune cell function, changes within the TME regarding tissue-specific microbial populations may initiate changes in T cell function within neoplasms (Nejman et al., 2020). Intriguingly, *Rotter-Maskowitz et al.* recently identified correlations between intratumoral bacterial presence and predicted clinical presentation and response to anticancer treatment (Nejman et al., 2020). Similar concepts can be traced back to William Coley in the late 19th century, who showed that injecting killed bacterial species into tumor tissue resulted in tumor regression, which we now believe is due to adjuvant effects *via* activation of innate immune receptors and engagement of subsequent immune responses within the tumor (Kopenhaver et al., 2020). Further investigations are needed to determine if bacterial presence in cancer impacts cancer progression *via* changes in immune cell function or if metabolites preferred by certain bacteria that are present as a result of cancer progression provide a niche for those found in specific tumor tissues, the presence of particular bacterial species in tumor tissue compared to healthy tissue indicates a potential avenue for understanding better what factors impact cancer progression within the TME (Thompson et al., 2017).

Gut microbiome associations with malignancies

Investigations during cancer progression and treatment suggest certain gut microbial presence outside the TME correlate with

systemic inflammatory processes that affect the TME, including the upregulation of pro-inflammatory cytokines such as tumor necrosis factor (TNF) caused by increased immune cell responses, resulting in more significant tumor regression (Iida et al., 2013). These systemic alterations could be associated with secreted metabolites from specific bacteria, noting that metagenomic studies concluded that the enrichment of anabolic pathways resulting from cellular metabolism and differences in pro-inflammatory cytokines caused by some bacteria's presence affect tumor response (Gopalakrishnan et al., 2018). Additionally, several studies suggest that gut microbiome composition plays a role in cancer progression at mucosal sites and in tumors not confined to mucosal tissue, which may be partly due to metabolites produced by microbes in the gut (Zhuang et al., 2019; Sánchez-Alcoholado et al., 2020).

Transport of microbial-derived metabolites to T immune cells

Metabolites can be produced by gut-specific or organ-specific bacteria and passively or actively transported to other locations impacting organ and cellular function at local and peripheral sites (Cummings et al., 1987; Kamp and Hamilton, 2006). For this reason changes in microbiome composition will also impact metabolite presence. For instance, during dysbioisis, certain SCFAs or other metabolites may be reduced in quantity if the bacteria that produce them are no longer present. Active diffusion of metabolites in T cells can occur *via* membrane transporters, including MCT1 (monocarboxylate transporter-1/Slc16a1) and SMCT1 (sodium-coupled monocarboxylate transporter-1/Slc5 a8) (Park et al., 2014). Once inside the cell, SCFAs are known to act *via* G-protein-coupled receptor (GPCR) signaling, inhibition of histone deacetylase (HDAC), production of acetyl-CoA, and further changes in the metabolism of the cell resulting in increased or decreased functionality (Kim, 2021). Evidence shows that SCFA presence activates mTOR, and STAT3 in T cells *via* GPCR41, GPCR43, GPCR109a resulting in Blimp-1 expression, which triggers the expression of many downstream signaling cascades (Zhao et al., 2018). Studies in germ-free mice suggest that changes in microbiota can directly impact the expression of toll-like receptors (TLRs) (Lundin et al., 2008) and can affect antigen-presenting cell presence, T cell differentiation, and systemic immunity (Rangan and Mondino, 2022).

Molecular effects of dysbiosis on CD4 T cell signaling and function

Recent studies showed C2, C3, and C4 metabolites, which can be produced by microbiota, could exhibit immunomodulatory functions by altering CD4 T cell differentiation in a concentration-dependent manner, as high concentrations of C2 and C3 drove expression of IL-17A, IL-17F, RORα, RORγt, T-bet, and IFN-γ, cytokines associated with Th17 and Th1 profiles (Figure 2A). (Park et al., 2014). More specifically, the natural killer group 2, member D (NKG2D) ligand system is a central immunomodulatory system in which immune cells recognize NKG2DL on stressed or infected cells through NKG2DR, present on immune cells such as CD4 T cells, CD8 T cells, and NK cells, activating effector functions (Groh et al., 2003). C3 produced by *propionibacteria* during cellular metabolism can induce the expression of NKG2D ligands MICA/B on both activated T lymphocytes and cancer cells in an intracellular calcium-dependent manner, allowing for proper immune effector function and potential prophylaxis of malignant cells (Figure 2B). (Andresen et al., 2009). This finding also implies the absence of C3 during the elimination of microbial populations that produce it can limit the degree of NKG2D ligands and, therefore, limit subsequent NKG2D/NKG2D interactions, resulting in a reduced overall T cell effector function within the TME. Further, C4 presence was shown to drive Treg differentiation in a concentration-dependent manner both *in vitro* and *in vivo* (Figure 2C). (Furusawa et al., 2013; Kespohl et al., 2017). Additional studies have indicated issues with the development of regulatory T cells (Tregs) in antibiotic-treated mice, indicating a requirement for healthy intestinal microbiota to achieve even normal Treg development (Han et al., 2021).

Molecular impact of dysbiosis on CD8 T cell signaling and function

In pre-clinical mouse models, microbial dysbiosis induced by maternal antibiotic treatment found that the offspring had altered $CD8^+$ T Cell receptor signaling apparent by an inability to sustain interferon-gamma (IFN-γ) production *in vivo* after vaccination and *in vitro* upon T cell receptor (TCR) stimulation. Resultantly, these cells did not maintain protein tyrosine phosphorylation and Erk1/2 activation, which are necessary for the proper functioning of $CD8^+$ T Cells (Brownlie and Zamoyska, 2013). Further, SCFA presence was shown to enhance the function of cytotoxic T lymphocytes through an increased function of mTOR post-treatment of T cells with pentanoate and C4 and driving supplementary inhibition of class I histone deacetylase activity. mTOR typically drives differentiation into Th1, Th2, and Th17 but is also a critical regulator in CD8 T cell differentiation through the regulation of cytolytic effector molecules (Finlay et al., 2012; Liu et al., 2015). Effector molecules such as CD25, IFN-γ, and TNF-α were then elevated in these treated cells, demonstrating enhanced cytotoxic activity and potential for pentanoate and C4 as supplements to cancer treatment for some malignancies (Figure 2D). (Schiweck et al., 2022).

Elimination of microbial species by antibiotics and the resulting effect on T cell populations

Considering the impact of microbial species on T-cell function, clinicians should be cognizant of the immunopharmacological behavior of antibiotics regarding microbial species-specific elimination based on antibiotic type. For instance, neomycin, which predominantly eradicates facultative gram-negative species, and vancomycin which predominantly eradicates gram-positive species, have both been associated with the reduced expansion of T cells in mouse models during antibiotic administration (Duan et al., 2010; Cheng et al., 2017). Cocktails of ampicillin, vancomycin,

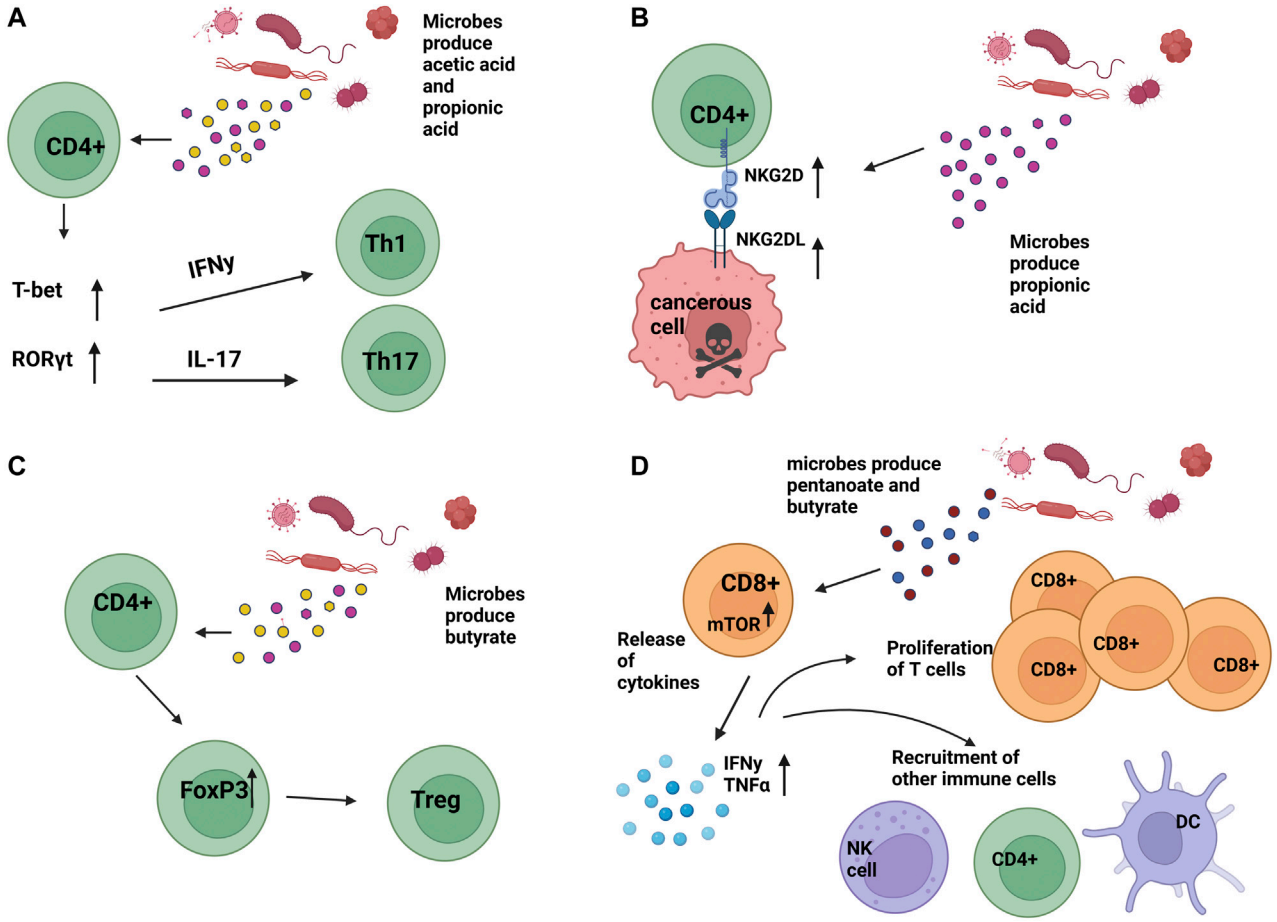

FIGURE 2
Impact of microbial secreted metabolites on T cell function **(A)**. Bacterial-produced acetic acid (C2) and propionic acid (C3) can result in upregulation of T-bet and RORγt, causing upregulation of IFNγ and IL-17 and subsequent differentiation into Th1 and Th17 T cells, respectively. **(B)**. Microbial produced propionic acid (C3) has resulted in the upregulation of NKG2D and NKG2DL on CD4 T cells, CD8 T cells, and NK cells, contributing to an anti-tumoral environment and death of cancer cells. **(C)**. Microbial-produced butyrate has been shown to cause the differentiation of T-regulatory cells (Tregs) in a concentration-dependent manner. **(D)**. Microbial-produced pentanoate and butyrate have been shown to contribute to increased CD8 T cell effector functions. *Image created with BioRender.com*.

neomycin and metronidazole (AVNM) have been associated with lower immune function and decreased concentrations of bacterial metabolites C3 and C4 in mice (Ubeda and Pamer, 2012). γδ T cells are a part of the Th17 subset and are a source of the pro-inflammatory cytokine IL-17. Antibiotics have also been shown to modulate γδ T cell populations, with variations depending on the antibiotic type used and species of bacteria eliminated (Duan et al., 2010). Understanding which antibiotics eliminate microbial populations may be critical when designing patient treatment regimens to reduce the chances of anomalous immune cell function.

Interactions of dysbiosis on T cell-based immunotherapy in the TME

Both CD4 and CD8 T cells play an essential role in the clearance of malignancies. The impact of microbiome dysbiosis on immune cell function generates a challenging dilemma, considering that many immunotherapies in pre-clinical and clinical use, such as immune checkpoint blockade and adoptive cellular therapies, are T-cell-based.

Impact of dysbiosis on T cell dependent immune checkpoint blockade

It has been documented that specific microbes in the gut can impact ICB immunotherapy approaches across several cancers (McCulloch et al., 2022). For instance, anti-CTLA-4 treatment for melanoma relies on the presence of *Bacteroides* species; additionally, the treatment showed no effect on germ-free and antibiotic-treated mice (Vétizou et al., 2015). *Bacteroides* species can be propionogenic, having the capacity to generate the SCFA C3. Therefore, the absence of C3 can alter the proper function of T cells, which anti-CTLA-4 treatment relies on (Louis et al., 2014). In a separate study, the introduction and restoration of propionogenic bacteria during antibiotic-induced dysbiosis resulted in the restoration of C3 levels, indicating that the re-establishment of

propionogenic bacteria could counteract decreases in SCFAs required for the efficacy of particular immunotherapy (El Hage et al., 2019). In mice and humans, high C4 concentrations in the blood were associated with resistance to anti-CTLA-4 therapies, evidenced by restrained upregulation of B7 on T cells (Coutzac et al., 2020). Similar results of reliance of immunotherapy efficacy on bacterial presence were seen in anti-PD-L1 treatment for melanoma, which depended on the presence of *Bifidobacterium* (Sivan et al., 2015). *Bifidobacterium* species produce SCFAs C2, C3, and C4 SCFAs that contribute to immune cell function (Louis et al., 2014). Strikingly, more recent investigations have shown fecal transplants from ICB responders to non-responders for melanoma treatment saw that more than one-third of human patients previously unresponsive to treatment become responsive after transplants (Davar et al., 2021).

Impact of dysbiosis on adoptive T-cell therapy

Recent studies in mice have further shown that differences in gut microbiome composition and dysbiosis due to antibiotic administration could alter the efficacy of adoptive T-cell cancer treatments. However, these changes in response to treatment are likely species-specific since they vary based on the type of antibiotic administered. In fact, some mice treated with vancomycin displayed an increase in CD8α+ dendritic cells (DCs) with supplemental decreases in tumor burden in an IL-12-dependent manner. At the same time, alternative antibiotics did not produce the same effect (Uribe-Herranz et al., 2018). Additional studies have indicated that severe cytokine release syndrome during CAR- T cell therapy is associated with particular microbiome alterations and a higher abundance of *Bifidobacterium*, *Leuconostoc*, *Stenotrophomonas*, and *Staphylococcus* and that desired responses may require specific gut microbial presence (Hu et al., 2022; Smith et al., 2022). These results demonstrate that microbiome composition can also impact T-cell therapies whose efficacy relies on proper immune cell function.

The impact of diet on T cell based cancer therapies

It is well known that diet can influence the establishment of microbial communities within a host (David et al., 2013). Since we now know that microbial community composition can impact T cell function, investigations regarding how diet can impact cancer treatments that rely on T cell function have been recently investigated. In mice, the western diet of high fat, high carbohydrate, and low fiber diet can decrease downstream production of short-chain fatty acids (SCFA), which originate from microbiota; this could impact T cell function and efficacy of treatments that rely on T cells. (Statovci et al., 2017). Other findings have shown that ketogenic diets can increase antitumor immunosurveillance by reducing PD-L1 expression on tumor cells in a malignant glioma model. (Lussier et al., 2016). Additionally, when placed on a ketogenic diet, mice have displayed enhancement of the anticancer effects of PD-1 blockade. (Ferrere et al., 2021). More recent studies have shown that when fucoidan, a polysaccharide naturally derived from brown algae, was co-administered with ICB treatment, it significantly improved the antitumoral activity of PD-1 antibodies in a murine melanoma model *in vivo* through consistent activation of tumor-infiltrating $CD8^+$ T cells. (Yang et al., 2021). Recent studies also indicate that calorie restriction can increase the antitumoral ability of T cells (Pietrocola and Kroemer, 2019), an approach that, when applied to a murine triple-negative breast cancer model, augmented radiation efficacy (Saleh et al., 2013). Further, clinical trials analyzing melanoma patients showed that patients who consumed a high-fiber diet were five times more likely to respond to PD-1 therapy (Spencer et al., 2019). Modulation of diet serves as a potential interventional strategy that can be used to enhance T cell-based immunotherapies therapies in the future.

Discussion

With the onset of technological advancements such as molecular sequencing and sophisticated Metabolic-network modeling, insights into how microbiome composition impacts health, disease, immune cell function disease, cancer, and immune cell function have been recently better illuminated. Further insights into cohesion between microbiome composition and proper immune cell function may be a helpful resource that will eventually allow scientists to regulate the immune response to and clearance of malignancies. Further, broadening our understanding of T cell function in the TME concerning microbiome composition may improve our understanding of how best to administer current antineoplastic drugs and therapies.

There also exists potential to exploit the dysbiotic microbiome's impact on immune cell function as an augmentation to anti-tumoral therapies for some cancers originating in common lymphoid progenitors (CLP). For instance, patients with cutaneous T-cell lymphoma (CTCL) reported decreased overall tumor burden when treated with an aggressive antibiotic regimen. Upon immunohistochemistry analysis, samples displayed a decrease in interleukin-2 high-affinity receptors in T cells at these sites, indicating a decline in mechanisms that allow for T cell proliferation (Lindahl et al., 2019). Similar regimens have also been pursued in Mucosal Associated Lymphoid Tissue (MALT) lymphomas, improving 5- year survival rates (Ferreri et al., 2018). These incidents demonstrate the importance of identifying how antibiotics can affect immune cells and the specific malignant cells clinicians might target during treatment.

Though the composition of the microbiome and microbial dysbiosis can impact immune cell function and subsequent immune response to malignancy, research gaps still exist that must be pursued to mitigate the adverse effects of microbial dysbiosis and immune system dysfunction. Further research is needed into what comprises the "optimal" microbiome and whether this composition differs for particular diseases and malignancies. Current data indicate a requirement for the presence of specific microbial species' therapeutic efficiency (Sivan et al., 2015), but this also tends to vary from cancer to cancer, making it difficult to classify what an optimal microbiome for patients might look like. For this reason, a more significant

effort is needed to determine what microbes may offer benefits during cancer treatment and what causes these microbiomes to be beneficial or harmful in particular organ systems. Since both gut and tumor-specific microbial composition can potentially permute disease progression, identifying what comprises microbial populations both locally and systemically across specific cancers may serve as a helpful resource for future diagnostic approaches. Several microbial signatures between blood and tissues have already been identified across cancers, indicating a potential diagnostic approach to cancer treatment that may soon be available (Poore et al., 2020).

Further research into how the dysbiotic state of the microbiome can impact T cell function can lead to potentially better treatments for cancer patients through the modulation of microbes present in the host. Knowledge pertaining to which bacterial communities and their associated mechanisms are needed for proper immune cell function would allow physicians to be aware of the implications associated with specific antibiotic use and subsequent species-specific elimination of bacterial communities in conjunction with ICB treatments. Overall, expanding our knowledge about the microbiomes' interconnection with the immune system and T-cell function during cancer progression and treatment can improve our knowledge of how to design best and administer cancer treatments and ultimately improve patient outcomes.

Author contributions

MD conceived, designed, and wrote the first draft of this manuscript. MD and JB read and critically revised the manuscript.

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References

Ahmadzadeh, M., Johnson, L. A., Heemskerk, B., Wunderlich, J. R., Dudley, M. E., White, D. E., et al. (2009). Tumor antigen–specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. *Blood* 114, 1537–1544. doi:10.1182/BLOOD-2008-12-195792

Al-Shibli, K. I., Donnem, T., Al-Saad, S., Persson, M., Bremnes, R. M., and Busund, L. T. (2008). Prognostic effect of epithelial and stromal lymphocyte infiltration in non-small cell lung cancer. *Clin. Cancer Res.* 14, 5220–5227. doi:10.1158/1078-0432.CCR-08-0133

Andresen, L., Hansen, K. A., Jensen, H., Pedersen, S. F., Stougaard, P., Hansen, H. R., et al. (2009). Propionic acid secreted from propionibacteria induces NKG2D ligand expression on human-activated T lymphocytes and cancer cells. *J. Immunol.* 183, 897–906. doi:10.4049/JIMMUNOL.0803014

Apostolou, P., Tsantsaridou, A., Papasotiriou, I., Toloudi, M., Chatziioannou, M., and Giamouzis, G. (2011). Bacterial and fungal microflora in surgically removed lung cancer samples. *J. Cardiothorac. Surg.* 6, 137. doi:10.1186/1749-8090-6-137

Arpaia, N., Campbell, C., Fan, X., Dikiy, S., Van Der Veeken, J., Deroos, P., et al. (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nat* 504, 451–455. doi:10.1038/nature12726

Banerjee, S., Tian, T., Wei, Z., Shih, N., Feldman, M. D., Peck, K. N., et al. (2018). Distinct microbial signatures associated with different breast cancer types. *Front. Microbiol.* 9, 951. doi:10.3389/fmicb.2018.00951

Banerjee, S., Wei, Z., Tian, T., Bose, D., Shih, N. N. C., Feldman, M. D., et al. (2021). Prognostic correlations with the microbiome of breast cancer subtypes. *Cell Death Dis.* 129 (12), 831. doi:10.1038/s41419-021-04092-x

Brownlie, R. J., and Zamoyska, R. (2013). T cell receptor signalling networks: Branched, diversified and bounded. *Nat. Rev. Immunol.* 13, 257–269. doi:10.1038/nri3403

Castellarin, M., Warren, R. L., Freeman, J. D., Dreolini, L., Krzywinski, M., Strauss, J., et al. (2012). Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. *Genome Res.* 22, 299–306. doi:10.1101/GR.126516.111

Chávez-Galán, L., Arenas-Del Angel, M. C., Zenteno, E., Chávez, R., and Lascurain, R. (2009). Cell death mechanisms induced by cytotoxic lymphocytes. *Cell. Mol. Immunol.* 6, 15–25. doi:10.1038/cmi.2009.3

Cheng, R. Y., Li, M., Li, S. S., He, M., Yu, X. H., Shi, L., et al. (2017). Vancomycin and ceftriaxone can damage intestinal microbiota and affect the development of the intestinal tract and immune system to different degrees in neonatal mice. *Pathog. Dis.* 75, 104. doi:10.1093/FEMSPD/FTX104

Claesson, M. J., Jeffery, I. B., Conde, S., Power, S. E., O'connor, E. M., Cusack, S., et al. (2012). Gut microbiota composition correlates with diet and health in the elderly. *Nat* 488, 178–184. doi:10.1038/nature11319

Costantini, L., Magno, S., Albanese, D., Donati, C., Molinari, R., Filippone, A., et al. (2018). Characterization of human breast tissue microbiota from core needle biopsies through the analysis of multi hypervariable 16S-rRNA gene regions. *Sci. Rep.* 8, 16893–16899. doi:10.1038/s41598-018-35329-z

Coutzac, C., Jouniaux, J. M., Paci, A., Schmidt, J., Mallardo, D., Seck, A., et al. (2020). Systemic short chain fatty acids limit antitumor effect of CTLA-4 blockade in hosts with cancer. *Nat. Commun.* 11, 2168. doi:10.1038/s41467-020-16079-x

Cummings, J. H., Pomare, E. W., Branch, H. W. J., Naylor, C. P. E., and MacFarlane, G. T. (1987). Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* 28, 1221–1227. doi:10.1136/GUT.28.10.1221

Czesnikiewicz-Guzik, M., and Müller, D. N. (2018). Scientists on the Spot: Salt, the microbiome, and cardiovascular diseases. *Cardiovasc. Res.* 114, e72–e73. doi:10.1093/cvr/cvy171

Davar, D., Dzutsev, A. K., McCulloch, J. A., Rodrigues, R. R., Chauvin, J. M., Morrison, R. M., et al. (2021). Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science* 371, 595–602. doi:10.1126/science.abf3363

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., et al. (2013). Diet rapidly and reproducibly alters the human gut microbiome. *Nat* 505, 559–563. doi:10.1038/nature12820

Duan, J., Chung, H., Troy, E., and Kasper, D. L. (2010). Microbial colonization drives expansion of IL-1 receptor 1-expressing and IL-17-producing gamma/delta T cells. *Cell Host Microbe* 7, 140–150. doi:10.1016/j.chom.2010.01.005

El Hage, R., Hernandez-Sanabria, E., Calatayud Arroyo, M., Props, R., and Van De Wiele, T. (2019). Propionate-producing consortium restores antibiotic-induced dysbiosis in a dynamic *in vitro* model of the human intestinal microbial ecosystem. *Front. Microbiol.* 10, 1206. doi:10.3389/fmicb.2019.01206

Ferrere, G., Alou, M. T., Liu, P., Goubet, A. G., Fidelle, M., Kepp, O., et al. (2021). Ketogenic diet and ketone bodies enhance the anticancer effects of PD-1 blockade. *JCI Insight* 6, e145207. doi:10.1172/JCI.INSIGHT.145207

Ferreri, A. J. M., Cecchetti, C., Kiesewetter, B., Sassone, M., Calimeri, T., Perrone, S., et al. (2018). Clarithromycin as a "repurposing drug" against MALT lymphoma. *Br. J. Haematol.* 182, 913–915. doi:10.1111/BJH.14878

Finlay, D. K., Rosenzweig, E., Sinclair, L. V., Carmen, F. C., Hukelmann, J. L., Rolf, J., et al. (2012). PDK1 regulation of mTOR and hypoxia-inducible factor 1 integrate metabolism and migration of CD8+ T cells. *J. Exp. Med.* 209, 2441–2453. doi:10.1084/JEM.20112607

Freilich, S., Kreimer, A., Borenstein, E., Yosef, N., Sharan, R., Gophna, U., et al. (2009). Metabolic-network-driven analysis of bacterial ecological strategies. *Genome Biol.* 10, R61–R68. doi:10.1186/gb-2009-10-6-r61

Furusawa, Y., Obata, Y., Fukuda, S., Endo, T. A., Nakato, G., Takahashi, D., et al. (2013). Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nat* 504, 446–450. doi:10.1038/nature12721

Gopalakrishnan, V., Spencer, C. N., Nezi, L., Reuben, A., Andrews, M. C., Karpinets, T. V., et al. (2018). Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* 359, 97–103. doi:10.1126/science.aan4236

Greathouse, K. L., White, J. R., Vargas, A. J., Bliskovsky, V. V., Beck, J. A., von Muhlinen, N., et al. (2018). Interaction between the microbiome and TP53 in human lung cancer. *Genome Biol.* 19, 123. doi:10.1186/s13059-018-1501-6

Groh, V., Brühl, A., El-Gabalawy, H., Nelson, J. L., and Spies, T. (2003). Stimulation of T cell autoreactivity by anomalous expression of NKG2D and its MIC ligands in rheumatoid arthritis. *Proc. Natl. Acad. Sci.* 100, 9452–9457. doi:10.1073/PNAS.1632807100

Haabeth, O. A. W., Tveita, A. A., Fauskanger, M., Schjesvold, F., Lorvik, K. B., Hofgaard, P. O., et al. (2014). How do CD4+ T cells detect and eliminate tumor cells that either lack or express MHC class II molecules? *Front. Immunol.* 5, 174. doi:10.3389/fimmu.2014.00174

Han, H., Yan, H., and King, K. Y. (2021). Broad-spectrum antibiotics deplete bone marrow regulatory T cells. *Cells* 10, 1–9. doi:10.3390/CELLS10020277

Hu, Y., Li, J., Ni, F., Yang, Z., Gui, X., Bao, Z., et al. (2022). CAR-T cell therapy-related cytokine release syndrome and therapeutic response is modulated by the gut microbiome in hematologic malignancies. *Nat. Commun.* 13, 5313–5314. doi:10.1038/s41467-022-32960-3

Iida, N., Dzutsev, A., Stewart, C. A., Smith, L., Bouladoux, N., Weingarten, R. A., et al. (2013). Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science* 342, 967. doi:10.1126/science.1240527

Janssen, E. M., Lemmens, E. E., Wolfe, T., Christen, U., Von Herrath, M. G., and Schoenberger, S. P. (2003). CD4+ T cells are required for secondary expansion and memory in CD8+ T lymphocytes. *Nat* 421, 852–856. doi:10.1038/nature01441

Kamp, F., and Hamilton, J. A. (2006). How fatty acids of different chain length enter and leave cells by free diffusion. *Prostagl. Leukot. Essent. Fat. Acids* 75, 149–159. doi:10.1016/J.PLEFA.2006.05.003

Kespohl, M., Vachharajani, N., Luu, M., Harb, H., Pautz, S., Wolff, S., et al. (2017). The microbial metabolite butyrate induces expression of Th1- associated factors in cD4+ T cells. *Front. Immunol.* 8, 1036. doi:10.3389/fimmu.2017.01036

Kim, C. H. (2021). Control of lymphocyte functions by gut microbiota-derived short-chain fatty acids. *Cell. Mol. Immunol.* 185 (18), 1161–1171. doi:10.1038/s41423-020-00625-0

Kopenhaver, J., Carlson, R. D., and Snook, A. E. (2020). Mobilizing toxins for cancer treatment: Historical perspectives and current strategies. *Toxins* 12, 416. doi:10.3390/TOXINS12060416

Lindahl, L. M., Willerslev-Olsen, A., Gjerdrum, L. M. R., Nielsen, P. R., Blümel, E., Rittig, A. H., et al. (2019). Antibiotics inhibit tumor and disease activity in cutaneous T-cell lymphoma. *Blood* 134, 1072–1083. doi:10.1182/BLOOD.2018888107

Liu, C., Chapman, N. M., Karmaus, P. W. F., Zeng, H., and Chi, H. (2015). mTOR and metabolic regulation of conventional and regulatory T cells. *J. Leukoc. Biol.* 97, 837–847. doi:10.1189/JLB.2RI0814-408R

Louis, P., Hold, G. L., and Flint, H. J. (2014). The gut microbiota, bacterial metabolites and colorectal cancer. *Nat. Rev. Microbiol.* 1210 (12), 661–672. doi:10.1038/nrmicro3344

Lundin, A., Bok, C. M., Aronsson, L., Björkholm, B., Gustafsson, J. Å., Pott, S., et al. (2008). Gut flora, toll-like receptors and nuclear receptors: A tripartite communication that tunes innate immunity in large intestine. *Cell. Microbiol.* 10, 1093–1103. doi:10.1111/J.1462-5822.2007.01108.X

Lundin, K. U., Screpanti, V., Omholt, H., Hofgaard, P. O., Yagita, H., Grandien, A., et al. (2004). CD4+ T cells kill Id+ B-lymphoma cells: FasLigand-fas interaction is dominant *in vitro* but is redundant *in vivo*. *Cancer Immunol. Immunother.* 53, 1135–1145. doi:10.1007/s00262-004-0538-4

Lussier, D. M., Woolf, E. C., Johnson, J. L., Brooks, K. S., Blattman, J. N., and Scheck, A. C. (2016). Enhanced immunity in a mouse model of malignant glioma is mediated by a therapeutic ketogenic diet. *BMC Cancer* 16, 310–10. doi:10.1186/s12885-016-2337-7

Luu, M., Pautz, S., Kohl, V., Singh, R., Romero, R., Lucas, S., et al. (2019). The short-chain fatty acid pentanoate suppresses autoimmunity by modulating the metabolic-epigenetic crosstalk in lymphocytes. *Nat. Commun.* 10, 760. doi:10.1038/s41467-019-08711-2

McCulloch, J. A., Davar, D., Rodrigues, R. R., Badger, J. H., Fang, J. R., Cole, A. M., et al. (2022). Intestinal microbiota signatures of clinical response and immune-related adverse events in melanoma patients treated with anti-PD-1. *Nat. Med.* 28, 545–556. doi:10.1038/s41591-022-01698-2

Methé, B. A., Nelson, K. E., Pop, M., Creasy, H. H., Giglio, M. G., Huttenhower, C., et al. (2012). A framework for human microbiome research. *Nat* 486, 215–221. doi:10.1038/nature11209

Nejman, D., Livyatan, I., Fuks, G., Gavert, N., Zwang, Y., Geller, L. T., et al. (2020). The human tumor microbiome is composed of tumor type-specific intracellular bacteria. *Science* 368, 973–980. doi:10.1126/science.aay9189

Ossendorp, F., Mengedé, E., Camps, M., Filius, R., and Melief, C. J. M. (1998). Specific T helper cell requirement for optimal induction of cytotoxic T lymphocytes against major histocompatibility complex class II negative tumors. *J. Exp. Med.* 187, 693–702. doi:10.1084/JEM.187.5.693

Park, J., Kim, M., Kang, S. G., Jannasch, A. H., Cooper, B., Patterson, J., et al. (2014). Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR–S6K pathway. *Mucosal Immunol.* 81 (8), 80–93. doi:10.1038/mi.2014.44

Petersen, C., and Round, J. L. (2014). Defining dysbiosis and its influence on host immunity and disease. *Cell. Microbiol.* 16, 1024–1033. doi:10.1111/CMI.12308

Pham, T. A. N., and Lawley, T. D. (2014). Emerging insights on intestinal dysbiosis during bacterial infections. *Curr. Opin. Microbiol.* 17, 67–74. doi:10.1016/J.MIB.2013.12.002

Pietrocola, F., and Kroemer, G. (2019). Caloric restriction promotes the stemness and antitumor activity of T lymphocytes. *Oncoimmunology* 8, e1616153. doi:10.1080/2162402X.2019.1616153

Poore, G. D., Kopylova, E., Zhu, Q., Carpenter, C., Fraraccio, S., Wandro, S., et al. (2020). Microbiome analyses of blood and tissues suggest cancer diagnostic approach. *Nat* 579, 567–574. doi:10.1038/s41586-020-2095-1

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nat* 464, 59–65. doi:10.1038/nature08821

Rangan, P., and Mondino, A. (2022). Microbial short-chain fatty acids: A strategy to tune adoptive T cell therapy. *J. Immunother. Cancer* 10, e004147. doi:10.1136/JITC-2021-004147

Riiser, A. (2015). The human microbiome, asthma, and allergy. *Allergy, Asthma Clin. Immunol.* 11, 35–37. doi:10.1186/S13223-015-0102-0

Riquelme, E., Zhang, Y., Zhang, L., Montiel, M., Zoltan, M., Dong, W., et al. (2019). Tumor microbiome diversity and composition influence pancreatic cancer outcomes. *Cell* 178, 795–806. doi:10.1016/J.CELL.2019.07.008

Russo, E., Taddei, A., Ringressi, M. N., Ricci, F., and Amedei, A. (2016). The interplay between the microbiome and the adaptive immune response in cancer development. *Ther. Adv. Gastroenterol.* 9, 594–605. doi:10.1177/1756283X16635082

Ryu, T. Y., Kim, K., Han, T. S., Lee, M. O., Lee, J., Choi, J., et al. (2022). Human gut-microbiome-derived propionate coordinates proteasomal degradation via HECTD2 upregulation to target EHMT2 in colorectal cancer. *ISME J.* 165 (16), 1205–1221. doi:10.1038/s41396-021-01119-1

Saleh, A. D., Simone, B. A., Palazzo, J., Savage, J. E., Sano, Y., Dan, T., et al. (2013). Caloric restriction augments radiation efficacy in breast cancer. *Cell Cycle* 12, 1955–1963. doi:10.4161/CC.25016

Sánchez-Alcoholado, L., Ramos-Molina, B., Otero, A., Laborda-Illanes, A., Ordóñez, R., Medina, J. A., et al. (2020). The role of the gut microbiome in colorectal cancer development and therapy response. *Cancers* 12, 1406. doi:10.3390/CANCERS12061406

Schiweck, C., Edwin Thanarajah, S., Aichholzer, M., Matura, S., Reif, A., Vrieze, E., et al. (2022). Regulation of CD4+ and CD8+ T cell Biology by short-chain fatty acids and its relevance for autoimmune pathology. *Int. J. Mol. Sci.* 23, 8272. doi:10.3390/IJMS23158272

Sender, R., Fuchs, S., and Milo, R. (2016). Revised estimates for the number of human and bacteria cells in the body. *PLOS Biol.* 14, e1002533. doi:10.1371/JOURNAL.PBIO.1002533

Sfanos, K. S., Sauvageot, J., Fedor, H. L., Dick, J. D., De Marzo, A. M., and Isaacs, W. B. (2008). A molecular analysis of prokaryotic and viral DNA sequences in prostate tissue

from patients with prostate cancer indicates the presence of multiple and diverse microorganisms. *Prostate* 68, 306–320. doi:10.1002/PROS.20680

Sivan, A., Corrales, L., Hubert, N., Williams, J. B., Aquino-Michaels, K., Earley, Z. M., et al. (2015). Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science* 350, 1084–1089. doi:10.1126/science.aac4255

Smith, M., Dai, A., Ghilardi, G., Amelsberg, K. V., Devlin, S. M., Pajarillo, R., et al. (2022). Gut microbiome correlates of response and toxicity following anti-CD19 CAR T cell therapy. *Nat. Med.* 28, 713–723. doi:10.1038/s41591-022-01702-9

Spencer, C. N., Gopalakrishnan, V., McQuade, J., Andrews, M. C., Helmink, B., Khan, M. A. W., et al. (2019). Abstract 2838: The gut microbiome (GM) and immunotherapy response are influenced by host lifestyle factors. *Cancer Res.* 79, 2838. doi:10.1158/1538-7445.AM2019-2838

Statovci, D., Aguilera, M., MacSharry, J., and Melgar, S. (2017). The impact of Western diet and nutrients on the microbiota and immune response at mucosal interfaces. *Front. Immunol.* 8, 838. doi:10.3389/fimmu.2017.00838

Tan, J., McKenzie, C., Potamitis, M., Thorburn, A. N., Mackay, C. R., and Macia, L. (2014). The role of short-chain fatty acids in health and disease. *Adv. Immunol.* 121, 91–119. doi:10.1016/B978-0-12-800100-4.00003-9

Taylor, R. C., Patel, A., Panageas, K. S., Busam, K. J., and Brady, M. S. (2007). Tumor-infiltrating lymphocytes predict sentinel lymph node positivity in patients with cutaneous melanoma. *J. Clin. Oncol.* 25, 869–875. doi:10.1200/JCO.2006.08.9755

Thaiss, C. A., Zmora, N., Levy, M., and Elinav, E. (2016). The microbiome and innate immunity. *Nat* 535, 65–74. doi:10.1038/nature18847

Thompson, K. J., Ingle, J. N., Tang, X., Chia, N., Jeraldo, P. R., Walther-Antonio, M. R., et al. (2017). A comprehensive analysis of breast cancer microbiota and host gene expression. *PLoS One* 12, e0188873. doi:10.1371/JOURNAL.PONE.0188873

Trujillo, J. A., Sweis, R. F., Bao, R., and Luke, J. J. (2018). T cell–inflamed versus non-T cell–inflamed tumors: A conceptual framework for cancer immunotherapy drug development and combination therapy selection. *Cancer Immunol. Res.* 6, 990–1000. doi:10.1158/2326-6066.CIR-18-0277

Tuddenham, S., and Sears, C. L. (2015). The intestinal microbiome and health. *Curr. Opin. Infect. Dis.* 28, 464–470. doi:10.1097/QCO.0000000000000196

Ubeda, C., and Pamer, E. G. (2012). Antibiotics, microbiota, and immune defense. *Trends Immunol.* 33, 459–466. doi:10.1016/J.IT.2012.05.003

Uribe-Herranz, M., Bittinger, K., Rafail, S., Guedan, S., Pierini, S., Tanes, C., et al. (2018). Gut microbiota modulates adoptive cell therapy via CD8α dendritic cells and IL-12. *JCI insight* 3, e94952. doi:10.1172/JCI.INSIGHT.94952

Vétizou, M., Pitt, J. M., Daillère, R., Lepage, P., Waldschmitt, N., Flament, C., et al. (2015). Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* 350, 1079–1084. doi:10.1126/science.aad1329

Willing, B. P., Dicksved, J., Halfvarson, J., Andersson, A. F., Lucio, M., Zheng, Z., et al. (2010). A pyrosequencing study in twins shows that gastrointestinal microbial profiles vary with inflammatory bowel disease phenotypes. *Gastroenterology* 139, 1844–1854. doi:10.1053/J.GASTRO.2010.08.049

Yang, J., Yang, X., Pan, W., Wang, M., Lu, Y., Zhang, J., et al. (2021). Fucoidan-supplemented diet potentiates immune checkpoint blockage by enhancing antitumor immunity. *Front. Cell Dev. Biol.* 9, 2198. doi:10.3389/fcell.2021.733246

Yang, W., Yu, T., Huang, X., Bilotta, A. J., Xu, L., Lu, Y., et al. (2020). Intestinal microbiota-derived short-chain fatty acids regulation of immune cell IL-22 production and gut immunity. *Nat. Commun.* 11, 4457. doi:10.1038/s41467-020-18262-6

Zhang, L., Conejo-Garcia, J. R., Katsaros, D., Gimotty, P. A., Massobrio, M., Regnani, G., et al. (2003). Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N. Engl. J. Med.* 348, 203–213. doi:10.1056/NEJMoa020177

Zhao, Y., Chen, F., Wu, W., Sun, M., Bilotta, A. J., Yao, S., et al. (2018). GPR43 mediates microbiota metabolite SCFA regulation of antimicrobial peptide expression in intestinal epithelial cells via activation of mTOR and STAT3. *Mucosal Immunol.* 11, 752–762. doi:10.1038/MI.2017.118

Zhou, X., Liu, X., Huang, L., Zhou, X., Huang, L., and Liu, X. (2021). Macrophage-Mediated tumor cell phagocytosis: Opportunity for nanomedicine intervention. *Adv. Funct. Mater.* 31, 2006220. doi:10.1002/ADFM.202006220

Zhuang, H., Cheng, L., Wang, Y., Zhang, Y. K., Zhao, M. F., Liang, G. Da, et al. (2019). Dysbiosis of the gut microbiome in lung cancer. *Front. Cell. Infect. Microbiol.* 9, 112. doi:10.3389/FCIMB.2019.00112

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Modulation of T-cell function by myeloid-derived suppressor cells in hematological malignancies

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Myeloid-derived suppressor cells (MDSCs) are pathologically activated neutrophils and monocytes that negatively regulate the immune response to cancer and chronic infections. Abnormal myelopoiesis and pathological activation of myeloid cells generate this heterogeneous population of myeloid-derived suppressor cells. They are characterized by their distinct transcription, phenotypic, biochemical, and functional features. In the tumor microenvironment (TME), myeloid-derived suppressor cells represent an important class of immunosuppressive cells that correlate with tumor burden, stage, and a poor prognosis. Myeloid-derived suppressor cells exert a strong immunosuppressive effect on T-cells (and a broad range of other immune cells), by blocking lymphocyte homing, increasing production of reactive oxygen and nitrogen species, promoting secretion of various cytokines, chemokines, and immune regulatory molecules, stimulation of other immunosuppressive cells, depletion of various metabolites, and upregulation of immune checkpoint molecules. Additionally, the heterogeneity of myeloid-derived suppressor cells in cancer makes their identification challenging. Overall, they serve as a major obstacle for many cancer immunotherapies and targeting them could be a favorable strategy to improve the effectiveness of immunotherapeutic interventions. However, in hematological malignancies, particularly B-cell malignancies, the clinical outcomes of targeting these myeloid-derived suppressor cells is a field that is still to be explored. This review summarizes the complex biology of myeloid-derived suppressor cells with an emphasis on the immunosuppressive pathways used by myeloid-derived suppressor cells to modulate T-cell function in hematological malignancies. In addition, we describe the challenges, therapeutic strategies, and clinical relevance of targeting myeloid-derived suppressor cells in these diseases.

KEYWORDS

myeloid-derived suppressor cells, hematological malignancies, tumor microenvironment, t cells, immunosuppression

1 Introduction

Myeloid cells are highly diverse cells that are essential for the efficient functioning of innate and adaptive immunity. These cells are divided into two categories (a) monocytic myeloid cells which include monocytes, macrophages, and dendritic cells (DC), and (b) granulocytic/polymorphonuclear (PMN) myeloid cells including neutrophils, eosinophils, basophils, and mast cells. Under steady-state conditions, hematopoiesis is a well-regulated process that includes a series of cell lineage commitments, defined steps of cell differentiation (dedicated transition of hematopoietic stem cells to lymphoid cells/myeloid cells), and lastly,

maturation/circulation of immune cells. During stress/pathological conditions such as cancer or infection, certain signals derived from the hematopoietic stem cell (HSC) niche modify the fate of HSCs, known as "emergency hematopoiesis". This emergency hematopoiesis results in increased demand for both lymphoid and myeloid cells. The persistence of emergency myelopoiesis results in the accumulation of immature myeloid cells/neutrophils/monocytes which are characterized by their immune suppressor activity, now known as myeloid-derived suppressor cells (MDSCs). The immunosuppressive activity of neutrophils and monocytes was first reported around 30 years ago and these immunosuppressive cells were later named MDSCs (Gabrilovich et al., 2007). In healthy individuals, MDSCs are present in very low numbers and are involved in regulating immune responses and tissue repair (Gabrilovich and Nagaraj, 2009). However, MDSCs rapidly expand attesting to their critical role during pathological conditions such as chronic infections, autoimmune disorders, and cancer.

Given the adverse effects of MDSCs, the question arises as to why this population is evolutionary conserved. The first report by Köstlin et al. (2017) reported the expansion of polymorphonuclear MDSCs (PMN-MDSCs) during human pregnancy and the modulation of T-cell response. This provides maternal tolerance for the allogenic fetus. During pregnancy PMN-MDSCs accumulate in the peripheral blood of the mother and the human placenta/cord blood of the newborns. However, after giving birth, the mother's MDSCs revert to their normal levels with the polarization of T-cell association with MDSCs (Köstlin et al., 2016). Additionally, Ostrand-Rosenberg and Fenselau (2018) found that MDSCs serve as a frontline barrier (in placenta) and facilitate maternal-fetal tolerance. These studies attest to the fact that MDSC's play an important role in pregnancy and neonates (Veglia et al., 2018; Dorhoi et al., 2020).

On the other hand, activated MDSCs participate in several aspects of angiogenesis, tumor growth and metastasis, premetastatic niche formation, and epithelial-mesenchymal transition (EMT). The tumor microenvironment (TME) plays a critical role in supporting the growth and differentiation of MDSCs. The TME is a complex network of cellular and non-cellular components around the tumor cells which consists of lymphocytes, other immune cells, stromal cells, and extracellular matrix (ECM). The TME provides a permissive environment for tumor growth, invasion, and metastasis. The composition of TME in B-cell malignancies in divided into two parts: The immune microenvironment and the non-immune microenvironment (discussed below in section 4.1). The bidirectional interaction of MDSCs and tumor cells creates a haven in TME for the tumor growth. Accumulating evidence supports a pivotal role of MDSCs in cancer progression and immune suppression. Recent studies have reported the importance of the TME and the expansion of MDSCs in the pathogenesis of B-cell lymphoma (Höpken and Rehm, 2019; Liu et al., 2021). Briefly, B-cell lymphomas are a group of hematological malignancies that are characterized by complex clinical and biological heterogeneity. Lymphomas comprises Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). Further, B-cell lymphoma constitutes for almost 95% of the total NHL cases. (Ennishi et al., 2020; Sehn and Salles, 2021).

In this review, we will discuss the phenotypic characteristics of MDSC, along with their genomic and metabolic profiles. We also describe how the characteristic features of MDSCs can be potentially used for therapeutic purposes in B-cell lymphomas. However, due to the short lifespan (Condamine et al., 2014) and immune escape properties of MDSCs, the therapeutic strategies in B-cell malignancies are currently somewhat limited. Therefore, we highlight the gaps in the literature which can be explored in emerging studies that will help to limit the severity of the disease. In summary, we define the challenges and clinical relevance of targeting MDSCs in hematological malignancies.

2 Biology, development and differentiation of myeloid-derived suppressor cells

Hematopoietic stem cells (HSPCs) reside in bone marrow and differentiate into all cell types including lymphoid cells, myeloid cells, and red blood cells (RBCs.) Common myeloid progenitor cells in bone marrow generate myeloid-derived suppressor cells (MDSCs). The generation of MDSCs is considered as a two-phased, somewhat overlapping process,—(a) expansion of myeloid cells and (b) procurement of MDSCs features by myeloid cells. Generally, MDSCs develop in bone marrow, however, their generation may also take place in other organs including spleen or liver as demonstrated in tumor bearing mice (TB mice) and in patients diagnosed with cancer (Tavukcuoglu et al., 2020).

Classically, bone marrow derived immature myeloid cells (IMCs) differentiate into granulocytes, dendritic cells and macrophages. However, during demand adapted myelopoiesis (or emergency myelopoiesis), there is robust expansion of IMCs. If the inflammation is resolved, the mechanism of natural myelopoiesis is restored. However, a persistent low strength stimulus during chronic inflammation or cancer causes substantial changes in the biology of these IMCs resulting in their accumulation. During diseased conditions like cancer, the mechanism of emergency myelopoiesis is hijacked by cancer cells, releasing cytokines, chemokines, reactive radicals, immune checkpoint population, and metabolites. These secreted factors strongly contribute to tumor niche formation and to activation of immunosuppressive mechanisms.

The development of MDSC is a complex signalling network which can be understood by dividing the process into two categories: Firstly, signals which promote the accumulation of IMCs and secondly, signals which facilitate pathological activation of IMCs (Condamine et al., 2015a). The accumulation of MDSC in tumor bearing hosts is reported in many clinical studies (Sinha et al., 2008; Porembka et al., 2012; Capietto et al., 2013; Cassetta et al., 2020). Pathological activation of MDSC arises with the persistent stimulation of myeloid cells during prolonged exposure to inflammatory molecules and myeloid growth factors. These activation signals include granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), IL-6, IL-1β, VEGF, adenosine, hypoxia-inducible factor (HIF1α) and many more (Gabrilovich and Nagaraj, 2009). Once activated, these MDSC actively participate in various aspects of tumor

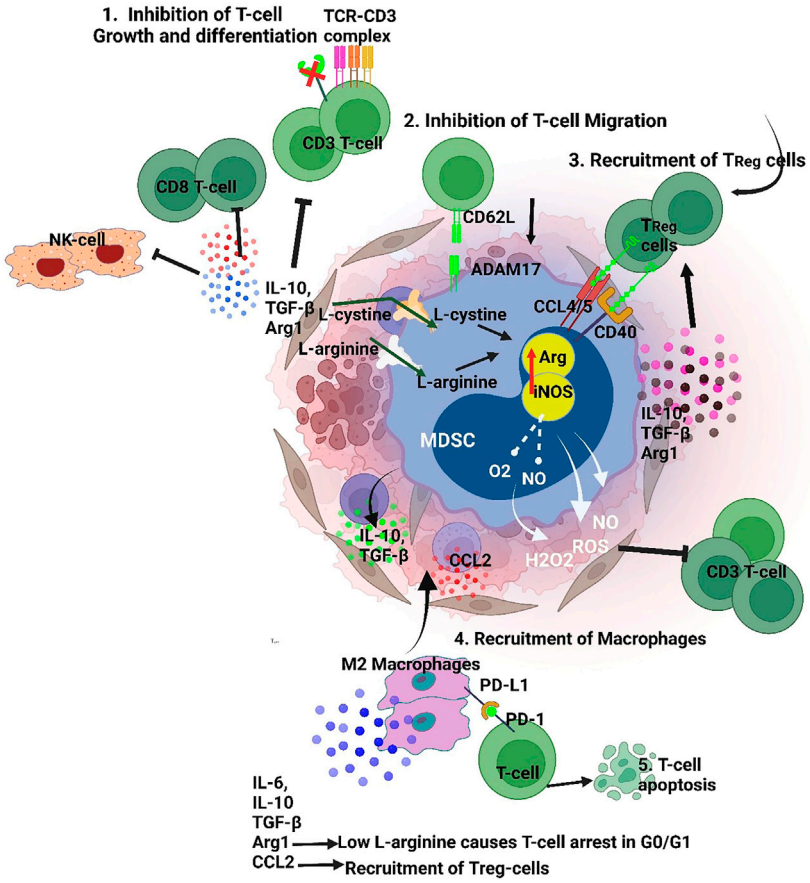

FIGURE 1
MDSCs suppressive mechanism targets both innate and adaptive immunity 1. Targeting T cell and NK cell function by TGFβ/IL10 induced inhibition. Also, MDSCs release ROS, H_2O_2 and peroxynitrite which dysregulate the TCR Z chain inhibiting T-cell/MDSC interaction 2. MDSCs maintain a pre-metastatic niche inhibiting T-cell migration, 3. MDSCs interact with Tregs *via* CCL4/5 and CD40 that recruitments them to the TME, 4. MDSCs induce a M2 macrophage phenotype by secretion of IL-10, thereby promoting immune escape for tumor cells. Furthermore, secretion of factors like IL-10 and TGF-b, and reduction of L-arginine by MDSCs induce Treg polarization. (iNOS-Inducible nitric oxide synthase, Arg- Arginase, PD-L1- Programmed death-ligand 1, TGFβ- Transforming growth factor beta, IL-10- Interleukin 10, 1L-6- Interleukin 6,CCL-2- C-C Motif Chemokine Ligand 2, CCL4- C-C Motif Chemokine Ligand 2, ADAM17-a disintegrin and metalloprotease domain).

progression including tumor invasion, angiogenesis, tumor microenvironment formation and epithelial-mesenchymal transition (Karakhanova et al., 2015; Li et al., 2017; Fleming et al., 2018). Furthermore, immune cell suppression is also a defining feature of MDSCs. Despite their profound effects, the characterization standards of MDSC are not very well defined and the defining phenotypic, morphological, and functional features of MDSC are still an area of intensive research.

The major subgroups of MDSCs in humans and mice are granulocytic/polymorphonuclear MDSC (PMN-MDSCs) and monocytic MDSC (M-MDSCs). The classification of PMN-MDSC and M-MDSC is based on their origin in granulocytic and monocytic cell lineages, respectively. Bronte *et al.* in 2016 reported another small group of MDSC in humans termed as early-MDSC (eMDSC) (Bronte et al., 2016). However, the precise characteristic features of the three subtypes of MDSC is still unclear. The three main populations of MDSCs are found at different frequencies in various cancer subtypes and every pathological condition such as cancer, autoimmunity, and infection may affect their existence (Youn and Gabrilovich, 2010). For instance, the fate and differentiation of hematopoietic cells in the bone marrow is affected by tumor cells and is clearly distinct from the effect of infection and sepsis related myelopoiesis. Specifically, the cues that lead to the expansion and mobilization of myeloid cells specially MDSCs, namely, endotoxins, cytokines, chemokines, and hormones, play an important role to define the plasticity and longevity and cytokine profile of MDSCs.

2.1 Phenotypic characteristic features of MDSC

In mice, the MDSCs were first characterized as cells expressing Gr-1 and CD11b. These markers were subsequently improved by identifying the existence of subpopulations of MDSCs, namely, PMN-MDSCs ($CD11b^+$ $Ly6G^+$ $Ly6C^{low}$) and M-MDSC ($CD11b^+$ $Ly6G^-$ $Ly6C^{hi}$). Ginderachter and others validated the two distinct MDSC subfractions with a detailed description of their

TABLE 1 Phenotype and genotype of MDSCs in human cancer.

MDSCs	Tumor entity	Sample type	Phenotypic markers	Genotypic markers
PMN-MDSCs (Lin^- HLA^-DR^- $CD11b^+$ $CD33^+$ $CD15^+/CD66b^+$	NSCLC, head and neck cancer	PB	LOX1 Condamine and Gabrilovich. (2011); Condamine et al. (2016)	
	NSCLC	PB	FATP2 Veglia et al. (2019)	
	Head and neck cancer	PB	DR5 Condamine et al. (2014)	
	Breast cancer	PB	CD84 Alshetaiwi et al. (2020)	WFDC17, CD84, ARG2, IL1B Alshetaiwi et al. (2020)
	Multiple myeloma	BM		PTGS2, CSF1, IL-8, IRF1, IL4R, STAT1, STAT3 and STAT6, TGFB1 Perez et al. (2020)
	NSCLC	PB		AQP9, LILRA5, CXCL3, BCL2A1, LILRA6, C19orf59, LIRRB2, CCL18 LYN, CCL20 MEFV, CD300E MMP12, CTSC NCF2, CXCL1 OSM, CXCL2 PLAUR, CXCL3 PTAFR, CXCL5 S100A12, CXCL6 S100A8, CXCR1 S100A9, CXCR2 SERPINB8, EMR2 SLC11A1, EMR3 SOD2, FCGR3B TREM1, FGR, FPR1, GPR97, HCK, HK3, ICAM1, IL15RA, IL1B, IL1R2, IL1R2, IL6, IL2RA, IL8, LILRA3, TREM1 Veglia et al. (2021)
	NSCLC	Lung cancer tissue		IL6, OLR1, and TGFB1
	MM	BM and PB	ROS, ARG Görgün et al. (2013)	
M-MDSC (Lin^- HLA^-DR^- $CD11b^+$ $CD33^+$ $CD14^+$	MM	BM and PB	ROS, ARG Görgün et al. (2013)	
	B-cell non-Hodgkin lymphoma	PB	Arg-1, IL-10, PD-L1 and S100A12 Lin et al. (2011); Tadmor et al. (2013); Khalifa et al. (2014); Wu et al. (2015); Xiu et al. (2015); Azzaoui et al. (2016)	
	DLBCL	PB	IL-10 and S100A12 Azzaoui et al. (2016)	
	Head and neck cancer	PB	DR5 Condamine et al. (2014)	
	Ovarian cancer	Blood, ascites, and tissue	PD-L1 Okła et al. (2020)	PD-L1 Okła et al. (2020)
	NSCLC	PB		S100A8, S100A9 Feng et al. (2012)
	Breast cancer	PB	CD84 Alshetaiwi et al. (2020)	WFDC17, CD84, ARG2, IL1B Alshetaiwi et al. (2020)
	NSCLC	Lung cancer tissue		IL10, CD14, and VEGFA
	Melanoma	Spleen	IL-6, IL-8, Tobin et al. (2019)	CXCR1

NSCLC, Non-Small Cell Lung Cancer; PB, peripheral blood; BM, bone marrow; LOX1, Lectin-like oxidized low-density lipoprotein (LDL) receptor-1; FATP2, Fatty acid transport protein; DR5, Death receptor 5; WFDC17, whey acidic protein-type four-disulfide core domain 17; ARG2, Arginase 2; IL1B, Interleukin-1beta; PTGS2, Prostaglandin-endoperoxide synthase; CSF1, Colony Stimulating Factor 1; IL-8, Interleukin 8; IRF1, Interferon Regulatory Factor 1; IL4R, Interleukin four Receptor; STAT1, Signal transducer and activator of transcription 1; STAT3, Signal transducer and activator of transcription 3; STAT6, Signal transducer and activator of transcription 6; TGFB1, transforming growth factor-beta; AQP9, Aquaporin 9; LILRA5, Leukocyte Immunoglobulin Like Receptor A5; CXCL3, Chemokine (C-X-C motif) ligand 3; BCL2A1, BCL2 Related Protein A1; LILRA6, Leukocyte Immunoglobulin Like Receptor A6; C19orf59, chromosome 19 open reading frame 59; LIRRB2, Leukocyte Immunoglobulin Like Receptor B2; CCL18, C-C Motif Chemokine Ligand 18; LYN, LYN, proto-oncogene; CCL20, C-C Motif Chemokine Ligand 20; MEFV, MEFV, innate immunity regulator, pyrin, MMP12, (Matrix Metallopeptidase 12; CTSC, Cathepsin C; NCF2, Neutrophil Cytosolic Factor 2; OSM, Oncostatin M; PLAUR, plasminogen activator, Urokinase Receptor; PTAFR, platelet activating factor receptor; CXCL5, C-X-C Motif Chemokine Ligand 5; S100A12, S100 Calcium Binding Protein A12; S100A9, S100 Calcium Binding Protein A9; CXCR2, C-X-C Motif Chemokine Receptor 2; SERPINB8, Serpin Family B Member 8; EMR2, EGF-Like Module-Containing Mucin-Like Hormone Receptor-Like 2); SLC11A1, Solute Carrier Family 11A Member 1; EMR3, EGF-like module-containing mucin-like hormone receptor-like 3; SOD2, Superoxide Dismutase 2; FCGR3B, Fc Gamma Receptor IIIb; TREM1, Triggering Receptor Expressed On Myeloid Cells 1; FGR, FGR, Proto-Oncogene, Src Family Tyrosine Kinase; FPR1, Formyl peptide receptor 1; GPR97, G protein-coupled receptor 97; HCK, HCK, Proto-Oncogene, Src Family Tyrosine Kinase; HK3, Hexokinase 3; ICAM1, Intercellular Adhesion Molecule 1; IL15RA, Interleukin 15 Receptor Subunit Alpha; IL1B, Interleukin-1beta; IL1R2, Interleukin one Receptor Type 2; IL6, Interleukin 6; IL2RA, Interleukin two Receptor Subunit Alpha; LILRA3, Leukocyte Immunoglobulin Like Receptor A3; TREM1, Triggering Receptor Expressed On Myeloid Cells 1; TGFB1, Transforming Growth Factor Beta 1; ROS, Reactive oxidation species; ARG, Arginase; DLBCL, Diffuse large B-cell lymphoma; MM, multiple myeloma.

morphological, molecular and functional complexity (Movahedi et al., 2008). These cell surface characteristics have defined an initial framework for the MDSC characterization.

With the increased relevance of MDSCs in tumor immunology, uniformity in the classification of MDSC has gained interest. This challenge was explored by the Cancer Immuno guiding Program

under the umbrella of the Association of Cancer Immunotherapy to establish the foundation of MDSC phenotypic characteristics. An interlaboratory collaboration of 23 laboratories (15 Europe and 8 US) coordinated a proficiency panel program which aimed at harmonizing the accepted MDSC phenotype. The results documented high variability in MDSC phenotyping which was associated with the variance of gating strategy. Therefore, a strong recommendation to harmonize the marker combinations and gating strategies for the identification of MDSC subsets was proposed (Mandruzzato et al., 2016). This collaborative research successfully described a specific gating strategy for the identification of human MDSC through flow cytometry.

Human peripheral mononuclear blood (PBMC) contains PMN-MDSC which are defined as $CD11b^+$ $CD14^-$ $CD15^+$ or $CD11b^+$ $CD14^-$ $CD66b^+$ cells and M-MDSC defined as $CD11b^+$ $CD14^+$ $CD15^-$ cells. CD33, another myeloid marker can also be used instead of $CD11b^+$, since a very few $CD15^+$ cells are $CD11b^-$. Three markers, namely, Lin^- (CD3, CD14, CD15, CD19, CD56), $HLA\text{-}DR^{low}$ and $CD33^+$ define an immature progenitor of MDSCs. Another immature subset of MDSC is now termed early MDSCs (eMDSC) which are $CD11b^+$ and $CD33^+$ but $CD14^-/CD15^-$. This group of cells comprises of myeloid progenitors and precursors, representing less than 5% of the MDSC population (Bronte et al., 2016). A recent study also reported a monocytic lineage termed monocyte-like precursors of granulocytes (MLPGs) which differentiates into PMN-MDSCs (Mastio et al., 2019). However, the possibility of differentiating PMN-MDSC from precursor cells requires additional validation.

Despite specific gating criteria for the identification of MDSC (both in human and mouse), discrimination of monocytes from M-MDSC and neutrophils from PMN-MDSCs is difficult because unique phenotypic markers for MDSCs. (Bronte et al., 2016). Functional studies using MDSCs are performed on the entire cell population, therefore the precise nature of M-MDSCs and PMN-MDSCs remains unclear.

Additionally, a major challenge is also observed in recognizing MDSC in tissue samples by immunohistochemistry (IHC). Gr-1 and Ly6G markers are used for identification of MDSC in mice by IHC. However, it is impossible to differentiate between neutrophils and monocytes in frozen and paraffin-embedded tissue samples. Similarly, in human samples, distinction of MDSC from monocytes and neutrophils is difficult. Generally, CD33 is considered for the identification of MDSC in human samples but this does not allow for the discrimination of MDSC from macrophages, dendritic cells (DC) and other myeloid cells. Therefore, a combination of CD33 and S100A9 is suggested for MDSC identification as this excludes DC and macrophages. Some recent studies have also suggested lectin-type oxidized LDL receptor 1 (LOX1; encoded by ORL1 gene) as a specific marker to identify human PMN-MDSCs in peripheral blood and patients with cancer (Condamine et al., 2016; Nan et al., 2018; Kim et al., 2019a; Chai et al., 2019). Similarly, M-MDSC can be distinguished from monocytes by the low expression of MHC class II. The challenge for future studies is the identification of unique cell surface markers of MDSCs so that, their function in various diseases can be explored.

Briefly, in other cancers, evidence suggests that M-MDSCs from tumor bearing (TB) mice can differentiate into PMN-MDSCs, attesting to the transition of monocytic cells to differentiated granulocytes (Youn et al., 2012; Youn et al., 2013). Transcriptional silencing of retinoblastoma (Rb1) gene by histone deacetylase 2 (HDAC-2) has been implicated in this process. Involvement of the Rb1 gene in PMN-MDSC differentiation is also supported by recent preclinical studies in breast cancer (Casbon et al., 2015). However, only a certain percentage of M-MDSCs population can be differentiated into PMN-MDSCs. This evidence raises a discussion as to whether M-MDSCs may constitute granulocytic progenitors. The phenotype and nature of these cells was explored by Mastio and others in 2019. They clearly demonstrated the existence of monocytic precursors of granulocytes which only expanded in mouse models but also in cancer patients. The mechanistic studies showed the myeloid differentiation pathway is controlled by the downregulation of Rb1 which is an important factor for the accumulation of PMN-MDSCs. However, the exact mechanism for abnormal myelopoiesis and reason for expansion of PMN-MDSCs in cancer patients requires additional study.

Phenotypic characteristics of hematological malignancies based on similar patterns of MDSCs appear to be shared by other cancers. For instance, in patients with B cell non-Hodgkin lymphoma (B-NHL) or T-NHL, M-MDSCs were increased as compared to healthy donors, andis associated with advanced stage of lymphoma (Wang et al., 2022). M-MDSC dependent T cell suppression correlated with the overexpression of Arg-1, IL-10, PD-L1 and S100A12 (Lin et al., 2011; Tadmor et al., 2013; Khalifa et al., 2014; Wu et al., 2015; Xiu et al., 2015; Azzaoui et al., 2016). In other lymphoid malignancies an enrichment has been also described in myeloma (Görgün et al., 2013), chronic lymphoid leukemia (CLL) (Jitschin et al., 2014), DLBCL (Tadmor et al., 2013; Azzaoui et al., 2016) and T-cell lymphoma (Movahedi et al., 2008). An expanded immunosuppressive population of $CD66b^+CD33^{low}$ $HLA\text{-}DR^-$ cells was observed within PBMCs from patients with Hodgkin and non-Hodgkin lymphoma. This was the first report on the presence of G-MDSCs in lymphomas. The study defined the G-MDSCs as $CD66b^+CD33^{dim}HLA\text{-}DR^-CD11b^+CD16^+$ (Marini et al., 2016) and co-related with unfavorable prognosis and disease aggressiveness. M- and G-MDSCs were also reported in peripheral blood of DLBCL patients. Myeloid dependent T-cell suppression was co-related with the release of IL-10 and S100A12 and increase in PD-L1 expression. *In vitro* experiments noted the restoration of T-cell proliferation with the depletion of the M-MDSC population (Azzaoui et al., 2016). In multiple myeloma, MDSCs showed a strong tumor promoting and immune-suppressive activity. Both, M- and G-MDSCs were significantly increased with high expression of ROS and ARG1 in peripheral blood and bone marrow of MM patients as compared to healthy donors (Görgün et al., 2013).

3 Functions of MDSC

3.1 Immunological function

The important characteristic that defines MDSC is their suppression of immune cells including B-cells (Li et al., 2014), NK cells (Elkabets et al., 2010; Hongo et al., 2014) and T-cells. Both M-MDSC and PMN-MDSC share similar biochemical features

which enables the suppression of the immune response that includes upregulation of signal transducer and activator of transcription 3 (STAT3), expression of Arginase one and S100A8/A9, and induction of ER stress. Specifically, PMN-MDSC upregulate reactive oxygen species (ROS), peroxynitrite, prostaglandin E_2 (PGE_2) and arginase 1. Additionally, M-MDSC upregulate nitric oxide (NO), cytokines such as IL10 and TGFβ (Figure 1), and increase expression of PD-L1 (Gabrilovich, 2017). Therefore, MDSCs in the TME strongly contribute to the formation of a pre-metastatic niche and subsequent development of metastatic lesions. MDSCs also facilitate the escape of tumor cells from the primary site by suppressing the immune system *via* multiple signaling pathways which directly corelate to poor patient prognosis. Therefore, MDSC-directed cancer therapies usher in a new era of research for establishing novel anticancer therapies.

3.1.1 T-cell suppressive function

MDSCs affect T-cell function by depleting the fundamental amino acids such as L-arginine and cysteine. Also, MDSC drive ROS production by increasing NADPH oxidase activity. Studies have found that administration of ROS inhibitors effectively regains T-cell activation (Corzo et al., 2009; Wei et al., 2015). The VEGF receptors on MDSC also support expansion and recruitment into theTME (Kusmartsev et al., 2008; Corzo et al., 2009). Other MDSC receptors such as adisintegrinandmetalloproteinase17 (ADAM17), downregulate L-selectin levels on CD4/CD8 T-cells preventing T-cell homing (Hanson et al., 2009). MDSCs have also been shown to increase PD-L1 expression which downregulates T cell mediated antitumor reactivity (Weber et al., 2018). Fuse et al. (2016) effectively blocked PD-L1 and reported a significant decrease in MDSC suppressing T-cell activity (Figure 1). MDSCs produce CCR5 ligands (CCL4 and CCL5) which recruit Tregs and promote immune escape for tumors (Figure 1). Schlecker et al. (2012) have shown that intratumoral injection of CCL4/CCL5 increased tumor infiltration of Treg cells (Figure 1). Similarly, lack of CCR5 reduced the recruitment of Tregs into the TME.

3.1.2 B-cell suppressive function

In 2015, Crook and others reported for the first time B-cell inhibition by MDSCs in a murine model of rheumatoid arthritis (Crook et al., 2015). Despite extensive research, limited studies attest to MDSC and B cell crosstalk. In human PBMC, PMN-MDSCs were shown to modulate B-cell function by suppressing cell proliferation. It was further demonstrated that arginase-1, NO, ROS were involved in the suppression of B-cells (Lelis et al., 2017). A novel finding revealed an interaction between MDSCs and B-cells in tumor bearing mice. It was observed that MDSCs accumulated in the germinal center of the spleens of tumor bearing mice and co-localized with B-cells. *In vitro* co-culture of MDSCs with B-cells promoted proliferation and differentiation of B-cells into plasma cells producing IgA. Further experimental validations suggested that IL-10 and TGF-β1 play an important role in promoting MDSCs mediated IgA production (Xu et al., 2017a).

Wang et al. (2018) and others found that MDSCs suppress B cell function in a murine lung cancer model. It was reported that B-cells were significantly reduced in the bone marrowand spleen of tumor bearing mice. Mechanistic studies revealed that IL-7 and STAT5, key regulators for B cell commitment, were also downregulated. The B-cell impairment was positively co-related with MDSC infiltration and cancer prognosis. Furthermore, IgG levels were decreased with B-cell dysfunction. MDSCs suppressed B-cell proliferation in an arginase-dependent manner, indicating cell-cell interactions between MDSCs and B-cells.

3.1.3 Suppression of other cells

MDSCs are further known to inhibit NK cell cytotoxicity. Expression of indoleamine 2,3-dioxygenase (IDO) expression by MDSCs reduces tryptophan concentration in the TME. This not only stimulates Treg cells (Arandi et al., 2018) but also induces apoptosis of NK cells (Fang et al., 2022). Murine studies have reported MDSC related inhibition of NK cells mediated by TGFβ (Li et al., 2012). Membrane bound TGFβ was shown to impair NK cell cytotoxic activity, reduce NKG2D expression and decrease INF-γ production (Li et al., 2009). Ligands such as IL-33 (Fournié and Poupot, 2018; Shen et al., 2018)- produced by epithelial andendothelial cells and IL-1β (Elkabets et al., 2010) expressed by MDSCs have greater inhibitory properties for NK cells than other immune cells. In humans, MDSC inhibit the effects on INF-γ *via* NKp30-dependent mechanism (Hoechst et al., 2009) and interfere with NK FcR-mediated cytotoxicity which impairs NK cytotoxic and cytokine production (Stiff et al., 2018).

INF-γ and other molecules present in the TME promote MDSC expansion which results in the release of IL-10. The increased amount of IL-10, an anti-inflammatory molecule, inhibits the release of inflammatory cytokines thereby promoting a pro-tumorigenic microenvironment. The IL-10 mechanism strongly suppresses NK-cell and $CD8^+$ T-lymphocyte activation (Figure 1) (Ibrahim et al., 2018; Yaseen et al., 2020). Additionally, NO production by MDSCs also arrests NK function and the secretion of INF-γ, granzyme B and TNF-α (Zhang et al., 2018). MDSCs produce IDO, which impairs development and activation of NK cells *via* STAT3 and TGF-β signaling (Sun et al., 2013; Sui et al., 2014). On the other hand, *in-vitro* studies on murine NK cells have shown Jak-3 inhibition and activation of STAT5 when co-cultured with MDSC (Hoechst et al., 2009). Furthermore, other NK-MDSCs cells interactions include TIGIT-CD155 (Sarhan et al., 2016) interactions and IL-1R8 (Molgora et al., 2017) signaling induced by interactions with MDSCs.

3.2 Non-immunological function

MDSCs also contribute to the progression of primary tumors by promoting angiogenesis and metastasis. Yang et al. (2004) demonstrated a tumor-promoting role for Gr+CD11b+ cells in tumor bearing animals. Co-injection of tumor cells with Gr+CD11b+ cells increased angiogenesis, reduced tumor necrosis and enhanced tumor growth. It was also reported that MMP9 produced by Gr+CD11b+ cells contributes to tumor angiogenesis and growth. In another study, MDSCs isolated from mouse tumors displayed STAT3 activation and induced angiogenesis by expressing VEGF (Kujawski et al., 2008). MDSCs also promote a mesenchymal phenotype in tumor cells by secreting several inflammatory factors such as TGF-β, IL-6, IL1-β and Hepatocyte Growth Factor (HGF) (Figure 1). These factors

reduce E-cadherin expression in tumor cells which drives epithelial-mesenchymal transition (Toh et al., 2011; Ouzounova et al., 2017; Pastaki Khoshbin et al., 2019).

Studies in the literature have reported a critical role for MDSCs in establishing a pre-metastatic niche. Generally, primary tumors release signaling molecules before the onset of metastasis to regulate secondary tumor formation and recruitment of cells including neutrophils, macrophages, T_{reg} and MDSCs. These cells communicate *via* signals that prepare healthy tissues at secondary sites to act as "soil" that encourage the inhabitation of circulating tumor cells (CTCs) (Peinado et al., 2017; Nasrollahzadeh et al., 2020; Wu et al., 2020). It has been reported that VEGFR1$^+$ hematopoietic bone marrow progenitors promote the formation of a pre-metastatic niche that supports tumor metastasis. VEGFR1$^+$ cells express VLA-4 (also known as integrin alpha4beta1) which can act in concert to promote a supportive niche for tumor growth (Kaplan et al., 2005). A study by Yan et al. (2010) reported an increased population of Gr-1+CD11b+ cells (immature myeloid cells) in the lungs of mice bearing mammary adenocarcinoma. The immature myeloid cells in the premetastatic lungs showed significant decrease in INF-gamma production with increased production of proinflammatory cytokines. Also, these cells expressed high levels of matrix metalloproteinase 9 (MMP9) and promoted vascular remodeling. The deletion of MMP9 diminished lung metastasis. Further studies have shown MDSC derived exosomes, containing TGF- β, VEGF and S100A8/A9, significantly induced metastasis, immunosuppression and promotes PMN-MDSC expansion (Wang et al., 2019a; Hsu et al., 2019).

3.3 Gene expression profiles of MDSC from solid tumors

The MDSCs genotype has benn widely studied in several cancers, namely, breast cancer, colorectal cancer, lung adenocarcinoma, etc. However, research on hematological malignancies is much more limited as indicated by gaps in the literature. For instance, an RNA sequencing analysis on non-small cell lung carcinoma (NSCLC) patients by Mastio and others defined of M-MDSCs and monocytes with distinct gene signature profiles. The gene expression profile of HLA-DR$^{-/LOW}$ CD14$^+$ CD15$^-$ showed 619 differentially expressed genes as compared to monocytes. A notable upregulation of neutrophil-associated genes was observed including IL-8, MNDA (Myeloid cell nuclear differentiation antigen), CSF3R (G-CSF receptor), LYZ (lysozyme), NCF4 and NCF2 (neutrophil cytosolic factors 4 and 2). Additionally, downregulation of CSF1R (M-CSF receptor), NR4A1 (Nur77), CXCR1, ITGA4 (CD49d) and CD52 was observed (Table 1) (Mastio et al., 2019). The higher expression of neutrophil function genes in HLA-DR$^{-/LOW}$ CD14$^+$ CD15$^-$ cells suggests that these cells may have neutrophil precursors. Further extensive analysis showed an upregulation of the *CXCR1* gene, which is a chemokine receptor on neutrophils and is not associated with monocytes. Experimental validation on prostate cancer in the same study showed that 20% of M-MDSCs were *CXCR1* positive and had potent immunosuppressive activity. Furthermore, these CXCR1$^+$ M-MDSCs not only had the ability to differentiate into neutrophils but also shared features of macrophages (Mastio et al., 2019). This study therefore strongly attests to the enrichment of MLPGs (MDSC originating from M-MDSC and differentiating into PMN-MDSC) in humans.

Another study on pancreatic ductal carcinoma (PDAC) showed distinct cytological features (small size) and T cell suppressive function in M- MDSCs. Genome wide mRNA expression profile on M-MDSCs from three suppressive and four non-suppressive PDAC patients demonstrated an overall cancer-related gene signature including increased TNFα signaling *via* NF-κB, inflammatory response, IL6 JAK/STAT3 signaling and apoptosis categories. The suppressive CD14$^+$ cells showed higher expression of FBN2, TSPAN16, LEPR, CLTA and CD163 which are genes associated with classic monocytes and differential expression patterns of MAP3K3, PRKRA, JAK2, as well as differential expression of components of the STAT family (STAT1, STAT2, STAT3, STAT5A, STAT5B and STAT6) (Trovato et al., 2019). This study also reported upregulation of metabolic genes linked to immunosuppression including fatty acid and lipoprotein metabolism-related genes, namely, CD36, LYPLA1 and CERS5, ATP and glucose metabolism genes such as ATP51C, ATP5G2, SDHB and PDK4, GXYLT1. Additionally, the BM-MDSCs and cancer patient immunosuppressive M-MDSCs shared a common gene signature profile, i.e., non-differentially expressed genes such as PTGS2, TNF, CD38, ARG1, AKT3, JAK1, JAK3, STAT1, STAT4, STAT5, STAT6 and STAT3, suggesting a common regulatory mechanism among the myeloid cells. Briefly, this study states an important immunosuppressive phenotype of M-MDSCs (Table 1).

Other studies have also reported CD33 as a specific marker expressed on MDSCs (Ortiz et al., 2015), which is one of the potential targets for cancer therapies (Jitschin et al., 2018). Other genes associated with gene ontology (GO) analysis included chemokine receptor 2 (CXCR2) (Veglia et al., 2019; Ugolini et al., 2020), colony Stimulating Factor 1 Receptor (CSF1R) (Zhou et al., 2018), and C-C Motif chemokine receptor 1(CCR1) (Cai et al., 2017), suggesting that MDSCs might communicate with recruitment signals from other sites of inflammation including the primary tumor or metastatic sites. This study also validated MDSC gene signatures from spleens of healthy and tumor bearing mice the upregulation of CD11B + Gr1+ cells (Alshetaiwi et al., 2020).

Leader et al. applied scRNA to corelate transcriptome profiles of tumor early-stage NSCLC and normal tissues from early-stage NSCLC. The interrogation into immune ecosystem revealed a remarkable heterogeneity and plasticity in the myeloid cells. According to analyses, M-MDSCs trajectory analysis M-MDSCs and PMN-MDSCs transitioned along the monocyte-to-M2 macrophage path. However, MDSCs subsets did not show any root or branch-level enrichment. Gene expression analysis of M-MDSCs and PMN-MDSCs showed a distinct gene signatures of IL-10, CD14, VEGFA and IL-6, OLR1, TGFβ1, respectively (Leader et al., 2021).

Studies in the literature attest to distinct transcriptome profiles of MDSCs with the upregulation of genes characterized by immunosuppression, high transcriptional activity, and increased pro-inflammatory pathways. These hallmark genes can be further explored in hematological malignancies and their functions can be studied. However, we also face challenges to clearly distinguish MDSCs from monocytes and neutrophils. Therefore, a more comprehensive analysis the gene expression signatures of the

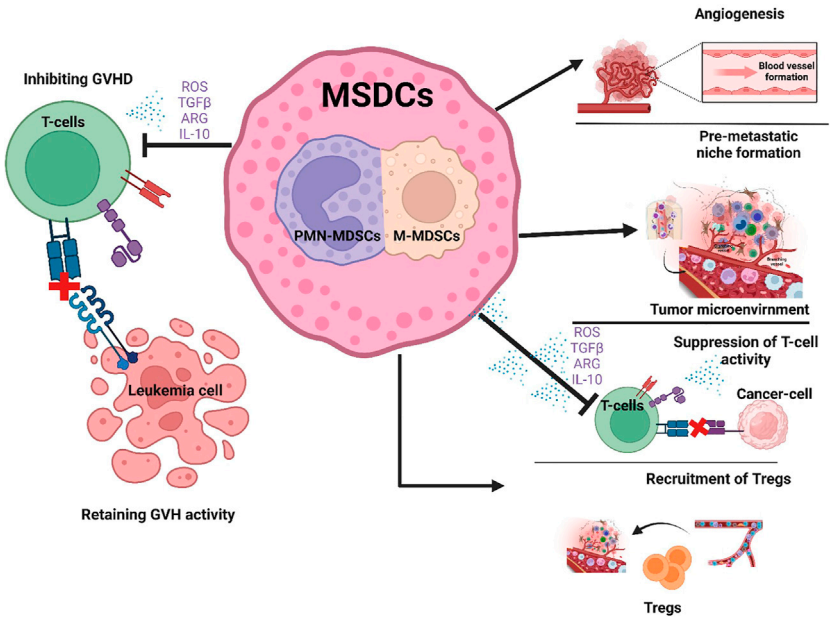

FIGURE 2
Role of MDSCs in hematological malignancies and Hematopoietic stem cell transplantation. MDSCs have a favorable role in that they suppress T cell activity thereby preventing GVHD after HSCT (Left side of figure). In contrast, the unfavorable expansion of MDSCs promotes tumor growth by increasing angiogenesis, creating a pre-metastatic niche, suppressing T-cell activity and recruiting Tregs in the TME (Right side of figure).

MDSCs subtypes will create new opportunities for selective targeting MDSCs populations and thereby designing new therapies.

4 MDSCs in hematological malignancies

Mostexperimental and clinical studies on solid tumors have reported dysregulation of MDSCs However, recent studies have defined their role in immune dysregulation in hematologic malignancies, immune-mediated cytopenias and allogeneic hemopoietic stem cell transplantation. Also, MDSCs potential role as biomarkers and therapeutic targets have started gaining attention in B-cell malignancies. The signaling pathways associated with the differentiation, expansion, circulation, and function of MDSCs, as well as the crosstalk between MDSCs and tumor cells make therapeutic strategies challenging. This section will summarize the research studies on the MDSCs in hematological malignancies and their tumor microenvironment.

4.1 MDSCs in tumor microenvironment

The TME of B-cell lymphomas is highly variable, which consists of immune cells, stromal cells, blood vessels and extracellular matrix. Genetic aberrations harbored by tumor cells, their proliferation, increased angiogenesis, and an immune response by the host, contributes to the complex spatial cellular distribution of TME. Therefore, crosstalk between the TME elements is distinct in different types of lymphoma. Lymphomas can have variable amounts of tumor cell content, which ranges from a minimum of ~1% in Hodgkin's lymphoma to a maximum of 90+ % in Burkitt's lymphoma (BL). Generally, the TME in hematological malignancies can be divided into three models based on the interaction between tumor cells and the TME: 1. 'recruitment', 2. 're-education,' and 3. 'effacement.'

Briefly, the 're-education' pattern is commonly seen in FL, CLL, MM, and MALT lymphomas, which primarily affect lymph nodes. In this pattern, the malignant cells have a substantial degree of dependency on the TME for survival. Proliferation of lymphoma cells is partially supported by external stimuli in the TME, such as cytokines, chemokines, growth factors and signaling *via* stromal cells. The spatial arrangement of the tumor tissue is very similar to the normal lymphoid tissue, comprising of residual reactive germinal centers with distinct follicular dendritic cell (FDC) meshworks and follicular T-helper cells (T_{FH} cells) (Scott and Gascoyne, 2014; Nathan et al., 2016).The second pattern is one of 'recruitment' and is typically observed in classical Hodgkin's lymphoma (CHL). Here, the tumor comprises of a supportive non-malignant milieu which surrounds the infrequent malignant Hodgkin Reed-Sternberg cells (HRS cells). In this pattern, the tumor cells rely completely on the TME for survival and growth (Scott and Gascoyne, 2014; Nathan et al., 2016). Lastly, the third pattern is 'effacement' and includes BL where malignant cells express genetic aberrations such as MYC translocations and cooperative mutations (Schmitz et al., 2012). These changes remove the dependency of the malignant cell on the microenvironment for growth and proliferation, causing cell-autonomous survival (Scott and Gascoyne, 2014; Nathan et al., 2016).

As discussed earlier, the composition of TME in B-cell malignancies in divided into two parts: Immune

microenvironment and non-immune microenvironment. The immune microenvironment consists of immune cells including T and B lymphocytes, MDSCs, tumor-associated macrophages (TAMs), tumor associated neutrophils (TANs), NK cells, dendritic cells, and many other cells. Furthermore, the non-immune microenvironment mostly includes fibroblasts, stromal cells, secretory molecules including growth factors, exosomes, cytokines, and chemokines.

TAMs are the most intensive immunosuppressive cells in TME. These cells inhibits T-cell activation and recruits at the tumor site by secreting cytokines, chemokines and other factors (Pathria et al., 2019). Based on immunomodulatory functions, TAMs are classified into M1 and M2 phenotypes. M1 macrophages are cytotoxic and play a critical role in tumor development. However, during the process of tumor growth M1-like macrophages are transformed into M2-like macrophages (Mantovani et al., 2017). M2-like macrophages produce cytokines and growth factors which contribute to tumor development, angiogenesis, inflammation and immunosuppression (Wang et al., 2019b) (Figure 1). As discussed in previous sections, the TME cells express specific cell surface markers which is associated with poor prognosis in B cell lymphoma patients. The cytokines and chemokines secreted by TME cells creates an immunosuppressive environment which modulates the activity of immune cells such as suppression of T cell function. These cytokines are divided into two groups. The first group includes VEGF, granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF) and granulocyte colony-stimulating factor (G-CSF).Similarly, the second group of cytokines includes interferon-γ (INF- γ), IL-6, IL-4, IL13, high-mobility group box 1 (HMGB1), and tumor necrosis factor (TNF) (Sevko et al., 2013; Condamine et al., 2015b).

A direct relationship between MDSC and TME is reported in many cancers including prostate, breast, colon, melanoma and in B-cell malignancies. As an important immunosuppressive cell, MDSCs effectively suppress T-cell, B-cell, and NK cell activation, and recruits Tregs at the site of tumor. Therefore, tumor cells escape by the immune surveillance and increases tumor growth and metastasis. A recent report demonstrated the secretion of exosomes in TME. These tumor derived exosomes accelerate the activation and expansion of MDSCs (Tian et al., 2019) delivering molecules and proteins such as miRNAs (Ren et al., 2019), IL-10, 1L-16, PEG2 and TGF-β.

4.2 Lymphomas

A preclinical study on murine A20 B-cell lymphoma model, Serafini and others identified a population of cells with a unique phenotype. These cells showed expression of Gr1, F4/80 and IL-4Rα, with low expression of MHC class I and II molecules. In subsequent studies, the population was confirmed to be MDSCs, and these cells suppressed $CD8^+$ T cell activity and induced recruitment and expansion of Treg cells (Serafini et al., 2008) (Figure 1). The immunosuppressive activity of MDSCs was inhibited by lenalidomide, in that the drug reduced the frequency of MDSCs in A20 lymphoma mice. (Sakamaki et al., 2014). Furthermore, inflammatory changes promoted infiltration of MDSCs in the spleens of EL4-luc2 lymphoma mice model. These mice demonstrated elevated numbers of neutrophils resulting in abnormal myelopoiesis, which stimulated MDSCs thereby supporting tumor development (Abedi-Valugerdi et al., 2016). In murine B- cell lymphoma, the expression of mir-30 further promoted the development of MDSCs. Activation of JAK/STAT3 signaling pathway by miR-30 decreased the expression of suppressor of cytokine signaling-3 (SOCS3), thereby promoting differentiation of MDSCs and releasing immunosuppressive factors such as ARG-1, IL-10 and ROS (Xu et al., 2017b). To confirm increased levels of PDL-1 expression and expansion of MDSCs (at the tumor site) in DLBCL patients, PDL-1 expression was evaluated in an A20 B-cell lymphoma mouse model. It was observed that anti-PDL-1 treatment decreased MDSCs at the tumor site (Lu et al., 2021). Further, another study identified calmodulin kinase 2 (Camkk2) as an important target responsible for the accumulation and expansion of MDSCs in, E.G.,7-OVA TB mice (a mouse lymphoma model derived by electroporating T lymphoblast, EL4 cells). Knockdown of Camkk2 in mice decreased the accumulation of MDSCs, enhanced the anti-tumor response of T-cells and slowed tumor growth. Tumor growth was restored in $Camkk2^{-/-}$ mice when transplanted with MDSCs. This study confirmed the immune suppressive function of MDSCs and potential role of Camkk2 in targeting MDSCs expansion (Huang et al., 2021). Also, M-MDSC accumulation *via* an IL-35 mechanism was significantly decreased with the anti-IL-35 treatment in the Ly8DLBCL murine model (Wang et al., 2021a).

Clinically, Romano et al. (2015) reported an increase in M-MDSCs, PMN-MDSCs and $CD34^+$ MDSCs in peripheral blood of Hodgkin's lymphoma (HL) patients as compared to healthy individuals. It was observed that the levels of MDSCs were much lower in complete remission patients when compared to the patients who did not have a complete remission after chemotherapy. Amini et al. (2019) also observed increased numbers of PMN-MDSCs in the peripheral blood of 19 HL patients when compared to healthy individuals. A similar study found that PMN-MDSCs ($CD66b^+$ $CD33^{dim}$ $HLADR^-$) were higher in 124 B-cell lymphoma patients which included HL and B-NHL. The study found that depletion of $CD66b^+$ MDSCs restored the T-cell response and proliferation (Marini et al., 2016). Furthermore, in DLBCL patients, there was a significant increase in MDSCs in the peripheral blood and this was a poor prognostic factor. However, only the increased number of M-MDSCs correlated with the international prognostic index (IPI), high numbers of circulating Treg cells and elevated expression of IL10, PDL-1 and S100A12. These factors were associated with the immunosuppressive mechanism of MDSCs, and inhibition of IL-10, PDL-1 and S100A12 increased T-cell activation and proliferation (Azzaoui et al., 2016). Some recent studies have reported that high levels of M-MDSCs in newly diagnosed and relapsed patients correlated with tumor progression and patient survival. Additionally, a clinical trial with lenalidomide and R-GDP (Rituximab plus gemcitabine, cisplatin and dexamethasone) chemotherapy in relapsed DLBCL patients showed decreased levels of MDSCs and Treg cells in individuals with a favorable 24-month survival rate. This study also demonstrated that Vit-D deficient DLBCL patients had increased numbers of MDSCs and Treg cells, suggesting that an enhanced treatment outcomes could be achieved by Vit-D

supplementation in DLBCL-patients (Jiménez-Cortegana et al., 2021a; Jiménez-Cortegana et al., 2021b). Additionally, patients with NK/T-cell lymphoma (ENKL) had high MDSCs levels and specially M-MDSCs were responsible for a poorer disease-free survival and overall survival. Studies have found that IL-17 produced by CD4+Th17 cells in ENKL patients increases MDSC expansion and inhibits T cell proliferation (Zhang et al., 2015). The effect of MDSCs on other types of NHL including cell lymphoma and follicular lymphoma is still unexplored.

4.3 Multiple myeloma

MDSCs expansion in bone marrow was observed during early stages of multiple myeloma (MM) in the 5TMM mouse model and in later stages, MDSCs could be detected in peripheral blood. Also, myeloid leukemia cell differentiation protein (Mcl-1) was reported as a potential target promoting MDSCs survival. *In vitro* studies have shown that mesenchymal stem cell (BMSC)- derived exosomes induced the survival of MM MDSCs and increased NO production in MDSCs by activating STAT3 and STAT1 signaling. These bone marrow-derived mesenchymal stem/stromal cells (BMSC) also increased the expression of anti-apoptotic proteins, namely, Bcl-xL and Mcl-1 suppressing T-cell activation and contributing to bortezomib and melphalan resistance (Wang et al., 2015a; Veirman et al., 2015; De Veirman et al., 2019). A mechanistic study reported the presence of S100A9 and its receptor TLR4 in MM MDSCs model mice which triggered MDSCs expansion and secreted inflammatory molecules such as TNF- α, IL-6 and IL-10 (Figure 1). However, blocking S100A9 did not affect the MDSCs expansion but rather decreased the cytokine production (De Veirman et al., 2017). Further, in a clinical study, a pro-tumorigenic affect of MM derived Galectin-1 was observed on the expansion of M-MDSCs with CD304 interaction, thereby facilitating MM progression following autologous stem cell transplant (ASCT) (Lim et al., 2021). These studies attest that MDSC in the MM microenvironment play an important role in interaction between cells, cytokines and other factors present in the tumor niche which together influence MM progression.

Clinically, for the first time in 2010, Brimnes et al. (2010) reported an increase in M-MDSCs in peripheral blood of MM patients as compared to controls. In later studies Wang et al. (2015b) showed a positive correlation of M-MDSCs with MM recurrence and negative correlation with treatment outcomes. A similar study found out that neutrophils from bone marrow were immune suppressive and had MDSCs like activity. Furthermore, it was found that $CD11b^+$ $CD113^+$ $CD16^+$ neutrophils have G-MDSCs (Perez et al., 2020) phenotype.

Several studies have reported an accumulation of G-MDSCs in the bone marrow and peripheral blood of MM patients as compared to healthy individuals and the expansion is corelated with the disease pathogenesis (Ramachandran et al., 2013; Favaloro et al., 2014; Giallongo et al., 2016). In context to treatment with bortezomib, lenalidomide (immunomodulatory agent) and DC vaccination there are inconsistent results on its effect on MDSCs and MM patients (Gunes et al., 2020). A study by Kuwahara-Ota et al. (2020) identified lenalidomide and pomalidomide as potential inhibitors for MDSCs induction, which downregulated CCL5 and MIF in MM patients. This study also found out that downregulation of CCL5, and induction of interferon regulatory factor 8 (IRF8) affected MDSCs expansion. Additionally, it was also observed that high amount of M-MDSCs in MM patients inhibited the cytotoxicity of pre-autologous stem cell transplantation (ASCT) and was associated with poor clinical outcome (Lee et al., 2019). A co-culture study of MPC11 cells (B lymphocyte cell line) with MDSCs of MM patients showed high proliferation of MPC11 cells and reduction in T cell immune response when compared to no MDSCs, and decitabine (DAC) treated bone marrow cells (Zhou et al., 2019).

A study by Nakamura et al. (2018) have reported the high expression of IL-18 in the bone marrow microenvironment of MM patients. It was observed that, IL-18 driven MDSCs immune suppression *via* C/EBβ was associated with poor prognosis. The migratory inhibitor factor in the MM bone marrow microenvironment is an important factor which leads to the expansion and differentiation of MDSCs, increases levels of PDL-1 expression on MDSCs and suppresses T-cell function (Lewinsky et al., 2021). But now we know that some MM targeting drugs such as Daratumumab (targeting CD38) successfully diminished the immune suppressive cells, namely, Tregs, Bregs and MDSCs (van de Donk and Usmani, 2018). Additionally, estrogen has also been associated with MM disease progression (Ozerova and Nefedova, 2019). Together, all the studies identified new molecules which promotes MDSC formation/expansion and targeting them could improve drug therapeutics for MM.

4.4 Leukemia

In earlier studies, Sun et al. (2015) found expansion of MDSCs in acute myeloid leukemia (AML) and a complete remission group of patients as compared to partial and no remission group of patients. This study also reported the overexpression of WT1 (Wilms tumor gene) gene in the bone marrow samples of AML patients which had positive correlation with MDSCs expansion. Another study identified higher amounts of M-MDSCs and e-MDSCs in the peripheral blood of AML patients (Lv et al., 2021). Further, an elevated expression of MDSCs corelated with the poor remission, higher relapse rates and reduced long term survival (Wang et al., 2020).

In AML, it was also observed the cytarabine (Ara-C)- triggered increased expression of TNFα from the AML cells resulting in the expansion and survival of the MDSC by activating IL-6/STAT3 and NFKB signaling (Bai et al., 2022). It has also been observed that the combination of Ara-C, Plerixafor (CXCR4 inhibitor) and PDL-1 in AML murine model decreased the number of Tregs and MDSCs in peripheral blood and bone marrow (Hwang et al., 2019). Additionally, MDSC expansion was observed in B-cell acute lymphoblastic leukemia (B-ALL) (Zahran et al., 2021). Hohtari et al. (2019) noted that in all the AML bone marrow (n = 52) there was a decrease in M1-type macrophages and effector T cells with an increase in M2-type macrophages and MDSCs as compared to healthy individuals. They also observed an elevation of PD-1 and CTLA-4 (negative immune check point) which was responsible for immune suppressive role in AML patients. Furthermore, in acute promyelocytic leukemia (APL) patients innate lymphoid cells (ILC2s) were increased and hyper-activated which in turn

TABLE 2 Clinical trials performed worldwide for MDSCs (listed in clinicaltrials.gov).

Study	Single agents targeting MDSCs	Cellular/immune therapy (including TRIKEs)	Combination approaches	Status	Clinical Trials.gov identifier	Key outcomes	Sponsor information
Myeloid-derived suppressor cells (MDSCs) in OSCC patients	Dietary supplement: *ß*-glucan	NA	Beta-glucan administration in oral squamous cell carcinoma patients	Unknown	NCT04387682	β-glucan increasing anti-tumor immunity for OSCCs reduces the recurrence rate or improves survival	National Taiwan University Hospital
Targeting myeloid-derived suppressor cells in recurrent glioblastoma: Phase 0/1 trial of low dose capecitabine + bevacizumab in patients with recurrent glioblastoma	Capecitabine and Bevacizumab	Chemotherapy with immunotherapy	Capecitabine given orally, and the cycle length was 28 days Treatment continued until disease progressed Bevacizumab (10 mg/kg) given every 28 days, until progression	Active. Not recruiting Start Date: 21 January 2016 Estimated end Date: January 2023	NCT02669173	To achieve a 20-fold MDSC reduction in the concentration of circulating MDSCs after treatment with low-dose capecitabine	Case Comprehensive Cancer Center
IMMUNeOCT study: Octreotide LAR in the induction of immunologic response in patient with neuroendocrine tumors: An interventional pharmacological study	Octreotide Acetate	Chemotherapy	Octreotide Acetate administration every 28 days	Completed Start Date: 16 October 2019 Estimated end Date 10 September 2020	NCT04129255	To observe the impact of OCTREOTIDE LAR on the immune response by studying T-Reg and MDSC and the immunoregulatory cell population in peripheral blood of patients with neuroendocrine tumors G1/G2 treated with OCT LAR.	Hangzhou Sumgen Biotech Co., Ltd
Tadalafil to overcome immunosuppression during chemoradiotherapy for IDH-wildtype Grade III-IV Astrocytoma	Tadalafil	Chemotherapy, radiation therapy and adjuvant therapy	Tadalafil given orally once daily for a total of 60 days Radiation therapy (RT) to 60 Gy in 30 daily fractions will be administered in this study And adjuvant TMZ will be initiated for 4–6 weeks after completion of RT (6 cycles at 150–200 mg/m2 PO per day on Days 1–5 of every 28-day cycle)	Active, not recruiting Start Date: 17 February 2021 Estimated end Date 10 June 2022	NCT04757662	Relative change of MDSCs in peripheral blood	Washington University School of Medicine
SX-682 treatment in subjects with metastatic melanoma concurrently treated with pembrolizumab	SX-682 in combination with pembrolizumab	Chemotherapy SX-682- a selective inhibitor of C-X-C Motif Chemokine Receptor 1 (CXCR1) and C-X-C Motif Chemokine Receptor 2 (CXCR2)	SX-682 Maximum Tolerated Dose (MTD) during Monotherapy Stage [Time Frame: Up to 21 Days in 21-day Cycle 1 of Monotherapy Stage.]	Recruiting Start Date: 19 May 2017	NCT03161431	Biomarker measurement including, tumor MDSC, Tregs and CD69/CD8 T cells, and in the circulation, T- and B-cell subpopulations, neutrophils, the neutrophil-to-lymphocyte ratio (NLR),	Syntrix Biosystems, Inc

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TABLE 2 (*Continued*) Clinical trials performed worldwide for MDSCs (listed in clinicaltrials.gov).

Study	Single agents targeting MDSCs	Cellular/immune therapy (including TRIKEs)	Combination approaches	Status	Clinical Trials.gov identifier	Key outcomes	Sponsor information
		Pembrolizumab- targets the programmed cell death 1 receptor (PD-1)		Estimated end Date 8 February 2023		Tregs, the CD4:CD8 ratio, chemokines, cytokines, and LDH	
Study evaluating the influence of LV5FU2 bevacizumab plus anakinra association on metastatic colorectal cancer (IRAFU)	LV5FU2 Bevacizumab plus anakinra	Chemotherapy and anti-angiogenic therapy		Completed	NCT02090101	1.Median progression-free and overall survival were 5.4 (95% CI, 3.6–6.6) and 14.5 months (95% CI, 9–20.6), respectively	Centre Georges Francois Leclerc
			2-week cycles of bevacizumab + LV5FU2 (folinic acid + 5-FU) + anakinra	Start Date: 18 March 2014		2.Common grade 3 adverse events were neutropenia in 25% of patients, digestive side effects in 21.9 patients, and hypertension in 18.75%	
		IL-1β and *a* inhibitor		Estimated end Date: 9 August 2018			
RTA 408 capsules in patients with melanoma—REVEAL	Combination With Nivolumab/ ipilimumab	Chemotherapy Omaveloxolone targets Nrf2 pathway	Omaveloxolone Capsules (2.5 mg/capsule)		NCT02259231	Omaveloxolone was considered up to 150 mg in combination with checkpoint inhibitors	Reata Pharmaceuticals, Inc
			Ipilimumab (3 mg/kg)	Completed			
			Nivolumab (240 mg)	Start Date: October 2014			
			Omaveloxolone Capsules (10 mg/capsule)	Estimated end Date: 24 June 2021			
			Omaveloxolone Capsules (50 mg/capsule)				
Ipilimumab and All-trans retinoic acid combination treatment of advanced melanoma	Ipilimumab (anti-CTLA-4 and PD-1) and VESANOID (all-trans retinoic acid)	Chemotherapy	Ipilimumab- 4 doses of either 3 or 10 mg/kg ipilimumab every 3 weeks	Active, not recruiting	NCT02403778	1. Number of Adverse Events	University of Colorado, Denver
			VESANOID- 150 mg/ m2 orally for 3 days	Start Date: 17 December 2015		2. MDSC frequency and suppressive function	
PDE5 Inhibition *Via* tadalafil to enhance anti-tumor mucin 1 (muc1) vaccine efficacy in patients with HNSCC	Tadalafil	Chemotherapy	Tadalafil-Course 1–10–20 mg tablets orally for 19 consecutive days. In Courses 2 to 4- Tablets were administered daily for 14 consecutive days	Completed Start Date: 9 September 2015 Estimated end Date: 15 June 2021	NCT02544880	1.Adverse events and/or treatment limiting-toxicities after receiving protocol therapy. 2. Rate of tumor-specific immune response to protocol therapy	Donald T. Weed, MD, FACS
	Biological: Anti-MUC1 Vaccine						
	Biological: Anti-Influenza Vaccine						
	Other: Tadalafil Placebo						

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TABLE 2 (*Continued*) Clinical trials performed worldwide for MDSCs (listed in clinicaltrials.gov).

Study	Single agents targeting MDSCs	Cellular/immune therapy (including TRIKEs)	Combination approaches	Status	Clinical Trials.gov identifier	Key outcomes	Sponsor information
	Other: Anti-MUC1 Vaccine Placebo						
	Other: Standard of Care Treatment						
	Other: Anti-Influenza Vaccine Placebo						
Tolerability and safety of HF1K16 injection in patients with refractory solid tumors	HF1K16	Chemotherapy	Dose escalation cohort of HF1K16 given QOD at 45 mg/m2, 90 mg/m2, 120 mg/m2, 160 mg/m2	Recruiting Start Date: 24 May 2022	NCT05388487	1.Incidence of Adverse Events 2. Incidence of dose-limiting toxicities 3.Whole blood profiling 4. MDSC expression	High Field Biopharmaceuticals Corporation

activated M-MDSCs *via* IL-13 secretion. After treatment with all trans retinoic acid (ATRA), the levels of PGD2, NKp30, ILC2s, IL-13 and M-MDSCs was restored (Trabanelli et al., 2017).

In chronic granulocytic leukemia (CML), an increased number of MDSCs and its immune suppressive markers such as IL10 and ARG1 was observed. Further, it was noted that imatinib and dasatinib (tyrosine kinase inhibitor) treatment reduced MDSCs numbers to the normal range (Giallongo et al., 2014; Christiansson et al., 2015). Additional studies also reported the tyrosine kinase inhibitor (TKI) treatments reduced G-MDSCs in patients, however, only dasatinib showed a significant reduction in M-MDSCs. Therefore, dasatinib can serve as an important targeting agent for M-MDSCs in chronic myeloid lymphoma (CML) patients (Giallongo et al., 2018). Also, an elevated number of M-MDSCs was observed in the peripheral blood of fifty CLL patients and was associated with poor survival (Zahran et al., 2020). It was also seen that Tregs and MDSCs can be reduced to normal range with ibrutinib treatment in 1–2 years (Solman et al., 2021). Furthermore, PMN-MDSCs showed greater immunosuppressive impact on M-MDSCs in CLL patients. However, ibrutinib successfully lowered the PMN-MDSCs, targeted MDSCs differentiation, induced naïve T cells and improve the tumor microenvironment (Ferrer et al., 2021).

4.5 Myeloproliferative syndromes

The first study on MDSC expansion in myeloproliferative syndromes (MDS) was shared by Chen et al. (2013). Using multiple cell transfection models, it was noted that the expansion of MDSCs was driven by S100A9 and CD33. These two important proteins form functional ligand/receptor pairs which suppress immunoreceptor tyrosine-based inhibition motif and induce secretion of suppressive cytokines such as IL-10 and TGFβ. In MDS, MDSCs suppress T cells by recruitment and proliferation of Tregs in the bone marrow (Kittang et al., 2016). Also, Gal-9, an important ligand for immune checkpoint molecule TIM3 is highly expressed on the MDSCs of MDS patients. This Gal9 was observed to bind TIM3 on CD8+T cells, suppressing their immune function and cause T-cell exhaustion (Tao et al., 2020). Studies have also shown that ARG1, an immune suppressive molecule suppresses the ani-tumor response of $CD8^+$ T cells *via* STAT3 signaling pathway (Qi et al., 2021) (Figure 1). Therefore, targeting these negatively corelated immune checkpoint molecules of MDSCs in MDS patients could suppress the activation and proliferation of MDSCs.

4.6 Hematopoietic stem cell transplantation

In previous sections we have discussed the immune suppressive and tumor promoting role of MDSCs in hematological malignancies. However, in hematological stem cell transplantation (HSCT), the relationship between graft versus leukemia (GVL), graft versus host disease (GVHD) and MDSCs is complicated (Figure 2).

In mice and humans, the expression of MDSCs have been detected in the peripheral blood during allogenic hematopoietic stem cell transplantation (allo-HSCT) (Luyckx et al., 2012). A

clinical study by Fan et al. (2017) showed that G-CSF mobilization improved relapse free survival and GVHD in bone marrow hematopoietic transplantation (G-BM) as compared to G-PBMC transplantation. A similar study also showed that mobilization with pegylated-G-CSF in allo-HSCT elevates M-MDSCs in the donors and improved the rates of severe GVHD (Li et al., 2021).

A transcriptomic study by Andrea et al. reported that G-CSF mobilized PMN-MDSCs had upregulation of genes promoting DNA replication, cell cycle, and cell division, namely, Ki-67, topoisomerase II alpha, and cyclin B, respectively. This study showed that G-CSF-mobilized PMN-MDSCs enrichment in the graft displayed remarkable molecular characteristics with significant inhibition of donor NK cells (Pelosi et al., 2021). A retrospective study reported encouraging improvement in ani-leukemic affect of donor lymphocyte infusion (DLI) after G-CSF treatment as compared to standard DLI after relapsed allo-HCT (Schneidawind et al., 2019). Wang et al. (2021b) described that human umbical cord mesenchymal stem cells causes MDSCs expansion and prevents GVHD after HSCT by secreting CXCL1 and HLA-G (Yang et al., 2020). The mechanistic study on GVHD mice models revealed that MDSCs suppresses GVHD and preserves GVL activities by inducing NKG2D expression on T cells and suppresses GVHD by increasing Tregs (Zhang et al., 2019). It was also observed that the delay in M-MDSCs recovery and invariant natural killer cells (iNKT) after transplantation was associated with a high incidence of grade III-IV acute GVHD. However, low levels of M-MDSCs and high levels of iNKT cells significantly reduced leukemic relapse (Kim et al., 2019b). This study suggests that the balance between MDSCs and other immune cells is critical for achieving good outcomes in HSCT.

The major challenges in the field of GVHD during transfusion of immune suppressive cells is the inefficient immune system, dysregulation in growth of immune cells and the risk of infection. Pre-transplantation measures together with stem cell infusion and inflammation in the host causes M-MDSCs expansion, and this expansion of MDSCs causes non-relapse mortality (Lee et al., 2018). In recent studies treatment with cyclophosphamide (an immunosuppressant) after allo-HSCT caused early differentiation of MDSCs with reduced GVHD incidence (D'Aveni et al., 2020). It was observed that post-transplant cyclophosphamide (PTCy) patients showed healthy MDSC recovery, particularly PMN-MDSCs than standard of care (SOC) recipients, with active T-cell suppressive function (Oshrine et al., 2022). For the first time, it was also found that mitochondrial permeability transition pore (MPTP) opened in the PMN-MDSCs is due to the intense inflammatory environment of GVHD causing mitochondrial damage, oxidative stress, and apoptosis of PMN-MDSCs. By obstructing MPTP opening by cyclosporine A (CsA) (an immunosuppressant), restored the immunosuppressive function and viability of PMN-MDSCs *in vitro* and *in vivo*. Therefore, MPTP blockade by CsA can preserve/active PMN-MDSCs which can improve efficiency bone marrow transplantation. Also, restoration of immunosuppressive function of PMN-MDSCs by enhancing mitochondrial health could serve as a novel therapeutic strategy for aGVHD (Figure 2).

Cyclosporin A blocks mitochondrial permeability transition pore (MPTP) of PMN-MDSCs therefore, inhibiting MDSCs damage in GVHD inflammatory environment (Li et al., 2022). These studies strongly attest that MDSCs could enhance the function of immune suppressive drug in GVHD. Therefore, more studies are required to confirm whether MDSCs regulating GVHD incidence regulates tumor suppression and whether MDSCs can cause tumor recurrence?

5 Targeting MDSCs in B-cell malignancies

Although MDSCs have a short lifespan, their continuous recruitment to the sites of inflammation enables a significantly long-lasting affect at that TME site. Also, as the life span of MDSCs in the tissue is short, it is very difficult to reverse its pathological state. Therefore, targeting MDSCs for effective therapies could be achieved by 1. Targeting MDSC recruitment, 2. MDSC depletion and 3. reprograming MDSCs to enhance antitumor immunity.

Strategies to target MDSCs in hematological malignancies have provided some initially promising results. STAT3 and cyclooxygenase 2 (COX2)/PGE2 plays an important role in MDSCs formation, differentiation, and accumulation. Veltman et al. (2010) reported 'celecoxib', an inhibitor of COX2, as a potential tool to improve dendritic cell based immunotherapy and can also effectively suppress MDSC function. A retrospective population-based study on DLBCL patients showed a survival benefit to COX-2 inhibitors prior to chemo-immunotherapy treatment (Smyth et al., 2020). A pre-clinical study by De Henau et al. (2016) showed that resistance to immune checkpoint blockade (ICB) is corelated with the infiltration of myeloid cells in various tumors. These findings strongly suggest the inhibition of PI3Kγ (gamma isoforms of phosphoinositide 3-kinase, which are highly expressed in myeloid cells) could be a potential target to overcome immune checkpoint blockade resistance in tumor immune landscape. Furthermore, in HL, it has been reported that RP6530, a PI3Kδ/γ inhibitor decreases the MDSC percentage, and downregulates iNOS which results in tumor regression (Locatelli et al., 2019).

Additionally, miRNAs are also known to affect MDSC function. In a B-cell lymphoma mouse model, mir-30a was upregulated M- and G-MDSCs which further increased the immunosuppressive function of MDSCs by inhibiting the expression of the suppressor of cytokine signaling (SOCS3) gene (Xu et al., 2017c). Li et al. (2020) reported R96A, 'c-Rel', which is a member of NF-B family, as a myeloid checkpoint for cancer immunotherapy. A deficiency of c-Rel in MDSCs was reported to inhibit cancer growth in mice which was further confirmed by pharmaceutical inhibition of c-Rel. Furthermore, a combinational therapy of c-Rel and PD1 blockade was more effective in cancer treatment than either strategy alone.

Chimeric antigen receptor (CAR) T-cell therapy, also known as a "living drug", works on the principle of 'engineering the patient's T-cells for the treatment of cancer'. This treatment is routinely used in blood cancers, including lymphomas, some forms of leukemia, and, most recently, multiple myeloma (Neelapu et al., 2017; Schuster et al., 2017; Maude et al., 2018). However, Jain et al. (2021) reported that CAR T-cell failure in large B-cell lymphoma (LBCL) was associated with increased numbers of circulating M-MDSC and tumor associated IFN signaling. Some more clinical studies ongoing are listed in Table 2.

6 Conclusion

Emerging evidence suggests a significant role for MDSCs in maintaining homeostasis in the immune system. Research studies have explored the immunosuppressive function of MDSCs and identified their pro-tumor characteristics as well as their negative prognostic impact for patients with malignant hematological diseases. In contrast, MDSCs may also have a favorable role in hematological diseases, for example, by preventing GVHD after HSCT (Figure 2). With the clear understanding of the origin, development, and differentiation of MDSCs, the genomic and metabolic mechanisms could be manipulated to optimize the relationship between MDSCs and other cells in the tumor microenvironment and utilize their function to improve patient outcomes. Aside from the role of MDSCs in suppressing the antitumor immune response in the TME, MDSCs also substantially impact the efficacy of immunotherapy, particularly CAR-T cell treatment (Lindo et al., 2021; Tumino et al., 2021). Several studies have developed ways to inhibit or remove MDSCs by modulating their differentiation (Luo et al., 2022), or by developing NK cells expressing chimeric activated receptors that eliminate MDSCs and improve the anti-tumor affects of CAR-T cells (Parihar et al., 2019). Therefore, treating hematological malignancies by targeting MDSCs will be an important future therapeutic direction. However, a critical challenge will be how best to identify and then target specific subsets of MDSC to improve the clinical outcome of patients. Clearly, substantial additional research is needed to achieve this goal.

Author contributions

VB and SA write the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Abedi-Valugerdi, M., Wolfsberger, J., Pillai, P. R., Zheng, W., Sadeghi, B., Zhao, Y., et al. (2016). Suppressive effects of low-dose 5-fluorouracil, busulfan or treosulfan on the expansion of circulatory neutrophils and myeloid derived immunosuppressor cells in tumor-bearing mice. *Int. Immunopharmacol.* 40, 41–49. doi:10.1016/j.intimp.2016.08.023

Alshetaiwi, H., Pervolarakis, N., McIntyre, L. L., Ma, D., Nguyen, Q., Rath, J. A., et al. (2020). Defining the emergence of myeloid-derived suppressor cells in breast cancer using single-cell transcriptomics. *Sci. Immunol.* 5 (44), eaay6017. doi:10.1126/sciimmunol.aay6017

Amini, R. M., Enblad, G., Hollander, P., Laszlo, S., Eriksson, E., Ayoola Gustafsson, K., et al. (2019). Altered profile of immune regulatory cells in the peripheral blood of lymphoma patients. *BMC Cancer* 19 (1), 316. doi:10.1186/s12885-019-5529-0

Arandi, N., Ramzi, M., Safaei, F., and Monabati, A. (2018). Overexpression of indoleamine 2,3-dioxygenase correlates with regulatory T cell phenotype in acute myeloid leukemia patients with normal karyotype. *Blood Res.* 53 (4), 294–298. doi:10.5045/br.2018.53.4.294

Azzaoui, I., Uhel, F., Rossille, D., Pangault, C., Dulong, J., Le Priol, J., et al. (2016). T-cell defect in diffuse large B-cell lymphomas involves expansion of myeloid-derived suppressor cells. *Blood* 128 (8), 1081–1092. doi:10.1182/blood-2015-08-662783

Bai, H., Peng, Y., Li, Y., Duan, J., Fu, W., Liang, X., et al. (2022). Cytarabine-induced TNFα promotes the expansion and suppressive functions of myeloid-derived suppressor cells in acute myeloid leukaemia. *Scand. J. Immunol.* 95 (6), e13158. doi:10.1111/sji.13158

Brimnes, M. K., Vangsted, A. J., Knudsen, L. M., Gimsing, P., Gang, A. O., Johnsen, H. E., et al. (2010). Increased level of both CD4+FOXP3+ regulatory T cells and CD14+HLA-DR−/low myeloid-derived suppressor cells and decreased level of dendritic cells in patients with multiple myeloma. *Scand. J. Immunol.* 72 (6), 540–547. doi:10.1111/j.1365-3083.2010.02463.x

Bronte, V., Brandau, S., Chen, S. H., Colombo, M. P., Frey, A. B., Greten, T. F., et al. (2016). Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat. Commun.* 7, 12150. doi:10.1038/ncomms12150

Cai, T. T., Ye, S. B., Liu, Y. N., He, J., Chen, Q. Y., Mai, H. Q., et al. (2017). LMP1-mediated glycolysis induces myeloid-derived suppressor cell expansion in nasopharyngeal carcinoma. *PLoS Pathog.* 13 (7), e1006503. doi:10.1371/journal.ppat.1006503

Capietto, A. H., Kim, S., Sanford, D. E., Linehan, D. C., Hikida, M., Kumosaki, T., et al. (2013). Down-regulation of PLCγ2-β-catenin pathway promotes activation and expansion of myeloid-derived suppressor cells in cancer. *J. Exp. Med.* 210 (11), 2257–2271. doi:10.1084/jem.20130281

Casbon, A. J., Reynaud, D., Park, C., Khuc, E., Gan, D. D., Schepers, K., et al. (2015). Invasive breast cancer reprograms early myeloid differentiation in the bone marrow to generate immunosuppressive neutrophils. *Proc. Natl. Acad. Sci. U. S. A.* 112 (6), E566–E575. doi:10.1073/pnas.1424927112

Cassetta, L., Bruderek, K., Skrzeczynska-Moncznik, J., Osiecka, O., Hu, X., Rundgren, I. M., et al. (2020). Differential expansion of circulating human MDSC subsets in patients with cancer, infection and inflammation. *J. Immunother. Cancer* 8 (2), e001223. doi:10.1136/jitc-2020-001223

Chai, E., Zhang, L., and Li, C. (2019). LOX-1+ PMN-MDSC enhances immune suppression which promotes glioblastoma multiforme progression. *Cancer Manag. Res.* 11, 7307–7315. doi:10.2147/CMAR.S210545

Chen, X., Eksioglu, E. A., Zhou, J., Zhang, L., Djeu, J., Fortenbery, N., et al. (2013). Induction of myelodysplasia by myeloid-derived suppressor cells. *J. Clin. Investigation* 123 (11), 4595–4611. doi:10.1172/JCI67580

Christiansson, L., Söderlund, S., Mangsbo, S., Hjorth-Hansen, H., Höglund, M., Markevärn, B., et al. (2015). The tyrosine kinase inhibitors imatinib and dasatinib reduce myeloid suppressor cells and release effector lymphocyte responses. *Mol. Cancer Ther.* 14 (5), 1181–1191. doi:10.1158/1535-7163.MCT-14-0849

Condamine, F. L., Nagalingum, N. S., Marshall, C. R., and Morlon, H. (2015). Origin and diversification of living cycads: A cautionary tale on the impact of the branching process prior in bayesian molecular dating. *BMC Evol. Biol.* 15 (1), 65. doi:10.1186/s12862-015-0347-8

Condamine, T., Dominguez, G. A., Youn, J. I., Kossenkov, A. V., Mony, S., Alicea-Torres, K., et al. (2016). Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. *Sci. Immunol.* 1 (2), aaf8943. doi:10.1126/sciimmunol.aaf8943

Condamine, T., and Gabrilovich, D. I. (2011). Molecular mechanisms regulating myeloid-derived suppressor cell differentiation and function. *Trends Immunol.* 32 (1), 19–25. doi:10.1016/j.it.2010.10.002

Condamine, T., Kumar, V., Ramachandran, I. R., Youn, J. I., Celis, E., Finnberg, N., et al. (2014). ER stress regulates myeloid-derived suppressor cell fate through TRAIL-R-mediated apoptosis. *J. Clin. Invest.* 124 (6), 2626–2639. doi:10.1172/JCI74056

Condamine, T., Mastio, J., and Gabrilovich, D. I. (2015). Transcriptional regulation of myeloid-derived suppressor cells. *J. Leukoc. Biol.* 98 (6), 913–922. doi:10.1189/jlb.4RI0515-204R

Corzo, C. A., Cotter, M. J., Cheng, P., Cheng, F., Kusmartsev, S., Sotomayor, E., et al. (2009). Mechanism regulating reactive oxygen species in tumor-induced myeloid-derived suppressor cells. *J. Immunol.* 182 (9), 5693–5701. doi:10.4049/jimmunol.0900092

Crook, K. R., Jin, M., Weeks, M. F., Rampersad, R. R., Baldi, R. M., Glekas, A. S., et al. (2015). Myeloid-derived suppressor cells regulate T cell and B cell responses during autoimmune disease. *J. Leukoc. Biol.* 97 (3), 573–582. doi:10.1189/jlb.4A0314-139R

D'Aveni, M., Notarantonio, A. B., Bertrand, A., Boulangé, L., Pochon, C., and Rubio, M. T. (2020). Myeloid-derived suppressor cells in the context of allogeneic hematopoietic stem cell transplantation. *Front. Immunol.* 11, 989. doi:10.3389/fimmu.2020.00989

De Henau, O., Rausch, M., Winkler, D., Campesato, L. F., Liu, C., Cymerman, D. H., et al. (2016). Overcoming resistance to checkpoint blockade therapy by targeting PI3Kγ in myeloid cells. *Nature* 539 (7629), 443–447. doi:10.1038/nature20554

De Veirman, K., De Beule, N., Maes, K., Menu, E., De Bruyne, E., De Raeve, H., et al. (2017). Extracellular S100A9 protein in bone marrow supports multiple myeloma survival by stimulating angiogenesis and cytokine secretion. *Cancer Immunol. Res.* 5 (10), 839–846. doi:10.1158/2326-6066.CIR-17-0192

De Veirman, K., Menu, E., Maes, K., De Beule, N., De Smedt, E., Maes, A., et al. (2019). Myeloid-derived suppressor cells induce multiple myeloma cell survival by activating the AMPK pathway. *Cancer Lett.* 442, 233–241. doi:10.1016/j.canlet.2018.11.002

Dorhoi, A., Kotzé, L. A., Berzofsky, J. A., Sui, Y., Gabrilovich, D. I., Garg, A., et al. (2020). Therapies for tuberculosis and AIDS: Myeloid-derived suppressor cells in focus. *J. Clin. Invest.* 130 (6), 2789–2799. doi:10.1172/JCI136288

Elkabets, M., Ribeiro, V. S., Dinarello, C. A., Ostrand-Rosenberg, S., Di Santo, J. P., Apte, R. N., et al. (2010). IL-1β regulates a novel myeloid-derived suppressor cell subset that impairs NK cell development and function. *Eur. J. Immunol.* 40 (12), 3347–3357. doi:10.1002/eji.201041037

Ennishi, D., Hsi, E. D., Steidl, C., and Scott, D. W. (2020). Toward a new molecular taxonomy of diffuse large B-cell lymphoma. *Cancer Discov.* 10 (9), 1267–1281. doi:10.1158/2159-8290.CD-20-0174

Fan, Q., Liu, H., Liang, X., Yang, T., Fan, Z., Huang, F., et al. (2017). Superior GVHD-free, relapse-free survival for G-BM to G-PBSC grafts is associated with higher MDSCs content in allografting for patients with acute leukemia. *J. Hematol. Oncol.* 10 (1), 135. doi:10.1186/s13045-017-0503-2

Fang, X., Guo, L., Xing, Z., Shi, L., Liang, H., Li, A., et al. (2022). Ido1 can impair NK cells function against non-small cell lung cancer by downregulation of NKG2D Ligand via ADAM10. *Pharmacol. Res.* 177, 106132. doi:10.1016/j.phrs.2022.106132

Favaloro, J., Liyadipitiya, T., Brown, R., Yang, S., Suen, H., Woodland, N., et al. (2014). Myeloid derived suppressor cells are numerically, functionally and phenotypically different in patients with multiple myeloma. *Leukemia Lymphoma* 55 (12), 2893–2900. doi:10.3109/10428194.2014.904511

Feng, P. H., Lee, K. Y., Chang, Y. L., Chan, Y. F., Kuo, L. W., Lin, T. Y., et al. (2012). CD14(+)S100A9(+) monocytic myeloid-derived suppressor cells and their clinical relevance in non-small cell lung cancer. *Am. J. Respir. Crit. Care Med.* 186 (10), 1025–1036. doi:10.1164/rccm.201204-0636OC

Ferrer, G., Jung, B., Chiu, P. Y., Aslam, R., Palacios, F., Mazzarello, A. N., et al. (2021). Myeloid-derived suppressor cell subtypes differentially influence T-cell function, T-helper subset differentiation, and clinical course in CLL. *Leukemia* 35 (11), 3163–3175. doi:10.1038/s41375-021-01249-7

Fleming, V., Hu, X., Weber, R., Nagibin, V., Groth, C., Altevogt, P., et al. (2018). Targeting myeloid-derived suppressor cells to bypass tumor-induced immunosuppression. *Front. Immunol.* 9, 398. doi:10.3389/fimmu.2018.00398

Fournié, J. J., and Poupot, M. (2018). The pro-tumorigenic IL-33 involved in antitumor immunity: A yin and yang cytokine. *Front. Immunol.* 9, 2506. doi:10.3389/fimmu.2018.02506

Fuse, H., Tomihara, K., Heshiki, W., Yamazaki, M., Akyu-Takei, R., Tachinami, H., et al. (2016). Enhanced expression of PD-L1 in oral squamous cell carcinoma-derived CD11b(+)Gr-1(+) cells and its contribution to immunosuppressive activity. *Oral Oncol.* 59, 20–29. doi:10.1016/j.oraloncology.2016.05.012

Gabrilovich, D. I., Bronte, V., Chen, S. H., Colombo, M. P., Ochoa, A., Ostrand-Rosenberg, S., et al. (2007). The terminology issue for myeloid-derived suppressor cells. *Cancer Res.* 67 (1), 425; author reply 426. author reply 6. doi:10.1158/0008-5472.CAN-06-3037

Gabrilovich, D. I. (2017). Myeloid-derived suppressor cells. *Cancer Immunol. Res.* 5 (1), 3–8. doi:10.1158/2326-6066.CIR-16-0297

Gabrilovich, D. I., and Nagaraj, S. (2009). Myeloid-derived suppressor cells as regulators of the immune system. *Nat. Rev. Immunol.* 9 (3), 162–174. doi:10.1038/nri2506

Giallongo, C., Parrinello, N., Tibullo, D., La Cava, P., Romano, A., Chiarenza, A., et al. (2014). Myeloid derived suppressor cells (MDSCs) are increased and exert immunosuppressive activity together with polymorphonuclear leukocytes (PMNs) in chronic myeloid leukemia patients. *PLOS ONE* 9 (7), e101848. doi:10.1371/journal.pone.0101848

Giallongo, C., Parrinello, N. L., La Cava, P., Camiolo, G., Romano, A., Scalia, M., et al. (2018). Monocytic myeloid-derived suppressor cells as prognostic factor in chronic myeloid leukaemia patients treated with dasatinib. *J. Cell. Mol. Med.* 22 (2), 1070–1080. doi:10.1111/jcmm.13326

Giallongo, C., Tibullo, D., Parrinello, N. L., Cava, P. L., Rosa, M. D., Bramanti, V., et al. (2016). Granulocyte-like myeloid derived suppressor cells (G-MDSC) are increased in multiple myeloma and are driven by dysfunctional mesenchymal stem cells (MSC). *Oncotarget* 7 (52), 85764–85775. doi:10.18632/oncotarget.7969

Görgün, G. T., Whitehill, G., Anderson, J. L., Hideshima, T., Maguire, C., Laubach, J., et al. (2013). Tumor-promoting immune-suppressive myeloid-derived suppressor cells in the multiple myeloma microenvironment in humans. *Blood* 121 (15), 2975–2987. doi:10.1182/blood-2012-08-448548

Gunes, E. G., Rosen, S. T., and Querfeld, C. (2020). The role of myeloid-derived suppressor cells in hematologic malignancies. *Curr. Opin. Oncol.* 32 (5), 518–526. doi:10.1097/CCO.0000000000000662

Hanson, E. M., Clements, V. K., Sinha, P., Ilkovitch, D., and Ostrand-Rosenberg, S. (2009). Myeloid-derived suppressor cells down-regulate L-selectin expression on CD4+ and CD8+ T cells. *J. Immunol.* 183 (2), 937–944. doi:10.4049/jimmunol.0804253

Hoechst, B., Voigtlaender, T., Ormandy, L., Gamrekelashvili, J., Zhao, F., Wedemeyer, H., et al. (2009). Myeloid derived suppressor cells inhibit natural killer cells in patients with hepatocellular carcinoma via the NKp30 receptor. *Hepatology* 50 (3), 799–807. doi:10.1002/hep.23054

Hohtari, H., Brück, O., Blom, S., Turkki, R., Sinisalo, M., Kovanen, P. E., et al. (2019). Immune cell constitution in bone marrow microenvironment predicts outcome in adult ALL. *Leukemia* 33 (7), 1570–1582. doi:10.1038/s41375-018-0360-1

Hongo, D., Tang, X., Baker, J., Engleman, E. G., and Strober, S. (2014). Requirement for interactions of natural killer T cells and myeloid-derived suppressor cells for transplantation tolerance. *Am. J. Transpl.* 14 (11), 2467–2477. doi:10.1111/ajt.12914

Höpken, U. E., and Rehm, A. (2019). Targeting the tumor microenvironment of leukemia and lymphoma. *Trends Cancer* 5 (6), 351–364. doi:10.1016/j.trecan.2019.05.001

Hsu, Y. L., Yen, M. C., Chang, W. A., Tsai, P. H., Pan, Y. C., Liao, S. H., et al. (2019). CXCL17-derived CD11b(+)Gr-1(+) myeloid-derived suppressor cells contribute to lung metastasis of breast cancer through platelet-derived growth factor-BB. *Breast Cancer Res.* 21 (1), 23. doi:10.1186/s13058-019-1114-3

Huang, W., Liu, Y., Luz, A., Berrong, M., Meyer, J. N., Zou, Y., et al. (2021). Calcium/calmodulin dependent protein kinase kinase 2 regulates the expansion of tumor-induced myeloid-derived suppressor cells. *Front. Immunol.* 12, 754083. doi:10.3389/fimmu.2021.754083

Hwang, H-S., Han, A. R., Lee, J. Y., Park, G. S., Min, W-S., and Kim, H-J. (2019). Enhanced anti-leukemic effects through induction of immunomodulating microenvironment by blocking CXCR4 and PD-L1 in an AML mouse model. *Immunol. Investig.* 48 (1), 96–105. doi:10.1080/08820139.2018.1497057

Ibrahim, M. L., Klement, J. D., Lu, C., Redd, P. S., Xiao, W., Yang, D., et al. (2018). Myeloid-derived suppressor cells produce IL-10 to elicit DNMT3b-dependent IRF8 silencing to promote colitis-associated colon tumorigenesis. *Cell Rep.* 25 (11), 3036–3046.e6. doi:10.1016/j.celrep.2018.11.050

Jain, M. D., Zhao, H., Wang, X., Atkins, R., Menges, M., Reid, K., et al. (2021). Tumor interferon signaling and suppressive myeloid cells are associated with CAR T-cell failure in large B-cell lymphoma. *Blood* 137 (19), 2621–2633. doi:10.1182/blood.2020007445

Jiménez-Cortegana, C., Palazón-Carrión, N., Martin Garcia-Sancho, A., Nogales-Fernandez, E., Carnicero-González, F., Ríos-Herranz, E., et al. (2021). Circulating myeloid-derived suppressor cells and regulatory T cells as immunological biomarkers in refractory/relapsed diffuse large B-cell lymphoma: Translational results from the R2-GDP-GOTEL trial. *J. Immunother. Cancer* 9 (6), e002323. doi:10.1136/jitc-2020-002323

Jiménez-Cortegana, C., Sánchez-Martínez, P. M., Palazón-Carrión, N., Nogales-Fernández, E., Henao-Carrasco, F., Martín García-Sancho, A., et al. (2021). Lower survival and increased circulating suppressor cells in patients with relapsed/refractory diffuse large B-cell lymphoma with deficit of vitamin D levels using R-GDP plus lenalidomide (R2-GDP): Results from the R2-GDP-GOTEL trial. *Cancers* 13 (18), 4622. doi:10.3390/cancers13184622

Jitschin, R., Braun, M., Büttner, M., Dettmer-Wilde, K., Bricks, J., Berger, J., et al. (2014). CLL-cells induce IDOhi CD14+HLA-DRlo myeloid-derived suppressor cells that inhibit T-cell responses and promote TRegs. *Blood* 124 (5), 750–760. doi:10.1182/blood-2013-12-546416

Jitschin, R., Saul, D., Braun, M., Tohumeken, S., Völkl, S., Kischel, R., et al. (2018). CD33/CD3-bispecific T-cell engaging (BiTE®) antibody construct targets monocytic AML myeloid-derived suppressor cells. *J. Immunother. Cancer* 6 (1), 116. doi:10.1186/s40425-018-0432-9

Kaplan, R. N., Riba, R. D., Zacharoulis, S., Bramley, A. H., Vincent, L., Costa, C., et al. (2005). VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature* 438 (7069), 820–827. doi:10.1038/nature04186

Karakhanova, S., Link, J., Heinrich, M., Shevchenko, I., Yang, Y., Hassenpflug, M., et al. (2015). Characterization of myeloid leukocytes and soluble mediators in pancreatic

cancer: Importance of myeloid-derived suppressor cells. *Oncoimmunology* 4 (4), e998519. doi:10.1080/2162402X.2014.998519

Khalifa, K. A., Badawy, H. M., Radwan, W. M., Shehata, M. A., and Bassuoni, M. A. (2014). CD14(+) HLA-DR low/(-) monocytes as indicator of disease aggressiveness in B-cell non-Hodgkin lymphoma. *Int. J. Lab. Hematol.* 36 (6), 650–655. doi:10.1111/ijlh.12203

Kim, H. R., Park, S. M., Seo, S. U., Jung, I., Yoon, H. I., Gabrilovich, D. I., et al. (2019). The ratio of peripheral regulatory T cells to lox-1(+) polymorphonuclear myeloid-derived suppressor cells predicts the early response to anti-PD-1 therapy in patients with non-small cell lung cancer. *Am. J. Respir. Crit. Care Med.* 199 (2), 243–246. doi:10.1164/rccm.201808-1502LE

Kim, T. W., Park, S-S., Lim, J-Y., Min, G. J., Park, S., Jeon, Y-W., et al. (2019). Predictive role of circulating immune cell subtypes early after allogeneic hematopoietic stem cell transplantation in patients with acute leukemia. *IJSC* 12 (1), 73–83. doi:10.15283/ijsc18094

Kittang, A. O., Kordasti, S., Sand, K. E., Costantini, B., Kramer, A. M., Perezabellan, P., et al. (2016). Expansion of myeloid derived suppressor cells correlates with number of T regulatory cells and disease progression in myelodysplastic syndrome. *OncoImmunology* 5 (2), e1062208. doi:10.1080/2162402X.2015.1062208

Köstlin, N., Hofstädter, K., Ostermeir, A. L., Spring, B., Leiber, A., Haen, S., et al. (2016). Granulocytic myeloid-derived suppressor cells accumulate in human placenta and polarize toward a Th2 phenotype. *J. Immunol.* 196 (3), 1132–1145. doi:10.4049/jimmunol.1500340

Köstlin, N., Ostermeir, A. L., Spring, B., Schwarz, J., Marmé, A., Walter, C. B., et al. (2017). HLA-G promotes myeloid-derived suppressor cell accumulation and suppressive activity during human pregnancy through engagement of the receptor ILT4. *Eur. J. Immunol.* 47 (2), 374–384. doi:10.1002/eji.201646564

Kujawski, M., Kortylewski, M., Lee, H., Herrmann, A., Kay, H., and Yu, H. (2008). Stat3 mediates myeloid cell-dependent tumor angiogenesis in mice. *J. Clin. Invest.* 118 (10), 3367–3377. doi:10.1172/JCI35213

Kusmartsev, S., Eruslanov, E., Kübler, H., Tseng, T., Sakai, Y., Su, Z., et al. (2008). Oxidative stress regulates expression of VEGFR1 in myeloid cells: Link to tumor-induced immune suppression in renal cell carcinoma. *J. Immunol.* 181 (1), 346–353. doi:10.4049/jimmunol.181.1.346

Kuwahara-Ota, S., Shimura, Y., Steinebach, C., Isa, R., Yamaguchi, J., Nishiyama, D., et al. (2020). Lenalidomide and pomalidomide potently interfere with induction of myeloid-derived suppressor cells in multiple myeloma. *Br. J. Haematol.* 191 (5), 784–795. doi:10.1111/bjh.16881

Leader, A. M., Grout, J. A., Maier, B. B., Nabet, B. Y., Park, M. D., Tabachnikova, A., et al. (2021). Single-cell analysis of human non-small cell lung cancer lesions refines tumor classification and patient stratification. *Cancer cell.* 12, 1594–1609.e12. doi:10.1016/j.ccell.2021.10.009

Lee, S-E., Lim, J-Y., Kim, T. W., Jeon, Y-W., Yoon, J-H., Cho, B-S., et al. (2018). Matrix metalloproteinase-9 in monocytic myeloid-derived suppressor cells correlate with early infections and clinical outcomes in allogeneic hematopoietic stem cell transplantation. *Biol. Blood Marrow Transplant.* 24 (1), 32–42. doi:10.1016/j.bbmt.2017.08.017

Lee, S-E., Lim, J-Y., Kim, T. W., Ryu, D-B., Park, S. S., Jeon, Y-W., et al. (2019). Different role of circulating myeloid-derived suppressor cells in patients with multiple myeloma undergoing autologous stem cell transplantation. *J. Immunother. Cancer* 7 (1), 35. doi:10.1186/s40425-018-0491-y

Lelis, F. J. N., Jaufmann, J., Singh, A., Fromm, K., Teschner, A. C., Pöschel, S., et al. (2017). Myeloid-derived suppressor cells modulate B-cell responses. *Immunol. Lett.* 188, 108–115. doi:10.1016/j.imlet.2017.07.003

Lewinsky, H., Gunes, E. G., David, K., Radomir, L., Kramer, M. P., Pellegrino, B., et al. (2021). CD84 is a regulator of the immunosuppressive microenvironment in multiple myeloma. *JCI Insight* 6 (4), e141683. doi:10.1172/jci.insight.141683

Li, C., Liu, T., Bazhin, A. V., and Yang, Y. (2017). The sabotaging role of myeloid cells in anti-angiogenic therapy: Coordination of angiogenesis and immune suppression by hypoxia. *J. Cell Physiol.* 232 (9), 2312–2322. doi:10.1002/jcp.25726

Li, H., Han, Y., Guo, Q., Zhang, M., and Cao, X. (2009). Cancer-expanded myeloid-derived suppressor cells induce anergy of NK cells through membrane-bound TGF-beta 1. *J. Immunol.* 182 (1), 240–249. doi:10.4049/jimmunol.182.1.240

Li, L., Yin, J., Li, Y., Wang, C., Mao, X., Wei, J., et al. (2021). Allogeneic hematopoietic stem cell transplantation mobilized with pegylated granulocyte colony-stimulating factor ameliorates severe acute graft-versus-host disease through enrichment of monocytic myeloid-derived suppressor cells in the graft: A real world experience. *Front. Immunol.* 12, 801313. doi:10.3389/fimmu.2021.801313

Li, T., Li, X., Zamani, A., Wang, W., Lee, C. N., Li, M., et al. (2020). c-Rel is a myeloid checkpoint for cancer immunotherapy. *Nat. Cancer* 1 (5), 507–517. doi:10.1038/s43018-020-0061-3

Li, X., Kong, D., Yu, Q., Si, X., Yang, L., Zeng, X., et al. (2022). Cyclosporine A regulates PMN-MDSCs viability and function through MPTP in acute GVHD: Old medication, new target. *Transplant. Cell. Ther.* 28 (7), 411.e1–411411.e9. doi:10.1016/j.jtct.2022.04.010

Li, Y., Tu, Z., Qian, S., Fung, J. J., Markowitz, S. D., Kusner, L. L., et al. (2014). Myeloid-derived suppressor cells as a potential therapy for experimental autoimmune myasthenia gravis. *J. Immunol.* 193 (5), 2127–2134. doi:10.4049/jimmunol.1400857

Li, Z., Pang, Y., Gara, S. K., Achyut, B. R., Heger, C., Goldsmith, P. K., et al. (2012). Gr-1+CD11b+ cells are responsible for tumor promoting effect of TGF-β in breast cancer progression. *Int. J. Cancer* 131 (11), 2584–2595. doi:10.1002/ijc.27572

Lim, J-Y., Kim, T-W., Ryu, D-B., Park, S-S., Lee, S-E., Kim, B-S., et al. (2021). Myeloma-secreted galectin-1 potently interacts with CD304 on monocytic myeloid-derived suppressor cells. *Cancer Immunol. Res.* 9 (5), 503–513. doi:10.1158/2326-6066.CIR-20-0663

Lin, Y., Gustafson, M. P., Bulur, P. A., Gastineau, D. A., Witzig, T. E., and Dietz, A. B. (2011). Immunosuppressive CD14+HLA-DR(low)/- monocytes in B-cell non-Hodgkin lymphoma. *Blood* 117 (3), 872–881. doi:10.1182/blood-2010-05-283820

Lindo, L., Wilkinson, L. H., and Hay, K. A. (2021). Befriending the hostile tumor microenvironment in CAR T-cell therapy. *Front. Immunol.* 11, 618387. doi:10.3389/fimmu.2020.618387

Liu, Y., Zhou, X., and Wang, X. (2021). Targeting the tumor microenvironment in B-cell lymphoma: Challenges and opportunities. *J. Hematol. Oncol.* 14 (1), 125. doi:10.1186/s13045-021-01134-x

Locatelli, S. L., Careddu, G., Serio, S., Consonni, F. M., Maeda, A., Viswanadha, S., et al. (2019). Targeting cancer cells and tumor microenvironment in preclinical and clinical models of Hodgkin lymphoma using the dual PI3Kδ/γ inhibitor RP6530. *Clin. Cancer Res.* 25 (3), 1098–1112. doi:10.1158/1078-0432.CCR-18-1133

Lu, F., Zhao, Y., Pang, Y., Ji, M., Sun, Y., Wang, H., et al. (2021). NLRP3 inflammasome upregulates PD-L1 expression and contributes to immune suppression in lymphoma. *Cancer Lett.* 497, 178–189. doi:10.1016/j.canlet.2020.10.024

Luo, W., Napoleon, J. V., Zhang, F., Lee, Y. G., Wang, B., Putt, K. S., et al. (2022). Repolarization of tumor-infiltrating myeloid cells for augmentation of CAR T cell therapies. *Front. Immunol.* 13, 816761. doi:10.3389/fimmu.2022.816761

Luyckx, A., Schouppe, E., Rutgeerts, O., Lenaerts, C., Fevery, S., Devos, T., et al. (2012). G-CSF stem cell mobilization in human donors induces polymorphonuclear and mononuclear myeloid-derived suppressor cells. *Clin. Immunol.* 143 (1), 83–87. doi:10.1016/j.clim.2012.01.011

Lv, J., Zhao, Y., Zong, H., Ma, G., Wei, X., and Zhao, Y. (2021). Increased levels of circulating monocytic- and early-stage myeloid-derived suppressor cells (MDSC) in acute myeloid leukemia. *Clin. Lab.* 67 (3). doi:10.7754/Clin.Lab.2020.200719

Mandruzzato, S., Brandau, S., Britten, C. M., Bronte, V., Damuzzo, V., Gouttefangeas, C., et al. (2016). Toward harmonized phenotyping of human myeloid-derived suppressor cells by flow cytometry: Results from an interim study. *Cancer Immunol. Immunother.* 65 (2), 161–169. doi:10.1007/s00262-015-1782-5

Mantovani, A., Marchesi, F., Malesci, A., Laghi, L., and Allavena, P. (2017). Tumour-associated macrophages as treatment targets in oncology. *Nat. Rev. Clin. Oncol.* 14 (7), 399–416. doi:10.1038/nrclinonc.2016.217

Marini, O., Spina, C., Mimiola, E., Cassaro, A., Malerba, G., Todeschini, G., et al. (2016). Identification of granulocytic myeloid-derived suppressor cells (G-MDSCs) in the peripheral blood of Hodgkin and non-Hodgkin lymphoma patients. *Oncotarget* 7 (19), 27676–27688. doi:10.18632/oncotarget.8507

Mastio, J., Condamine, T., Dominguez, G., Kossenkov, A. V., Donthireddy, L., Veglia, F., et al. (2019). Identification of monocyte-like precursors of granulocytes in cancer as a mechanism for accumulation of PMN-MDSCs. *J. Exp. Med.* 216 (9), 2150–2169. doi:10.1084/jem.20181952

Maude, S. L., Laetsch, T. W., Buechner, J., Rives, S., Boyer, M., Bittencourt, H., et al. (2018). Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N. Engl. J. Med.* 378 (5), 439–448. doi:10.1056/NEJMoa1709866

Molgora, M., Bonavita, E., Ponzetta, A., Riva, F., Barbagallo, M., Jaillon, S., et al. (2017). IL-1R8 is a checkpoint in NK cells regulating anti-tumour and anti-viral activity. *Nature* 551 (7678), 110–114. doi:10.1038/nature24293

Movahedi, K., Guilliams, M., Van den Bossche, J., Van den Bergh, R., Gysemans, C., Beschin, A., et al. (2008). Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T cell–suppressive activity. *Blood* 111 (8), 4233–4244. doi:10.1182/blood-2007-07-099226

Nakamura, K., Kassem, S., Cleynen, A., Chrétien, M-L., Guillerey, C., Putz, E. M., et al. (2018). Dysregulated IL-18 is a key driver of immunosuppression and a possible therapeutic target in the multiple myeloma microenvironment. *Cancer Cell* 33 (4), 634–648.e5. doi:10.1016/j.ccell.2018.02.007

Nan, J., Xing, Y. F., Hu, B., Tang, J. X., Dong, H. M., He, Y. M., et al. (2018). Endoplasmic reticulum stress induced LOX-1(+) CD15(+) polymorphonuclear myeloid-derived suppressor cells in hepatocellular carcinoma. *Immunology* 154 (1), 144–155. doi:10.1111/imm.12876

Nasrollahzadeh, E., Razi, S., Keshavarz-Fathi, M., Mazzone, M., and Rezaei, N. (2020). Pro-tumorigenic functions of macrophages at the primary, invasive and metastatic tumor site. *Cancer Immunol. Immunother.* 69 (9), 1673–1697. doi:10.1007/s00262-020-02616-6

Nathan, H. F., Chan Yoon, C., Randy, D. G., John, G., Sattva, S. N., Paolo, G., et al. (2016). Role of the tumor microenvironment in mature B-cell lymphoid malignancies. *Haematologica* 101 (5), 531–540. doi:10.3324/haematol.2015.139493

Neelapu, S. S., Locke, F. L., Bartlett, N. L., Lekakis, L. J., Miklos, D. B., Jacobson, C. A., et al. (2017). Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N. Engl. J. Med.* 377 (26), 2531–2544. doi:10.1056/NEJMoa1707447

Okła, K., Rajtak, A., Czerwonka, A., Bobiński, M., Wawruszak, A., Tarkowski, R., et al. (2020). Accumulation of blood-circulating PD-L1-expressing M-MDSCs and monocytes/macrophages in pretreatment ovarian cancer patients is associated with soluble PD-L1. *J. Transl. Med.* 18 (1), 220. doi:10.1186/s12967-020-02389-7

Ortiz, M. L., Kumar, V., Martner, A., Mony, S., Donthireddy, L., Condamine, T., et al. (2015). Immature myeloid cells directly contribute to skin tumor development by recruiting IL-17-producing CD4+ T cells. *J. Exp. Med.* 212 (3), 351–367. doi:10.1084/jem.20140835

Oshrine, B., Innamarato, P., Branthoover, H., Nagle, L., Verdugo, P., Pilon-Thomas, S., et al. (2022). Early recovery of myeloid-derived suppressor cells after allogeneic hematopoietic transplant: Comparison of post-transplantation cyclophosphamide to standard graft-versus-host disease prophylaxis. *Transplant. Cell. Ther.* 28 (4), 203.e1–203203.e7. doi:10.1016/j.jtct.2021.12.019

Ostrand-Rosenberg, S., and Fenselau, C. (2018). Myeloid-derived suppressor cells: Immune-suppressive cells that impair antitumor immunity and are sculpted by their environment. *J. Immunol.* 200 (2), 422–431. doi:10.4049/jimmunol.1701019

Ouzounova, M., Lee, E., Piranlioglu, R., El Andaloussi, A., Kolhe, R., Demirci, M. F., et al. (2017). Monocytic and granulocytic myeloid derived suppressor cells differentially regulate spatiotemporal tumour plasticity during metastatic cascade. *Nat. Commun.* 8, 14979. doi:10.1038/ncomms14979

Ozerova, M., and Nefedova, Y. (2019). Estrogen promotes multiple myeloma through enhancing the immunosuppressive activity of MDSC. *Leukemia Lymphoma* 60 (6), 1557–1562. doi:10.1080/10428194.2018.1538511

Parihar, R., Rivas, C., Huynh, M., Omer, B., Lapteva, N., Metelitsa, L. S., et al. (2019). NK cells expressing a chimeric activating receptor eliminate MDSCs and rescue impaired CAR-T cell activity against solid tumors. *Cancer Immunol. Res.* 7 (3), 363–375. doi:10.1158/2326-6066.CIR-18-0572

Pastaki Khoshbin, A., Eskian, M., Keshavarz-Fathi, M., and Rezaei, N. (2019). Roles of myeloid-derived suppressor cells in cancer metastasis: Immunosuppression and beyond. *Arch. Immunol. Ther. Exp. Warsz.* 67 (2), 89–102. doi:10.1007/s00005-018-0531-9

Pathria, P., Louis, T. L., and Varner, J. A. (2019). Targeting tumor-associated macrophages in cancer. *Trends Immunol.* 40 (4), 310–327. doi:10.1016/j.it.2019.02.003

Peinado, H., Zhang, H., Matei, I. R., Costa-Silva, B., Hoshino, A., Rodrigues, G., et al. (2017). Pre-metastatic niches: Organ-specific homes for metastases. *Nat. Rev. Cancer* 17 (5), 302–317. doi:10.1038/nrc.2017.6

Pelosi, A., Besi, F., Tumino, N., Merli, P., Quatrini, L., Li Pira, G., et al. (2021). NK cells and PMN-MDSCs in the graft from G-CSF mobilized haploidentical donors display distinct gene expression profiles from those of the non-mobilized counterpart. *Front. Immunol.* 12, 657329. doi:10.3389/fimmu.2021.657329

Perez, C., Botta, C., Zabaleta, A., Puig, N., Cedena, M-T., Goicoechea, I., et al. (2020). Immunogenomic identification and characterization of granulocytic myeloid-derived suppressor cells in multiple myeloma. *Blood* 136 (2), 199–209. doi:10.1182/blood.2019004537

Porembka, M. R., Mitchem, J. B., Belt, B. A., Hsieh, C. S., Lee, H. M., Herndon, J., et al. (2012). Pancreatic adenocarcinoma induces bone marrow mobilization of myeloid-derived suppressor cells which promote primary tumor growth. *Cancer Immunol. Immunother.* 61 (9), 1373–1385. doi:10.1007/s00262-011-1178-0

Qi, X., Jiang, H., Liu, P., Xie, N., Fu, R., Wang, H., et al. (2021). Increased myeloid-derived suppressor cells in patients with myelodysplastic syndromes suppress CD8+ T lymphocyte function through the STAT3-ARG1 pathway. *Leukemia Lymphoma* 62 (1), 218–223. doi:10.1080/10428194.2020.1817431

Ramachandran, I. R., Martner, A., Pisklakova, A., Condamine, T., Chase, T., Vogl, T., et al. (2013). Myeloid-derived suppressor cells regulate growth of multiple myeloma by inhibiting T cells in bone marrow. *J. Immunol.* 190 (7), 3815–3823. doi:10.4049/jimmunol.1203373

Ren, W., Zhang, X., Li, W., Feng, Q., Feng, H., Tong, Y., et al. (2019). Exosomal miRNA-107 induces myeloid-derived suppressor cell expansion in gastric cancer. *Cancer Manag. Res.* 11, 4023–4040. doi:10.2147/CMAR.S198886

Romano, A., Parrinello, N. L., Vetro, C., Forte, S., Chiarenza, A., Figuera, A., et al. (2015). Circulating myeloid-derived suppressor cells correlate with clinical outcome in Hodgkin Lymphoma patients treated up-front with a risk-adapted strategy. *Br. J. Haematol.* 168 (5), 689–700. doi:10.1111/bjh.13198

Sakamaki, I., Kwak, L. W., Cha, S. C., Yi, Q., Lerman, B., Chen, J., et al. (2014). Lenalidomide enhances the protective effect of a therapeutic vaccine and reverses immune suppression in mice bearing established lymphomas. *Leukemia* 28 (2), 329–337. doi:10.1038/leu.2013.177

Sarhan, D., Cichocki, F., Zhang, B., Yingst, A., Spellman, S. R., Cooley, S., et al. (2016). Adaptive NK cells with low TIGIT expression are inherently resistant to myeloid-derived suppressor cells. *Cancer Res.* 76 (19), 5696–5706. doi:10.1158/0008-5472.CAN-16-0839

Schlecker, E., Stojanovic, A., Eisen, C., Quack, C., Falk, C. S., Umansky, V., et al. (2012). Tumor-infiltrating monocytic myeloid-derived suppressor cells mediate CCR5-dependent recruitment of regulatory T cells favoring tumor growth. *J. Immunol.* 189 (12), 5602–5611. doi:10.4049/jimmunol.1201018

Schmitz, R., Young, R. M., Ceribelli, M., Jhavar, S., Xiao, W., Zhang, M., et al. (2012). Burkitt lymphoma pathogenesis and therapeutic targets from structural and functional genomics. *Nature* 490 (7418), 116–120. doi:10.1038/nature11378

Schneidawind, C., Jahnke, S., Schober-Melms, I., Schumm, M., Handgretinger, R., Faul, C., et al. (2019). G-CSF administration prior to donor lymphocyte apheresis promotes anti-leukaemic effects in allogeneic HCT patients. *Br. J. Haematol.* 186 (1), 60–71. doi:10.1111/bjh.15881

Schuster, S. J., Svoboda, J., Chong, E. A., Nasta, S. D., Mato, A. R., Anak, Ö., et al. (2017). Chimeric antigen receptor T cells in refractory B-cell lymphomas. *N. Engl. J. Med.* 377 (26), 2545–2554. doi:10.1056/NEJMoa1708566

Scott, D. W., and Gascoyne, R. D. (2014). The tumour microenvironment in B cell lymphomas. *Nat. Rev. Cancer* 14 (8), 517–534. doi:10.1038/nrc3774

Sehn, L. H., and Salles, G. (2021). Diffuse large B-cell lymphoma. *N. Engl. J. Med.* 384 (9), 842–858. doi:10.1056/NEJMra2027612

Serafini, P., Mgebroff, S., Noonan, K., and Borrello, I. (2008). Myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells. *Cancer Res.* 68 (13), 5439–5449. doi:10.1158/0008-5472.CAN-07-6621

Sevko, A., Michels, T., Vrohlings, M., Umansky, L., Beckhove, P., Kato, M., et al. (2013). Antitumor effect of paclitaxel is mediated by inhibition of myeloid-derived suppressor cells and chronic inflammation in the spontaneous melanoma model. *J. Immunol.* 190, 2464–2471. doi:10.4049/jimmunol.1202781

Shen, J. X., Liu, J., and Zhang, G. J. (2018). Interleukin-33 in malignancies: Friends or foes? *Front. Immunol.* 9, 3051. doi:10.3389/fimmu.2018.03051

Sinha, P., Okoro, C., Foell, D., Freeze, H. H., Ostrand-Rosenberg, S., and Srikrishna, G. (2008). Proinflammatory S100 proteins regulate the accumulation of myeloid-derived suppressor cells. *J. Immunol.* 181 (7), 4666–4675. doi:10.4049/jimmunol.181.7.4666

Smyth, L., Blunt, D. N., Gatov, E., Nagamuthu, C., Croxford, R., Mozessohn, L., et al. (2020). Statin and cyclooxygenase-2 inhibitors improve survival in newly diagnosed diffuse large B-cell lymphoma: A large population-based study of 4913 subjects. *Br. J. Haematol.* 191 (3), 396–404. doi:10.1111/bjh.16635

Solman, I. G., Blum, L. K., Burger, J. A., Kipps, T. J., Dean, J. P., James, D. F., et al. (2021). Impact of long-term ibrutinib treatment on circulating immune cells in previously untreated chronic lymphocytic leukemia. *Leukemia Res.* 102, 106520. doi:10.1016/j.leukres.2021.106520

Stiff, A., Trikha, P., Mundy-Bosse, B., McMichael, E., Mace, T. A., Benner, B., et al. (2018). Nitric oxide production by myeloid-derived suppressor cells plays a role in impairing fc receptor-mediated natural killer cell function. *Clin. Cancer Res.* 24 (8), 1891–1904. doi:10.1158/1078-0432.CCR-17-0691

Sui, Q., Zhang, J., Sun, X., Zhang, C., Han, Q., and Tian, Z. (2014). NK cells are the crucial antitumor mediators when STAT3-mediated immunosuppression is blocked in hepatocellular carcinoma. *J. Immunol.* 193 (4), 2016–2023. doi:10.4049/jimmunol.1302389

Sun, H., Li, Y., Zhang, Z., Ju, Y., Li, L., Zhang, B., et al. (2015). Increase in myeloid-derived suppressor cells (MDSCs) associated with minimal residual disease (MRD) detection in adult acute myeloid leukemia. *Int. J. Hematol.* 102 (5), 579–586. doi:10.1007/s12185-015-1865-2

Sun, X., Sui, Q., Zhang, C., Tian, Z., and Zhang, J. (2013). Targeting blockage of STAT3 in hepatocellular carcinoma cells augments NK cell functions via reverse hepatocellular carcinoma-induced immune suppression. *Mol. Cancer Ther.* 12 (12), 2885–2896. doi:10.1158/1535-7163.MCT-12-1087

Tadmor, T., Fell, R., Polliack, A., and Attias, D. (2013). Absolute monocytosis at diagnosis correlates with survival in diffuse large B-cell lymphoma-possible link with monocytic myeloid-derived suppressor cells. *Hematol. Oncol.* 31 (2), 65–71. doi:10.1002/hon.2019

Tao, J., Han, D., Gao, S., Zhang, W., Yu, H., Liu, P., et al. (2020). CD8+ T cells exhaustion induced by myeloid-derived suppressor cells in myelodysplastic syndromes patients might be through TIM3/Gal-9 pathway. *J. Cell. Mol. Med.* 24 (1), 1046–1058. doi:10.1111/jcmm.14825

Tavukcuoglu, E., Horzum, U., Yanik, H., Uner, A., Yoyen-Ermis, D., Nural, S. K., et al. (2020). Human splenic polymorphonuclear myeloid-derived suppressor cells (PMN-MDSC) are strategically located immune regulatory cells in cancer. *Eur. J. Immunol.* 50 (12), 2067–2074. doi:10.1002/eji.202048666

Tian, X., Shen, H., Li, Z., Wang, T., and Wang, S. (2019). Tumor-derived exosomes, myeloid-derived suppressor cells, and tumor microenvironment. *J. Hematol. Oncol.* 12 (1), 84. doi:10.1186/s13045-019-0772-z

Tobin, R. P., Jordan, K. R., Kapoor, P., Spongberg, E., Davis, D., Vorwald, V. M., et al. (2019). IL-6 and IL-8 are linked with myeloid-derived suppressor cell accumulation and

correlate with poor clinical outcomes in melanoma patients. *Front. Oncol.* 9, 1223. doi:10.3389/fonc.2019.01223

Toh, B., Wang, X., Keeble, J., Sim, W. J., Khoo, K., Wong, W. C., et al. (2011). Mesenchymal transition and dissemination of cancer cells is driven by myeloid-derived suppressor cells infiltrating the primary tumor. *PLoS Biol.* 9 (9), e1001162. doi:10.1371/journal.pbio.1001162

Trabanelli, S., Chevalier, M. F., Martinez-Usatorre, A., Gomez-Cadena, A., Salomé, B., Lecciso, M., et al. (2017). Tumour-derived PGD2 and NKp30-B7H6 engagement drives an immunosuppressive ILC2-MDSC axis. *Nat. Commun.* 8 (1), 593. doi:10.1038/s41467-017-00678-2

Trovato, R., Fiore, A., Sartori, S., Canè, S., Giugno, R., Cascione, L., et al. (2019). Immunosuppression by monocytic myeloid-derived suppressor cells in patients with pancreatic ductal carcinoma is orchestrated by STAT3. *J. Immunother. Cancer* 7 (1), 255. doi:10.1186/s40425-019-0734-6

Tumino, N., Weber, G., Besi, F., Del Bufalo, F., Bertaina, V., Paci, P., et al. (2021). Polymorphonuclear myeloid-derived suppressor cells impair the anti-tumor efficacy of GD2.CAR T-cells in patients with neuroblastoma. *J. Hematol. Oncol.* 14 (1), 191. doi:10.1186/s13045-021-01193-0

Ugolini, A., Tyurin, V. A., Tyurina, Y. Y., Tcyganov, E. N., Donthireddy, L., Kagan, V. E., et al. (2020). Polymorphonuclear myeloid-derived suppressor cells limit antigen cross-presentation by dendritic cells in cancer. *JCI Insight* 5 (15), e138581. doi:10.1172/jci.insight.138581

van de Donk, N. W. C. J., and Usmani, S. Z. (2018). CD38 antibodies in multiple myeloma: Mechanisms of action and modes of resistance. *Front. Immunol.* 9, 2134. doi:10.3389/fimmu.2018.02134

Veglia, F., Hashimoto, A., Dweep, H., Sanseviero, E., De Leo, A., Tcyganov, E., et al. (2021). Analysis of classical neutrophils and polymorphonuclear myeloid-derived suppressor cells in cancer patients and tumor-bearing mice. *J. Exp. Med.* 218 (4), e20201803. doi:10.1084/jem.20201803

Veglia, F., Perego, M., and Gabrilovich, D. (2018). Myeloid-derived suppressor cells coming of age. *Nat. Immunol.* 19 (2), 108–119. doi:10.1038/s41590-017-0022-x

Veglia, F., Tyurin, V. A., Blasi, M., De Leo, A., Kossenkov, A. V., Donthireddy, L., et al. (2019). Fatty acid transport protein 2 reprograms neutrophils in cancer. *Nature* 569 (7754), 73–78. doi:10.1038/s41586-019-1118-2

Veirman, K. D., Van Ginderachter, J. A., Lub, S., Beule, N. D., Thielemans, K., Bautmans, I., et al. (2015). Multiple myeloma induces Mcl-1 expression and survival of myeloid-derived suppressor cells. *Oncotarget* 6 (12), 10532–10547. doi:10.18632/oncotarget.3300

Veltman, J. D., Lambers, M. E. H., van Nimwegen, M., Hendriks, R. W., Hoogsteden, H. C., Aerts, J. G. J. V., et al. (2010). COX-2 inhibition improves immunotherapy and is associated with decreased numbers of myeloid-derived suppressor cells in mesothelioma. Celecoxib influences MDSC function. *BMC Cancer* 10 (1), 464. doi:10.1186/1471-2407-10-464

Wang, H., Tao, Q., Wang, Z., Zhang, Q., Xiao, H., Zhou, M., et al. (2020). Circulating monocytic myeloid-derived suppressor cells are elevated and associated with poor prognosis in acute myeloid leukemia. *J. Immunol. Res.* 2020, 7363084. doi:10.1155/2020/7363084

Wang, J., Li, D., Cang, H., and Guo, B. (2019). Crosstalk between cancer and immune cells: Role of tumor-associated macrophages in the tumor microenvironment. *Cancer Med.* 8 (10), 4709–4721. doi:10.1002/cam4.2327

Wang, J., Veirman, K. D., Beule, N. D., Maes, K., Bruyne, E. D., Valckenborgh, E. V., et al. (2015). The bone marrow microenvironment enhances multiple myeloma progression by exosome-mediated activation of myeloid-derived suppressor cells. *Oncotarget* 6 (41), 43992–44004. doi:10.18632/oncotarget.6083

Wang, R., Wang, X., Yang, S., Xiao, Y., Jia, Y., Zhong, J., et al. (2021). Umbilical cord-derived mesenchymal stem cells promote myeloid-derived suppressor cell enrichment by secreting CXCL1 to prevent graft-versus-host disease after hematopoietic stem cell transplantation. *Cytotherapy* 23 (11), 996–1006. doi:10.1016/j.jcyt.2021.07.009

Wang, Y., Ding, Y., Guo, N., and Wang, S. (2019). MDSCs: Key criminals of tumor pre-metastatic niche formation. *Front. Immunol.* 10, 172. doi:10.3389/fimmu.2019.00172

Wang, Y., Schafer, C. C., Hough, K. P., Tousif, S., Duncan, S. R., Kearney, J. F., et al. (2018). Myeloid-derived suppressor cells impair B cell responses in lung cancer through IL-7 and STAT5. *J. Immunol.* 201 (1), 278–295. doi:10.4049/jimmunol.1701069

Wang, Y., Wang, J., Zhu, F., Wang, H., Yi, L., Huang, K., et al. (2022). Elevated circulating myeloid-derived suppressor cells associated with poor prognosis in B-cell non-Hodgkin's lymphoma patients. *Immun. Inflamm. Dis.* 10 (5), e616. doi:10.1002/iid3.616

Wang, Z., Jiang, R., Li, Q., Wang, H., Tao, Q., and Zhai, Z. (2021). Elevated M-MDSCs in circulation are indicative of poor prognosis in diffuse large B-cell lymphoma patients. *J. Clin. Med.* 10 (8), 1768. doi:10.3390/jcm10081768

Wang, Z., Zhang, L., Wang, H., Xiong, S., Li, Y., Tao, Q., et al. (2015). Tumor-induced CD14+HLA-DR−/low myeloid-derived suppressor cells correlate with tumor progression and outcome of therapy in multiple myeloma patients. *Cancer Immunol. Immunother.* 64 (3), 389–399. doi:10.1007/s00262-014-1646-4

Weber, R., Fleming, V., Hu, X., Nagibin, V., Groth, C., Altevogt, P., et al. (2018). Myeloid-derived suppressor cells hinder the anti-cancer activity of immune checkpoint inhibitors. *Front. Immunol.* 9, 1310. doi:10.3389/fimmu.2018.01310

Wei, J., Zhang, M., and Zhou, J. (2015). Myeloid-derived suppressor cells in major depression patients suppress T-cell responses through the production of reactive oxygen species. *Psychiatry Res.* 228 (3), 695–701. doi:10.1016/j.psychres.2015.06.002

Wu, C., Wu, X., Zhang, X., Chai, Y., Guo, Q., Li, L., et al. (2015). Prognostic significance of peripheral monocytic myeloid-derived suppressor cells and monocytes in patients newly diagnosed with diffuse large b-cell lymphoma. *Int. J. Clin. Exp. Med.* 8 (9), 15173–15181.

Wu, M., Ma, M., Tan, Z., Zheng, H., and Liu, X. (2020). Neutrophil: A new player in metastatic cancers. *Front. Immunol.* 11, 565165. doi:10.3389/fimmu.2020.565165

Xiu, B., Lin, Y., Grote, D. M., Ziesmer, S. C., Gustafson, M. P., Maas, M. L., et al. (2015). IL-10 induces the development of immunosuppressive CD14(+)HLA-DR(low/-) monocytes in B-cell non-Hodgkin lymphoma. *Blood Cancer J.* 5 (7), e328. doi:10.1038/bcj.2015.56

Xu, X., Meng, Q., Erben, U., Wang, P., Glauben, R., Kühl, A. A., et al. (2017). Myeloid-derived suppressor cells promote B-cell production of IgA in a TNFR2-dependent manner. *Cell. Mol. Immunol.* 14 (7), 597–606. doi:10.1038/cmi.2015.103

Xu, Z., Ji, J., Xu, J., Li, D., Shi, G., Liu, F., et al. (2017). MiR-30a increases MDSC differentiation and immunosuppressive function by targeting SOCS3 in mice with B-cell lymphoma. *FEBS J.* 284 (15), 2410–2424. doi:10.1111/febs.14133

Xu, Z., Ji, J., Xu, J., Li, D., Shi, G., Liu, F., et al. (2017). MiR-30a increases MDSC differentiation and immunosuppressive function by targeting SOCS3 in mice with B-cell lymphoma. *Febs J.* 284 (15), 2410–2424. doi:10.1111/febs.14133

Yan, H. H., Pickup, M., Pang, Y., Gorska, A. E., Li, Z., Chytil, A., et al. (2010). Gr-1+CD11b+ myeloid cells tip the balance of immune protection to tumor promotion in the premetastatic lung. *Cancer Res.* 70 (15), 6139–6149. doi:10.1158/0008-5472.CAN-10-0706

Yang, L., DeBusk, L. M., Fukuda, K., Fingleton, B., Green-Jarvis, B., Shyr, Y., et al. (2004). Expansion of myeloid immune suppressor Gr+CD11b+ cells in tumor-bearing host directly promotes tumor angiogenesis. *Cancer Cell* 6 (4), 409–421. doi:10.1016/j.ccr.2004.08.031

Yang, S., Wei, Y., Sun, R., Lu, W., Lv, H., Xiao, X., et al. (2020). Umbilical cord blood-derived mesenchymal stromal cells promote myeloid-derived suppressor cell proliferation by secreting HLA-G to reduce acute graft-versus-host disease after hematopoietic stem cell transplantation. *Cytotherapy* 22 (12), 718–733. doi:10.1016/j.jcyt.2020.07.008

Yaseen, M. M., Abuharfeil, N. M., Darmani, H., and Daoud, A. (2020). Mechanisms of immune suppression by myeloid-derived suppressor cells: The role of interleukin-10 as a key immunoregulatory cytokine. *Open Biol.* 10 (9), 200111. doi:10.1098/rsob.200111

Youn, J. I., Collazo, M., Shalova, I. N., Biswas, S. K., and Gabrilovich, D. I. (2012). Characterization of the nature of granulocytic myeloid-derived suppressor cells in tumor-bearing mice. *J. Leukoc. Biol.* 91 (1), 167–181. doi:10.1189/jlb.0311177

Youn, J. I., and Gabrilovich, D. I. (2010). The biology of myeloid-derived suppressor cells: The blessing and the curse of morphological and functional heterogeneity. *Eur. J. Immunol.* 40 (11), 2969–2975. doi:10.1002/eji.201040895

Youn, J. I., Kumar, V., Collazo, M., Nefedova, Y., Condamine, T., Cheng, P., et al. (2013). Epigenetic silencing of retinoblastoma gene regulates pathologic differentiation of myeloid cells in cancer. *Nat. Immunol.* 14 (3), 211–220. doi:10.1038/ni.2526

Zahran, A. M., Moeen, S. M., Thabet, A. F., Rayan, A., Abdel-Rahim, M. H., Mohamed, W. M. Y., et al. (2020). Monocytic myeloid-derived suppressor cells in chronic lymphocytic leukemia patients: A single center experience. *Leukemia Lymphoma* 61 (7), 1645–1652. doi:10.1080/10428194.2020.1728747

Zahran, A. M., Shibl, A., Rayan, A., Mohamed, M. A. E. H., Osman, A. M. M., Saad, K., et al. (2021). Increase in polymorphonuclear myeloid-derived suppressor cells and regulatory T-cells in children with B-cell acute lymphoblastic leukemia. *Sci. Rep.* 11 (1), 15039. doi:10.1038/s41598-021-94469-x

Zhang, H., Li, Z-L., Ye, S-B., Ouyang, L-Y., Chen, Y-S., He, J., et al. (2015). Myeloid-derived suppressor cells inhibit T cell proliferation in human extranodal NK/T cell lymphoma: A novel prognostic indicator. *Cancer Immunol. Immunother.* 64 (12), 1587–1599. doi:10.1007/s00262-015-1765-6

Zhang, J., Chen, H-M., Ma, G., Zhou, Z., Raulet, D., Rivera, A. L., et al. (2019). The mechanistic study behind suppression of GVHD while retaining GVL activities by myeloid-derived suppressor cells. *Leukemia* 33 (8), 2078–2089. doi:10.1038/s41375-019-0394-z

Zhang, J., Han, X., Hu, X., Jin, F., Gao, Z., Yin, L., et al. (2018). Ido1 impairs NK cell cytotoxicity by decreasing NKG2D/NKG2DLs via promoting miR-18a. *Mol. Immunol.* 103, 144–155. doi:10.1016/j.molimm.2018.09.011

Zhou, J., Nefedova, Y., Lei, A., and Gabrilovich, D. (2018). Neutrophils and PMN-MDSC: Their biological role and interaction with stromal cells. *Semin. Immunol.* 35, 19–28. doi:10.1016/j.smim.2017.12.004

Zhou, J., Shen, Q., Lin, H., Hu, L., Li, G., and Zhang, X. (2019). Decitabine shows potent anti-myeloma activity by depleting monocytic myeloid-derived suppressor cells in the myeloma microenvironment. *J. Cancer Res. Clin. Oncol.* 145 (2), 329–336. doi:10.1007/s00432-018-2790-6

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Interrogating the CD27:CD70 axis in αCD40-dependent control of pancreatic adenocarcinoma

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Immune checkpoint blockade immunotherapy has radically changed patient outcomes in multiple cancer types. Pancreatic cancer is one of the notable exceptions, being protected from immunotherapy by a variety of mechanisms, including the presence of a dense stroma and immunosuppressive myeloid cells. Previous studies have demonstrated that CD40 stimulation can remodel the tumor microenvironment in a manner that promotes effector immune cell responses and can cooperate with immune checkpoint inhibition for durable tumor control mediated by T cells. Here we confirm the capability of this combination therapy to dramatically, and durably, control pancreatic cancer growth in an orthotopic model and that the immune memory to this cancer is primarily a function of $CD4^+$ T cells. We extend this understanding by demonstrating that recruitment of recently primed T cells from the draining lymph nodes is not necessary for the observed control, suggesting that the pre-existing intra-tumoral cells respond to the combination therapy. Further, we find that the efficacy of CD40 stimulation is not dependent upon CD70, which is commonly induced on dendritic cells in response to CD40 agonism. Finally, we find that directly targeting the receptor for CD70, CD27, in combination with the TLR3 agonist polyIC, provides some protection despite failing to increase the frequency of interferon gamma-secreting T cells.

KEYWORDS

CD40, CD27, CD70, TIL activation, PDAC, KPC, CPI therapy

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is notoriously resistant towards immune checkpoint inhibitor therapy (CPI) (Royal et al., 2010; Bian and Almhanna, 2021). Several features have been implicated in the lack of response to CPI, including a dense fibrous stroma that limits immune cell and molecule penetration; the presence of immunosuppressive myeloid cells; and a low mutational burden limiting the availability of antigenic epitopes available to effector T cells (Balli et al., 2017). Therefore, it is imperative to explore alternative approaches to activate and enhance the immune system's response in this context.

CD40 is a tumor necrosis factor receptor superfamily member that is expressed on dendritic cells (DC), macrophages, monocytes, and B cells(Bullock, 2022). CD40 can be

engaged by CD40 ligand (CD40L), which is expressed by activated $CD4^+$ T cells and NKT cells and leads to cellular activation. This process drives B cell survival and contributes to class switching, and is the critical activation step for licensing DC to initiate $CD8^+$ T cell responses to cross-presented antigens (Bennett et al., 1998; Ridge et al., 1998; Schoenberger et al., 1998; Pérez-Melgosa et al., 1999). Agonist αCD40 monoclonal antibodies (mAbs) have been used to simulate CD40L engagement *in vivo* and circumvent the need for $CD4^+$ T cell help (Rowley and Al-Shamkhani, 2004; Bullock and Yagita, 2005; French et al., 2007; McWilliams et al., 2010; Vonderheide and Glennie, 2013). Moreover, CD40 has been combined with various therapies, including TLR agonists, chemotherapy, radiotherapy, and checkpoint inhibitors, to increase the performance of cancer vaccines and enable immunological control of tumors (Vonderheide, 2020; Burrack et al., 2021).

Despite earlier studies that have shown that CD40 agonism is extremely potent against PDAC, the mechanistic basis for these anti-tumor capabilities is poorly understood. On the one hand, CD40 stimulation can drive a T cell-independent activation of macrophages that is sufficient to curtail tumor growth (Beatty et al., 2011). On the other, subsequent studies have implicated a role for the CD40-mediated activation of conventional DC (cDC) leading to T cell-dependent control of experimental PDAC (Winograd et al., 2015; Byrne and Vonderheide, 2016; Rech et al., 2018; Morrison et al., 2020; Byrne et al., 2021). The basis by which CD40-mediated activation of DC drives T cell responses in the context of PDAC is being elucidated. Various immunologically-relevant molecules are produced by cDC after the agonism of CD40, and blocking innate components, such as type-1 interferons, modestly limits the therapeutic activity of CD40 stimulation (Morrison et al., 2020). Pertaining to this, the CD70:CD27 axis has been shown to be a critical component in bridging CD40-mediated activation of DC and adaptive immunity in vaccination and cancer (Rowley and Al-Shamkhani, 2004; Bullock and Yagita, 2005; Feau et al., 2012). Stimulation of CD27 drives the expression of Eomesodermin, and critical cytokine receptors associated with T cell differentiation and survival (Dong et al., 2012). Further, stimulation of CD27 in combination with the IFNαβ receptor results in a synergistic expansion of $CD8^+$ T cells and is associated with the induction of the effector T cell transcription factor, T-bet. Studies in other systems have implicated a role for CD70 in the anti-tumor activity of CD40-stimulation (French et al., 2007; Oba et al., 2020). Thus, we hypothesized that the effectiveness of CD40 stimulation at limiting the growth of PDAC is mediated, in part, by the induction of CD70 on cDC.

Here, we employed a syngeneic murine LSL-$Kras^{G12D/+}$, LSL-$Trp^{53R172H/+}$, $Pdx1^{Cre}$ (KPC) cell-implantation model to investigate the underlying mechanism for αCD40-induced tumor control (Byrne and Vonderheide, 2016; Long et al., 2016). We validate previous findings that αCD40 and CPI treatment results in tumor regressions and immunological memory(Morrison et al., 2020). We find that the intratumoral reservoir of T cells present at the time of CD40 activation is sufficient to mediate tumor control, without recruitment of newly primed T cells from the tumor-draining lymph node. However, surprisingly we find that the activity of αCD40 is independent of CD70, but can in part be replicated by the provision of CD27 stimulation in combination with the TLR agonist polyIC (pIC), which potently induces the expression of interferons.

Materials and methods

Mice

6–8 week-old C57BL/6J mice were purchased from Jackson Laboratories. Mice were housed in a controlled environment that was free of specific pathogens, and their treatment was conducted in compliance with the Animal Care and Use Committee's guidelines at the University of Virginia (Charlottesville, Virginia).

Tumor implantation models

Two LSL-$Kras^{G12D/+}$, LSL-$Trp^{53R172H/+}$, $Pdx1^{Cre}$ (KPC) cell lines were used as indicated. KPC4662 was kindly provided by the Vonderheide laboratory (Byrne and Vonderheide, 2016). KPC7940b was procured from the Beatty laboratory (Long et al., 2016). For subcutaneous (s.c.) implantations, 250k tumor cells were implanted in 100ul of 1:1 matrigel (Corning) and DPBS (Gibco). Tumor volume estimates were based off length and height measurements *via* calipers as described (Sápi et al., 2015). Mice were humanely euthanized to collect tumors at specified time points, once they had grown to their maximum permitted size, or if wet ulcers had developed.

For the orthotopic experiments, we implanted 500k KPC4662 cells in a 25 µL suspension of 1:1 matrigel and DPBS or RPMI (as indicated), using the same procedure as previously described (Stokes et al., 2011). Tumor measurements were taken *via* ultrasonography and volume was calculated as described (Faustino-Rocha et al., 2013).

For rechallenge experiments, mice were implanted with tumor cells on the opposing rear flank for the initial rechallenge, and on either fore flanks for tertiary and quaternary implantations.

Blocking antibodies: αPD1 (200ug; RMP1-14), αCTLA4 (200ug; 9H10), αCD70 (600ug; FR70), agonist antibodies: αCD40-agonist (100ug; FGK45), or αCD27-agonist (100ug; AT124) (BioXcell, Ichor Biosciences, or Celldex), and depletion antibodies: αCD8 (250 µg; 2.43) and αCD4 (250 µg; GK1.5) were all administered intraperitoneally (i.p.) as indicated.

Brefeldin A (BFA) (250ug; Selleckchem), was injected i.p. 6 h prior to euthanasia. pIC (100ug) were administered i.p. as indicated. Fingolimod (FTY720; Sigma) was administered as previously described (Stevens and Bullock, 2021).

Flow cytometry

Flow cytometry was performed using an Aurora Northern Lights (Cytek) or Attune™ NxT Acoustic Focusing cytometers (Thermo Fisher). Data were collected using Spectroflo or Attune NxT software and analyzed using FlowJo (version 10.8). After titrating for optimal resolution, fluorescent mAbs from BD Biosciences, BioLegend, Invitrogen, and Phitonex were used to

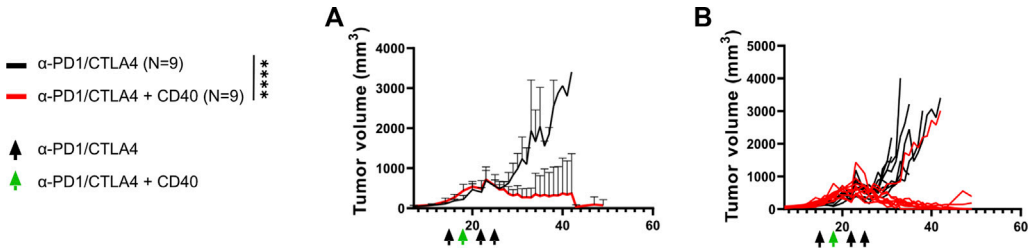

FIGURE 1
CPI40 treatment promotes robust PDAC tumor regression. 250k KPC-4662 tumor cells were implanted s.c. into C57BL/6mice. **(A)** Average and **(B)** individual tumor growth curves over time. Black arrows denote administration of αPD1 (200 ug) and αCTLA4 (200 ug), the green arrow denotes administration of the former plus αCD40 (100 ug). Mice were treated on days 15, 18, 22, and 25. $N = 9$ per group. For average curves, maximal tumor volume was repeated in this experiment for mice that reached endpoint before day 35. Mixed-effects analysis was performed, $p < 0.0001$ across time and treatment. This experiment was performed once.

stain for CD45 (30-F11), MHCII (2G9), CD3 (145-2C11), CD127 (SB/199), CD44 (IM7), TCRγδ (GL3), CD11c (HL3), CD27 (LG 3A10), CD8α (5H10), Ly6G (1A8), XCR1 (ZET), F4/80 (T45-2342), CD11b (M1/70), CD4 (GK1.5), CD172a (P84), CD19 (6D5), Ly49G2 (4D11), NKp46 (AF700), NK1.1 (PK136), EOMES (Dan11mag), FOXP3 (FJK-16s), IFN-γ (XMG1.2). LIVE/DEAD fixable dye from Thermo Fisher Scientific was also used to evaluate viability of cells.

Freshly harvested tumors were minced, homogenized, and underwent lympholyte-M (Cedarlane) gradient centrifugation for immune cell isolation. Samples were viability and surface stained prior to fixation with the Cytofix/Cytoperm, or FOXP3 fixation, kit (BD) for intracellular staining.

Statistical analysis

GraphPad Prism (GraphPad Software, Inc.) was employed to compute all statistical analyses. Mantel-Cox test was employed for survival analysis. Two-way ANOVA or a mixed effects model was used to determine significance of difference in tumor growth among multiple treatment arms. The Holm-Šídák multiple comparison, or Brown-Forsythe and Welch ANOVA tests, were used to define significance among groups. Symbols (*, **, ***, ****) are used to denote p values < 0.05, 0.01, 0.001, or 0.0001, respectively.

Results

Checkpoint inhibition and CD40 diminish discreet T_{reg} cell populations in the tumor microenvironment (TME)

We initially evaluated the effectiveness of PD1 and CTLA4 checkpoint inhibitors and CD40 (CPI40) treatment in suppressing KPC4662 tumors in a C57BL/6J mouse model. The treatment with CPI40 produced a noticeable reduction in tumor growth, both in orthotopically and subcutaneously implanted tumors (Supplementary Figure S1; Figures 1A, B). These data suggest that CPI40 treatment can lead to robust tumor control independent of additional treatment interventions and is effective against well-established tumors.

CPI40 has the potential to expand a T cell response within the tumor TME. Thus, we characterized immune cell infiltrate 6 days after CPI40 treatment *via* flow cytometry to understand alterations in lymphocyte representation and function. We initially confirmed that $CD4^+$ and $CD8^+$ T cells and innate lymphoid (ILC) cells were increased in frequency (Figure 2A). Interestingly, there was no indication of an increase in lymphocyte numbers, suggesting that other hematopoietic cells were reduced within the TME as a function of treatment (Figure 2B).

IFN-γ is a hallmark cytokine released by effector tumor infiltrating lymphocytes(TIL) populations. We assessed native TIL cytokine production within the TME by injecting animals with brefeldin A 6 h prior to sacrifice (Figures 2C–E). Although we found that $CD4^+$ and $CD8^+$ T cells and ILCs skewed towards enhanced IFN-γ production in the CPI40 treated group, the most robust phenotype was associated with $CD8^+$ T cells. This suggests that CPI40 has the potential to enable $CD8^+$ T cells to elicit anti-tumor functions within the TME.

Studies have indicated that $CD4^+$ T_{reg} cells are diminished within the TME of mice treated with α-CTLA4 and α-CD40 in the context of PDAC. (Morrison et al., 2020). NK cell-associated MHC I receptors have been shown to be expressed by $CD8^+$ T_{reg} cell populations (Kim et al., 2011). However, $CD8^+$ T_{reg} cells have never been characterized in the context of tumor immunity. Ly49G2 is an MHC I receptor commonly expressed on NK cells and has been shown to be expressed on a discreet population of $CD8^+$ T_{reg} cells (Kim et al., 2011). As expected, Ly49G2 can be found within the ILC compartment in the TME (Figures 2F–H). Interestingly, there were fewer $Ly49G2^+$ $CD8^+$ T cells in the CPI40 treated group, suggesting there is a higher $CD8^+$ effector to $CD8^+$ T_{reg} cell ratio in CPI40 treated mice. These data suggest that CPI40 treatment alters the $CD8^+$ effector to $CD8^+$ T_{reg} ratio.

We repeated the experiment in an orthotopic setting to verify that our initial findings would hold true in this context. CPI40 treatment had a robust effect on orthotopically implanted tumor outgrowth, as had been seen in subcutaneously implanted tumors (Supplementary Figures S1A, B). Additionally, we observed a similar increase in TIL frequency in the TME (Supplementary Figures S2). Despite similar numbers of total TILs (Supplementary Figures S2) there is clearly an increase in the density of $CD8^+$ effector cells when normalizing cell number to the mass of the tumor

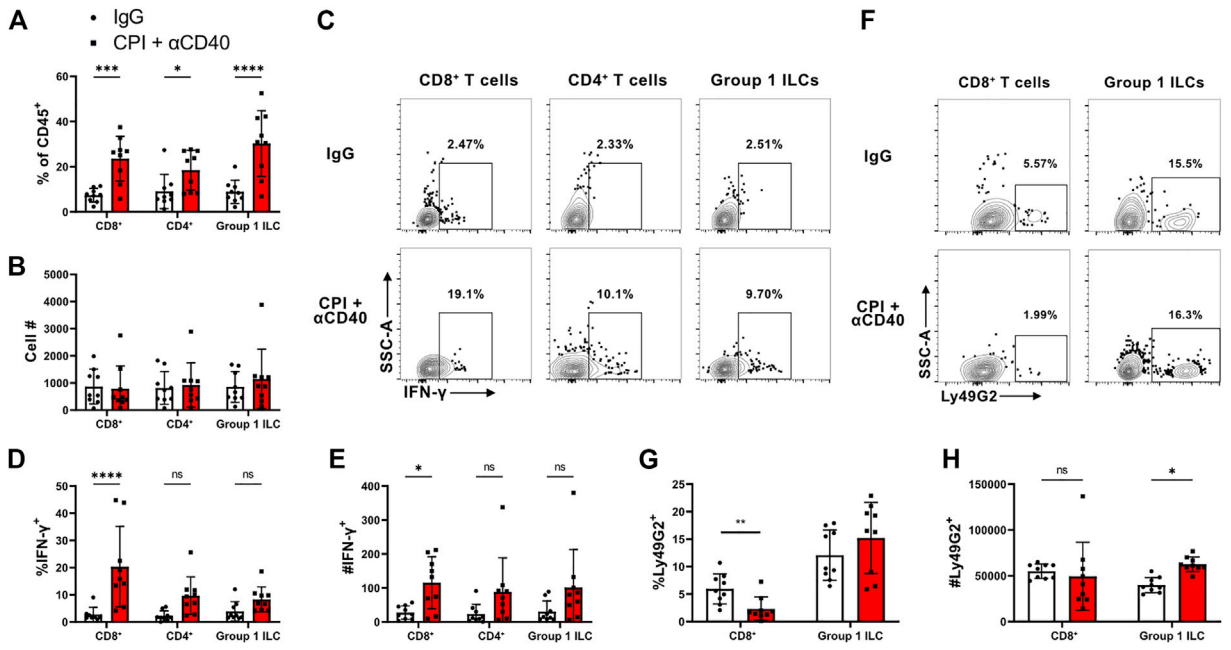

FIGURE 2
CPI40 treatment promotes maintenance and activation of TILs in the TME. Characterization of TILs 27* days post-implantation (*N* = 9 per group). Mice were injected i.p. with BFA 6 h prior to harvest. Figure shows the frequency and number of **(A, B)** TIL lineage, **(C–E)** IFN-γ producers, and **(F–H)** CD8$^+$ T_{reg} cells. Holm-Šídák multiple comparison test was run in all cases. This experiment was performed once.

(Supplementary Figures S2). These data suggest that CD8$^+$ T cells are maintained during control of tumor outgrowth.

The presence of T_{reg} cells was analyzed after treatment with CPI40 in an orthotopic setting. Results showed a reduced representation of both conventional CD4$^+$ CD25$^+$ T_{reg} cells and CD8$^+$ Ly49G2$^+$ T_{reg} cells in mice treated with CPI40. (Supplementary Figures S2). Again, this suggests that CPI40 therapy polarizes the TME immune landscape to be comprised of more effector, rather than suppressive, constituent cell populations.

Regulation of PD1 expression in response to CPI40

PD-1 is a well-known inhibitory receptor linked to both lymphoid cell activation and exhaustion. The expression level of PD-1 on a per cell basis correlates with the activation state of a cell, with higher expression associated with acute activation and lower expression associated with exhaustion (Wherry and Kurachi, 2015). To determine which cells are responding to PD-1 blockade and how they respond to CPI40, PD-1 expression was analyzed on TIL populations. (Supplementary Figures S2). CPI40 therapy led to no change in the proportion of PD-1$^+$ CD8$^+$ T cells, but there was a sharp decrease in expression on a per-cell basis. Additionally, there was a higher representation of PD-1$^+$ CD4$^+$ T cells, with no change in expression on a per-cell basis. These findings suggest that CD8$^+$ T cell expression may reflect acute activation rather than exhaustion during CPI40 treatment, whereas CD4$^+$ T cells have a higher frequency of PD-1 expression, which could potentially limit their effector potential in the absence of PD-1 blockade.

CPI40 induces CD4$^+$ T cell-dependent immune memory to PDAC

Our subcutaneous CPI40 experiment (Figure 1) resulted in 6 of 8 CPI40 survivors that fully controlled tumors (no palpable mass) (Figure 3A). Two of 6 of these mice redeveloped tumors naturally 15- and 28-day post observations of no palpable mass (i.e., transiently dormant tumor cells were still present). We investigated whether CPI40 treatment induced immune memory and rechallenged the remaining mice with KPC4662 on their opposing flank. 2/4 mice showed no signs of tumor development after rechallenge 36 days post-implantation (data not shown), suggesting that CPI40 treatment can induce tumor-specific memory. We then tested whether adaptive immunity was dependent upon CD8$^+$ T cells by depleting the remaining mice with CD8$^+$ T cell depleting antibody. Both mice were able to reject the tertiary implanted tumors (Figure 3B). We then tested whether CD4$^+$ T cells were involved using a similar approach. Interestingly, both mice developed tumors after a fourth rechallenge (Figure 3C). These data suggest that CD4$^+$ T cells are necessary for CPI40-induced adaptive immunity against KPC4662 tumor cells.

Circulating lymphoid cells are dispensable for PDAC growth control

We have previously shown that αCD40-mediated, T cell dependent tumor control does not require the recruitment of newly primed effector T cells in well-infiltrated melanomas

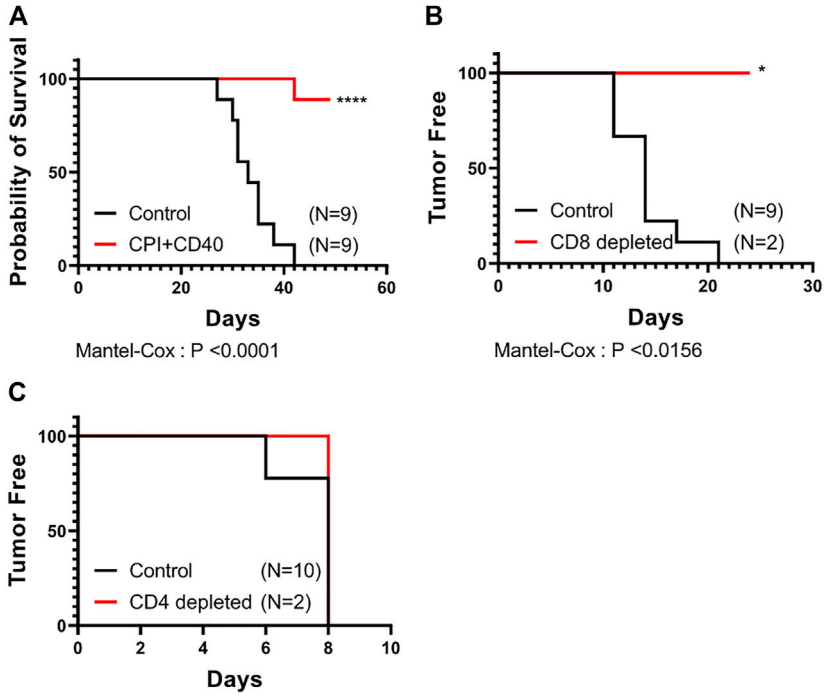

FIGURE 3
CPI40 treatment leads to prolonged survival that is dependent upon CD4$^+$ T cells. **(A)** Survival curves of treated groups (N = 9 per group). Tumor free survival after depletion of **(B)** CD8$^+$ then **(C)** CD4$^+$ T cells in naïve or CPI40 survivors. Depletions were performed sequentially in two independent experiments with the same subjects from the CPI40 treated group. Mantel-Cox analysis was performed in all cases. These experiments were performed once.

(Stevens and Bullock, 2021). However, PDACs are reported as being immunologically "cold" due to a paucity of TILs with limited effector activity in the tumor microenvironment. This suggests that CPI40's efficacy may depend on freshly primed T cells. We therefore tested whether a *de novo* T cell response is responsible for CPI40-driven control of pancreatic tumors. FTY720 is a drug that activates S1P1 receptor which induces its downregulation and subsequently prevents T cell egress from lymphoid tissues. FTY720 had no impact on tumor control in the context of CPI40 (Supplementary Figures S3A, B). These data suggest that a *de novo* induced response, driven by T cells that emigrate from the draining lymph node, is unnecessary for tumor control and that the cells intrinsic to the tumor at time of therapy are sufficient for the observed activity of the combination therapy.

Role of the CD27:CD70 axis in CD40-dependent growth control

Our previous studies have indicated an ability for CD40 stimulation along with pIC to enhance T cell responses within melanomas without the need for checkpoint blockade (Stevens and Bullock, 2021). Further, we have demonstrated that a major component of the efficacy of CD40 stimulation occurs *via* the induction of CD70 on cDCs (Bullock and Yagita, 2005). Others have demonstrated that the CD70:CD27 axis potentiates tumor specific T cell expansion after CD40 stimulation (Oba et al., 2020). Thus, we also hypothesized that blockade of CD70 would abrogate CD40-mediated control of subcutaneous PDAC tumors. Accordingly, we treated mice with just CD40 agonist mAb with, or without, a CD70 antagonist mAb. As we have previously demonstrated that the combination of CD27 (the receptor for CD70) and IFNαβR stimulation can substitute for CD40 stimulation, we reasoned that circumventing CD70-signaling with a CD27-agonist mAb would promote tumor control. Therefore, we also included a group that received plC and an agonistic CD27 mAb. Mice that were given αCD40 exhibited robust tumor control, regardless of blocking CD70 (Figure 4). While the degree of tumor control induced by the combination of plC and αCD27 is significant, it does not reach a comparable level to that provided by αCD40 treatment. These results indicate that CD40-specific stimulation is required for optimal PDAC tumor control, independent of its downstream signaling through CD70.

After initiation of immunotherapeutic treatments, we measured tumors daily until there was either clear tumor control or a lack thereof. Upon reaching this point, we characterized the immune cell infiltration of tumors *via* flow cytometry to evaluate immunological changes in the TME. Changes in the numbers of specific cell types were not informative regarding comparison between groups due to the small size of immune populations present in resolved tumors (data not shown). However, the tumors of mice that received treatments display a three-fold increase in the frequency of T cells present, indicating that the various immunotherapies can mobilize a T cell response (Figure 5A). CD8$^+$ T cells are often considered vital mediators of the antitumor response, however, the

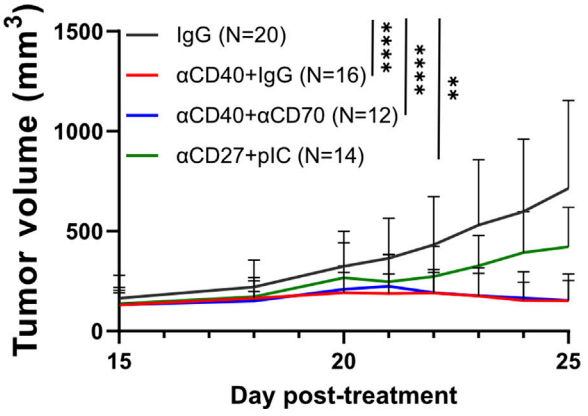

FIGURE 4
Interrogating the role of the CD27:CD70 axis in CD40-mediated tumor control. 250k KPC-7940b tumor cells were implanted s.c into C57BL/6 mice. Mice were treated with clg (600 ug) (D-1, D2, D5), CD70-blockade (600 ug) (D-1,D2, D5 (N = 4), or D-2 and D3 (N = 8)), CD40-agonist (100 ug) (D0), or CD27-agonist (100 ug) and pIC (100 ug) (D0, D3, D9). Tumor volumes over time are shown for each treated group. Standard deviations are shown for each time point. Mixed-effects analysis was performed, $p < 0.0001$ across time and treatment. These are aggregate data from two independent experiments.

αCD27 and pIC treated mice exhibit suboptimal tumor control, despite having the highest representation of $CD8^+$ T cells (Figure 5B). On the contrary, the frequency of $CD4^+$ T cells is highest among the well-controlled αCD40 treated tumors, even when CD70-signaling is blocked (Figure 5C). However, CPI40 does not induce the same pattern, perhaps due to a CPI specific mechanism (Figure 2A). The proportion of $CD4^+$ T_{reg} cells among $CD4^+$ T cells and total immune cells declined similarly among all treated groups, suggesting that differences in the degree of tumor control may be due to the accumulation of $CD4^+$ T cells (Figures 5D, E). This does not extend to the ILC compartment, as the frequency of NK1.1 + NKp46 + ILCs does not change after αCD40 monotherapy, despite an increase after CPI40 (Figure 5F).

Considered a benchmark for measuring the antitumor immune response, the ratio of $CD8^+$ T cells to T_{reg} cells only modestly increased in the tumors of αCD40 treated mice but increased more significantly in the pIC and αCD27 treated tumors (Figure 5G). Conversely, the ratio of $CD4^+$ T cells to $CD4^+$ T_{reg} cells trended towards being the highest among the αCD40 groups and moderate in the pIC and αCD27 group (Figure 5H). This further supports a role for CD40 stimulation in skewing T cell infiltration of tumors towards $CD4^+$ T cells, even in the absence of signaling through CD70.

Beyond examining αCD40-specific changes in tumor infiltration by T cells, we wanted to determine if there are αCD40 dependent changes in T cell activity. To assess *in vivo* IFN-γ production, we injected mice with brefeldin A 6 hours before sacrificing. This yielded evidence of improved IFN-γ production by $CD4^+$ but not $CD8^+$ T cells, even in mice treated with the αCD70 antagonist. (Figures 5I, J). pIC and αCD27 treatment did not change IFN-γ production by $CD4^+$ T cells (Figures 5I, J). Thus, the enhancement of $CD4^+$ T cell function requires CD40 stimulation but occurs independent of CD70-signaling. Immunotherapeutic treatments did not alter $CD8^+$ T cell production of IFN-γ, further suggesting that a $CD4^+$ T cell effector signature specifically coincides with robust tumor control (Figure. 6A and C).

Discussion

In this study, we have provided further evidence that targeting the stimulation of CD40, with or without concomitant use of immune checkpoint inhibition, is a potent mechanism for improving pancreatic cancer control, both in orthotopic and subcutaneous settings. The combination therapy promotes the presence of activated T cell effector populations with increased cytokine production, and this therapy remains effective when T cells are prevented from leaving the draining lymph nodes. We further find that the combination therapy results in substantial immunological protective memory that is remarkably dependent of $CD4^+$ T cells, not $CD8^+$ T cells. Finally, the efficacy of CD40 stimulation appears independent of its ability to induce the expression of CD70, though targeting CD27 and IFNαβR can modestly replicate the efficacy of CD40 agonism.

In previous studies we determined that CD40-mediated stimulation of melanoma intratumoral T cells is sufficient to promote tumor control without further contribution of T cells primed in draining lymph nodes (Stevens and Bullock, 2021). However, in that study, we needed to provide antigen to achieve consistent tumor control. In this study of pancreatic cancer, we find CD40 stimulation is sufficient, without further need to provide antigen. This suggests that the makeup of CD40-expressing cells and their ability to either present already acquired antigen or respond with direct tumoricidal activity to release more antigen is distinct in different models of cancer. Further, in the melanoma model control was transient, and the activation of T cells rapidly diminished. Conversely, only a single infusion of αCD40 was necessary to drive durable tumor control in this current study. Thus, resistance mechanisms that arise after CD40 stimulation in these models appear to be different. This becomes particularly relevant when considering the inconsistent outcomes for the PRINCE combination therapy clinical trial, in which only a subset of pancreatic cancer patients received a benefit (Padrón et al., 2022). Understanding how well mouse models correlate with the human experience, and whether they can reveal distinct resistance mechanisms, will be imperative for designing next-generation trials with increased efficacy.

Consistent with previous studies, we find that resolution of tumors after combination therapy provides potent resistance to rechallenge, demonstrating the establishment of memory against pancreatic tumor antigens. While initial control of these tumors depended upon either $CD4^+$ or $CD8^+$ T cells, rejection by memory T cells was attributed to the $CD4^+$ T cell subset. Given that the mice were challenged at distal sites, this argues that central, rather than tissue-resident, memory $CD4^+$ T cells are responsible for this protection. This outcome suggests that the $CD4^+$ T cell responses within patients should be examined as a potential biomarker of efficacy, and that strategies that promote

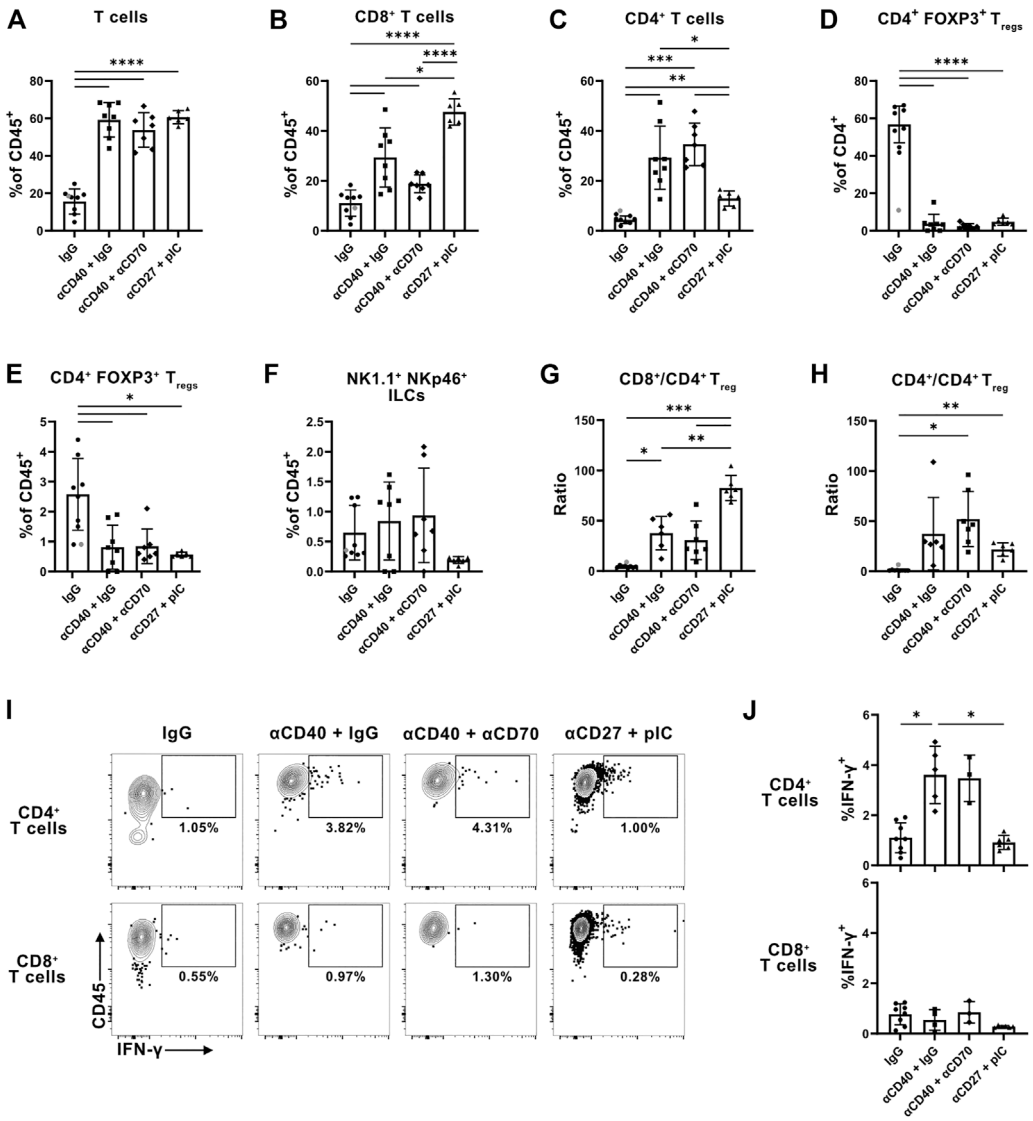

FIGURE 5
Stimulation of CD40 improves representation and cytokine production of CD4⁺ T cells within tumors independent of CD70-signaling.
Evaluation of T cell infiltration into KPC-7940b tumors 23 days post implantation. **(A)** Frequency of total T cells among CD45⁺ immune cells in the TME. **(B)** Frequency of CD8⁺ and **(C)** CD4⁺ T cells among all CD45⁺ immune cells. **(D)** Frequency of FOXP3⁺ T_{reg} cells among CD4⁺ T cells and **(E)** total CD45⁺ immune cells. **(F)** Frequency of NK1.1⁺ NKp46⁺ ILCs among CD45⁺ immune cells. **(G)** Ratio of CD8⁺ **(H)** and CD4⁺ T cells to CD4⁺ FOXP3⁺ T_{reg} cells. **(I)** Representative flow plots of IFN-γ production by CD4⁺ and CD8⁺ T cells. **(J)** Frequency of IFN-γ⁺ events among CD4⁺ and CD8⁺ T cells. Samples with fewer than 100 CD4⁺ or CD8⁺ T cells were insufficient for analysis of IFN-γ production and subsequently excluded. Grey dot denotes statistically determined outlier which was excluded from the analysis. Brown-Forsythe and Welch ANOVA tests were performed in all cases. This experiment was performed once.

CD4⁺ T cell immunity may be particularly effective in pancreatic cancer. Due to our prior experience with CD40-mediated vaccination, we directly tested the hypothesis that the effectiveness of CD40 agonists was due to their capacity to drive CD70 expression, with the resultant engagement of CD27 on effector T cell populations. Counter to our hypothesis, we find that tumor control and T cell presence and activation in the TME are independent of CD70, consistent with a recent study genetically showing that CD70 is dispensable for the activation of T cells by DC in a tumor setting (Wu et al., 2022).

To some degree, targeting CD27 along with IFNαβR, which we have previously shown to be a potent driver of CD8⁺ T cell responses to vaccination (Dong et al., 2012; Dong et al., 2019) and to promote tumor control (Roberts et al., 2010; van de Ven and Borst, 2015; Riccione et al., 2018), had limited success in this model of pancreatic cancer. This held true, even though αCD27+pIC led to a large expansion of CD8⁺ T cells within the pancreatic TME and a reduction in T_{reg} cells. Notably, αCD27 and pIC did not improve the proportion of T cells producing IFN-γ compared to that achieved by αCD40. This suggests that there are suppressive mechanisms in the TME that are subverted by CD40 but not

CD27 stimulation (which likely directly acts on intratumoral T cells). Immature myeloid cells are an obvious candidate, and future studies that are intended to identify the therapeutic target of CD40 stimulation will help tease apart this distinction. Further, given the T cell dependency of the tumor control achieved with CD40 stimulation, we conclude that CD40 stimulation is promoting T cell responses by a pathway independent of CD70. CD40 stimulation can promote a variety of costimulatory molecules on cDC, including 4-1BB and OX40, and cytokines such as IL-12 and Type-I interferons, each of which could provide the link to expanded T cell function. It is of special interest to note that OX40 (CD134) stimulation has been shown to strongly promote $CD4^+$ T cell responses (Croft, 2010; Kurche et al., 2010). Future studies will examine the contribution of these molecules with respect to the tumor control achieved by CD40 stimulation.

It is of note that we observe that the vast majority of the efficacy of the combination therapy is driven by CD40 stimulation, and that CPI therapy on its own was ineffective (data not shown). Studies from other labs have argued that increased tumor control is achieved by the inclusion of CPI (Rech et al., 2018; Morrison et al., 2020)) Reasons for this difference are not readily apparent, but the nature of the immune cell infiltration and myeloid cell makeup can differ considerably between institutions, often as a function of different microbiomes. This possibility is currently being studied.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

This animal study was reviewed and approved by The University of Virginia Institutional Animal Care and Use Committee.

Author contributions

AG, CC, FP, and TNJB contributed to conception and design of the study. AG, CC, and SA conducted the experiments. AG, CC, and TNJB wrote the manuscript. SA, TB, and FP edited the manuscript. SA and TB assisted with developing the methodology. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2023.1173686/full#supplementary-material

References

Balli, D., Rech, A. J., Stanger, B. Z., and Vonderheide, R. H. (2017). Immune cytolytic activity stratifies molecular subsets of human pancreatic cancer. *Clin. Cancer Res.* 23 (12), 3129–3138. doi:10.1158/1078-0432.CCR-16-2128

Beatty, G. L., Chiorean, E. G., Fishman, M. P., Saboury, B., Teitelbaum, U. R., Sun, W., et al. (2011). CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science* 331 (6024), 1612–1616. doi:10.1126/science.1198443

Bennett, S. R., Carbone, F. R., Karamalis, F., Flavell, R. A., Miller, J. F., and Heath, W. R. (1998). Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature* 393 (6684), 478–480. doi:10.1038/30996

Bian, J., and Almhanna, K. (2021). Pancreatic cancer and immune checkpoint inhibitors-still a long way to go. *Transl. gastroenterology hepatology* 6, 6. doi:10.21037/tgh.2020.04.03

Bullock, T. N. J. (2022). CD40 stimulation as a molecular adjuvant for cancer vaccines and other immunotherapies. *Cell. Mol. Immunol.* 19 (1), 14–22. doi:10.1038/s41423-021-00734-4

Bullock, T. N. J., and Yagita, H. (2005). Induction of CD70 on dendritic cells through CD40 or TLR stimulation contributes to the development of CD8+ T cell responses in the absence of CD4+ T cells. *J. Immunol.* 174 (2), 710–717. doi:10.4049/jimmunol.174.2.710

Burrack, A. L., Rollins, M. R., Spartz, E. J., Mesojednik, T. D., Schmiechen, Z. C., Raynor, J. F., et al. (2021). CD40 agonist overcomes T cell exhaustion induced by chronic myeloid cell IL-27 production in a pancreatic cancer preclinical model. *J. Immunol.* 206 (6), 1372–1384. doi:10.4049/jimmunol.2000765

Byrne, K. T., Betts, C. B., Mick, R., Sivagnanam, S., Bajor, D. L., Laheru, D. A., et al. (2021). Neoadjuvant selicrelumab, an agonist CD40 antibody, induces changes in the

tumor microenvironment in patients with resectable pancreatic cancer. *Clin. Cancer Res.* 27 (16), 4574–4586. doi:10.1158/1078-0432.CCR-21-1047

Byrne, K. T., and Vonderheide, R. H. (2016). CD40 stimulation obviates innate sensors and drives T cell immunity in cancer. *Cell Rep.* 15 (12), 2719–2732. doi:10.1016/j.celrep.2016.05.058

Croft, M. (2010). Control of immunity by the TNFR-related molecule OX40 (CD134). *Annu. Rev. Immunol.* 28, 57–78. doi:10.1146/annurev-immunol-030409-101243

Dong, H., Buckner, A., Prince, J., and Bullock, T. (2019). Frontline Science: Late CD27 stimulation promotes IL-7Rα transcriptional re-expression and memory T cell qualities in effector CD8+ T cells. *J. Leukoc. Biol.* 106 (5), 1007–1019. doi:10.1002/JLB.1HI0219-064R

Dong, H., Franklin, N. A., Roberts, D. J., Yagita, H., Glennie, M. J., and Bullock, T. N. J. (2012). CD27 stimulation promotes the frequency of IL-7 receptor-expressing memory precursors and prevents IL-12-mediated loss of CD8(+) T cell memory in the absence of CD4(+) T cell help. *J. Immunol.* 188 (8), 3829–3838. doi:10.4049/jimmunol.1103329

Faustino-Rocha, A., Oliveira, P. A., Pinho-Oliveira, J., Teixeira-Guedes, C., Soares-Maia, R., da Costa, R. G., et al. (2013). Estimation of rat mammary tumor volume using caliper and ultrasonography measurements. *Lab. Anim.* 42 (6), 217–224. doi:10.1038/laban.254

Feau, S., Garcia, Z., Arens, R., Yagita, H., Borst, J., and Schoenberger, S. P. (2012). The CD4$^+$ T-cell help signal is transmitted from APC to CD8$^+$ T-cells via CD27-CD70 interactions. *Nat. Commun.* 3, 948. doi:10.1038/ncomms1948

French, R. R., Taraban, V. Y., Crowther, G. R., Rowley, T. F., Gray, J. C., Johnson, P. W., et al. (2007). Eradication of lymphoma by CD8 T cells following anti-CD40 monoclonal antibody therapy is critically dependent on CD27 costimulation. *Blood* 109 (11), 4810–4815. doi:10.1182/blood-2006-11-057216

Kim, H.-J., Wang, X., Radfar, S., Sproule, T. J., Roopenian, D. C., and Cantor, H. (2011). CD8+ T regulatory cells express the Ly49 Class I MHC receptor and are defective in autoimmune prone B6-Yaa mice. *Proc. Natl. Acad. Sci. U. S. A.* 108 (5), 2010–2015. doi:10.1073/pnas.1018974108

Kurche, J. S., Burchill, M. A., Sanchez, P. J., Haluszczak, C., and Kedl, R. M. (2010). Comparison of OX40 ligand and CD70 in the promotion of CD4+ T cell responses. *J. Immunol.* 185 (4), 2106–2115. doi:10.4049/jimmunol.1000172

Long, K. B., Gladney, W. L., Tooker, G. M., Graham, K., Fraietta, J. A., and Beatty, G. L. (2016). IFNγ and CCL2 cooperate to redirect tumor-infiltrating monocytes to degrade fibrosis and enhance chemotherapy efficacy in pancreatic carcinoma. *Cancer Discov.* 6 (4), 400–413. doi:10.1158/2159-8290.CD-15-1032

McWilliams, J. A., Sanchez, P. J., Haluszczak, C., Gapin, L., and Kedl, R. M. (2010). Multiple innate signaling pathways cooperate with CD40 to induce potent, CD70-dependent cellular immunity. *Vaccine* 28 (6), 1468–1476. doi:10.1016/j.vaccine.2009.11.071

Morrison, A. H., Diamond, M. S., Hay, C. A., Byrne, K. T., and Vonderheide, R. H. (2020). Sufficiency of CD40 activation and immune checkpoint blockade for T cell priming and tumor immunity. *Proc. Natl. Acad. Sci. U. S. A.* 117 (14), 8022–8031. doi:10.1073/pnas.1918971117

Oba, T., Hoki, T., Yamauchi, T., Keler, T., Marsh, H. C., Cao, X., et al. (2020). A critical role of CD40 and CD70 signaling in conventional type 1 dendritic cells in expansion and antitumor efficacy of adoptively transferred tumor-specific T cells. *J. Immunol.* 205 (7), 1867–1877. doi:10.4049/jimmunol.2000347

Padrón, L. J., Maurer, D. M., O'Hara, M. H., O'Reilly, E. M., Wolff, R. A., Wainberg, Z. A., et al. (2022). Sotigalimab and/or nivolumab with chemotherapy in first-line metastatic pancreatic cancer: Clinical and immunologic analyses from the randomized phase 2 PRINCE trial. *Nat. Med.* 28 (6), 1167–1177. doi:10.1038/s41591-022-01829-9

Pérez-Melgosa, M., Hollenbaugh, D., and Wilson, C. B. (1999). Cutting edge: CD40 ligand is a limiting factor in the humoral response to T cell-dependent antigens. *J. Immunol.* 163 (3), 1123–1127. doi:10.4049/jimmunol.163.3.1123

Rech, A. J., Dada, H., Kotzin, J. J., Henao-Mejia, J., Minn, A. J., Twyman-Saint Victor, C., et al. (2018). Radiotherapy and CD40 activation separately augment immunity to checkpoint blockade in cancer. *Cancer Res.* 78 (15), 4282–4291. doi:10.1158/0008-5472.CAN-17-3821

Riccione, K. A., He, L. Z., Fecci, P. E., Norberg, P. K., Suryadevara, C. M., Swartz, A., et al. (2018). CD27 stimulation unveils the efficacy of linked class I/II peptide vaccines in poorly immunogenic tumors by orchestrating a coordinated CD4/CD8 T cell response. *Oncoimmunology* 7 (12), e1502904. doi:10.1080/2162402X.2018.1502904

Ridge, J. P., Di Rosa, F., and Matzinger, P. (1998). A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. *Nature* 393 (6684), 474–478. doi:10.1038/30989

Roberts, D. J., Franklin, N. A., Kingeter, L. M., Yagita, H., Tutt, A. L., Glennie, M. J., et al. (2010). Control of established melanoma by CD27 stimulation is associated with enhanced effector function and persistence, and reduced PD-1 expression of tumor infiltrating CD8(+) T cells. *J. Immunother.* 33 (8), 769–779. doi:10.1097/CJI.0b013e3181ee238f

Rowley, T. F., and Al-Shamkhani, A. (2004). Stimulation by soluble CD70 promotes strong primary and secondary CD8+ cytotoxic T cell responses *in vivo*. *J. Immunol.* 172 (10), 6039–6046. doi:10.4049/jimmunol.172.10.6039

Royal, R. E., Levy, C., Turner, K., Mathur, A., Hughes, M., Kammula, U. S., et al. (2010). Phase 2 trial of single agent Ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. *J. Immunother.* 33 (8), 828–833. doi:10.1097/CJI.0b013e3181eec14c

Sápi, J., Kovacs, L., Drexler, D. A., Kocsis, P., Gajari, D., and Sapi, Z. (2015). Tumor volume estimation and quasi-continuous administration for most effective bevacizumab therapy. *Plos One* 10 (11), e0142190. doi:10.1371/journal.pone.0142190

Schoenberger, S. P., Toes, R. E., van der Voort, E. I., Offringa, R., and Melief, C. J. (1998). T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature* 393 (6684), 480–483. doi:10.1038/31002

Stevens, A. D., and Bullock, T. N. J. (2021). Therapeutic vaccination targeting CD40 and TLR3 controls melanoma growth through existing intratumoral CD8 T cells without new T cell infiltration. *Cancer Immunol. Immunother.* 70 (8), 2139–2150. doi:10.1007/s00262-020-02841-z

Stokes, J. B., Adair, S. J., Slack-Davis, J. K., Walters, D. M., Tilghman, R. W., Hershey, E. D., et al. (2011). Inhibition of focal adhesion kinase by PF-562,271 inhibits the growth and metastasis of pancreatic cancer concomitant with altering the tumor microenvironment. *Mol. Cancer Ther.* 10 (11), 2135–2145. doi:10.1158/1535-7163.MCT-11-0261

van de Ven, K., and Borst, J. (2015). Targeting the T-cell co-stimulatory CD27/CD70 pathway in cancer immunotherapy: Rationale and potential. *Immunotherapy* 7 (6), 655–667. doi:10.2217/imt.15.32

Vonderheide, R. H. (2020). CD40 agonist antibodies in cancer immunotherapy. *Annu. Rev. Med.* 71, 47–58. doi:10.1146/annurev-med-062518-045435

Vonderheide, R. H., and Glennie, M. J. (2013). Agonistic CD40 antibodies and cancer therapy. *Clin. Cancer Res.* 19 (5), 1035–1043. doi:10.1158/1078-0432.CCR-12-2064

Wherry, E. J., and Kurachi, M. (2015). Molecular and cellular insights into T cell exhaustion. *Nat. Rev. Immunol.* 15 (8), 486–499. doi:10.1038/nri3862

Winograd, R., Byrne, K. T., Evans, R. A., Odorizzi, P. M., Meyer, A. R. L., Bajor, D. L., et al. (2015). Induction of T-cell immunity overcomes complete resistance to PD-1 and CTLA-4 blockade and improves survival in pancreatic carcinoma. *Cancer Immunol. Res.* 3 (4), 399–411. doi:10.1158/2326-6066.CIR-14-0215

Wu, R., Ohara, R. A., Jo, S., Liu, T. T., Ferris, S. T., Ou, F., et al. (2022). Mechanisms of CD40-dependent cDC1 licensing beyond costimulation. *Nat. Immunol.* 23 (11), 1536–1550. doi:10.1038/s41590-022-01324-w

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The predictive effect of immune therapy and chemotherapy under T cell-related gene prognostic index for Gastric cancer

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Background: Gastric cancer (GC) is one of the most common malignancies in the human digestive tract. CD4+T cells can eliminate tumor cells directly through the mechanism of cytolysis, they can also indirectly attack tumor cells by regulating the tumor TME. A prognostic model of CD4+T cells is urgently needed to improve treatment strategies and explore the specifics of this interaction between CD4+T cells and gastric cancer cells. Methods: The detailed data of GC samples were downloaded from the Cancer Genome Atlas (TCGA), GSE66229, and GSE84437 datasets. $CD4^+$ T cell-related genes were identified to construct a risk-score model by using the Cox regression method and validated with the Gene Expression Omnibus (GEO) dataset. In addition, postoperative pathological tissues of 139 gastric cancer patients were randomly selected for immunohistochemical staining, and their prognostic information were collected for external verification. Immune and molecular characteristics of these samples and their predictive efficacy in immunotherapy and chemotherapy were analysed.

Results: The training set and validation set had consistent results, with GC patients of high PROC and SERPINE1 expression having poorer prognosis. In order to improve their clinical application value, we constructed a risk scoring model and established a high-precision nomogram. Low-risk patients had a better overall survival (OS) than high-risk patients, consistent with the results from the GEO cohort. Furthermore, the risk-score model can predict infiltration of immune cells in the tumor microenvironment of GC, as well as the response of immunotherapy. Correlations between the abundance of immune cells with PROC and SERPINE1 genes were shown in the prognostic model according to the training cohort. Finally, sensitive drugs were identified for patients in different risk subgroup.

Conclusion: The risk model not only provides a basis for better prognosis in GC patients, but also is a potential prognostic indicator to distinguish the molecular and immune characteristics of the tumor, and its response to immune checkpoint inhibitor (ICI) therapy and chemotherapy.

KEYWORDS

T-cell score, T Cell related gene, gastric cancer, immune therapy, immune microenvironment infiltration

Introduction

Gastric cancer (GC) is one of the most common malignancies in the human digestive tract. According to the global cancer statistical analysis data, GC has become the sixth most diagnosed cancer and the third largest cause of cancer death, and thus it is a major global health crisis (Sung et al., 2021). Clinically, GC is mainly treated with surgical resection, chemotherapy, radiotherapy, targeted therapy, or in combination. In spite of this, patients still face many life-threatening issues like recurrence, metastasis, drug resistance, lack of corresponding drug targets, side effects and so on. The 5-year survival rate is as low as 10%–15% (Li Y. et al., 2020; Smyth et al., 2020), hence exploring new and more effective treatment methods have become a hot spot of research.

Immunotherapy is based on the study of the mechanism of immune escape, which can reactivate the anti-tumor immune response and overcome the escape pathway by "manipulating" the immune system (Kennedy and Salama, 2020). In recent years, it has been used to treat malignancies as a new treatment model, and has shown good therapeutic effects. Tumor microenvironment (TME), due to its key role in cancer progression and drug resistance, has become a potential immunotherapy target for many kinds of malignant tumors, including GC (Rihawi et al., 2021).

TME consists of different types of cells, including immune and inflammatory cells (lymphocytes and macrophages), stromal cells (fibroblasts, adipocytes and pericytes), small organelles, RNA, blood and lymphatic vessels, extracellular matrix (extra cellular matrix, ECM) and secreted proteins (Arneth, 2019). Many studies have reported that the occurrence and development of any tumor and the clinical prognosis of patients are closely related to the level of infiltration of tumor immune cells (Hainaut and Plymoth, 2013; Dashti et al., 2016; Kono et al., 2020). As a member of the immune regulatory network, the changes in immune-related genes (IRGs) will cause cascade reactions, thus promoting the progression of tumors (Zhang S. et al., 2020). Studies have shown that IRGs are closely related to the occurrence, development and metastasis of tumors. For example, studies have shown that the overexpression of YKT6 is closely related to the poor prognosis of OSCC, and its low expression is associated with the high level of CD8+T cells in OSCC and the potential response to immunotherapy (Yang et al., 2021). The high expression of HCST is closely related to the level of tumor infiltrating immune cells, especially dendritic cells, and is closely related to the clinicopathology and poor prognosis in renal clear cell carcinoma as well (Zhou et al., 2021). Gene ANGPT1 can increase T Cell infiltration and improve the prognosis of EC patients (Nong et al., 2021). CD4+T cells are a special type of T cells that can target tumor cells in many ways. On the one hand, they can eliminate tumor cells directly through the mechanism of cytolysis, while on the other, they can indirectly attack tumor cells by regulating the tumor TME (Kennedy and Celis, 2008; Melssen and Slingluff, 2017). Additionally, $CD4^+$ T cells can also kill tumor cells by increasing the number and quality of B Cells and CTL (Cytotoxic T Lymphocytes) responses (Bevan, 2004; Castellino and Germain, 2006).

Lately, the emergence of immune checkpoint inhibitor (ICI) represented by PD-1 (programmed cell death protein-1) has brought about a new dawn of treatment for tumor patients. Tumor tissue disables our T cells by expressing programmed death molecules such as PD-1 and B7-1 and subsequently binding them to PD-L1 (programmed death ligand-1) and CTLA-4 (cytotoxic T lymphocyte antigen-4) receptors on said T cells (Sharma and Allison, 2015). By blocking this binding, ICI keeps T cells alert and capable of searching and destroying tumor cells (Ganesh et al., 2019). ICI therapy is effective in the treatment of melanoma and non-small cell lung cancer, for example, and can be as effective as 50% in advanced melanomas (Mushti et al., 2018; Carlino et al., 2021). Hence, seeking for more potential targets for immunotherapy is understandably prioritized in many bleeding-edge studies. At present, there are many studies on immunotherapy for gastric cancer, but most of these studies only focus on one or two genetic biomarkers related to the prognosis of gastric cancer, which is far from enough. At the same time, more evidences are required to detect specific characteristics in GC patients which makes immunotherapy effective.

Therefore, in this study, WGCNA (weighted gene co-expression network analysis) was used to construct a network map of co-expression of immune cells, from which the key marker related to gastric cancer was screened. Then the risk-score model was established and verified in clinical practice in the aspects of prognosis, immune microenvironment and drug sensitivity of patients with gastric cancer. Thus, it provides important insights and strategies for individualized treatment of patients with gastric cancer.

Methods

Datasets collection of GC and preprocessing

The flow chart (Supplementary Figure S1) shows sample utilization at each stage of the statistical analysis. Data such as somatic mutation, gene expression, and corresponding clinical information of gastric cancer (GC) samples were collected for further analysis from The Cancer Genome Atlas (TCGA) database (https://tcga-data.nci.nih.gov/tcga/), which includes detailed information of tumor and para tumor samples. In addition, detailed characteristics and survival time of 433 gastric cancer samples in South Korea (GSE84437) and 300 gastric cancer samples in the ACRG (Asian Cancer Research Group) study (GSE66229) were obtained from the GEO database (https://www.ncbi.nlm.nih.gov/geo/).

Evaluation of immune cell infiltration

CIBERSORT is used to dissect the mixture of data from unknown content and noise. The algorithm can statistically estimate the relative proportions of subtype populations in complex tissue expression profiles and is a useful tool for estimating specific cell abundances in mixed tissues. We used CIBERSORT algorithm to analyze RNA-seq data of GC patients to estimate the relative proportions of various immune-infiltrating

cells and its content. Then, each subtype of immune cells in these GC sample was used as trait data for WGCNA.

Construction of co-expression network

In this study, we have selected 493 genes to construct a weight co-expression network in order to identify the relationship between functional modules and immune cell infiltration in GC patients. According to the Pearson correlation value between paired genes, the expression levels of individual transcripts were transformed into a similarity matrix, and then to an adjacency matrix, as calculated by amn = |cmn| β (cmn = Pearson's correlation between paired genes; amn = adjacency between paired genes). Parameter β can enhance the strong correlation between genes and decrease the weak correlation. When the power of β is set to 6, the adjacency matrix was converted into a topological overlap matrix. To divide genes with similar expression patterns into different modules, we applied a dynamic hybrid cutting method by using a bottom-up algorithm with a minimum block size truncation of 60.

Identification of hub genes

The candidate hub genes were selected according to the module connectivity and immune cell relationship of each gene in hub module. Module connectivity is defined as the absolute value of the Pearson's correlation between genes (Module Membership). The immune cell relevance is defined as the absolute value of the Pearson's correlation between each gene and its trait (Gene Significance). For each gene, we define the MM by the correlation between the gene expression profile and the ME (Module Eigengenes) of a given module. For example, MMturquoise (a) = cor (xa, Eturquoise) measures the correlation between gene "a" and the ME of the turquoise module. If MMturquoise (a) is close to 1 or −1, it is highly connected to the ME of the turquoise module. On the other hand, if MMturquoise (a) is close to 0, the "a" gene is not part of the turquoise module. In this study, we selected the MEblue module that is highly relevant to activated memory $CD4^+$ T cells for further analysis.

Function enrichment analyses

To verify the biological functions of modules, we employed the gene ontology (GO) annotation and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis by clusterProfiler R package (Huang da et al., 2009; Yu et al., 2012). The parameters of clusterProfiler R package were set to default. The thresholds of the GO functions and KEGG pathways were set as p-value <0.05 and qvalue <0.05 respectively. Gene Set Enrichment Analysis (GSEA) is used to identify a set of basically defined genes which exhibit statistical differences between two biological states (Hänzelmann et al., 2013). "c2.cp.kegg.v7.4.symbols.gmt" gene set enrichment analysis was executed according to gene expression, with p-value <0.05 and q-value <0.05 as indicative of statistical significance. In this study, we applied GSEA to find the signaling pathways of core genes by R packages "ggplot2" and "clusterProfiler".

Construction and validation of prognostic model

In our research, we used Lasso-Cox analysis to minimize the risk of over-fitting by using the "glmnet" R package. Multivariate Cox analysis was used to select the candidate genes for establishing a prognostic risk-score in the training cohort. The risk-score was calculated as follows:

$$\text{Risk} - \text{score} = \Sigma i(\text{Expi} * \text{coefi})$$

where Coefi and Expi denote the risk coefficient and expression of each gene, respectively. The cut-off point was determined by the "survminer" package. According to the risk-score, we showed that the survival curve was used for visualization with both training and testing cohorts in the high- or low-risk group by Kaplan-Meier analysis. p values <0.05 were considered to be statistically significant.

In vitro validation and survival analyses

Surgically treated and pathologically confirmed GC patients (n = 139) between 2010 and 2012 from the First Affiliated Hospital of Sun Yat-sen University (FAHSYSU) were randomly selected. The follow-up period was up to January 2019.139 paraffin-embedded GC specimens were obtained from Department of Pathology of FAHSYSU, and their IHC staining and tissue microarrays were conducted using an anti-PROC antibody (1:200; Proteintech, Wuhan, China) and anti-SERPINE1 antibody (1:100; Proteintech, Wuhan, China) as previously described. IHC results were evaluated by two independent investigators blinded to the experiments, and a semiquantitative method was used to score the specimens (Zhai et al., 2018). Positive was defined as samples in which more than 10% of the tumor cells were stained. The staining intensity was defined as follows: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). Negative to weak staining indicated low PROC and SERPINE1 expression, and moderate to strong staining indicated high PROC and SERPINE1 expression. Patient consent and ethical approval from the Institutional Review Board of Seventh Affiliated Hospital of Sun Yat-sen University were obtained for this study. Statistical analyses were performed using SPSS 22.0. The chi-square test was used for numerical data. Survival curves were generated using the Graphpad Prism 8.0.

Assessment of immunotherapy

In further analysis, we showed the correlations between the abundance of immune cells and two genes, specifically PROC and SERPINE1 in the prognostic model according to the training cohort. Beside comparing the prognostic among the risk-score,

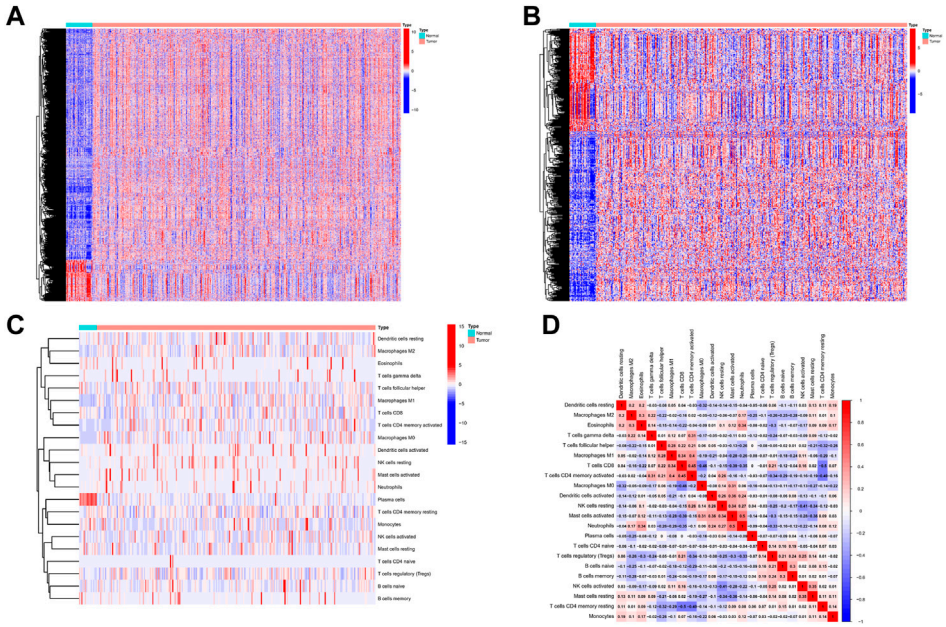

FIGURE 1
Differentially expressed immune-related genes and Analysis of Immune Cell Infiltration in GC. **(A)** Differentially expressed immune-related genes from the intersecting list. **(B)** Differentially expressed genes retrieved from the TCGA cohort. **(C)** The heat map of the immune cells between tumor and normal. **(D)** Correlation analysis of immune cells.

we also utilized the immunophenoscore (IPS) to predict the response of immune checkpoint inhibitors (ICIs) based on the expression of the main component in tumor immunity. On a scale of 0–10 based on representative z-scores of cell type gene expression, IPS was calculated where the immunogenicity was positively correlated to its IPS (Charoentong et al., 2017). The IPSs of patients with GC were derived from The Cancer Immunome Atlas (TCIA) (http://tcia.at/home). The result was obtained using the R package "ggpubr".

Establishment and validation of a nomogram scoring system

According to the independent prognosis outcome, a predictive nomogram was created by incorporating clinical characteristics and risk-score using the R package "rms". In the nomogram scoring system, each variable has a corresponding score and the total score is obtained by adding up the scores of all variables for each sample (Iasonos et al., 2008). The Nomogram was evaluated using ROC curves for 1-, 3-, and 5-year survival rates. The nomogram calibration plots were used to describe the predictive value of the anticipated 1-, 3-, and 5-year survival events in relation to the actual observed outcomes.

Assessment of drug sensitivity

The sensitivity of various drugs was predicted between high-risk and low-risk subgroups using the R package "pRRophetic" (Geeleher et al., 2014). The differences in IC50 between the two groups was compared using Wilcoxon signed-rank test. The results were plotted using the R package "ggplot2".

Statistical analysis

We used R software for statistical analyses (version 4.1.3; https://www. R-project.org). The significance of different immune cell infiltration and gene expression was calculated using Wilcoxon test analysis. Pearson correlation analysis was used to calculate the correlation between genes (Module Membership). When the $p < 0.05$, the result was considered statistically significant.

Results

Differentially expressed immune-related genes and analysis of immune cell infiltration in GC

The process flow chart of our study was clearly illustrated in Supplementary Figure S1. Initially, we obtained the transcriptome profiling data of Stomach adenocarcinoma (STAD) project from the Cancer Genome Atlas (TCGA) database, which included 375 tumor samples and 32 normal samples. Through differential expression genes analysis, there were 8,832 differentially expressed genes retrieved from the TCGA cohort, including 7,497 upregulated genes and 1,335 downregulated genes in the tumor samples when compared with normal samples (Supplementary Table S1A)

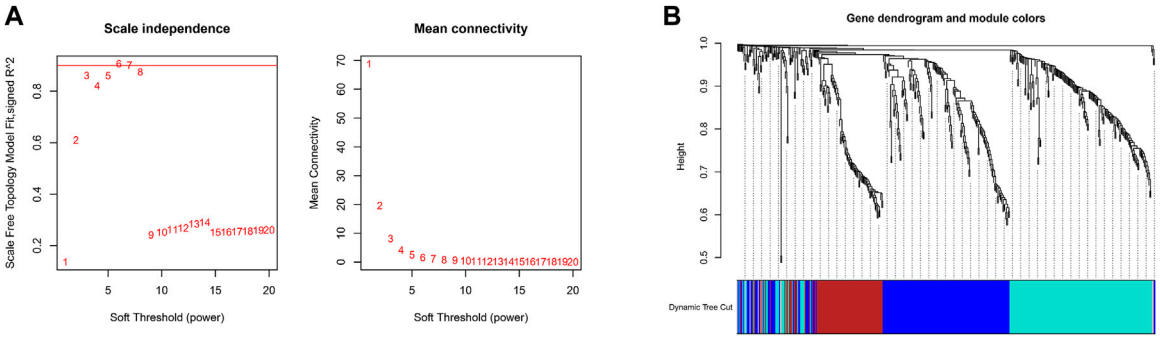

FIGURE 2
Construction Weighted Gene Co-Expression Network. **(A)** The scale-free fit index of the network topology in the WGCNA pipeline. **(B)** Dynamic hybrid cutting to construct hierarchical clustering tree.

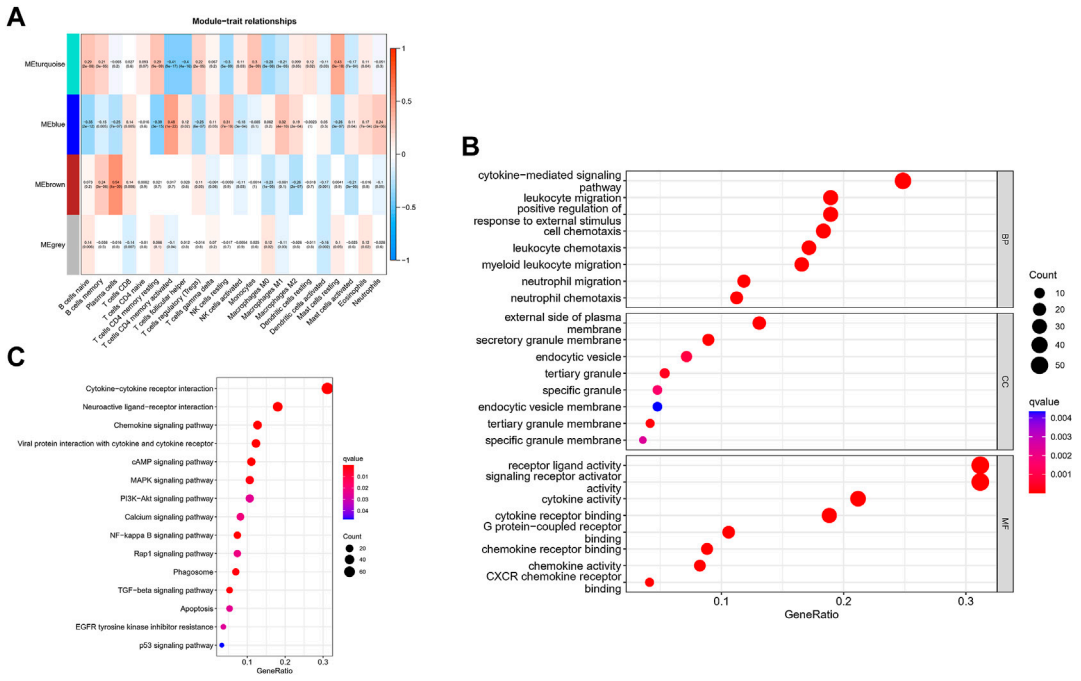

FIGURE 3
Identification of hub modules and Validation of enrichment analysis. **(A)** The correlation between the modules and immune cell. **(B)** The GO enrichment analysis of the MEblue module. **(C)** The ICEGG pathway enrichment analysis of the MEblue module.

(Figure 1A). Meanwhile, we cross-referenced these differential genes with the lists of immune-related genes retrieved from ImmPort and InnateDB. A total of 493 differentially expressed immune-related genes were obtained from the list, which can be broken down into 309 upregulated genes and 184 downregulated genes in the tumor samples when compared with normal samples (Supplementary Table S1B) (Figure 1B). In addition, we analyzed the different cell subtype abundance between GC and normal tissues by using the CIBERSORT algorithm. As a result, we identified the composition of immune cells in each GC sample (Supplementary Figure S2). The immune cells between tumor and normal sample are presented in the heat map (Figure 1C). The correlation analysis of immune cells can be found in Figure 1D. These results suggested that the aforementioned immune cells may be involved in the progression of GC.

Construction weighted gene Co-Expression network

The expression values of these 493 genes were utilized to build a co-expression network of GC using the R package "WGCNA". We clustered the samples of TCGA by estimating average linkage and Pearson's relation values. Soft threshold power analysis

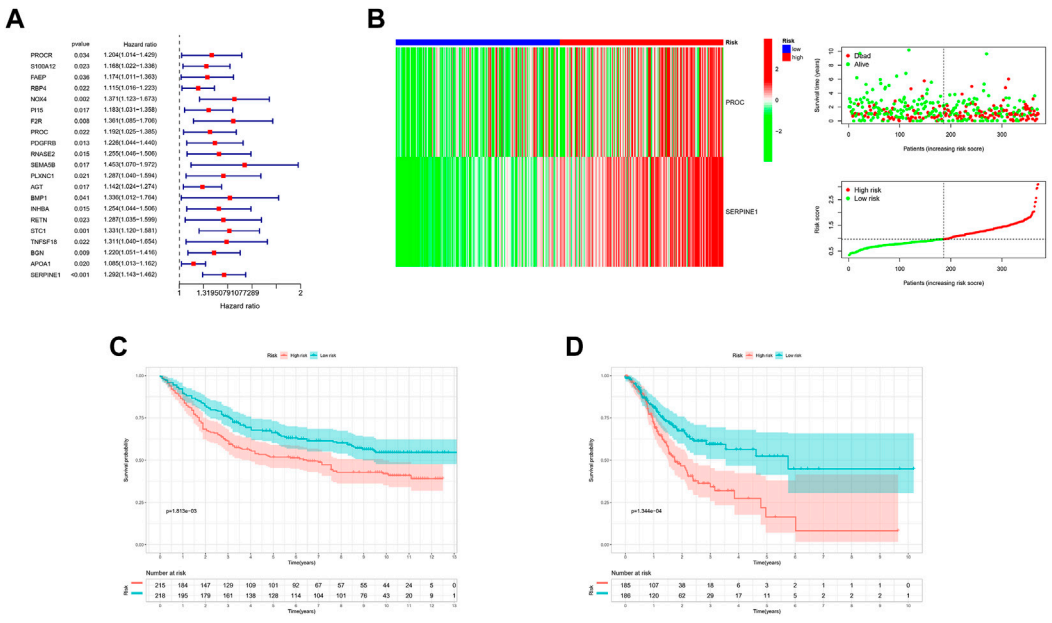

FIGURE 4
Establishment Risk Assessment Model and Survival outcomes in GC. **(A)** 21 CD4+ T cells-related hub genes Through a univariate Cox survival analysis in MEblue module. **(B)** The distribution plot of the Risk score with survival times. **(C, D)** Kaplan-Meier analysis of the OS between the two risk groups in the GSE84437 and TCGA cohort.

revealed the scale-free fit index of the network topology in the WGCNA pipeline. We used $\beta = 6$ as the soft thresholding value to build a scale-free network (Figure 2A). Dynamic hybrid cut was utilized to construct hierarchical clustering tree. Each leaf of the tree shows a single separate gene, in which genes with similar expression data are grouped close together to form a branch of the tree representing a gene module (Figure 2B).

Identification of hub modules and validation of enrichment analysis

The correlation between the modules and immune cell is showed in Figure 3A. Compared with other immune cells, the relationship between activated memory CD4$^+$ T cells and GC is less studied. In this study, the MEblue module was highly related to activated memory CD4$^+$ T cells. All genes in the MEblue module are shown in Supplementary Table S2. Hence, we selected MEblue module and activated memory CD4$^+$ T cells for further analysis. There was a very significant correlation between module membership and gene significance (cor = 0.48, p = 1E-22), suggesting that the 173 genes in the MEblue module tended to be significantly correlated with the infiltration level of activated memory CD4$^+$ T cells. For this reason, the MEblue module was considered to be a GC-related hub module. To illustrate the affected functions of the genes clustered in the MEblue module, GO and KEGG analysis was further performed. Based on the GO enrichment analysis, cellular response to biotic stimulus, cellular response to molecule of bacterial origin, response to lipopolysaccharide, neutrophil chemotaxis, and neutrophil migration were tagged as significantly enriched GO terms (Figure 3B). The KEGG pathway enrichment analysis revealed that most genes were mainly enriched in pathways including leukocyte chemotaxis, myeloid leukocyte migration, cell chemotaxis, and so on. (Figure 3C).

Establishing risk assessment model and survival outcomes in GC

Through a univariate Cox survival analysis, 21 CD4$^+$ T cell-related hub genes among the 173 genes in MEblue module deemed closely correlated with GC patient are set for follow-up analysis, as shown in Figure 4A ($p < 0.05$, log-rank test). To determine the best independent prognostic genes, lasso and multivariate Cox regression analysis for OS was performed among those 21 CD4$^+$ T cell-related hub genes (Supplementary Figure S3). There were only 2 genes (PROC, SERPINE1) that significantly affected the OS of GC patients. Meanwhile, based on the selected CD4+T cells-related hub genes, we constructed a prognostic index for all cancer samples. The risk model was established by multiplying expression data of hub genes by the Cox regression coefficient, such as follows: risk-score = expression level of PROC*(0.193,582) + expression level of SERPINE1*(0.267008). The distribution plot of the risk score demonstrated that the survival times were reduced while the risk-score increased in Figure 4B.

Finally, we used the identified cut-off point to re-distinguish high-risk groups from low-risk groups in the cohort for validation. As illustrated in Figures 4C,D, the patient within low-risk groups had a better OS than the high-risk patients ($p < 0.05$, log-rank test) no matter the TCGA cohort or GEO cohort.

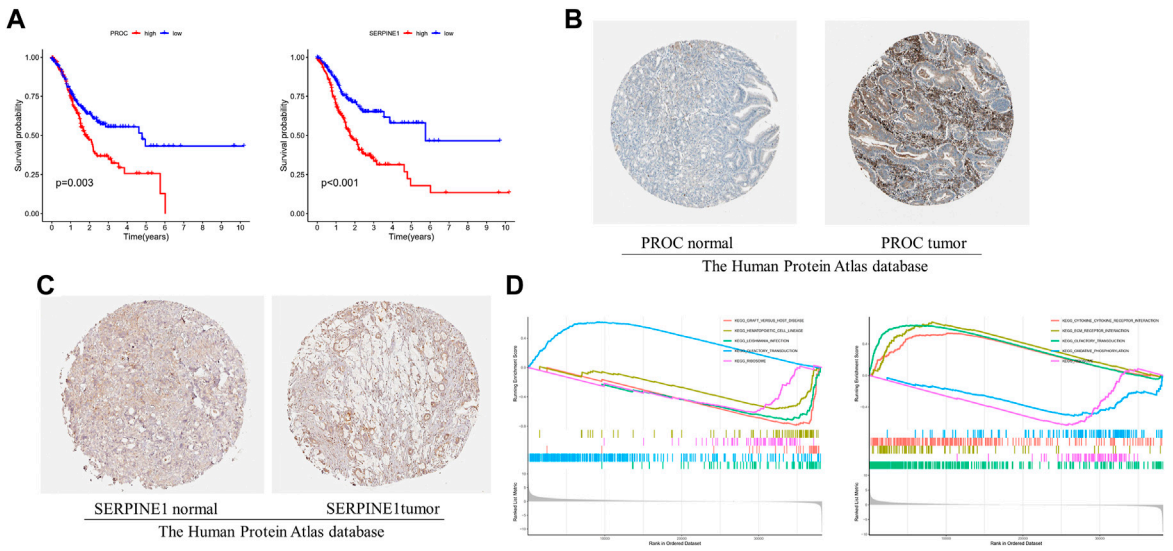

FIGURE 5
The 2 gcnc survival and GSEA of risk model in GC. **(A)** The relationship between gene expression of PROC and SERPINE I and OS. **(B, C)** Protein expression of PROC and SERPINEI in tumor and normal GC tissue. **(D)** The top 5 GSEA terms of PROC and SERPINEI. .

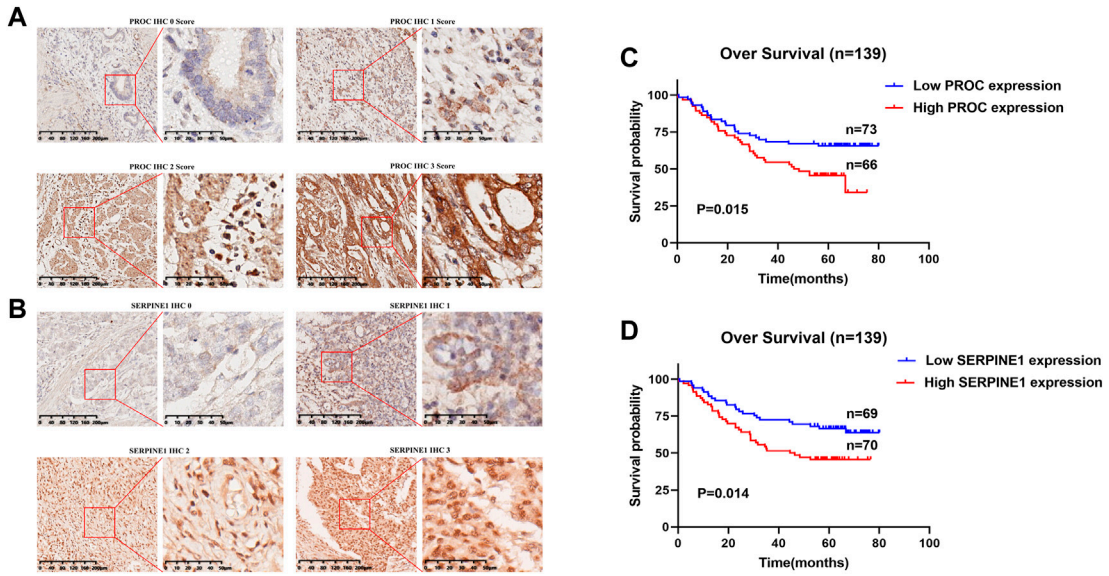

FIGURE 6
In Vitro Validation and Survival Analyses. **(A)** IHC staining of the PROC protein in GC tissues. **(B)** IHC staining of the SERPINEI protein in GC tissues. **(C)** Survival analyses of the PROC protein in GC patients. **(D)** Survival analyses of the SERPINEI protein in GC paticnts.

The 2 gene survival and GSEA of risk model in GC

The GC patient with low-expression of PROC and SERPINE1 had a better OS than the high-expression patients (Figures 5A,B). Consistent with our results, compared to normal tissues, the protein expression of PROC and SERPINE1 were both significantly higher in GC tissues (Figures 5C,D). In addition, GSEA was conducted to identify gene sets associated with the different risk subgroups. The top 5 GSEA terms of PROC and SERPINE1 were illustrated in Figures 5E,F, respectively. The genes of PROC were enriched in graft *versus* host disease, hematopoietic cell lineage, leishmania infection, olfactory transduction, and ribosome. While, the gene of SERPINE1 were enriched in aminoacyl-tRNA biosynthesis, cell cycle, DNA replication, pyrimidine metabolism, and spliceosome. Detailed GSEA results can be viewed in Supplementary Table S3.

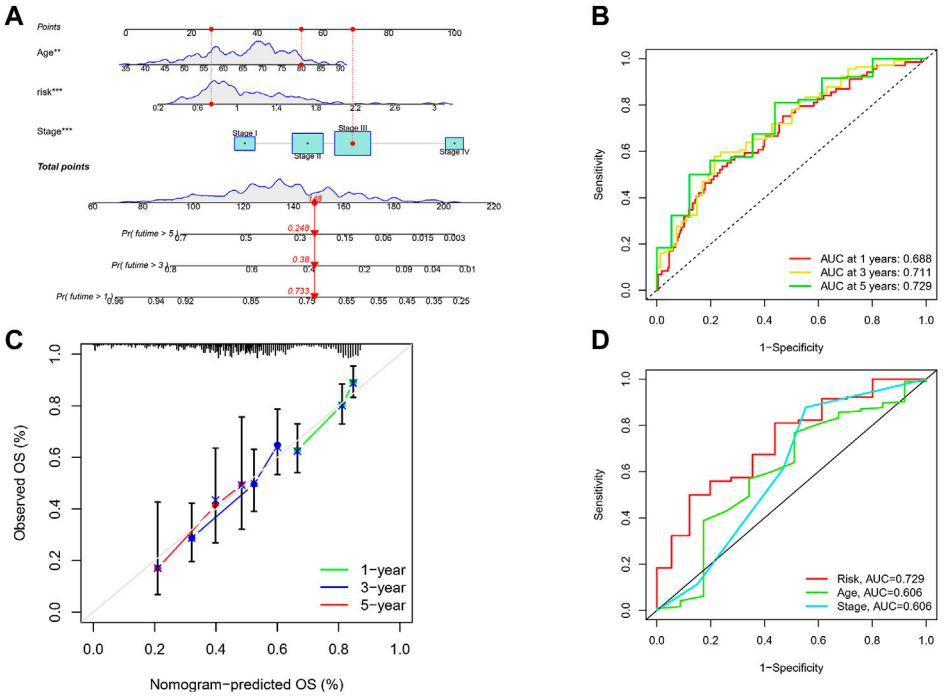

FIGURE 7
Construction of a nomogram to assess survival. **(A)** Nomogram for prcdicting the 1-, 3-, and 5-year OS of GC patients in TCGA cohort. **(B)** ROC curves for predicting the I-, 3-, and 5-year ROC curves in TCGA. **(C)** Calibration curves of the nomogram for predicting of I-, 3-, and 5-year OS in the TCGA. **(D)** The prediction accuracy of nomogram and the TNM stage in the TCGA.

In vitro validation and survival analyses

PROC and SERPINE1 genes were further validated *in vitro*. Immunohistochemistry and western blotting experiments on 139 paired GC patients and normal tissue samples showed that the protein expression of PROC and SERPINE1 were significantly higher in GC samples than in normal tissue samples (Figures 6A,B). Moreover, patients with high expression of PROC and SERPINE1 were found to have worse prognosis in our cohort (Figures 6C,D). These findings were consistent with the results obtained from the GC cohort of TCGA.

Construction of a nomogram to assess survival

Given the inconvenient clinical value of the risk-score in predicting OS in patients with GC, a nomogram incorporating risk-score and clinicopathological characteristics was developed to predict 1-, 3-, and 5-year OS rates in patients with GC (Figure 7A). For TCGA, our AUC studies on the nomogram model revealed a good accuracy for OS at 1-, 3-, and 5- years (Figure 7B). In TCGA, the proposed nomogram performed similarly to an ideal model according to the calibration plots (Figure 7C). Finally, we compared the nomogram's prediction accuracy to that of the TNM stage in the TCGA (Figure 7D). The results illustrated that the nomogram's AUC values were greater than the TNM stage in three cohorts.

Characteristics in clinicopathology and gene mutation in GC

Univariate Cox regression analysis illustrated that risk-score and stage were significantly associated with the prognosis of GC (Figure 8A). Multivariate Cox regression analysis showed that risk-score is an independent prognostic factor after adjusting for other clinicopathologic factors (Figure 8B). We analyzed the gene mutations to further understand the immunological nature in different risk subgroups. We identified the top 20 genes with the highest mutation rates in the high-risk subgroup (Figure 8C) and low-risk subgroup (Figure 8D). The results showed that missense mutation was the most common mutation type. The mutation rates of TTN, TP53, and MUC16 were not only higher than 25% in both groups, but are also the most common in both groups. Additionally, we analyzed the relationship between the risk-score and tumor mutational burden (TMB). The expression of TMB was significantly higher in the low-risk subgroup than in the high-risk subgroup (Figure 8E). Moreover, risk-score was correlated with TMB in gene subtypes ($r = -0.18$, $p < 0.05$), as revealed in Figure 8F. Finally, we found that high TMB was associated with longer survival time, with the effect of higher TMB on prognosis more obvious in the low-risk group. This may be related to the immune cells in the tumor microenvironment (Figures 8G,H).

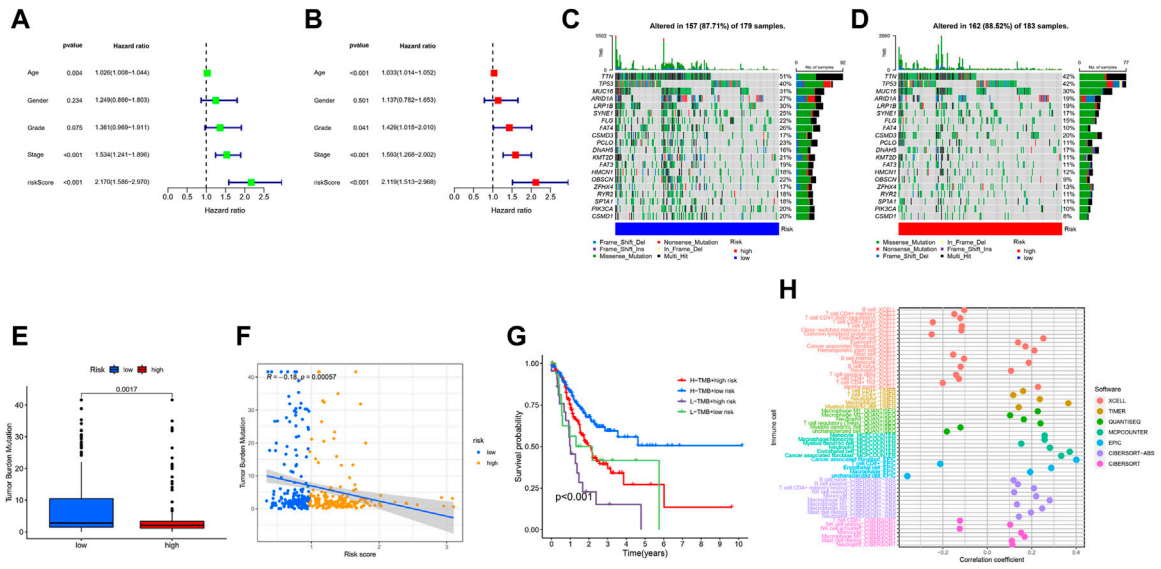

FIGURE 8
Characteristics in clinicopathology and gene mutation in GC. **(A, B)** The univariate and multivariate cox regression analysis in Risk-score subgroups. **(C, D)** Significantly mutated genes in the mutated GC samples of the high and the low risk groups, respectively. Mutated genes (rows, top 20) are ordered by mutation rate; samples (columns) are arranged to emphasize mutual exclusivity among mutations. The right shows mutation percentage, and the top shows the overall number of mutations. The color-coding indicates the mutation type. **(E)** The TMB of two different risk subgroups. **(F)** Relationships between Risk-score and TMB. **(G)** The prognosis of GC in different Risk-score and TMB subgroup. **(H)** The prognosis of GC in different Risk-score and TMB.

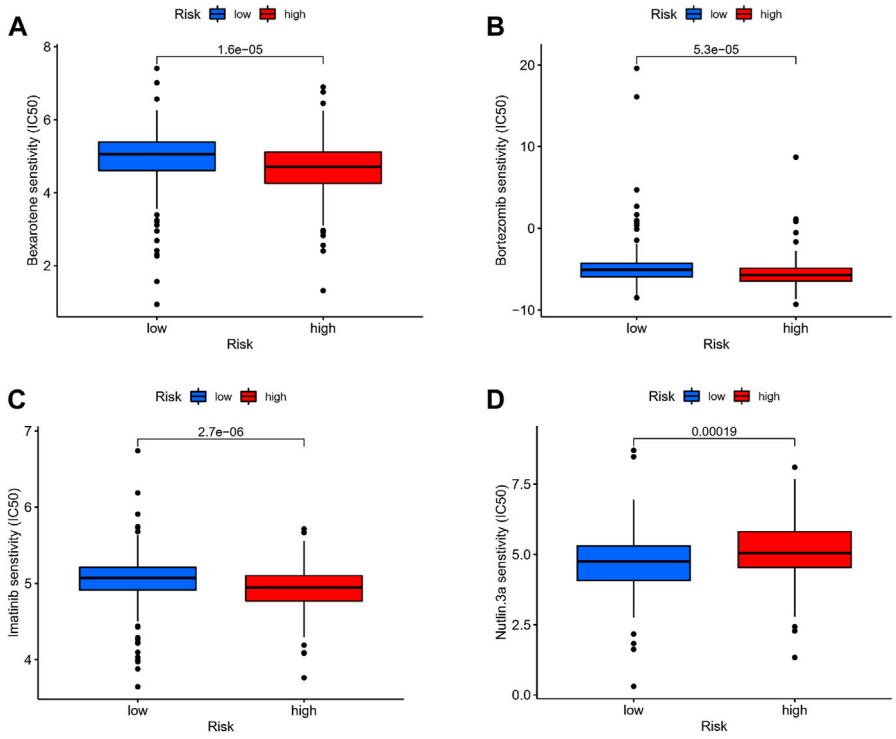

FIGURE 9
Drug sensitivity. **(A–D)** The relationship between different risk groups and the effectiveness of chemotherapy for treating GC in the TCGA cohort.

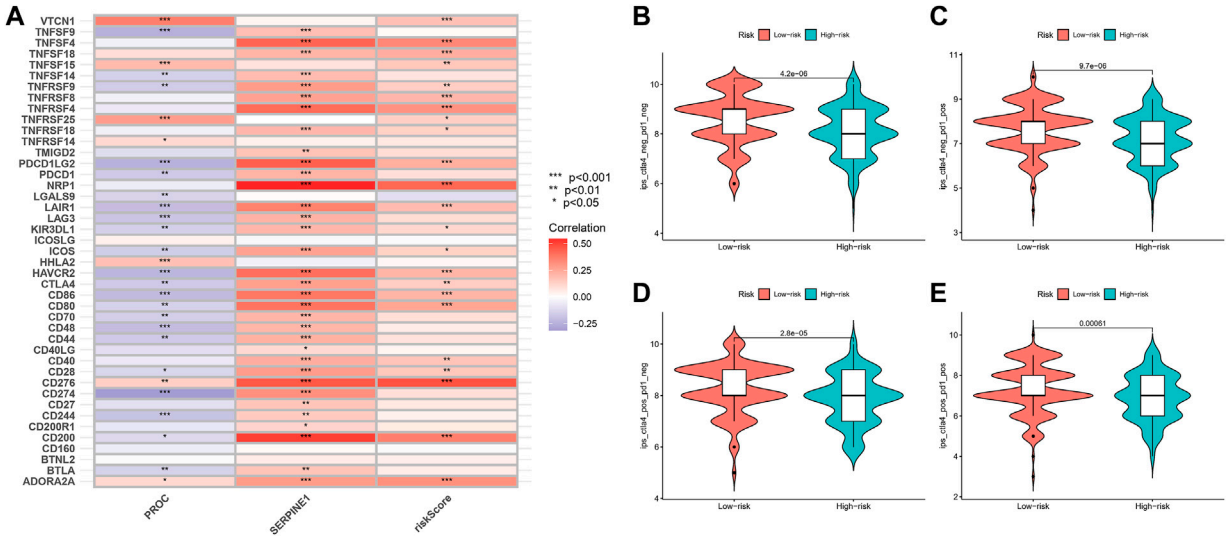

FIGURE 10
Relationship of Risk-Score with Immune checkpoint inhibitors and Immunotherapy prediction. **(A)** The relationship between PROC, SEP,PINEI, Risk-score and immune checkpoints. **(B–E)** The vioplot of the difference expression of Cf LA4 and PD- I between high- and low-risk groups.

Drug sensitivity

We attempted to discover the relationship between different risk groups and the effectiveness of chemotherapy for treating GC in the TCGA cohort. We elucidated that low risk was associated with a lower half maximal inhibitory concentration (IC50) of chemotherapeutics such as Nutlin.3a ($p < 0.05$), whereas high risk was associated with a lower IC50 in drugs such as Bexarotene, Imatinib, and Bortezomib ($p < 0.05$). Therefore, Figures 9A–D revealed that Risk-score acted as a potential predictor for chemo-sensitivity and the detail can be found in Supplementary Figure S4.

Relationship of risk-score with immune checkpoint inhibitors and immunotherapy prediction

In our study, we found an inverse relationship between the number of $CD8^+$ T cells and the degree of risk in the model (Supplementary Figure S5). In addition, we found that these two genes and risk-score are highly related to most immune checkpoints, such as CTLA4 and CD274 (PDL-1) (Figure 10A). As such, it is no surprise that immune checkpoint inhibitors have achieved good clinical results in recent years, even replacing and becoming the main treatment in some malignant tumors.

In order to assess the potential efficacy of clinical immunotherapy in different risk subgroups, we analyzed the correlation between the risk-score and IPS in GC patients to predict the response of ICIs. For IPS, the immune checkpoints were CTLA-4, PD-1 and PD-L1. Therefore, their immune checkpoints were utilized to evaluate the potential of ICI treatment (Figures 10B–E). As a result, we found that the immune response was significantly elevated in the low-risk group, which means that the ICIs will illicit more immunogenicity in the low-risk group. Collectively, these results suggested that the low-risk group will benefit more from immunotherapy due to a better immune response.

Discussion

GC is one of the human malignant tumors with the highest incidence in the world, but because the early symptoms are not obvious, most patients are diagnosed late stage (Nie et al., 2020), and the recurrence rate in these later stages are high, with poor prognosis and high rates of fatality (Galon and Bruni, 2020). In recent years, due to the incredibly complex and heterogeneous tumor microenvironment, more and more attention has been paid to the changes of tumor-associated immune cells. Many reports have pointed out that evading immune surveillance is one of the bases for the occurrence and development of cancer (Griss et al., 2019). Studies have concluded that the type and proportion of immune cell infiltration are closely related to the clinical outcome of patients (Zeng et al., 2019). IRGs participate in the regulation of the immune system and play an important role in the complex regulatory network of tumors. Thus, as our understanding grew, immunotherapy has sprouted as a new treatment method, and is now actively applied to a variety of tumors, with its efficacy verified (Das et al., 2020).

Therefore, in this study, we first analyzed the differentially expressed genes between gastric cancer samples and normal tissues in the TCGA database, and found hundreds of immune-related genes that may be meaningful. At the same time, we analyzed the types of immune cells in GC and explained the close correlation between them through CIBERSORT. Then, we selected the key modules from among them through WGCNA. Our study found that MEblue is a key module of gastric cancer, which contains gene groups related to the level of activated CD4 memory T Cell

infiltration. Currently, it is known that $CD4^+$ T cells have a variety of ways to kill tumor cells, and memory T cells are a subset of $CD4^+$ T cells. However, there are few studies on this kind of cells at present, so exploring the function of activated $CD4^+$ T cells has important research significance. Studies have shown that CD4 memory T cells are associated with better survival in patients with gastric cancer. Such as, Activin-A can enhance the anti-tumor ability of the body by preventing the depletion of CD4 T cells (Morianos et al., 2021). In mice, IL11 can inhibit the anti-tumor effect mediated by $CD4^+$ T cells (Huynh et al., 2021). Activated CD4 T cells can enhance antigen reactivity through NOTCH signaling pathway (Wilkens et al., 2022). GO and KEGG pathway enrichment analysis found that many of the above genes are related to leukocyte chemotaxis and neutrophil migration signal pathways, and these components play an important role in tumor microenvironment and affect tumor occurrence and development. According to study, MMP-9 is mainly secreted by neutrophils. By up-regulating the expression of MMP-9, SETDB1 can promote the occurrence and metastasis of gastric cancer (Shang et al., 2021). IL-17 can promote the proliferation and migration of gastric cancer cells by targeting SLP1 (Xu et al., 2020). CXCL5 can promote the metastasis of gastric cancer by inducing epithelial-mesenchymal transformation and activating neutrophils (Mao et al., 2020). Based on the regression analysis of the above genes, we found that only two genes (PROC and SERPINE1) had significant effects on OS in patients with gastric cancer.

SERPINE1 is an important inhibitor of serine protease and plays a major role in signal transduction, cell adhesion and cell migration (Simone et al., 2014; Zhang Q. et al., 2020). Plasminogen activator inhibitor-1 (PAI-1), the coding product of SERPINE1 gene, is a key regulator of tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA), and is a member of the plasminogen activator system. As a secretory protein, increased activity of PAI-1 increases the risk of metastasis in melanoma (Hanekom et al., 2002). In patients with breast cancer, elevated plasma PAI-1 level is a potential prognostic marker as well (Palmirotta et al., 2009; Ferroni et al., 2014). In GC, LncRNA NKX2-1-AS1 promotes tumor progression by up-regulating the expression of SERPINE1. SERPINE1 is an effective biomarker related to epithelial mesenchymal transformation of GC (Xu et al., 2019). In addition, the expression of PAI-1 is abnormal in ovarian cancer, glioma, renal clear cell carcinoma and other tissues, and is related to the poor prognosis of patients (Liao et al., 2018; Peng et al., 2019; Vachher et al., 2020; Ahluwalia et al., 2021; Ma et al., 2021; Zhao et al., 2021). Furthermore, as a protein encoded by the PROC gene, protein C is known to engage in hemostasis, inflammation, and signal transduction, and it has a protective effect on the endothelial barrier as well. In recent years, protein C has been recognized for its importance in a multitude of diseases, including sepsis, myocardial infarction, and cancer (Griffin et al., 2015). For example, activated protein C cross-activates sphingosine-1-phosphate receptor-1 (S1P1) in cancer thus leading to greater cell-to-cell junction stability, thereby decreasing extravasation (Van Sluis et al., 2009). In fact, addition of activated protein C *in vitro* decreases endothelial adhesion and transmigration of melanoma cells (Bezuhly et al., 2009). Other researches show that activated PROC-PROCR-F2R axis can stimulate the MAPK pathway via activation of epidermal growth factor receptor (EGFR) to promote the progression of breast cancer (Gramling et al., 2010). Additionally, activated protein C promotes anticoagulation of cancer cell microenvironment and upregulates cancer cell migration in ovarian cancer (Althawadi et al., 2015). However, at present, there is not much research on PROC gene in gastric cancer, so we predict that this gene may be associated with gastric cancer.

Our study found that age, risk-score and pathological stage were significantly associated with the prognosis of gastric cancer. Gastric cancer is an age-related disease because the overall survival outcome of elderly cancer patients is poor (Nelen et al., 2018). In the latest edition of AJCC, In et al. found that pathological stage was closely related to the prognosis of gastric cancer (In et al., 2017). Therefore, in order to better improve the accuracy of prognosis prediction, we combined risk-score with age and pathological stage to construct a line map to predict the OS of patients with gastric cancer. The results show that the line chart has high accuracy in OS prediction and is close to the ideal model after correction. And in the test of TCGA data set, the prediction accuracy of line chart risk-score is better than that of TMN stages. The results showed that risk-score was an independent prognostic factor. Chemotherapy is one of the important treatment methods for patients with gastric cancer, which generally needs to be evaluated after several cycles of treatment, so as to judge the efficacy of those chemotherapeutic drugs. In the calculation, we found that there are great differences in drug sensitivity among different risk groups, which may help patients with gastric cancer to select sensitive drugs before chemotherapy and reduce potential trial and error, potentially prolonging survival time.

A large number of studies on various tumors have shown that patients with high TMB tend to have a good survival rate (Cheng et al., 2022). Similarly, our study found that higher TMB was found in patients with low risk-score. The OS of patients with high TMB was significantly longer than that of patients with low TMB. In some literatures, MUC16 mutation is associated with better prognosis and higher TMB in gastric cancer, while TTN mutation is associated with immune checkpoint blockade in solid tumors (Li et al., 2018; Yang et al., 2020). Although TP53 is one of the most common mutant genes, it is not enough to correctly predict the prognosis of patients (Li L. et al., 2020; Wang et al., 2022). Therefore, gastric cancer patients with low risk-score benefit better from immunotherapy. Our study found that risk-score is closely related to many immune cells, such as macrophages, dendritic cells, NK cells, B Cells, T cells and so on, and these cells play an important role in the tumor microenvironment. For example, CD80 and CD86 are markers of M1 macrophages, which can inhibit tumor growth of gastric cancer (Xie et al., 2020). CD40 is a marker of dendritic cells. Its main roles in anti-tumor immune response are phagocytosis of dead tumor cells, capture and presentation of tumor-associated antigens and activation of various T cells, thus stimulating a series of immune responses to kill tumor cells (Murphy and Murphy, 2022). CD48 is a marker of NK cells. Inhibiting the function of NK cells can promote the growth of gastric cancer cells (Guo et al., 2021). Therefore, the TME score can reflect the prognosis of the tumor.

It has been reported that $CD8^+$ T cells are a highly destructive immune effector cell population in anti-tumor response. After activation, they form $CD8^+$ cytotoxic T cells, which circulate to the tumor site to induce immune clearance (Farhood et al., 2019).

Immune checkpoint blockade therapy uses immune checkpoint blocking agents to relieve the inhibitory pressure on $CD8^+$ T cells and restore their sensitivity and killing ability to tumor cells (Darvin et al., 2018). In our study, it was found that $CD8^+$ T Cell infiltration in the low-risk group was significantly higher than that in the high-risk group and had a stronger killing effect on the tumor. To find out which gastric cancer patients are sensitive to immunotherapy, we investigated the sensitivity of two risk subgroups to ICI. In our study, we used IPS to explore risk-score based on TME differences that may reflect the different immune benefits of ICI therapy. IPS is mainly associated with several immune checkpoints, including CTLA4, PD-1 and PD-L1. Our study shows that the low-risk group has a higher potential for ICI response. For clinical trials of immunotherapy, the literature shows that pd-1 has anti-tumor activity and is safe for patients with GC. It has been included in NCNN guidelines as an important treatment for advanced gastric cancer (Ajani et al., 2022). Consistent with our results, mortality was significantly lower in the low-risk group (classified according to Risk-score score). Based on the results of IPS, we found that risk-score can distinguish the different outcomes of individuals receiving immunotherapy. Risk-score as a predictive score is expected to provide a theoretical basis for the selection of ICI treatment in clinical trials. This means that further research can focus on the combination therapy of patients with gastric cancer, and the predictive model may help to accelerate the development of personalized cancer treatment.

In conclusion, our research shows that risk-score plays a very important role in analyzing the clinicopathological features, immune infiltration and clinical prognosis of patients with gastric cancer. In addition, this study also revealed the role of risk-score in predicting the prognosis, and provided a guide to immunotherapy combined with chemotherapy in GC patients. However, the interaction between these model genes and their biological mechanisms needs to be further studied.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of the Seventh Affiliated Hospital of Sun Yat-sen University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization: JC, XL, TM, and TH. Data curation: JC, XL, TM, and HR. Formal analysis: JC, XL, TM, and XW. Data analysis: JC, XL, TM, and KW. Investigation: JC, XL, TM, and WW. Methodology: JC, XL, TM, and ML. Project administration: YH, CZ, and TH. Resources: JC, XL, TM. Original draft: JC, XL, and TM. Writing, review, and editing: JC, XL, TM, and ZK. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2023.1161778/full#supplementary-material

References

Ahluwalia, P., Ahluwalia, M., Mondal, A.K., Sahajpal, N., Kota, V., Rojiani, M.V., et al. (2021). Prognostic and therapeutic implications of extracellular matrix associated gene signature in renal clear cell carcinoma. *Sci. Rep.* 11 (1), 7561. doi:10.1038/s41598-021-86888-7

Ajani, J.A., D'Amico, T.A., Bentrem, D.J., Chao, J., Cooke, D., Corvera, C., et al. (2022). Gastric cancer, version 2.2022, NCCN clinical practice guidelines in oncology. *J. Natl. Compr. Canc Netw.* 20 (2), 167–192. doi:10.6004/jnccn.2022.0008

Althawadi, H., Alfarsi, H., Besbes, S., Mirshahi, S., Ducros, E., Rafii, A., et al. (2015). Activated protein C upregulates ovarian cancer cell migration and promotes unclottability of the cancer cell microenvironment. *Oncol. Rep.* 34 (2), 603–609. doi:10.3892/or.2015.4061

Arneth, B. (2019). Tumor microenvironment. *Med. Kaunas.* 56 (1), 15. doi:10.3390/medicina56010015

Bevan, M.J. (2004). Helping the CD8(+) T-cell response. *Nat. Rev. Immunol.* 4 (8), 595–602. doi:10.1038/nri1413

Bezuhly, M., Cullen, R., Esmon, C.T., Morris, S.F., West, K.A., Johnston, B., et al. (2009). Role of activated protein C and its receptor in inhibition of tumor metastasis. *Blood* 113 (14), 3371–3374. doi:10.1182/blood-2008-05-159434

Carlino, M.S., Larkin, J., and Long, G.V. (2021). Immune checkpoint inhibitors in melanoma. *Lancet* 398 (10304), 1002–1014. doi:10.1016/s0140-6736(21)01206-x

Castellino, F., and Germain, R.N. (2006). Cooperation between CD4+ and CD8+ T cells: When, where, and how. *Annu. Rev. Immunol.* 24, 519–540. doi:10.1146/annurev.immunol.23.021704.115825

Charoentong, P., Finotello, F., Angelova, M., Mayer, C., Efremova, M., Rieder, D., et al. (2017). Pan-cancer immunogenomic analyses reveal genotype-immunophenotype relationships and predictors of response to checkpoint blockade. *Cell Rep.* 18 (1), 248–262. doi:10.1016/j.celrep.2016.12.019

Cheng, Y., Bu, D., Zhang, Q., Sun, R., Lyle, S., Zhao, G., et al. (2022). Genomic and transcriptomic profiling indicates the prognosis significance of mutational signature for TMB-high subtype in Chinese patients with gastric cancer. *J. Adv. Res.* 10, S2090. doi:10.1016/j.jare.2022.10.019

Darvin, P., Toor, S.M., Sasidharan Nair, V., and Elkord, E. (2018). Immune checkpoint inhibitors: Recent progress and potential biomarkers. *Exp. Mol. Med.* 50 (12), 1–11. doi:10.1038/s12276-018-0191-1

Das, S., Camphausen, K., and Shankavaram, U. (2020). Cancer-specific immune prognostic signature in solid tumors and its relation to immune checkpoint therapies. *Cancers (Basel)* 12 (9), 2476. doi:10.3390/cancers12092476

Dashti, A., Ebrahimi, M., Hadjati, J., Memarnejadian, A., and Moazzeni, S.M. (2016). Dendritic cell based immunotherapy using tumor stem cells mediates potent antitumor immune responses. *Cancer Lett.* 374 (1), 175–185. doi:10.1016/j.canlet.2016.01.021

Farhood, B., Najafi, M., and Mortezaee, K. (2019). CD8(+) cytotoxic T lymphocytes in cancer immunotherapy: A review. *J. Cell Physiol.* 234 (6), 8509–8521. doi:10.1002/jcp.27782

Ferroni, P., Roselli, M., Portarena, I., Formica, V., Riondino, S., Farina, F.L.A., et al. (2014). Plasma plasminogen activator inhibitor-1 (PAI-1) levels in breast cancer - relationship with clinical outcome. *Anticancer Res.* 34 (3), 1153–1161.

Galon, J., and Bruni, D. (2020). Tumor immunology and tumor evolution: Intertwined histories. *Immunity* 52 (1), 55–81. doi:10.1016/j.immuni.2019.12.018

Ganesh, K., Stadler, Z.K., Cercek, A., Mendelsohn, R.B., Shia, J., Segal, N.H., et al. (2019). Immunotherapy in colorectal cancer: Rationale, challenges and potential. *Nat. Rev. Gastroenterol. Hepatol.* 16 (6), 361–375. doi:10.1038/s41575-019-0126-x

Geeleher, P., Cox, N., and Huang, R.S. (2014). pRRophetic: an R package for prediction of clinical chemotherapeutic response from tumor gene expression levels. *PLoS One* 9 (9), e107468. doi:10.1371/journal.pone.0107468

Gramling, M.W., Beaulieu, L.M., and Church, F.C. (2010). Activated protein C enhances cell motility of endothelial cells and MDA-MB-231 breast cancer cells by intracellular signal transduction. *Exp. Cell Res.* 316 (3), 314–328. doi:10.1016/j.yexcr.2009.10.024

Griffin, J.H., Zlokovic, B.V., and Mosnier, L.O. (2015). Activated protein C: Biased for translation. *Blood* 125 (19), 2898–2907. doi:10.1182/blood-2015-02-355974

Griss, J., Bauer, W., Wagner, C., Simon, M., Chen, M., Grabmeier-Pfistershammer, K., et al. (2019). B cells sustain inflammation and predict response to immune checkpoint blockade in human melanoma. *Nat. Commun.* 10 (1), 4186. doi:10.1038/s41467-019-12160-2

Guo, S., Huang, C., Han, F., Chen, B., Ding, Y., Zhao, Y., et al. (2021). Gastric cancer mesenchymal stem cells inhibit NK cell function through mTOR signalling to promote tumour growth. *Stem Cells Int.* 2021, 9989790. doi:10.1155/2021/9989790

Hainaut, P., and Plymoth, A. (2013). Targeting the hallmarks of cancer: Towards a rational approach to next-generation cancer therapy. *Curr. Opin. Oncol.* 25 (1), 50–51. doi:10.1097/CCO.0b013e32835b651e

Hanekom, G.S., Stubbings, H.M., and Kidson, S.H. (2002). The active fraction of plasmatic plasminogen activator inhibitor type 1 as a possible indicator of increased risk for metastatic melanoma. *Cancer Detect Prev.* 26 (1), 50–59. doi:10.1016/s0361-090x(02)00002-8

Hänzelmann, S., Castelo, R., and Guinney, J. (2013). Gsva: Gene set variation analysis for microarray and RNA-seq data. *BMC Bioinforma.* 14, 7. doi:10.1186/1471-2105-14-7

Huang da, W., Sherman, B.T., and Lempicki, R.A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* 4 (1), 44–57. doi:10.1038/nprot.2008.211

Huynh, J., Baloyan, D., Chisanga, D., Shi, W., O'Brien, M., Afshar-Sterle, S., et al. (2021). Host IL11 signaling suppresses CD4(+) T cell-mediated antitumor responses to colon cancer in mice. *Cancer Immunol. Res.* 9 (7), 735–747. doi:10.1158/2326-6066.Cir-19-1023

Iasonos, A., Schrag, D., Raj, G.V., and Panageas, K.S. (2008). How to build and interpret a nomogram for cancer prognosis. *J. Clin. Oncol.* 26 (8), 1364–1370. doi:10.1200/jco.2007.12.9791

In, H., Solsky, I., Palis, B., Langdon-Embry, M., Ajani, J., and Sano, T. (2017). Validation of the 8th edition of the AJCC TNM staging system for gastric cancer using the national cancer database. *Ann. Surg. Oncol.* 24 (12), 3683–3691. doi:10.1245/s10434-017-6078-x

Kennedy, L.B., and Salama, A.K.S. (2020). A review of cancer immunotherapy toxicity. *CA Cancer J. Clin.* 70 (2), 86–104. doi:10.3322/caac.21596

Kennedy, R., and Celis, E. (2008). Multiple roles for CD4+ T cells in anti-tumor immune responses. *Immunol. Rev.* 222, 129–144. doi:10.1111/j.1600-065X.2008.00616.x

Kono, K., Nakajima, S., and Mimura, K. (2020). Current status of immune checkpoint inhibitors for gastric cancer. *Gastric Cancer* 23 (4), 565–578. doi:10.1007/s10120-020-01090-4

Li, L., Li, M., and Wang, X. (2020a). Cancer type-dependent correlations between TP53 mutations and antitumor immunity. *DNA Repair (Amst)* 88, 102785. doi:10.1016/j.dnarep.2020.102785

Li, X., Pasche, B., Zhang, W., and Chen, K. (2018). Association of MUC16 mutation with tumor mutation load and outcomes in patients with gastric cancer. *JAMA Oncol.* 4 (12), 1691–1698. doi:10.1001/jamaoncol.2018.2805

Li, Y., Wang, H.C., Wang, J.S., Sun, B., and Li, L.P. (2020b). Chemokine receptor 4 expression is correlated with the occurrence and prognosis of gastric cancer. *FEBS Open Bio* 10 (6), 1149–1161. doi:10.1002/2211-5463.12864

Liao, P., Li, W., Liu, R., Teer, J.K., Xu, B., Zhang, W., et al. (2018). Genome-scale analysis identifies SERPINE1 and SPARC as diagnostic and prognostic biomarkers in gastric cancer. *Onco Targets Ther.* 11, 6969–6980. doi:10.2147/ott.S173934

Ma, Z., Xu, J., Ru, L., and Zhu, W. (2021). Identification of pivotal genes associated with the prognosis of gastric carcinoma through integrated analysis. *Biosci. Rep.* 41 (4), BSR20203676. doi:10.1042/bsr20203676

Mao, Z., Zhang, J., Shi, Y., Li, W., Shi, H., Ji, R., et al. (2020). CXCL5 promotes gastric cancer metastasis by inducing epithelial-mesenchymal transition and activating neutrophils. *Oncogenesis* 9 (7), 63. doi:10.1038/s41389-020-00249-z

Melssen, M., and Slingluff, C.L., Jr. (2017). Vaccines targeting helper T cells for cancer immunotherapy. *Curr. Opin. Immunol.* 47, 85–92. doi:10.1016/j.coi.2017.07.004

Morianos, I., Tsitsopoulou, A., Potaris, K., Valakos, D., Fari, O., Vatsellas, G., et al. (2021). Activin-A impedes the establishment of CD4(+) T cell exhaustion and enhances anti-tumor immunity in the lung. *J. Exp. Clin. Cancer Res.* 40 (1), 295. doi:10.1186/s13046-021-02092-5

Murphy, T.L., and Murphy, K.M. (2022). Dendritic cells in cancer immunology. *Cell Mol. Immunol.* 19 (1), 3–13. doi:10.1038/s41423-021-00741-5

Mushti, S.L., Mulkey, F., and Sridhara, R. (2018). Evaluation of overall response rate and progression-free survival as potential surrogate endpoints for overall survival in immunotherapy trials. *Clin. Cancer Res.* 24 (10), 2268–2275. doi:10.1158/1078-0432.Ccr-17-1902

Nelen, S.D., Bosscha, K., Lemmens, V., Hartgrink, H.H., Verhoeven, R.H.A., de Wilt, J.H.W., et al. (2018). Morbidity and mortality according to age following gastrectomy for gastric cancer. *Br. J. Surg.* 105 (9), 1163–1170. doi:10.1002/bjs.10836

Nie, K., Shi, L., Wen, Y., Pan, J., Li, P., Zheng, Z., et al. (2020). Identification of hub genes correlated with the pathogenesis and prognosis of gastric cancer via bioinformatics methods. *Minerva Med.* 111 (3), 213–225. doi:10.23736/s0026-4806.19.06166-4

Nong, B., Su, T., Jin, M., Huang, J., Huang, A., Fang, D., et al. (2021). Immune-related gene ANGPT1 is an adverse biomarker for endometrial carcinoma. *Transl. Cancer Res.* 10 (6), 2962–2976. doi:10.21037/tcr-21-671

Palmirotta, R., Ferroni, P., Savonarola, A., Martini, F., Ciatti, F., Laudisi, A., et al. (2009). Prognostic value of pre-surgical plasma PAI-1 (plasminogen activator inhibitor-1) levels in breast cancer. *Thromb. Res.* 124 (4), 403–408. doi:10.1016/j.thromres.2009.02.014

Peng, Y., Kajiyama, H., Yuan, H., Nakamura, K., Yoshihara, M., Yokoi, A., et al. (2019). PAI-1 secreted from metastatic ovarian cancer cells triggers the tumor-promoting role of the mesothelium in a feedback loop to accelerate peritoneal dissemination. *Cancer Lett.* 442, 181–192. doi:10.1016/j.canlet.2018.10.027

Rihawi, K., Ricci, A.D., Rizzo, A., Brocchi, S., Marasco, G., Pastore, L.V., et al. (2021). Tumor-associated macrophages and inflammatory microenvironment in gastric cancer: Novel translational implications. *Int. J. Mol. Sci.* 22 (8), 3805. doi:10.3390/ijms22083805

Shang, W., Wang, Y., Liang, X., Li, T., Shao, W., Liu, F., et al. (2021). SETDB1 promotes gastric carcinogenesis and metastasis via upregulation of CCND1 and MMP9 expression. *J. Pathol.* 253 (2), 148–159. doi:10.1002/path.5568

Sharma, P., and Allison, J.P. (2015). The future of immune checkpoint therapy. *Science* 348 (6230), 56–61. doi:10.1126/science.aaa8172

Simone, T.M., Higgins, C.E., Czekay, R.P., Law, B.K., Higgins, S.P., Archambeault, J., et al. (2014). SERPINE1: A molecular switch in the proliferation-migration dichotomy in Wound-"Activated" keratinocytes. *Adv. Wound Care (New Rochelle)* 3 (3), 281–290. doi:10.1089/wound.2013.0512

Smyth, E.C., Nilsson, M., Grabsch, H.I., van Grieken, N.C., and Lordick, F. (2020). Gastric cancer. *Lancet* 396 (10251), 635–648. doi:10.1016/s0140-6736(20)31288-5

Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 71 (3), 209–249. doi:10.3322/caac.21660

Vachher, M., Arora, K., Burman, A., and Kumar, B. (2020). NAMPT, GRN, and SERPINE1 signature as predictor of disease progression and survival in gliomas. *J. Cell Biochem.* 121 (4), 3010–3023. doi:10.1002/jcb.29560

Van Sluis, G.L., Niers, T.M., Esmon, C.T., Tigchelaar, W., Richel, D.J., Buller, H.R., et al. (2009). Endogenous activated protein C limits cancer cell extravasation through sphingosine-1-phosphate receptor 1-mediated vascular endothelial barrier enhancement. *Blood* 114 (9), 1968–1973. doi:10.1182/blood-2009-04-217679

Wang, Z., Strasser, A., and Kelly, G.L. (2022). Should mutant TP53 be targeted for cancer therapy? *Cell Death Differ.* 29 (5), 911–920. doi:10.1038/s41418-022-00962-9

Wilkens, A.B., Fulton, E.C., Pont, M.J., Cole, G.O., Leung, I., Stull, S.M., et al. (2022). NOTCH1 signaling during CD4+ T-cell activation alters transcription factor networks and enhances antigen responsiveness. *Blood* 140 (21), 2261–2275. doi:10.1182/blood.2021015144

Xie, C., Guo, Y., and Lou, S. (2020). LncRNA ANCR promotes invasion and migration of gastric cancer by regulating FoxO1 expression to inhibit macrophage M1 polarization. *Dig. Dis. Sci.* 65 (10), 2863–2872. doi:10.1007/s10620-019-06019-1

Xu, B., Bai, Z., Yin, J., and Zhang, Z. (2019). Global transcriptomic analysis identifies SERPINE1 as a prognostic biomarker associated with epithelial-to-mesenchymal transition in gastric cancer. *PeerJ* 7, e7091. doi:10.7717/peerj.7091

Xu, J., Lv, S., Meng, W., and Zuo, F. (2020). LCN2 mediated by IL-17 affects the proliferation, migration, invasion and cell cycle of gastric cancer cells by targeting SLPI. *Cancer Manag. Res.* 12, 12841–12849. doi:10.2147/cmar.S278902

Yang, Y., Zhang, J., Chen, Y., Xu, R., Zhao, Q., and Guo, W. (2020). MUC4, MUC16, and TTN genes mutation correlated with prognosis, and predicted tumor mutation burden and immunotherapy efficacy in gastric cancer and pan-cancer. *Clin. Transl. Med.* 10 (4), e155. doi:10.1002/ctm2.155

Yang, Z., Yan, G., Zheng, L., Gu, W., Liu, F., Chen, W., et al. (2021). YKT6, as a potential predictor of prognosis and immunotherapy response for oral squamous cell carcinoma, is related to cell invasion, metastasis, and CD8+ T cell infiltration. *Oncoimmunology* 10 (1), 1938890. doi:10.1080/2162402x.2021.1938890

Yu, G., Wang, L.G., Han, Y., and He, Q.Y. (2012). clusterProfiler: an R package for comparing biological themes among gene clusters. *Omics* 16 (5), 284–287. doi:10.1089/omi.2011.0118

Zeng, D., Li, M., Zhou, R., Zhang, J., Sun, H., Shi, M., et al. (2019). Tumor microenvironment characterization in gastric cancer identifies prognostic and immunotherapeutically relevant gene signatures. *Cancer Immunol. Res.* 7 (5), 737–750. doi:10.1158/2326-6066.Cir-18-0436

Zhai, E., Liang, W., Lin, Y., Huang, L., He, X., Cai, S., et al. (2018). HSP70/HSP90-Organizing protein contributes to gastric cancer progression in an autocrine fashion and predicts poor survival in gastric cancer. *Cell Physiol. Biochem.* 47 (2), 879–892. doi:10.1159/000490080

Zhang, Q., Lei, L., and Jing, D. (2020a). Knockdown of SERPINE1 reverses resistance of triple-negative breast cancer to paclitaxel via suppression of VEGFA. *Oncol. Rep.* 44 (5), 1875–1884. doi:10.3892/or.2020.7770

Zhang, S., Zeng, Z., Liu, Y., Huang, J., Long, J., Wang, Y., et al. (2020b). Prognostic landscape of tumor-infiltrating immune cells and immune-related genes in the tumor microenvironment of gastric cancer. *Aging (Albany NY)* 12 (18), 17958–17975. doi:10.18632/aging.103519

Zhao, Q., Xie, J., Xie, J., Zhao, R., Song, C., Wang, H., et al. (2021). Weighted correlation network analysis identifies FN1, COL1A1 and SERPINE1 associated with the progression and prognosis of gastric cancer. *Cancer Biomark.* 31 (1), 59–75. doi:10.3233/cbm-200594

Zhou, Y., Wang, X., Zhang, W., Liu, H., Liu, D., Chen, P., et al. (2021). The immune-related gene HCST as a novel biomarker for the diagnosis and prognosis of clear cell renal cell carcinoma. *Front. Oncol.* 11, 630706. doi:10.3389/fonc.2021.630706

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Modulation of T cell function and survival by the tumor microenvironment

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Cancer immunotherapy is shifting paradigms in cancer care. T cells are an indispensable component of an effective antitumor immunity and durable clinical responses. However, the complexity of the tumor microenvironment (TME), which consists of a wide range of cells that exert positive and negative effects on T cell function and survival, makes achieving robust and durable T cell responses difficult. Additionally, tumor biology, structural and architectural features, intratumoral nutrients and soluble factors, and metabolism impact the quality of the T cell response. We discuss the factors and interactions that modulate T cell function and survive in the TME that affect the overall quality of the antitumor immune response.

KEYWORDS

T cell function, immunosuppression, metabolism, tumor microenvironment, immune checkpoint

Introduction

It is well-established that the immune system plays a major role in tumor control. Presentation of tumor-associated antigens (or lack thereof) can activate the immune system to recognize and eliminate dysfunctional and aberrant tumor cells. From decades of research, we appreciate the complexity of the immune machinery, which involves all aspects of the immune system from innate to adaptive responses, that acts as a defense mechanism against tumor growth. Dedicated and persistent efforts by many investigators have ushered in a new era for the clinical management of cancer and has brought the immune system to the forefront of clinical medicine for patients with cancer. Therapies targeting all components of the immune system, also known as immunotherapy and once significantly underappreciated, are now the standard of care for many patients with different types of tumors. The preponderance of evidence supports the CD8 T cell as the major subset of immune cells that is critically important for antitumor immunity and clinical efficacy in preventing disease progression and extending overall survival.

While the lymphoid compartments generally act as the sites for the initiation and development of antitumor immunity, the TME exerts significant influence on the quality of the immune response (Figure 1). In addition to the TME recruiting immune cells and supporting *de novo* T cell activation, it is generally an immunosuppressive environment. This suppressive environment poses challenges and limits the clinical efficacy of immunotherapy. In this review, we will discuss how T cells function and survive in the TME with an emphasis on CD8 T cells. We will also address challenges in maintaining an

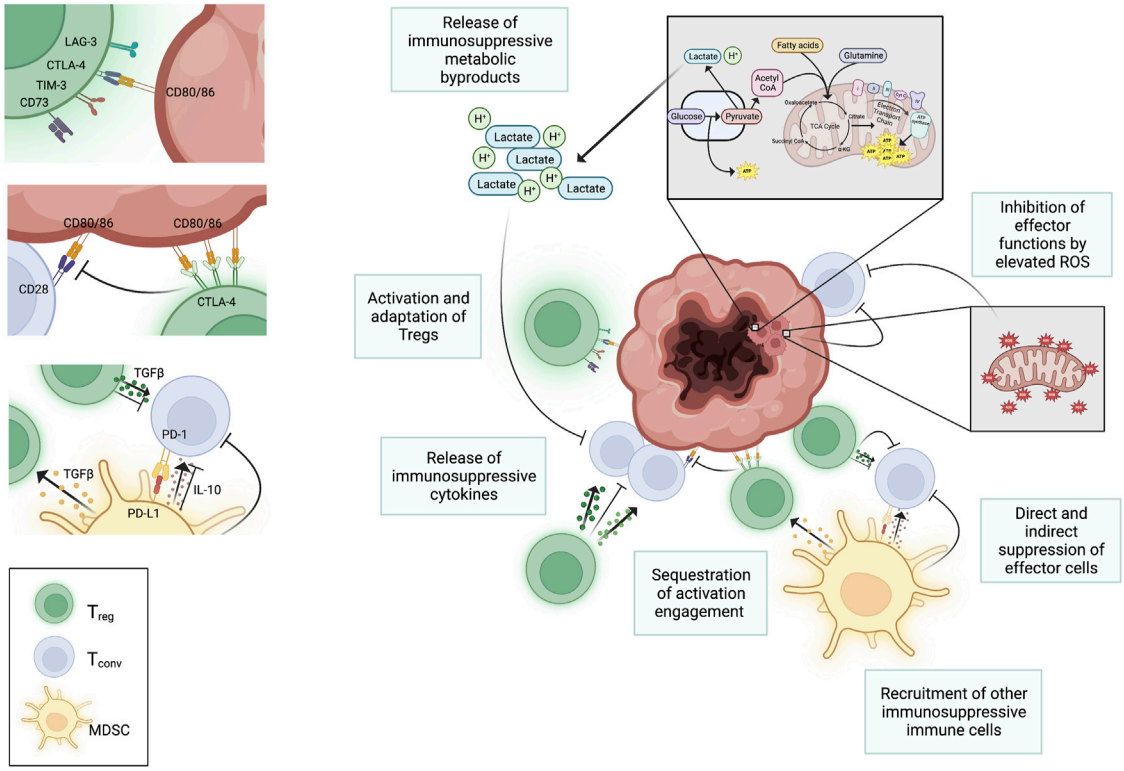

FIGURE 1
Negative regulation of T cell function in the TME. Several factors in the TME negatively impact T cell function. High metabolic activity of tumors metabolism and increased mitochondrial activity results in accumulation of harmful metabolic byproducts in the interstitial space that negatively impacts T cells in the TME. Regulatory immune cells, like T regs and myeloid derived suppressor cells (MDSCs), are recruited to the sites of tumors and suppress proliferation and function of conventional T cells through both the engagement of checkpoint inhibitors (e.g., PD-1 on conventional T cells) and through the release of suppressive cytokines (e.g., TGFβ, IL-10, and IL-35). These cytokines also serve to further activate T regs. Activated T regs upregulate CTLA-4, which selectively binds to CD80 and CD86 on APCs blocking interaction between CD80/86 and CD28 on conventional T cells. This hinders T cell activation. The binding of CTLA-4 to CD80 and CD86 can also initiate the endocytosis of these ligands further decreasing availability for binding to CD28 on conventional T cells. Despite the buildup of metabolic waste that suppress conventional T cells in the TME, regulatory immune cells are better able to tolerate the harsh conditions and can continue to extend their suppressive roles against the anti-tumor effector functions of conventional T cells. Created with Biorender.com.

effective T cell response and opportunities to improve the clinical efficacy of antitumor immunity.

T cell activation and development of effector function

Antigens derived from tumor cells may be first encountered in the interstitial fluid that drains from lymphatic vessels and into lymph nodes. Proteins and peptides are taken up by immune scavengers and specialized antigen presenting cells (APCs) such as dendritic cells (DCs). These APCs process and present the proteins and peptides derived from the tumor cells and the TME on major histocompatibility complexes (MHC) I and II by direct priming or through a process known as cross-presentation (Huang et al., 1994; Wolkers et al., 2001; Hargadon et al., 2006; McDonnell et al., 2010). Although macrophages (Pfeifer et al., 1993; Rock et al., 1993), neutrophils (Tvinnereim et al., 2004), B cells (Ke and Kapp, 1996), and DCs (Chung et al., 2005; McDonnell et al., 2010) can cross-present, the process is most efficiently carried out by DCs expressing CD8a (den Haan et al., 2000; Belz et al., 2004; Allan et al., 2006) and CD103 (Bedoui et al., 2009; del Rio et al., 2007). Recognition of the MHC-peptide complexes by naïve T cells and engagement of costimulatory receptors by ligands on the activated APCs results in a signaling cascade that leads to T cell activation, clonal expansion supported by interleukin 2 (IL-2), and the differentiation into effector T cells (Smith-Garvin et al., 2009). Cancer cells that have metastasized into lymph nodes can also directly present antigens to T cells, but this interaction is generally suboptimal in activating naïve T cells due to the lack of co-stimulatory ligand expression by the cancer cells. The TME can also serve as a site for the initial activation of naïve T cells. In experiments using mice that lack lymph nodes and adoptive transfer of naïve T cells, investigators have shown that the TME can support *de novo* naïve T cell activation and differentiation into effector T cells (Yu et al., 2004; Thompson et al., 2010).

T cell activation is negatively regulated by inhibitory molecules, the most extensively studied being cytotoxic T lymphocyte-associated antigen 4 (CTLA-4). It is constitutively expressed on T cells and binds to the same ligands (CD80 and CD86) as CD28 (Linsley et al., 1991; Azuma et al., 1993; Freeman et al., 1993; Hathcock et al., 1993; Linsley et al., 1994). The affinity

of CTLA-4 for CD80 and CD86 is significantly higher than that of CD28 and the general dogma is that CTLA-4 competes with CD28 to modulate the amplitude of TCR signaling during CD4 and CD8 T cell activation (Linsley et al., 1994; Egen and Allison, 2002; Riley et al., 2002; Schneider et al., 2002; Parry et al., 2005; Schneider et al., 2006). In binding to its ligands, CTLA-4 sequesters CD80 and CD86 and may actively remove the ligands from the surface of APCs further limiting CD28 engagement with the ligands (Qureshi et al., 2011). Aside from its inhibitory effects, CTLA-4 is a downstream transcriptional target of FOXP3 and its expression on regulatory T cells (T regs) enhances the suppressive activity of this subset of T cells (Fontenot et al., 2003; Hori et al., 2003; Wing et al., 2008; Peggs et al., 2009).

Tumor antigens

Recognition of antigens presented by tumor cells is critical for an effective T cell response. Tumors arise from normal cells in which genetic alterations lead to uncontrolled proliferation and transformation into malignant cells (Hanahan and Weinberg, 2000). As such antigens presented by non-viral tumors are of self-proteins or variations thereof. Identification of tumor antigens can be accomplished in several ways. They can be identified using serological analysis of recombinant tumor cDNA expression library (Kawakami et al., 1994) or synthetic peptide libraries (Townsend et al., 1986) by T cell clones. Alternatively, peptides can be isolated from MHC molecules and analyzed by mass spectrometry (Hunt et al., 1992). Over 170 antigenic peptides derived from 60 human antigens that are recognized by T cells have been identified (Renkvist et al., 2001). These antigens are generally classified into four groups: 1) cancer/testis or germline antigens, 2) tissue-specific differentiation proteins, 3) mutated self-proteins, and 4) those derived from post-translational modifications (PTMs) of proteins such as phosphopeptides (Boon and van der Bruggen, 1996; Zarling et al., 2000; Zarling et al., 2006; Mohammed et al., 2008; Depontieu et al., 2009; Zarling et al., 2014). Despite the fact that many tumor antigens are of unmutated self-proteins, CD8 (van der Bruggen et al., 1991; Cox et al., 1994; Van den Eynde et al., 1995) and CD4 (Topalian et al., 1994; Chaux et al., 1999; Pieper et al., 1999) T cell responses against antigens in all classes can be generated, sometimes spontaneously in humans (Bakker et al., 1994; Kawakami et al., 1994; Robbins et al., 1996).

The amount of tumor antigen present can modulate immune surveillance. Suboptimal activation of T cells may occur due to the lack of the necessary costimulatory molecules on tumor cells or because of induction of APCs that are ineffective at stimulating T cells (Gabrilovich et al., 1996; Menetrier-Caux et al., 1998; Munn et al., 2004; Hargadon et al., 2006). In situations where antigen is limited, highly avid CD8 T cells, which require only small amounts of antigen for activation, are needed to generate productive immune responses. Otherwise, those tumors go unnoticed by the immune system (Dudley et al., 1999; Zeh et al., 1999). Several studies in mice (Urban et al., 1986; Shankaran et al., 2001) and in humans (Slingluff et al., 2000; Yamshchikov et al., 2005) have shown that an ongoing immune response can place selective pressure that leads to the emergence of antigenic loss variants of tumors that escape immune recognition. This suggests that some tumor antigens are dispensable and not functionally important for tumor formation and/or metastasis. It also illuminates the need to identify and target functionally relevant tumor antigens for cancer therapy (Hirohashi et al., 2009). Antigens derived from proteins that regulate the malignant properties of tumors are necessary because such antigens are needed to maintain the malignant phenotype of tumors. Additionally targeting such antigens may counteract the emergence of antigenic loss variants of tumors as downregulation of the proteins would not be beneficial to the tumor. Systematic evaluation and careful selection of tumor antigens that are 1) differentially expressed in tumors and minimally expressed in normal cells, 2) functionally relevant to malignancy, and 3) antigenically distinct and immunogenic should provide a collection of peptides that will be more efficacious in clinical trials.

T cell differentiation and function

In the process of differentiation, T cells acquire certain properties and attributes that dictate their functional capacity. In the context of effector function, CD4 T cells can differentiate into several different subsets (Murphy and Stockinger, 2010; Zhu, 2018). T_H1 that produce interferon gamma (IFNγ) and tumor necrosis factor (TNF) to activate macrophages and recruit leukocytes. T_H2 subsets that secrete IL-4 and IL-13 and stimulate mast cells and eosinophils. Other subsets such as T regs hinder effective T cell response (Kim and Cantor, 2014). The differentiation of CD4 T cells into various subsets is heavily influenced by the cytokines present in the microenvironment. Polarization of CD4 T cells into the proinflammatory T_H1 phenotype is mediated by IL-12 and IFNγ (Hsieh et al., 1993; Perez et al., 1995) while T_H2 polarization is regulated by IL-2 and IL-4 (Le Gros et al., 1990; Swain et al., 1990). T_H17 CD4 T cell differentiation is mediated by transforming growth factor beta (TGFβ), IL-6, IL-21 and/or IL-23 (Veldhoen et al., 2006; Zhou et al., 2007). T regs comprise natural and inducible T regs cells that require TGFβ and IL-2 for differentiation (Chen et al., 2003; Davidson et al., 2007; Takeuchi and Nishikawa, 2016). Both natural and inducible T regs can suppress effector function of CD4 and CD8 T cells through direct cellular interactions and through the release of cytokines and growth factors such as IL-10, IL-35, and TGFβ that negatively regulate effector function. For example, IL-10 has been shown to suppress T_H17 effector cytokine production, while TGFβ dampens the effector response by regulating T_H1 and T_H17 differentiation and subsequent T cell tolerance (Li et al., 2007; Chaudhry et al., 2011). These cytokines have also been shown to block antigen presentation and interactions between conventional T cells and APCs in the TME. CD8 T cells differentiate into cytotoxic cells with the ability to directly kill tumor cell targets by producing cytokines and producing lytic granules including granzymes and perforin (Breart et al., 2008; Schietinger et al., 2013). Some subsets of T cells differentiate into circulatory effectors that move through tissues, lymphatics, and blood while others acquire features that lead to their retention within tissues. The acquisition of effector function renders some subsets of T cells terminally differentiated in that those cells become fully committed to a specific differentiation program and will not revert to a pluripotent or a state of plasticity.

In addition to the development of effector T cells, some T cells differentiate into memory cells that are highly efficient at recall

responses. There are several memory T cell subsets that have been described and these subsets can contribute to effective antitumor immunity. Central memory T cells have high proliferative capacity and are generally found in lymphoid tissues while effector memory T cells have limited proliferative capacity but swiftly gain effector function upon restimulation (Mueller et al., 2013). Within this subset are stem-cell memory cells identified in humans that express CD45RA, are multipotent, and have superior proliferative and self-renewing capacity (Gattinoni et al., 2009; Zehn et al., 2022). Tissue resident memory cells express tissue retention markers such as CD69 and CD103 and have a distinct transcriptional profile that is partly dictated by the tissue microenvironment (Schenkel and Masopust, 2014; Amsen et al., 2018; Pao et al., 2018; Szabo et al., 2019). The emergence and differentiation of memory T cells has traditionally been defined by a sufficient period of the absence of cognate antigen that allows for a resting period in memory formation. Memory cells are pluripotent with the ability to maintain their molecular signature and differentiate into effector cells when a specific antigen is subsequently recognized. Within the TME and in chronically infected tissues where antigen is constantly presented, new subsets of T cells with memory-like features that help to sustain the *in situ* immune response are being recognized.

T cell entry and infiltration into the TME

Migration of effector T cells into tumors is orchestrated by interactions between chemokines, chemokine receptors, and adhesion molecules. Egress from the lymph nodes after T cell activation requires downregulation of lymph node homing molecules such as CD62L and CCR7 (Guarda et al., 2007; Groom et al., 2012) and the upregulation of sphingosine 1-phosphate receptor 1 (S1PR1) (Cyster and Schwab, 2012; Baeyens et al., 2015) and other chemokine receptors such as CXCR3 and CXCR6 (Groom and Luster, 2011; Karaki et al., 2021) and tissue-specific adhesion molecules (Mora and von Andrian, 2006; Ferguson and Engelhard, 2010). These changes in adhesion molecule expression permits the egress of activated T cells from lymphoid tissues and migration to peripheral sites. T regs may be more readily recruited into the TME because they express higher levels of various chemokine receptors such as CCR4, CCR5, and CCR8 compared to other T cell types (De Simone et al., 2016; Nishikawa and Koyama, 2021; Sasidharan Nair et al., 2021). Additionally, tumors that secrete CCL1 the ligand for CCR8 preferentially promote the recruitment of T regs into the TME (Barsheshet et al., 2017).

T cells extravasate into tissues at points of low shear stress in postcapillary venules (Choi et al., 2004) and endothelial cells lining vessels at peripheral tissue sites are key mediators of T cell migration and extravasation into the TME. Under ideal conditions, local inflammatory cues in the TME including TNF-α and IL-1 enhance the expression of chemokines and adhesion molecules in the microenvironment and on endothelial cells (Bellone and Calcinotto, 2013; Slaney et al., 2014; Peske et al., 2015). These changes allow binding of effector T cells to the vasculature and their extravasation into the TME. Interaction between selectins (carbohydrate binding molecules) such as E-selectin (CD62E) and P-selectin (CD62P) expressed on endothelial cells and their respective ligands, cutaneous lymphocyte antigen 1 (CLA-1) and P-selectin glycoprotein ligand 1 (PSGL-1), that are expressed on effector T cells results in slow rolling of the T cells on the vascular surface (McEver, 2015; Krummel et al., 2016). The slow rolling further allows chemokine receptors on the T cells to engage chemokines secreted by the endothelial cells. Engagement of the chemokine receptors then leads to changes in the conformational state of adhesion molecules from low affinity to high affinity states that permit stronger adhesive bonds between the T cells and the vasculature to support endothelial transmigration of the effector T cells into the TME (Bellone and Calcinotto, 2013).

Modulation of adhesion molecules, chemokines, and chemokine receptors

Infiltration of T cells into the TME is dependent on the TME generating the appropriate inflammatory environment to active endothelial cells lining tumor associated vessels (Figure 2). However, even with the appropriate inflammatory signals in the TME, the tumor vasculature has been shown to be less responsive to inflammation leading to what has been described as endothelial anergy (Piali et al., 1995; Griffioen et al., 1996a; Weishaupt et al., 2007; Clark et al., 2008; Afanasiev et al., 2013).

Endothelial anergy has been partially attributed to angiogenic factors (Griffioen et al., 1996b; Dirkx et al., 2003) present in the TME that can be secreted by tumor, immune, and stromal cells (Hendry et al., 2016). Angiogenic factors such as vascular endothelial growth factor A (VEGF-A) and basic fibroblast growth factor (bFGF) are upregulated in many tumors partly because of hypoxic conditions in the TME (Keck et al., 1989; Leung et al., 1989). Intercellular adhesion molecule 1 (ICAM-1), ICAM-2 (Griffioen et al., 1996b), and vascular cell adhesion molecule 1 (VCAM-1) (Piali et al., 1995) expression on endothelial cells can be suppressed by VEGF-A and bFGF (Griffioen et al., 1998; Zhang and Issekutz, 2002). This results in the inhibition of T cell adhesion and extravasation. Consistent with this, investigators have shown that blocking antibodies against VEGF enhances infiltration of adoptively transferred T cells that translates into better tumor (Jain, 2005; Shrimali et al., 2010). Similarly, vascular E-selectin expression can be downregulated by angiogenic factors in the TME that results in decreased infiltration of T cells (Tromp et al., 2000; Maksan et al., 2004; Clark et al., 2008; Afanasiev et al., 2013).

The endothelin 1 and endothelin B receptor (ETBR) axis also interfere with ICAM-1 expression and T cell homing. Overexpression of ETBR on ovarian tumor endothelial cells suppresses ICAM-1 expression and induces nitric oxide synthase and nitric oxide (Tsukahara et al., 1994; Buckanovich et al., 2008). Increased nitric oxide in the endothelium also hinders ICAM-1 clustering thereby preventing T cell adhesion and extravasation (Buckanovich et al., 2008). Reactive nitrogen species in the TME promote chemokine nitration that also blocks T cell infiltration (Molon et al., 2011). Consequently, tumors with minimal to no TILs are associated with overexpression of ETBR and blocking ETBR leads to increased expression and clustering of ICAM-1 on endothelial cells and increased T cell infiltration into tumors (Buckanovich et al., 2008). In glioblastomas, vessels expressing ETBR have less T cell infiltrates in the surrounding area in comparison to areas with ETBR negative vasculature (Nakashima

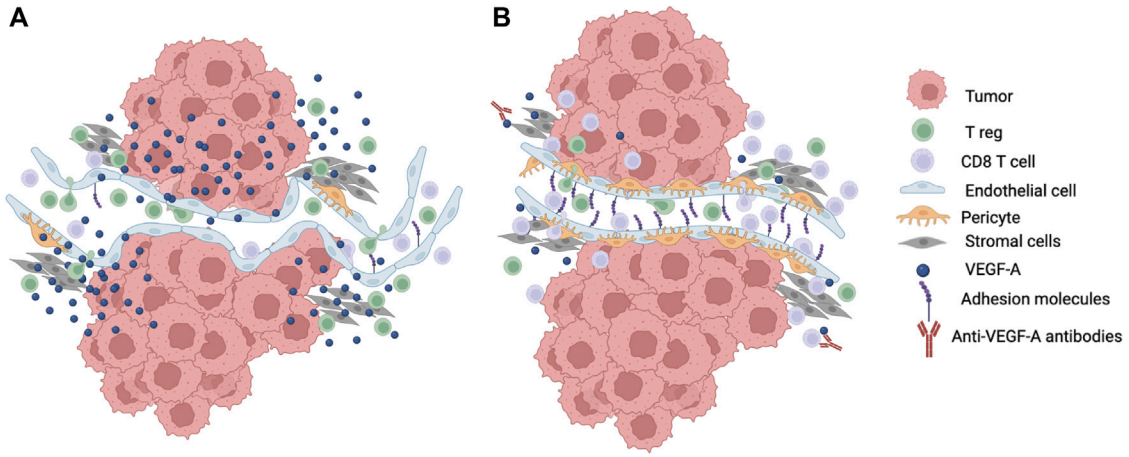

FIGURE 2
Modulation of T cell infiltration into the TME. **(A)** Growth and energetic demands of tumor cells creates a hypoxic TME that drive secretion of angiogenic factors such as VEGF-A into the microenvironment. This leads to neovascularization with new vessel formation that are immature, tortuous, easily compressed, and leaky with reduced pericytes and suboptimal surrounding basement membranes. The abnormal tumor vasculature contributes to the disruption of laminar flow and shear stress. Increased VEGF-A also leads to downregulation of adhesion molecules like ICAM-1 and VCAM-1 that are required for T cell adhesion and extravasation. Tumor cells may also preferentially secrete chemokines that attract T regs. These changes in the microenvironment lead to decreased effector T cell infiltration into the TME. **(B)** Reduction in the amount of VEGF-A in the TME such as through the blockade of VEGF-A using antibodies leads to normalization of the tumor vasculature with better perfusion and increased T cell adhesion to endothelial cells as well as less vascular compression and less mechanical stress that improve effector T cell infiltration into the TME. Created with Biorender.com.

et al., 2016). The association between ETBR expression and TILs may be dependent on the type of tumor as other studies have failed to find an association between T cell infiltration and ETBR expression in oral squamous cell carcinoma (Tanaka et al., 2014).

Structure and mechanics of tumor vasculature

The physical properties of vasculature in the TME also impacts T cells trafficking into tumors. New vessels are formed through a process called neovascularization in response to hypoxia and angiogenic factors secreted by tumor and stromal cells in the TME (Katayama et al., 2019). These vessels are generally immature, disorganized, tortuous, saccular, and leaky. Loose attachment or lack of pericytes, smooth muscle cells, thick or thin basement membrane around the vessels, increased fenestrations, and wide intercellular junctions between endothelial cells contribute to leakiness and abnormal tumor vasculature (Jain, 2005; Chung et al., 2010; Carmeliet and Jain, 2011; Farnsworth et al., 2014). Furthermore, tumor cells can mimic endothelial cells (Hendrix et al., 2003) creating pseudo-vessels that lack the properties of normal vasculature. The leakiness of the vasculature and compression of vessels by surrounding tumors also increase interstitial pressure in the microenvironment that disrupts perfusion thereby perpetuating a hypoxic environment (Heldin et al., 2004; Jain, 2013; Jain et al., 2014). The abnormal formation of vessels in the TME causes discontinuous laminar blood flow and points of low and no shear stress (Hendry et al., 2016). Additionally, endothelial cell polarity and the expression of adhesion molecules on endothelial cells is regulated in part by shear stress (Carmeliet and Jain, 2011). T cell rolling on endothelial cells requires a certain threshold of shear stress (Finger et al., 1996; Lawrence et al., 1997). Consequently, disruptions in shear stress negatively impact T cell rolling, adhesion, and extravasation.

Immune cell niches in the TME that support T cell function

Chemokines, cytokines, tumor vasculature, and environmental cues direct T cell migration and localization within the TME. Heterogeneous distribution of these factors likely lead to discrete areas of immune cell aggregates within the TME. Inflammatory signals from factors such as TNF-α can lead to the remodeling of tumor vasculature and the upregulation of adhesion molecules, chemokines, and chemokine receptors that attract T cells and other immune cells into those perivascular areas. The cascade of events results in the formation of discrete clusters of immune and stromal cells that contribute to the localized immune response in the TME.

Tertiary lymphoid structures

The presence of aggregates of immune cells in inflamed non-lymphoid tissues has been noted for many years. These aggregates are inducible, transient, and unencapsulated structures that range from simple clusters of lymphoid cells to well-organized immune structures with compartmentalized B and T cell areas intertwined in a stromal network and vasculature that closely resemble secondary lymphoid organs (SLOs) (Schumacher and Thommen, 2022). The well-organized aggregates are known as tertiary lymphoid structures (TLSs). While there is a range in the composition and organization

of immune aggregates in inflamed non-lymphoid tissues, the current accepted definition of TLSs is the presence organized lymphocyte and myeloid cell aggregates in inflamed tissues with discernable B and T cell-like areas within these structures (Ruddle, 1999). TLSs have been identified in several diseases and conditions including infection, cancer, autoimmunity, and transplanted tissues (Hjelmstrom, 2001; Aloisi and Pujol-Borrell, 2006; Sato et al., 2009; Pitzalis et al., 2014; Hiraoka et al., 2015). They are thought to form through a process akin to that which governs the ontogeny of SLOs (Ruddle, 1999) and CXCL13-expressing effector CD8 T cells seem to play a major role in the induction of TLS formation (Workel et al., 2019; Rodriguez et al., 2021). TLSs are also functionally similar to SLOs with compartmentalized B cell-rich and T cell-rich areas and evidence of T and B cell activation and differentiation (Murakami et al., 1999; Moyron-Quiroz et al., 2006) and have been implicated in perpetuating autoimmune disease and allograft rejection as well as in helping to control infection and tumor growth (Hjelmstrom, 2001; Aloisi and Pujol-Borrell, 2006; Sato et al., 2009; Pitzalis et al., 2014; Hiraoka et al., 2015).

The functional significance of TLSs has largely been inferred from phenotypic evidence of T cell function in TLSs and correlative studies linking the presence of TLSs with increased T cell infiltration, tumor control, and prognosis in human patients. In melanoma tumor samples, T cell activation markers are increased in TLSs (Helmink et al., 2020). Naïve T cells have also been observed to proliferate within TLSs (McMahon et al., 2005; Lee et al., 2006). Germinal centers and upregulation of AICD, BCL6, and other markers of germinal center formation, somatic hypermutation, and class switching have also been identified in TLSs (Armengol et al., 2001; Sims et al., 2001; Nielsen and Nelson, 2012; Germain et al., 2014).

In addition to the correlative analyses, studies in mice lacking SLOs have provided data that suggest functional relevance of TLSs in the TME and in infection. In such models, induction of TLS formation has been associated with the presence of antigen-specific T cells and some level of disease control. For example, induction of TLS by lymphotoxin-α leads to T cell effector function and tumor control in mice that lack lymph nodes (Schrama et al., 2001; Schrama et al., 2008). Furthermore, mice lacking spleen, lymph nodes, and Peyer's patches are capable of developing antigen-specific effector and memory T cells that are associated with inducible bronchus-associated lymphoid tissues, a type of TLS in the lung, when infected with influenza (Moyron-Quiroz et al., 2004; Moyron-Quiroz et al., 2006; Rangel-Moreno et al., 2007).

In support of the evidence suggesting the functional capacity and relevance of TLSs, several studies have shown an association between the presence of TLSs and overall survival and/or clinical responsiveness to different forms of cancer immunotherapy. While some studies have reported no effect or a negative impact of TLSs on immune response against cancer (Bento et al., 2015; Figenschau et al., 2015; Finkin et al., 2015; Joshi et al., 2015), there is strong and growing evidence to suggest that the presence of TLSs has a positive predictive value for patients. In several different tumor types, the presence of TLSs has been associated with responsiveness to immune checkpoint inhibition and better survival (Cabrita et al., 2020; Helmink et al., 2020; Petitprez et al., 2020). It is unclear whether immune checkpoint blockade drives *de novo* TLS formation; however, it can enhance the number of TLSs within tumors (Cottrell et al., 2018; van Dijk et al., 2020). On the other hand, vaccines and neoadjuvant chemotherapy can induce TLS formation in several tumor types (Lutz et al., 2014; Maldonado et al., 2014; Remark et al., 2016; Morcrette et al., 2019). In aggregate, the data strongly suggest that TLSs, immune niches within the TME, contribute to T cell function and the antitumor immune response.

T cell exhaustion

As effector CD8 T cells engage tumor cells in the TME, they become dysfunctional over time and lose the ability to produce effector molecules such as cytokines and cytotoxic granules (Barber et al., 2006; Wherry, 2011). This state results from continued and persistent antigenic stimulation and is referred to as T cell exhaustion. There are two main subsets of exhausted T cells (terminally differentiated exhausted and stem-like, also known as progenitor exhausted cells) that have been described (Zehn et al., 2022). The terminally differentiated exhausted T cells express many inhibitory receptors collectively known as immune checkpoint molecules (Virgin et al., 2009). T cells in this state have limited to no capacity to kill tumor cells thereby hampering the quality of the immune response (Bengsch et al., 2016). Loss of effector function is progressive with the loss of the ability to produce IL-2, reduced proliferative capacity, and ineffective target cell killing occurring first (Wherry, 2011). This is followed by the decreased capacity to produce other cytokines such as TNF-α and IFNγ and then cell death.

The programmed cell death 1 (PD-1) molecule is the major mediator of T cell exhaustion. It is upregulated on T cells upon T cell activation and the expression of PD-1 on the cell surface can be detected very early in the T cell activation phase (Ahn et al., 2018). The induction of PD-1 expression is dependent on TCR-antigen/MHC interaction and inhibition of PD-1 signaling in the early T cell activation phase results in enhanced mTOR signaling and increased production of effector molecules (Ahn et al., 2018). Repeated antigen exposure and T cell stimulation leads to high levels and persistent PD-1 expression on activated T cells. T regs constitutively express high levels of PD-1 and PD-1 expression serves to promote proliferation of T regs (Francisco et al., 2009). PD-1 has two ligands, PD-L1 that is expressed on a wide variety of cells including many cancer cells, and PD-L2 that is expressed mainly on DCs (Keir et al., 2008). Engagement of PD-1 on effector T cells leads to diminished proliferation and cytokine production (Freeman et al., 2000; Carter et al., 2002; Brown et al., 2003). Blocking the interaction between PD-1 and its ligands results in the reinvigoration of T cells as evidenced by an expansion of effector cells and the recovery of cytokine production and cytolytic ability (Barber et al., 2006; Huang et al., 2017). PD-1 also regulates metabolism in T cells. Engagement of the PD-1 pathway plays a role in metabolic reprogramming of T cells by promoting fatty acid oxidation and limiting glycolysis, amino acid metabolism, and glutaminolysis (Parry et al., 2005; Patsoukis et al., 2015). Since effector T cells depend on glycolysis to maintain proliferation and cytokines, this change in metabolism compromises effector function.

As effector cells progress through the process of exhaustion, additional inhibitory molecules such as lymphocyte activation gene 3 (LAG-3), T cell immunoglobulin and mucin-domain containing-3

(TIM-3), and T cell immunoglobulin and ITIM domain (TIGIT) are upregulated. LAG-3 is a type I transmembrane protein that is structurally like CD4 (Triebel et al., 1990) and binds to MHC class II (Baixeras et al., 1992), galectin-3 (Kouo et al., 2015), α-synuclein (Mao et al., 2016), LSECtin (Xu et al., 2014a), and fibrinogen-like protein 1 (Wang et al., 2019). It is expressed on activated T cells and other immune cells and exists in transmembrane and soluble forms (Li et al., 2004). It colocalizes with CD3 in immune synapses to regulate TCR and calcium channel signaling to leads to inhibition of proliferation and cytokine production (Maçon-Lemaître and Triebel, 2005; Guy et al., 2022). TIM-3 is expressed on IFNγ-secreting T cells and engagement of TIM-3 with its ligands inhibits T cell function and induces apoptosis by regulating intracellular calcium flux (Zhu et al., 2005; Kang et al., 2015; Wolf et al., 2020). TIGIT is mainly expressed on T and NK cells and has two known ligands, CD155 and CD112 (Yu et al., 2009). TIGIT suppresses immune function by competing with costimulatory molecules CD266 and CD96 (Dougall et al., 2017). Interaction with CD155 on DCs results in IL-10 production that reduces effector function (Yu et al., 2009). It also negatively regulates T cell proliferation and inhibits IFNγ production (Lozano et al., 2012). Knockdown of TIGIT using short hairpin RNA restores T cell proliferation and cytokine production (Lozano et al., 2012). Many of these inhibitory molecules (ligands and receptors) are upregulated in the TME and are associated with poor prognosis (Zhang et al., 2017).

Given the negative effects of immune checkpoint molecules on effector T cell function and negative prognosis for patients with cancer, immune checkpoint molecules are being targeted for immunotherapy using different approaches that block the negative regulation these molecules exert on T cell function. Blockade of the inhibitory pathways in T cells leads to recovery of some, if not all, of the effector T cell function and has been associated with improved clinical response and outcome for patients with cancer (Brahmer et al., 2012; Topalian et al., 2012; Lipson et al., 2013). Inihibitors of mmune checkpoint molecules such as blocking antibodies against PD-1 and CTLA-4 are currently FDA approved and used routinely for the clinical management of cancer as monotherapeutic agents or in combination with other treatments (Twomey and Zhang, 2021) and many more are currently in clinical trials.

Stem-like CD8 T cells

The second subset of exhausted CD8 T cells are known as stem-like CD8 T cells or progenitor exhausted cells and differentiate in chronic disease states such as chronic viral infections and cancer (He et al., 2016; Im et al., 2016; Utzschneider et al., 2016). These cells have pluripotent and memory-like features and are present in lymphoid and inflamed tissues as well as in the TME (Utzschneider et al., 2016; Im et al., 2020). They express PD-1, TCF-1, CD73, co-stimulatory molecules, low levels of CD127 and generally lack expression of other inhibitory molecules such as TIM-3 (Im et al., 2016). They have the capacity for self-renewal as well as the ability to differentiate into effector cells. As such, stem-like CD8 T cells sustain the terminally differentiated effector population in chronic infection and tumors and are essential for the maintenance of the effector response. Inhibition of the PD-1 pathway using blocking antibodies against PD-1 or its ligand, PD-L1, results in expansion of the stem-like CD8 T cells and differentiation into effector cells (Im et al., 2016; Hudson et al., 2019). This proliferative burst requires CD28 co-stimulation and stem-like CD8 T cells lacking CD28 fail to proliferate (Kamphorst et al., 2017). The expansion and generation of new effectors from stem-like CD8 T cells after PD-1 inhibition underscores the immunological and positive clinical responses seen in cancer patients treated with immune checkpoint inhibitors.

Of note, the T cell exhaustion molecular program, marked by upregulation of TOX and NR4A1, is maintained in stem-like CD8 T cells and is imprinted onto effector progeny derived from the stem-like cells (Utzschneider et al., 2013; Martinez et al., 2015; Pauken et al., 2016; Alfei et al., 2019; Khan et al., 2019; Scott et al., 2019; Seo et al., 2019; Yao et al., 2019). This programming cannot be overridden by using immune checkpoint inhibition to drive the differentiation of new effector progeny (Utzschneider et al., 2013; Pauken et al., 2016; Ghoneim et al., 2017; Philip et al., 2017). However, recent studies have shown that the combination of IL-2 and inhibition of the PD-1 pathway modifies the transcriptional program of effector progeny derived from stem-like CD8 T cells (Codarri Deak et al., 2022; Hashimoto et al., 2022). This effect is mediated by the high affinity IL-2 receptor, CD25. Stem-like CD8 T cells transferred into a new host that receive IL-2 stimulation in the presence of PD-1 blockade produce progeny with a molecular profile distinct from the exhaustion pathway with downregulation of Tox, Nr4a1, other genes in the exhaustion pathway, and several inhibitory receptors. The molecular profile of effector cells that differentiate in this context resemble highly functional effector cells that are generated from acute viral infections. Thus, the cytokine milieu during reactivation and expansion of stem-like CD8 T cells in lymphoid tissues and the TME can significantly impact the quality of the immune response.

Metabolism and nutrients in the TME

Several metabolic pathways regulate T cell differentiation and effector function. Resting T cells use oxidative phosphorylation to fuel ATP production. Once activated, downstream signaling pathway feeding through the P13K/AKT/mTOR pathway promotes Myc-dependent metabolic reprogramming to glycolysis (Araki et al., 2009; MacIver et al., 2011; Wang et al., 2011; Verbist et al., 2016). This switch in metabolism is required for the high energy demands for T cell expansion and the production of effector molecules such as IL-2, IFNγ, and granzyme B (Cham et al., 2008; Chang et al., 2015). Short-lived effector T cells such as terminally differentiated cells also depend on glycolysis. In contrast, T regs and memory T cells depend on oxidative phosphorylation and fatty acid metabolism. In T regs, fatty acid oxidation and oxidative phosphorylation promote immunosuppressive activity and these metabolic pathways are driven by FOXP3 (Michalek et al., 2011; Angelin et al., 2017). In fact, the expression of the enzymes required for glycolysis can be suppressed by FOXP3 (Angelin et al., 2017). T regs also upregulate molecules involved in fatty acid transport such as CD36 and sterol-regulatory-element binding protein that supports the use of lipids in the TME for proliferation and

immunosuppressive activity (Wang et al., 2020; Lim et al., 2021). Consistent with this, an accumulation of T regs has been noted in tumors that produce fatty acids such as gastric cancer cells with RHOA Y42 mutation (Kumagai et al., 2020). In TMEs with low levels of fatty acids, T regs upregulate fatty acid binding proteins to scavenge for nutrients to support the immunosuppressive function (Field et al., 2020).

High metabolic activity of tumor cells that maintains growth depletes nutrients in the TME leaving limited supply of glucose for activated T cells to maintain proliferation and adequate effector function (Kishton et al., 2017). Additionally, the abnormal vasculature in the TME results in heterogenous distribution of nutrients that further compromises effector T cell metabolism. Limited nutrients in the TME, in addition to chronic stimulation, result in changes in the metabolic profile of effector T cells that lead to reduced glucose uptake, increased expression of reactive oxygen species, loss of mitochondrial mass, and reduced effector function in T cells (Scharping et al., 2016; Siska et al., 2017). To adapt to the limited nutrients in the TME, activated T cells can incorporate other biomolecules like glutamine and amino acids into mitochondrial respiration to support proliferation and effector function (Michalek et al., 2011; Sinclair et al., 2013; Johnson et al., 2018). T cells can breakdown glutamine into a-ketoglutarate and other intermediates that feed into the TCA cycle (Johnson et al., 2016). However, some cancer cells do not express the enzymes required for glutamine synthesis (Cluntun et al., 2017) and therefore also compete with T cells for exogenous glutamine. Activated T cells can also utilize endogenous and exogenous lipids for β-oxidation to maintain proliferation and function (MacIver et al., 2013; Howie et al., 2017). However, the use of other metabolic pathways may not be as effective as glycolysis in promoting effector function in the TME as suggested by studies showing insufficient production of cytokines when T cells are forced to use other pathways such as oxidative phosphorylation (Chang et al., 2013). Other molecules such as methionine and arginine can also be depleted by cancer cells and myeloid-derived suppressor cells in the TME that negatively impact T cell proliferation effector function (Rodriguez et al., 2003; Bian et al., 2020; Grzywa et al., 2020).

Metabolic byproducts produced in the TME can also impact T cell proliferation and effector function. The most widely recognized metabolite in the TME to negatively impact T cells is lactate or lactic acid that is transported from cancer cells as a byproduct of glucose metabolism (Halestrap and Wilson, 2012). Lactate hinders proliferation and cytokine production by depleting intracellular levels of nicotinamide adenine dinucleotide (NAD^+) (Fischer et al., 2007). T regs are generally unaffected by the effects of lactic acid on intracellular NAD^+ because mitochondrial metabolism in T regs generates NAD^+ (Angelin et al., 2017). Additionally, PD-1 expression on T regs can be upregulated in response to lactic acid in the TME and this enhances the immunosuppressive activity of T regs (Kumagai et al., 2022). Increased levels of lactate in the TME also lowers the pH within tumors, which poses further restrictions on cytokine production and the cytolytic activity of T cells (Fischer et al., 2007; Erra Díaz et al., 2018). Consequently, neutralization of the acidic pH in murine tumors with sodium bicarbonate leads to increased sensitivity of tumors to immune checkpoint blockade therapies and reduction of tumor volume (Pilon-Thomas et al., 2016).

Hypoxia

Rapidly growing cancer cells have high oxygen consumption requirements that create hypoxic environments within tumors. Tortuous and abnormal tumor vasculature can also result in poor perfusion of the TME leading to low oxygen levels, especially in the central tumor bed, that can impact T cell function. Hypoxia triggers the activation of the hypoxia-inducible factor-1 (HIF-1) pathway and upregulation of HIF-1 proteins. Activation of this pathway influences the fate of differentiating CD4 T cells by promoting the polarization of the cells into the proinflammatory T_H1 and T_H17 subsets of helper cells (Dang et al., 2011). Additionally, HIF-1 can directly promote gene transcription of FOXP3 in a process mediated by TGF-β (Ren et al., 2016). In ovarian and hepatocellular tumors, hypoxia can drive the increased secretion of CCL28 that leads to increased recruitment of CCR10-expressing T regs into the TME (Facciabene et al., 2011; Ren et al., 2016). A similar effect of hypoxia on T reg migration involving CXCL12 and CXCR4 has also been reported in breast cancer (Yan et al., 2011). Within the TME, sustained exposure to hypoxia has been shown to promote the progression of effector CD8 T cells into terminally differentiated cells with an exhausted phenotype. In TMEs with severe hypoxia such as tumors with increased oxidative metabolism, the exhausted T cells are unresponsive to immune checkpoint inhibitors (Najjar et al., 2019). In hypoxic conditions, MHC class II and co-stimulatory molecules such as CD86 and CD40 expression on DCs have been found to be downregulated (Correale et al., 2012). Additionally, hypoxia negatively impacts chemotaxis to CCR5 and CCR4 ligands by monocyte-derived DCs (Labiano et al., 2015). As discussed earlier, increased levels of VEGF-A, which can be induced by hypoxia, in the TME inhibits DC maturation that results in ineffective T cell stimulation (Noman et al., 2015). Overall, hypoxia adds to the hostile microenvironment that limits effective T cell activation, effector function, and survival.

T cell survival in the TME

Maintenance of a robust antitumor immune T cell response is essential to control tumor growth. This can be accomplished by continued replenishment of effector T cells in the TME through the activation and migration of new effector cells from lymphoid tissues, the activation and differentiation of naïve T cells within the TME, and/or supporting T cell survival in the TME. Effector T cells upregulate pro-apoptotic receptors such as FAS and TNFRs upon activation, which leaves them vulnerable to apoptosis (Singer and Abbas, 1994; Ettinger et al., 1995; Zheng et al., 1995). Progressive differentiation of T cells also leads to the telomere shortening (Weng et al., 1995) that decreases lifespan and promotes senescence. However, the main factors that influence T cell longevity are cytokines and metabolism (Chang and Pearce, 2016; Kishton et al., 2017; Pan et al., 2017; Lin et al., 2020).

Several cytokines promote T cell survival. The IL-2 family of cytokines that includes IL-2, IL-4, IL-7, and IL-15 has a major influence on T cell lifespan. They are expressed by a variety of immune cells as well as epithelial and endothelial cells (Benczik and Gaffen, 2004) and they can regulate T cell survival independent of their capacity to induce proliferation and in

part, through the upregulation of anti-apoptotic genes such as BCL2 (Marrack et al., 1998; Vella et al., 1998; Zhang et al., 1998). However, T cells may respond to these environmental cues differently depending on their differentiation state. T cell sensitivity to cytokines can also vary between resting (naïve and memory) T cells and activated/effector T cells. While IL-6 supports the survival of resting T cells, it has little effect on the longevity of activated T cells (Teague et al., 1997). Activated T cells rely on IL-2 and IL-4 signaling for survival and withdrawal of these cytokines lead to T cell death (Marrack et al., 1998; Borthwick et al., 2003). Sequestration of IL-2 by T regs in the TME (Ku et al., 2000; Carmenate et al., 2018) limit the availability of the cytokine for other T cell subsets and thereby impede their survival.

Interestingly transmigration of activated T cells across endothelial cells can support T cell survival through the interaction between ICAM-1 and LFA-1 in the absence of IL-2 (Borthwick et al., 2003). Effector T cells are also more sensitive to survival cues from Type I interferons secreted in inflammatory conditions than resting cells (Marrack et al., 1998). In addition to supporting the survival of memory T cells, IL-15 can also rescue activated T cells from death (Vella et al., 1998; Zhang et al., 1998; Di Pilato et al., 2021). A subset of DCs known as DC3 or LAMP3+ DCs support T cell survival in the TME by secreting CXCL16 to attract CXCR6-expressing T cells into perivascular areas in the TME where CCR7+ DC3 are clustered (Di Pilato et al., 2021). In these clusters, the DCs provide IL-15 stimulation to the T cells to prevent activation-induced cell death. Notably, DC3s do not express XCR1, the receptor for XCL1 that is secreted by stem-like CD8 T cells (Im et al., 2016) and it is not known if other subsets of DCs such as conventional DC1s that express XCR1 are also capable of trans-presenting IL-15 to different subsets of T cells.

In addition to cytokines, specific metabolic and catabolic pathways can regulate T cell survival. Lipid uptake and fatty acid metabolism supports T cell survival. Resident memory T cells deficient in fatty acid-binding proteins 4 and 5 reduces lipid uptake and impairs survival (Pan et al., 2017). Blockade of mitochondrial fatty acid β-oxidation also reduces the lifespan of resident memory T cells. However, the survival of central memory T cells is not impacted in the absence of FABP4 and FABP5 suggesting that different subsets of T cells rely on distinct cues for survival. In contrast to memory T cells, chronic mTOR signaling in effector T cells enforces glycolysis and amino acid metabolism that render the cells vulnerable to cell death (Araki et al., 2009; MacIver et al., 2011; Pollizzi et al., 2016; Verbist et al., 2016). Consequently, inhibition of mTOR signaling improves T cell persistence (Araki et al., 2009). When exposed to high levels of L-arginine, metabolism in activated T cells shifts from glycolysis to oxidative phosphorylation that drives better survival (Geiger et al., 2016). Autophagy has also been shown to impact T cell longevity. T cell survival and memory formation are defective in T cells that lack autophagy related 4 and 5 genes (Xu et al., 2014b). These data show that the mechanisms that support T cell survival in the microenvironment are complex and distinct for different subsets of T cells in the TME. As investigators elucidate critical factors that regulate T cells in the TME, targeted therapeutic approaches that promote T cell survival may further enhance clinical efficacy of cancer immunotherapy.

Therapeutic applications and opportunities

Therapeutic strategies for cancer immunotherapy span all stages of the adaptive immune response and include vaccine development, adjuvant, immune modulators, and adoptive T cell therapy. Cancer vaccines involve regimens consisting of tumor antigens alone or in combination with adjuvants. Tumor antigens can be delivered as vaccines in a variety of formulations. These include whole tumor lysates, peptides, in DNA and mRNA-based constructs, and peptides loaded onto DCs (Saxena et al., 2021). There are also several therapeutic strategies targeting DC activation and maturation to induce robust antitumor T cell responses. Strategies and adjuvants that stimulate toll-like receptor pathways such as polyICLC and CpG that induced DC maturation and activation are being investigated (Chiang et al., 2013; Woo et al., 2014; Barber, 2015; Liau et al., 2018; Ramanjulu et al., 2018).

Previous efforts for cancer vaccines have been focused on shared tumor antigens derived from overexpressed self-antigens, differentiation proteins, and cancer testis antigens and these efforts have resulted in limited efficacy for a subset of cancer types (Comiskey et al., 2018; Pavlick et al., 2020). Although the intent of using shared tumor antigen is to develop vaccines that can be broadly used against several tumor types and for different patients, the tumor antigen profiles that can elicit strong and protective immune responses can be distinct between patients and tumor types. As such, there is now a focus on strategies utilizing individualized neoantigens as therapeutic vaccines (Carreno et al., 2015; Ott et al., 2017; Sahin et al., 2017; Keskin et al., 2019) as well as a combination of shared antigens and personalized neoantigens (Hilf et al., 2019; Saxena et al., 2021). High tumor mutational burden correlates with the presences of neoantigens and clinical response to immune checkpoint inhibitors (ICIs) (Maleki Vareki, 2018; Miao et al., 2018). Therapeutic approaches combining neoantigen vaccines with ICIs are being pursued (Ott et al., 2020; Saxena et al., 2021). Prophylactic vaccines targeting pathogen-mediated/driven tumors such as human papillomavirus-associated cervical cancer and oropharyngeal squamous cell carcinoma have also shown significant efficacy in reducing the risk of cancer in patients (Melief et al., 2015; Tomaić, 2016; van der Burg et al., 2016).

Immune modulators are a class of cancer immunotherapeutic agents that are used to induce and support antitumor responses. Immunomodulators include adjuvants, cytokines, chemokines, costimulatory agonists, and ICIs. IL-2 and IFNα were the first immunomodulators used to treat melanoma and renal cell carcinoma (Waldmann, 2018), and molecules that target the IL-2 and IFNAR pathways are FDA-approved for some cancer types. Other cytokines are being investigated to support T cell function and survival include IL-12, IL-15, and IL-21 (Floros and Tarhini, 2015). Co-stimulatory agonists for CD40, CD137 (4-1BB), OX-40 and others are currently in clinical trials to treat different cancer types (Mayes et al., 2018). Several ICIs against PD-1, PD-L1, CTLA-4, and LAG3 are FDA-approved therapies for several different types of cancers including melanoma, lung, gastrointestinal, breast, and renal cell cancer (Vaddepally et al., 2020). Other immunosuppressive factors including indoleamine 2, 3-dioxygenase (IDO) (Zhai et al., 2018; Kjeldsen et al., 2021) and the

adenosine pathway (Leone and Emens, 2018; Fong et al., 2020) are also being targeted for cancer therapy.

Adoptive T cell therapy involves the transfer of expanded tumor infiltrating lymphocytes or engineered T cells to reduce tumor burden. The isolation, *in vitro* expansion, and reinfusion of tumor infiltrating lymphocytes into patients as personalized therapy have resulted in good durable clinical responses (Hinrichs and Rosenberg, 2014) and adoptive T cell therapy is a viable approach to treat some types of cancers. T cells can also be engineered to express distinct tumor-specific T cell receptors that can then be infused into patients. Engineered T cells with a specific T cell receptor against the cancer testis antigen New York esophageal squamous cell carcinoma 1 (NY-ESO-1) have been used to treat solid tumors (D'Angelo et al., 2018; Robbins et al., 2011). Chimeric antigen receptor (CAR) T cells that are engineered to recognize tumors and specific proteins (June et al., 2018) have demonstrated strong clinical efficacy for hematologic cancers (Huang and Huang, 2022) and can be modified to target other tumor types.

Conclusion

T cell function in the TME is complex and influenced by a myriad of factors at every step of the life cycle of a T cell. These factors include direct cellular interactions with tumor, stromal, and other immune cells, structural and architectural features of the TME, soluble factors such as chemokines and cytokines, nutrients and metabolites, and metabolism in the TME. The resulting functional phenotype of effector T cells in the microenvironment is likely dictated by the sum and balance of opposing signals and cues in the TME. This poses challenges in developing robust therapeutic strategies with strong clinical efficacy. Despite these challenges, cancer immunotherapy has revolutionized clinical care and management of patients with cancer. There is an active and robust research community studying combinatorial approaches to bolster cancer treatment and many of the factors and cellular pathways that mediate T cell function provide new opportunities for innovation in cancer immunotherapy and in synergizing with other treatment modalities such as chemotherapy and radiation therapy.

Author contributions

Conceptualization and overall supervision: RO; Writing of manuscript and figure preparation: NM, DA, and RO; Editing: RO. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Afanasiev, O. K., Nagase, K., Simonson, W., Vandeven, N., Blom, A., Koelle, D. M., et al. (2013). Vascular E-selectin expression correlates with CD8 lymphocyte infiltration and improved outcome in Merkel cell carcinoma. *J. Invest. Dermatol* 133 (8), 2065–2073. doi:10.1038/jid.2013.36

Ahn, E., Araki, K., Hashimoto, M., Li, W., Riley, J. L., Cheung, J., et al. (2018). Role of PD-1 during effector CD8 T cell differentiation. *Proc. Natl. Acad. Sci. U. S. A.* 115 (18), 4749–4754. doi:10.1073/pnas.1718217115

Alfei, F., Kanev, K., Hofmann, M., Wu, M., Ghoneim, H. E., Roelli, P., et al. (2019). TOX reinforces the phenotype and longevity of exhausted T cells in chronic viral infection. *Nature* 571 (7764), 265–269. doi:10.1038/s41586-019-1326-9

Allan, R. S., Waithman, J., Bedoui, S., Jones, C. M., Villadangos, J. A., Zhan, Y., et al. (2006). Migratory dendritic cells transfer antigen to a lymph node-resident dendritic cell population for efficient CTL priming. *Immunity* 25 (1), 153–162. Research Support, Non-U.S. Gov't. doi:10.1016/j.immuni.2006.04.017

Aloisi, F., and Pujol-Borrell, R. (2006). Lymphoid neogenesis in chronic inflammatory diseases. *Nat. Rev. Immunol.* 6 (3), 205–217. doi:10.1038/nri1786

Amsen, D., van Gisbergen, K. P. J. M., Hombrink, P., and van Lier, R. A. W. (2018). Tissue-resident memory T cells at the center of immunity to solid tumors. *Nat. Immunol.* 19 (6), 538–546. doi:10.1038/s41590-018-0114-2

Angelin, A., Gil-de-Gómez, L., Dahiya, S., Jiao, J., Guo, L., Levine, M. H., et al. (2017). Foxp3 reprograms T cell metabolism to function in low-glucose, high-lactate environments. *Cell Metab.* 25 (6), 1282–1293. doi:10.1016/j.cmet.2016.12.018

Araki, K., Turner, A. P., Shaffer, V. O., Gangappa, S., Keller, S. A., Bachmann, M. F., et al. (2009). mTOR regulates memory CD8 T-cell differentiation. *Nature* 460 (7251), 108–112. doi:10.1038/nature08155

Armengol, M. P., Juan, M., Lucas-Martin, A., Fernandez-Figueras, M. T., Jaraquemada, D., Gallart, T., et al. (2001). Thyroid autoimmune disease: Demonstration of thyroid antigen-specific B cells and recombination-activating gene expression in chemokine-containing active intrathyroidal germinal centers. *Am. J. Pathol.* 159 (3), 861–873. doi:10.1016/s0002-9440(10)61762-2

Azuma, M., Ito, D., Yagita, H., Okumura, K., Phillips, J. H., Lanier, L. L., et al. (1993). B70 antigen is a second ligand for CTLA-4 and CD28. *Nature* 366 (6450), 76–79. doi:10.1038/366076a0

Baeyens, A., Fang, V., Chen, C., and Schwab, S. R. (2015). Exit strategies: S1P signaling and T cell migration. *Trends Immunol.* 36 (12), 778–787. doi:10.1016/j.it.2015.10.005

Baixeras, E., Huard, B., Miossec, C., Jitsukawa, S., Martin, M., Hercend, T., et al. (1992). Characterization of the lymphocyte activation gene 3-encoded protein. A new ligand for human leukocyte antigen class II antigens. *J. Exp. Med.* 176 (2), 327–337. doi:10.1084/jem.176.2.327

Bakker, A. B., Schreurs, M. W., de Boer, A. J., Kawakami, Y., Rosenberg, S. A., Adema, G. J., et al. (1994). Melanocyte lineage-specific antigen gp100 is recognized by melanoma-derived tumor-infiltrating lymphocytes. *J. Exp. Med.* 179 (3), 1005–1009. Research Support, Non-U.S. Gov't. doi:10.1084/jem.179.3.1005

Barber, D. L., Wherry, E. J., Masopust, D., Zhu, B., Allison, J. P., Sharpe, A. H., et al. (2006). Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 439 (7077), 682–687. doi:10.1038/nature04444

Barber, G. N. (2015). Sting: Infection, inflammation and cancer. *Nat. Rev. Immunol.* 15 (12), 760–770. doi:10.1038/nri3921

Barsheshet, Y., Wildbaum, G., Levy, E., Vitenshtein, A., Akinseye, C., Griggs, J., et al. (2017). CCR8+ foxp3+ T_{reg} cells as master drivers of immune regulation. *Proc. Natl. Acad. Sci. U. S. A.* 114 (23), 6086–6091. doi:10.1073/pnas.1621280114

Bedoui, S., Whitney, P. G., Waithman, J., Eidsmo, L., Wakim, L., Caminschi, I., et al. (2009). Cross-presentation of viral and self antigens by skin-derived CD103+ dendritic cells. *Nat. Immunol.* 10 (5), 488–495. Research Support, Non-U.S. Gov't. doi:10.1038/ni.1724

Bellone, M., and Calcinotto, A. (2013). Ways to enhance lymphocyte trafficking into tumors and fitness of tumor infiltrating lymphocytes. *Front. Oncol.* 3, 231. doi:10.3389/fonc.2013.00231

Belz, G. T., Smith, C. M., Kleinert, L., Reading, P., Brooks, A., Shortman, K., et al. (2004). Distinct migrating and nonmigrating dendritic cell populations are involved in MHC class I-restricted antigen presentation after lung infection with virus. *Proc. Natl. Acad. Sci. U. S. A.* 101 (23), 8670–8675. *In Vitro* Research Support, Non-U.S. Gov't. doi:10.1073/pnas.0402644101

Benczik, M., and Gaffen, S. L. (2004). The interleukin (IL)-2 family cytokines: Survival and proliferation signaling pathways in T lymphocytes. *Immunol. Invest.* 33 (2), 109–142. doi:10.1081/imm-120030732

Bengsch, B., Johnson, A. L., Kurachi, M., Odorizzi, P. M., Pauken, K. E., Attanasio, J., et al. (2016). Bioenergetic insufficiencies due to metabolic alterations regulated by the inhibitory receptor PD-1 are an early driver of CD8(+) T cell exhaustion. *Immunity* 45 (2), 358–373. doi:10.1016/j.immuni.2016.07.008

Bento, D. C., Jones, E., Junaid, S., Tull, J., Williams, G. T., Godkin, A., et al. (2015). High endothelial venules are rare in colorectal cancers but accumulate in extra-tumoral areas with disease progression. *Oncoimmunology* 4 (3), e974374. doi:10.4161/2162402x.2014.974374

Bian, Y., Li, W., Kremer, D. M., Sajjakulnukit, P., Li, S., Crespo, J., et al. (2020). Cancer SLC43A2 alters T cell methionine metabolism and histone methylation. *Nature* 585 (7824), 277–282. doi:10.1038/s41586-020-2682-1

Boon, T., and van der Bruggen, P. (1996). Human tumor antigens recognized by T lymphocytes. *J. Exp. Med.* 183 (3), 725–729. doi:10.1084/jem.183.3.725

Borthwick, N. J., Akbar, A. A., Buckley, C., Pilling, D., Salmon, M., Jewell, A. P., et al. (2003). Transendothelial migration confers a survival advantage to activated T lymphocytes: Role of LFA-1/ICAM-1 interactions. *Clin. Exp. Immunol.* 134 (2), 246–252. doi:10.1046/j.1365-2249.2003.02298.x

Brahmer, J. R., Tykodi, S. S., Chow, L. Q., Hwu, W. J., Topalian, S. L., Hwu, P., et al. (2012). Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N. Engl. J. Med.* 366 (26), 2455–2465. doi:10.1056/NEJMoa1200694

Breart, B., Lemaître, F., Celli, S., and Bousso, P. (2008). Two-photon imaging of intratumoral CD8+ T cell cytotoxic activity during adoptive T cell therapy in mice. *J. Clin. Invest.* 118 (4), 1390–1397. doi:10.1172/JCI34388

Brown, J. A., Dorfman, D. M., Ma, F. R., Sullivan, E. L., Munoz, O., Wood, C. R., et al. (2003). Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production. *J. Immunol.* 170 (3), 1257–1266. doi:10.4049/jimmunol.170.3.1257

Buckanovich, R. J., Facciabene, A., Kim, S., Benencia, F., Sasaroli, D., Balint, K., et al. (2008). Endothelin B receptor mediates the endothelial barrier to T cell homing to tumors and disables immune therapy. *Nat. Med.* 14 (1), 28–36. doi:10.1038/nm1699

Cabrita, R., Lauss, M., Sanna, A., Donia, M., Skaarup Larsen, M., Mitra, S., et al. (2020). Tertiary lymphoid structures improve immunotherapy and survival in melanoma. *Nature* 577 (7791), 561–565. doi:10.1038/s41586-019-1914-8

Carmeliet, P., and Jain, R. K. (2011). Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. *Nat. Rev. Drug Discov.* 10 (6), 417–427. doi:10.1038/nrd3455

Carmenate, T., Ortíz, Y., Enamorado, M., García-Martínez, K., Avellanet, J., Moreno, E., et al. (2018). Blocking IL-2 signal *in vivo* with an IL-2 antagonist reduces tumor growth through the control of regulatory T cells. *J. Immunol.* 200 (10), 3475–3484. doi:10.4049/jimmunol.1700433

Carreno, B. M., Magrini, V., Becker-Hapak, M., Kaabinejadian, S., Hundal, J., Petti, A. A., et al. (2015). Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. *Science* 348 (6236), 803–808. doi:10.1126/science.aaa3828

Carter, L., Fouser, L. A., Jussif, J., Fitz, L., Deng, B., Wood, C. R., et al. (2002). PD-1: PD-L inhibitory pathway affects both CD4(+) and CD8(+) T cells and is overcome by IL-2. *Eur. J. Immunol.* 32 (3), 634–643. doi:10.1002/1521-4141(200203)32:3<634::AID-IMMU634>3.0.CO;2-9

Cham, C. M., Driessens, G., O'Keefe, J. P., and Gajewski, T. F. (2008). Glucose deprivation inhibits multiple key gene expression events and effector functions in CD8+ T cells. *Eur. J. Immunol.* 38 (9), 2438–2450. doi:10.1002/eji.200838289

Chang, C. H., Curtis, J. D., Maggi, L. B., Faubert, B., Villarino, A. V., O'Sullivan, D., et al. (2013). Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell* 153 (6), 1239–1251. doi:10.1016/j.cell.2013.05.016

Chang, C. H., and Pearce, E. L. (2016). Emerging concepts of T cell metabolism as a target of immunotherapy. *Nat. Immunol.* 17 (4), 364–368. doi:10.1038/ni.3415

Chang, C. H., Qiu, J., O'Sullivan, D., Buck, M. D., Noguchi, T., Curtis, J. D., et al. (2015). Metabolic competition in the tumor microenvironment is a driver of cancer progression. *Cell* 162 (6), 1229–1241. doi:10.1016/j.cell.2015.08.016

Chaudhry, A., Samstein, R. M., Treuting, P., Liang, Y., Pils, M. C., Heinrich, J. M., et al. (2011). Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. *Immunity* 34 (4), 566–578. doi:10.1016/j.immuni.2011.03.018

Chaux, P., Vantomme, V., Stroobant, V., Thielemans, K., Corthals, J., Luiten, R., et al. (1999). Identification of MAGE-3 epitopes presented by HLA-DR molecules to CD4(+) T lymphocytes. *J. Exp. Med.* 189 (5), 767–778. Research Support, Non-U.S. Gov't. doi:10.1084/jem.189.5.767

Chen, W., Jin, W., Hardegen, N., Lei, K. J., Li, L., Marinos, N., et al. (2003). Conversion of peripheral CD4+CD25-naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J. Exp. Med.* 198 (12), 1875–1886. doi:10.1084/jem.20030152

Chiang, C. L., Kandalaft, L. E., Tanyi, J., Hagemann, A. R., Motz, G. T., Svoronos, N., et al. (2013). A dendritic cell vaccine pulsed with autologous hypochlorous acid-oxidized ovarian cancer lysate primes effective broad antitumor immunity: From bench to bedside. *Clin. Cancer Res.* 19 (17), 4801–4815. doi:10.1158/1078-0432.CCR-13-1185

Choi, J., Enis, D. R., Koh, K. P., Shiao, S. L., and Pober, J. S. (2004). T lymphocyte-endothelial cell interactions. *Annu. Rev. Immunol.* 22, 683–709. doi:10.1146/annurev.immunol.22.012703.104639

Chung, A. S., Lee, J., and Ferrara, N. (2010). Targeting the tumour vasculature: Insights from physiological angiogenesis. *Nat. Rev. Cancer* 10 (7), 505–514. doi:10.1038/nrc2868

Chung, Y., Chang, J. H., Kweon, M. N., Rennert, P. D., and Kang, C. Y. (2005). CD8alpha-11b+ dendritic cells but not CD8alpha+ dendritic cells mediate cross-tolerance toward intestinal antigens. *Blood* 106 (1), 201–206. Research Support, Non-U.S. Gov't. doi:10.1182/blood-2004-11-4240

Clark, R. A., Huang, S. J., Murphy, G. F., Mollet, I. G., Hijnen, D., Muthukuru, M., et al. (2008). Human squamous cell carcinomas evade the immune response by down-regulation of vascular E-selectin and recruitment of regulatory T cells. *J. Exp. Med.* 205 (10), 2221–2234. doi:10.1084/jem.20071190

Cluntun, A. A., Lukey, M. J., Cerione, R. A., and Locasale, J. W. (2017). Glutamine metabolism in cancer: Understanding the heterogeneity. *Trends Cancer* 3 (3), 169–180. doi:10.1016/j.trecan.2017.01.005

Codarri Deak, L., Nicolini, V., Hashimoto, M., Karagianni, M., Schwalie, P. C., Lauener, L., et al. (2022). PD-1-cis IL-2R agonism yields better effectors from stem-like $CD8^+$ T cells. *Nature* 610 (7930), 161–172. doi:10.1038/s41586-022-05192-0

Comiskey, M. C., Dallos, M. C., and Drake, C. G. (2018). Immunotherapy in prostate cancer: Teaching an old dog new tricks. *Curr. Oncol. Rep.* 20 (9), 75. doi:10.1007/s11912-018-0712-z

Correale, P., Rotundo, M. S., Botta, C., Del Vecchio, M. T., Ginanneschi, C., Licchetta, A., et al. (2012). Tumor infiltration by T lymphocytes expressing chemokine receptor 7 (CCR7) is predictive of favorable outcome in patients with advanced colorectal carcinoma. *Clin. Cancer Res.* 18 (3), 850–857. doi:10.1158/1078-0432.CCR-10-3186

Cottrell, T. R., Thompson, E. D., Forde, P. M., Stein, J. E., Duffield, A. S., Anagnostou, V., et al. (2018). Pathologic features of response to neoadjuvant anti-PD-1 in resected non-small-cell lung carcinoma: A proposal for quantitative immune-related pathologic response criteria (irPRC). *Ann. Oncol.* 29 (8), 1853–1860. doi:10.1093/annonc/mdy218

Cox, A. L., Skipper, J., Chen, Y., Henderson, R. A., Darrow, T. L., Shabanowitz, J., et al. (1994). Identification of a peptide recognized by five melanoma-specific human cytotoxic T cell lines. *Science* 264 (5159), 716–719. doi:10.1126/science.7513441

Cyster, J. G., and Schwab, S. R. (2012). Sphingosine-1-phosphate and lymphocyte egress from lymphoid organs. *Annu. Rev. Immunol.* 30, 69–94. doi:10.1146/annurev-immunol-020711-075011

D'Angelo, S. P., Melchiori, L., Merchant, M. S., Bernstein, D., Glod, J., Kaplan, R., et al. (2018). Antitumor activity associated with prolonged persistence of adoptively transferred NY-ESO-1 c259T cells in synovial sarcoma. *Cancer Discov.* 8 (8), 944–957. doi:10.1158/2159-8290.CD-17-1417

Dang, E. V., Barbi, J., Yang, H. Y., Jinasena, D., Yu, H., Zheng, Y., et al. (2011). Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. *Cell* 146 (5), 772–784. doi:10.1016/j.cell.2011.07.033

Davidson, T. S., DiPaolo, R. J., Andersson, J., and Shevach, E. M. (2007). Cutting edge: IL-2 is essential for TGF-beta-mediated induction of Foxp3+ T regulatory cells. *J. Immunol.* 178 (7), 4022–4026. doi:10.4049/jimmunol.178.7.4022

De Simone, M., Arrigoni, A., Rossetti, G., Gruarin, P., Ranzani, V., Politano, C., et al. (2016). Transcriptional landscape of human tissue lymphocytes unveils uniqueness of tumor-infiltrating T regulatory cells. *Immunity* 45 (5), 1135–1147. doi:10.1016/j.immuni.2016.10.021

del Rio, M. L., Rodriguez-Barbosa, J. I., Kremmer, E., and Forster, R. (2007). CD103- and CD103+ bronchial lymph node dendritic cells are specialized in presenting and cross-presenting innocuous antigen to CD4+ and CD8+ T cells. *J. Immunol.* 178 (11), 6861–6866. Research Support, Non-U.S. Gov't. doi:10.4049/jimmunol.178.11.6861

den Haan, J. M., Lehar, S. M., and Bevan, M. J. (2000). CD8(+) but not CD8(-) dendritic cells cross-prime cytotoxic T cells *in vivo*. *J. Exp. Med.* 192 (12), 1685–1696. doi:10.1084/jem.192.12.1685

Depontieu, F. R., Qian, J., Zarling, A. L., McMiller, T. L., Salay, T. M., Norris, A., et al. (2009). Identification of tumor-associated, MHC class II-restricted phosphopeptides as targets for immunotherapy. *Proc. Natl. Acad. Sci. U. S. A.* 106 (29), 12073–12078. doi:10.1073/pnas.0903852106

Di Pilato, M., Kfuri-Rubens, R., Pruessmann, J. N., Ozga, A. J., Messemaker, M., Cadilha, B. L., et al. (2021). CXCR6 positions cytotoxic T cells to receive critical survival signals in the tumor microenvironment. *Cell* 184 (17), 4512–4530.e22. doi:10.1016/j.cell.2021.07.015

Dirkx, A. E., Oude Egbrink, M. G., Kuijpers, M. J., van der Niet, S. T., Heijnen, V. V., Bouma-ter Steege, J. C., et al. (2003). Tumor angiogenesis modulates leukocyte-vessel

wall interactions *in vivo* by reducing endothelial adhesion molecule expression. *Cancer Res.* 63 (9), 2322–2329.

Dougall, W. C., Kurtulus, S., Smyth, M. J., and Anderson, A. C. (2017). TIGIT and CD96: New checkpoint receptor targets for cancer immunotherapy. *Immunol. Rev.* 276 (1), 112–120. doi:10.1111/imr.12518

Dudley, M. E., Nishimura, M. I., Holt, A. K., and Rosenberg, S. A. (1999). Antitumor immunization with a minimal peptide epitope (G9-209-2M) leads to a functionally heterogeneous CTL response. *J. Immunother.* 22 (4), 288–298. Comparative Study. doi:10.1097/00002371-199907000-00002

Egen, J. G., and Allison, J. P. (2002). Cytotoxic T lymphocyte antigen-4 accumulation in the immunological synapse is regulated by TCR signal strength. *Immunity* 16 (1), 23–35. doi:10.1016/s1074-7613(01)00259-x

Erra Díaz, F., Dantas, E., and Geffner, J. (2018). Unravelling the interplay between extracellular acidosis and immune cells. *Mediat. Inflamm.* 2018, 1218297. doi:10.1155/2018/1218297

Ettinger, R., Panka, D. J., Wang, J. K., Stanger, B. Z., Ju, S. T., and Marshak-Rothstein, A. (1995). Fas ligand-mediated cytotoxicity is directly responsible for apoptosis of normal CD4+ T cells responding to a bacterial superantigen. *J. Immunol.* 154 (9), 4302–4308. doi:10.4049/jimmunol.154.9.4302

Facciabene, A., Peng, X., Hagemann, I. S., Balint, K., Barchetti, A., Wang, L. P., et al. (2011). Tumour hypoxia promotes tolerance and angiogenesis via CCL28 and T(reg) cells. *Nature* 475 (7355), 226–230. doi:10.1038/nature10169

Farnsworth, R. H., Lackmann, M., Achen, M. G., and Stacker, S. A. (2014). Vascular remodeling in cancer. *Oncogene* 33 (27), 3496–3505. doi:10.1038/onc.2013.304

Ferguson, A. R., and Engelhard, V. H. (2010). CD8 T cells activated in distinct lymphoid organs differentially express adhesion proteins and coexpress multiple chemokine receptors. *J. Immunol.* 184 (8), 4079–4086. Comparative Study Research Support, N.I.H., Extramural. doi:10.4049/jimmunol.0901903

Field, C. S., Baixauli, F., Kyle, R. L., Puleston, D. J., Cameron, A. M., Sanin, D. E., et al. (2020). Mitochondrial integrity regulated by lipid metabolism is a cell-intrinsic checkpoint for treg suppressive function. *Cell Metab.* 31 (2), 422–437. doi:10.1016/j.cmet.2019.11.021

Figenschau, S. L., Fismen, S., Fenton, K. A., Fenton, C., and Mortensen, E. S. (2015). Tertiary lymphoid structures are associated with higher tumor grade in primary operable breast cancer patients. *BMC Cancer* 15, 101. doi:10.1186/s12885-015-1116-1

Finger, E. B., Puri, K. D., Alon, R., Lawrence, M. B., von Andrian, U. H., and Springer, T. A. (1996). Adhesion through L-selectin requires a threshold hydrodynamic shear. *Nature* 379 (6562), 266–269. doi:10.1038/379266a0

Finkin, S., Yuan, D., Stein, I., Taniguchi, K., Weber, A., Unger, K., et al. (2015). Ectopic lymphoid structures function as microniches for tumor progenitor cells in hepatocellular carcinoma. *Nat. Immunol.* 16 (12), 1235–1244. doi:10.1038/ni.3290

Fischer, K., Hoffmann, P., Voelkl, S., Meidenbauer, N., Ammer, J., Edinger, M., et al. (2007). Inhibitory effect of tumor cell-derived lactic acid on human T cells. *Blood* 109 (9), 3812–3819. doi:10.1182/blood-2006-07-035972

Floros, T., and Tarhini, A. A. (2015). Anticancer cytokines: Biology and clinical effects of interferon-α2, interleukin (IL)-2, IL-15, IL-21, and IL-12. *Semin. Oncol.* 42 (4), 539–548. doi:10.1053/j.seminoncol.2015.05.015

Fong, L., Hotson, A., Powderly, J. D., Sznol, M., Heist, R. S., Choueiri, T. K., et al. (2020). Adenosine 2A receptor blockade as an immunotherapy for treatment-refractory renal cell cancer. *Cancer Discov.* 10 (1), 40–53. doi:10.1158/2159-8290.CD-19-0980

Fontenot, J. D., Gavin, M. A., and Rudensky, A. Y. (2003). Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat. Immunol.* 4 (4), 330–336. doi:10.1038/ni904

Francisco, L. M., Salinas, V. H., Brown, K. E., Vanguri, V. K., Freeman, G. J., Kuchroo, V. K., et al. (2009). PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *J. Exp. Med.* 206 (13), 3015–3029. doi:10.1084/jem.20090847

Freeman, G. J., Gribben, J. G., Boussiotis, V. A., Ng, J. W., Restivo, V. A., Lombard, L. A., et al. (1993). Cloning of B7-2: A CTLA-4 counter-receptor that costimulates human T cell proliferation. *Science* 262 (5135), 909–911. doi:10.1126/science.7694363

Freeman, G. J., Long, A. J., Iwai, Y., Bourque, K., Chernova, T., Nishimura, H., et al. (2000). Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J. Exp. Med.* 192 (7), 1027–1034. doi:10.1084/jem.192.7.1027

Gabrilovich, D. I., Ciernik, I. F., and Carbone, D. P. (1996). Dendritic cells in antitumor immune responses. I. Defective antigen presentation in tumor-bearing hosts. *Cell. Immunol.* 170 (1), 101–110. Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. doi:10.1006/cimm.1996.0139

Gattinoni, L., Zhong, X. S., Palmer, D. C., Ji, Y., Hinrichs, C. S., Yu, Z., et al. (2009). Wnt signaling arrests effector T cell differentiation and generates CD8+ memory stem cells. *Nat. Med.* 15 (7), 808–813. doi:10.1038/nm.1982

Geiger, R., Rieckmann, J. C., Wolf, T., Basso, C., Feng, Y., Fuhrer, T., et al. (2016). L-arginine modulates T cell metabolism and enhances survival and anti-tumor activity. *Cell* 167 (3), 829–842. doi:10.1016/j.cell.2016.09.031

Germain, C., Gnjatic, S., Tamzalit, F., Knockaert, S., Remark, R., Goc, J., et al. (2014). Presence of B cells in tertiary lymphoid structures is associated with a protective immunity in patients with lung cancer. *Am. J. Respir. Crit. Care Med.* 189 (7), 832–844. doi:10.1164/rccm.201309-1611OC

Ghoneim, H. E., Fan, Y., Moustaki, A., Abdelsamed, H. A., Dash, P., Dogra, P., et al. (2017). De novo epigenetic programs inhibit PD-1 blockade-mediated T cell rejuvenation. *Cell* 170 (1), 142–157. doi:10.1016/j.cell.2017.06.007

Griffioen, A. W., Damen, C. A., Blijham, G. H., and Groenewegen, G. (1996). Tumor angiogenesis is accompanied by a decreased inflammatory response of tumor-associated endothelium. *Blood* 88 (2), 667–673. doi:10.1182/blood.v88.2.667.bloodjournal882667

Griffioen, A. W., Damen, C. A., Martinotti, S., Blijham, G. H., and Groenewegen, G. (1996). Endothelial intercellular adhesion molecule-1 expression is suppressed in human malignancies: The role of angiogenic factors. *Cancer Res.* 56 (5), 1111–1117.

Griffioen, A. W., Relou, I. A., Gallardo Torres, H. I., Damen, C. A., Martinotti, S., De Graaf, J. C., et al. (1998). The angiogenic factor bFGF impairs leukocyte adhesion and rolling under flow conditions. *Angiogenesis* 2 (3), 235–243. doi:10.1023/A:1009237324501

Groom, J. R., and Luster, A. D. (2011). CXCR3 in T cell function. *Exp. Cell Res.* 317 (5), 620–631. doi:10.1016/j.yexcr.2010.12.017

Groom, J. R., Richmond, J., Murooka, T. T., Sorensen, E. W., Sung, J. H., Bankert, K., et al. (2012). CXCR3 chemokine receptor-ligand interactions in the lymph node optimize CD4+ T helper 1 cell differentiation. *Immunity* 37 (6), 1091–1103. doi:10.1016/j.immuni.2012.08.016

Grzywa, T. M., Sosnowska, A., Matryba, P., Rydzynska, Z., Jasinski, M., Nowis, D., et al. (2020). Myeloid cell-derived arginase in cancer immune response. *Front. Immunol.* 11, 938. doi:10.3389/fimmu.2020.00938

Guarda, G., Hons, M., Soriano, S. F., Huang, A. Y., Polley, R., Martín-Fontecha, A., et al. (2007). L-selectin-negative CCR7-effector and memory CD8+ T cells enter reactive lymph nodes and kill dendritic cells. *Nat. Immunol.* 8 (7), 743–752. doi:10.1038/ni1469

Guy, C., Mitrea, D. M., Chou, P. C., Temirov, J., Vignali, K. M., Liu, X., et al. (2022). LAG3 associates with TCR-CD3 complexes and suppresses signaling by driving co-receptor-Lck dissociation. *Nat. Immunol.* 23 (5), 757–767. doi:10.1038/s41590-022-01176-4

Halestrap, A. P., and Wilson, M. C. (2012). The monocarboxylate transporter family-role and regulation. *IUBMB Life* 64 (2), 109–119. doi:10.1002/iub.572

Hanahan, D., and Weinberg, R. A. (2000). The hallmarks of cancer. *Cell* 100 (1), 57–70. doi:10.1016/s0092-8674(00)81683-9

Hargadon, K. M., Brinkman, C. C., Sheasley-O'neill, S. L., Nichols, L. A., Bullock, T. N., and Engelhard, V. H. (2006). Incomplete differentiation of antigen-specific CD8 T cells in tumor-draining lymph nodes. *J. Immunol.* 177 (9), 6081–6090. doi:10.4049/jimmunol.177.9.6081

Hashimoto, M., Araki, K., Cardenas, M. A., Li, P., Jadhav, R. R., Kissick, H. T., et al. (2022). PD-1 combination therapy with IL-2 modifies $CD8^+$ T cell exhaustion program. *Nature* 610 (7930), 173–181. doi:10.1038/s41586-022-05257-0

Hathcock, K. S., Laszlo, G., Dickler, H. B., Bradshaw, J., Linsley, P., and Hodes, R. J. (1993). Identification of an alternative CTLA-4 ligand costimulatory for T cell activation. *Science* 262 (5135), 905–907. doi:10.1126/science.7694361

He, R., Hou, S., Liu, C., Zhang, A., Bai, Q., Han, M., et al. (2016). Follicular CXCR5-expressing CD8(+) T cells curtail chronic viral infection. *Nature* 537 (7620), 412–428. doi:10.1038/nature19317

Heldin, C. H., Rubin, K., Pietras, K., and Ostman, A. (2004). High interstitial fluid pressure - an obstacle in cancer therapy. *Nat. Rev. Cancer* 4 (10), 806–813. doi:10.1038/nrc1456

Helmink, B. A., Reddy, S. M., Gao, J., Zhang, S., Basar, R., Thakur, R., et al. (2020). B cells and tertiary lymphoid structures promote immunotherapy response. *Nature* 577 (7791), 549–555. doi:10.1038/s41586-019-1922-8

Hendrix, M. J., Seftor, E. A., Hess, A. R., and Seftor, R. E. (2003). Vasculogenic mimicry and tumour-cell plasticity: Lessons from melanoma. *Nat. Rev. Cancer* 3 (6), 411–421. doi:10.1038/nrc1092

Hendry, S. A., Farnsworth, R. H., Solomon, B., Achen, M. G., Stacker, S. A., and Fox, S. B. (2016). The role of the tumor vasculature in the host immune response: Implications for therapeutic strategies targeting the tumor microenvironment. *Front. Immunol.* 7, 621. doi:10.3389/fimmu.2016.00621

Hilf, N., Kuttruff-Coqui, S., Frenzel, K., Bukur, V., Stevanović, S., Gouttefangeas, C., et al. (2019). Actively personalized vaccination trial for newly diagnosed glioblastoma. *Nature* 565 (7738), 240–245. doi:10.1038/s41586-018-0810-y

Hinrichs, C. S., and Rosenberg, S. A. (2014). Exploiting the curative potential of adoptive T-cell therapy for cancer. *Immunol. Rev.* 257 (1), 56–71. doi:10.1111/imr.12132

Hiraoka, N., Ino, Y., Yamazaki-Itoh, R., Kanai, Y., Kosuge, T., and Shimada, K. (2015). Intratumoral tertiary lymphoid organ is a favourable prognosticator in patients with pancreatic cancer. *Br. J. Cancer* 112 (11), 1782–1790. doi:10.1038/bjc.2015.145

Hirohashi, Y., Torigoe, T., Inoda, S., Kobayasi, J., Nakatsugawa, M., Mori, T., et al. (2009). The functioning antigens: Beyond just as the immunological targets. *Cancer Sci.* 100 (5), 798–806. Research Support, Non-U.S. Gov't Review. doi:10.1111/j.1349-7006.2009.01137.x

Hjelmstrom, P. (2001). Lymphoid neogenesis: De novo formation of lymphoid tissue in chronic inflammation through expression of homing chemokines. *J. Leukoc. Biol.* 69 (3), 331–339. doi:10.1189/jlb.69.3.331

Hori, S., Nomura, T., and Sakaguchi, S. (2003). Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299 (5609), 1057–1061. doi:10.1126/science.1079490

Howie, D., Ten Bokum, A., Necula, A. S., Cobbold, S. P., and Waldmann, H. (2017). The role of lipid metabolism in T lymphocyte differentiation and survival. *Front. Immunol.* 8, 1949. doi:10.3389/fimmu.2017.01949

Hsieh, C. S., Macatonia, S. E., Tripp, C. S., Wolf, S. F., O'Garra, A., and Murphy, K. M. (1993). Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science* 260 (5107), 547–549. doi:10.1126/science.8097338

Huang, A. C., Postow, M. A., Orlowski, R. J., Mick, R., Bengsch, B., Manne, S., et al. (2017). T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. *Nature* 545 (7652), 60–65. doi:10.1038/nature22079

Huang, A. Y., Golumbek, P., Ahmadzadeh, M., Jaffee, E., Pardoll, D., and Levitsky, H. (1994). Role of bone marrow-derived cells in presenting MHC class I-restricted tumor antigens. *Science* 264 (5161), 961–965. doi:10.1126/science.7513904

Huang, J., and Huang, X. (2022). CAR-T cell therapy for hematological malignancies: Limitations and optimization strategies. *Front. Immunol.* 13, 1019115. doi:10.3389/fimmu.2022.1019115

Hudson, W. H., Gensheimer, J., Hashimoto, M., Wieland, A., Valanparambil, R. M., Li, P., et al. (2019). Proliferating transitory T cells with an effector-like transcriptional signature emerge from PD-1+ stem-like CD8+ T cells during chronic infection. *Immunity* 51 (6), 1043–1058. doi:10.1016/j.immuni.2019.11.002

Hunt, D. F., Henderson, R. A., Shabanowitz, J., Sakaguchi, K., Michel, H., Sevilir, N., et al. (1992). Characterization of peptides bound to the class I MHC molecule HLA-A2.1 by mass spectrometry. *Science* 255 (5049), 1261–1263. doi:10.1126/science.1546328

Im, S. J., Hashimoto, M., Gerner, M. Y., Lee, J., Kissick, H. T., Burger, M. C., et al. (2016). Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. *Nature* 537 (7620), 417–421. doi:10.1038/nature19330

Im, S. J., Konieczny, B. T., Hudson, W. H., Masopust, D., and Ahmed, R. (2020). PD-1+ stemlike CD8 T cells are resident in lymphoid tissues during persistent LCMV infection. *Proc. Natl. Acad. Sci. U. S. A.* 117 (8), 4292–4299. doi:10.1073/pnas.1917298117

Jain, R. K., Martin, J. D., and Stylianopoulos, T. (2014). The role of mechanical forces in tumor growth and therapy. *Annu. Rev. Biomed. Eng.* 16, 321–346. doi:10.1146/annurev-bioeng-071813-105259

Jain, R. K. (2005). Normalization of tumor vasculature: An emerging concept in antiangiogenic therapy. *Science* 307 (5706), 58–62. doi:10.1126/science.1104819

Jain, R. K. (2013). Normalizing tumor microenvironment to treat cancer: Bench to bedside to biomarkers. *J. Clin. Oncol.* 31 (17), 2205–2218. doi:10.1200/JCO.2012.46.3653

Johnson, M. O., Siska, P. J., Contreras, D. C., and Rathmell, J. C. (2016). Nutrients and the microenvironment to feed a T cell army. *Semin. Immunol.* 28 (5), 505–513. doi:10.1016/j.smim.2016.09.003

Johnson, M. O., Wolf, M. M., Madden, M. Z., Andrejeva, G., Sugiura, A., Contreras, D. C., et al. (2018). Distinct regulation of Th17 and Th1 cell differentiation by glutaminase-dependent metabolism. *Cell* 175 (7), 1780–1795. doi:10.1016/j.cell.2018.10.001

Joshi, N. S., Akama-Garren, E. H., Lu, Y., Lee, D. Y., Chang, G. P., Li, A., et al. (2015). Regulatory T cells in tumor-associated tertiary lymphoid structures suppress anti-tumor T cell responses. *Immunity* 43 (3), 579–590. doi:10.1016/j.immuni.2015.08.006

June, C. H., O'Connor, R. S., Kawalekar, O. U., Ghassemi, S., and Milone, M. C. (2018). CAR T cell immunotherapy for human cancer. *Science* 359 (6382), 1361–1365. doi:10.1126/science.aar6711

Kamphorst, A. O., Wieland, A., Nasti, T., Yang, S., Zhang, R., Barber, D. L., et al. (2017). Rescue of exhausted CD8 T cells by PD-1-targeted therapies is CD28-dependent. *Science* 355 (6332), 1423–1427. doi:10.1126/science.aaf0683

Kang, C. W., Dutta, A., Chang, L. Y., Mahalingam, J., Lin, Y. C., Chiang, J. M., et al. (2015). Apoptosis of tumor infiltrating effector TIM-3+CD8+ T cells in colon cancer. *Sci. Rep.* 5, 15659. doi:10.1038/srep15659

Karaki, S., Blanc, C., Tran, T., Galy-Fauroux, I., Mougel, A., Dransart, E., et al. (2021). CXCR6 deficiency impairs cancer vaccine efficacy and $CD8^+$ resident memory T-cell recruitment in head and neck and lung tumors. *J. Immunother. Cancer* 9 (3), e001948. doi:10.1136/jitc-2020-001948

Katayama, Y., Uchino, J., Chihara, Y., Tamiya, N., Kaneko, Y., Yamada, T., et al. (2019). Tumor neovascularization and developments in therapeutics. *Cancers (Basel)* 11 (3), 316. doi:10.3390/cancers11030316

Kawakami, Y., Eliyahu, S., Delgado, C. H., Robbins, P. F., Rivoltini, L., Topalian, S. L., et al. (1994). Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. *Proc. Natl. Acad. Sci. U. S. A.* 91 (9), 3515–3519. doi:10.1073/pnas.91.9.3515

Ke, Y., and Kapp, J. A. (1996). Exogenous antigens gain access to the major histocompatibility complex class I processing pathway in B cells by receptor-mediated uptake. *J. Exp. Med.* 184 (3), 1179–1184. Research Support, U.S. Gov't, P.H.S. doi:10.1084/jem.184.3.1179

Keck, P. J., Hauser, S. D., Krivi, G., Sanzo, K., Warren, T., Feder, J., et al. (1989). Vascular permeability factor, an endothelial cell mitogen related to PDGF. *Science* 246 (4935), 1309–1312. doi:10.1126/science.2479987

Keir, M. E., Butte, M. J., Freeman, G. J., and Sharpe, A. H. (2008). PD-1 and its ligands in tolerance and immunity. *Annu. Rev. Immunol.* 26, 677–704. doi:10.1146/annurev.immunol.26.021607.090331

Keskin, D. B., Anandappa, A. J., Sun, J., Tirosh, I., Mathewson, N. D., Li, S., et al. (2019). Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. *Nature* 565 (7738), 234–239. doi:10.1038/s41586-018-0792-9

Khan, O., Giles, J. R., McDonald, S., Manne, S., Ngiow, S. F., Patel, K. P., et al. (2019). TOX transcriptionally and epigenetically programs CD8+ T cell exhaustion. *Nature* 571 (7764), 211–218. doi:10.1038/s41586-019-1325-x

Kim, H. J., and Cantor, H. (2014). CD4 T-Cell subsets and tumor immunity: The helpful and the not-so-helpful. *Cancer Immunol. Res.* 2 (2), 91–98. doi:10.1158/2326-6066.CIR-13-0216

Kishton, R. J., Sukumar, M., and Restifo, N. P. (2017). Metabolic regulation of T cell longevity and function in tumor immunotherapy. *Cell Metab.* 26 (1), 94–109. doi:10.1016/j.cmet.2017.06.016

Kjeldsen, J. W., Lorentzen, C. L., Martinenaite, E., Ellebaek, E., Donia, M., Holmstroem, R. B., et al. (2021). A phase 1/2 trial of an immune-modulatory vaccine against Ido/PD-L1 in combination with nivolumab in metastatic melanoma. *Nat. Med.* 27 (12), 2212–2223. doi:10.1038/s41591-021-01544-x

Kouo, T., Huang, L., Pucsek, A. B., Cao, M., Solt, S., Armstrong, T., et al. (2015). Galectin-3 shapes antitumor immune responses by suppressing CD8+ T cells via LAG-3 and inhibiting expansion of plasmacytoid dendritic cells. *Cancer Immunol. Res.* 3 (4), 412–423. doi:10.1158/2326-6066.CIR-14-0150

Krummel, M. F., Bartumeus, F., and Gérard, A. (2016). T cell migration, search strategies and mechanisms. *Nat. Rev. Immunol.* 16 (3), 193–201. doi:10.1038/nri.2015.16

Ku, C. C., Murakami, M., Sakamoto, A., Kappler, J., and Marrack, P. (2000). Control of homeostasis of CD8+ memory T cells by opposing cytokines. *Science* 288 (5466), 675–678. doi:10.1126/science.288.5466.675

Kumagai, S., Koyama, S., Itahashi, K., Tanegashima, T., Lin, Y. T., Togashi, Y., et al. (2022). Lactic acid promotes PD-1 expression in regulatory T cells in highly glycolytic tumor microenvironments. *Cancer Cell* 40 (2), 201–218.e9. doi:10.1016/j.ccell.2022.01.001

Kumagai, S., Togashi, Y., Sakai, C., Kawazoe, A., Kawazu, M., Ueno, T., et al. (2020). An oncogenic alteration creates a microenvironment that promotes tumor progression by conferring a metabolic advantage to regulatory T cells. *Immunity* 53 (1), 187–203. doi:10.1016/j.immuni.2020.06.016

Labiano, S., Palazon, A., and Melero, I. (2015). Immune response regulation in the tumor microenvironment by hypoxia. *Semin. Oncol.* 42 (3), 378–386. doi:10.1053/j.seminoncol.2015.02.009

Lawrence, M. B., Kansas, G. S., Kunkel, E. J., and Ley, K. (1997). Threshold levels of fluid shear promote leukocyte adhesion through selectins (CD62L,P,E). *J. Cell Biol.* 136 (3), 717–727. doi:10.1083/jcb.136.3.717

Le Gros, G., Ben-Sasson, S. Z., Seder, R., Finkelman, F. D., and Paul, W. E. (1990). Generation of interleukin 4 (IL-4)-producing cells *in vivo* and *in vitro*: IL-2 and IL-4 are required for *in vitro* generation of IL-4-producing cells. *J. Exp. Med.* 172 (3), 921–929. doi:10.1084/jem.172.3.921

Lee, Y., Chin, R. K., Christiansen, P., Sun, Y., Tumanov, A. V., Wang, J., et al. (2006). Recruitment and activation of naive T cells in the islets by lymphotoxin beta receptor-dependent tertiary lymphoid structure. *Immunity* 25 (3), 499–509. doi:10.1016/j.immuni.2006.06.016

Leone, R. D., and Emens, L. A. (2018). Targeting adenosine for cancer immunotherapy. *J. Immunother. Cancer* 6 (1), 57. doi:10.1186/s40425-018-0360-8

Leung, D. W., Cachianes, G., Kuang, W. J., Goeddel, D. V., and Ferrara, N. (1989). Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 246 (4935), 1306–1309. doi:10.1126/science.2479986

Li, M. O., Wan, Y. Y., and Flavell, R. A. (2007). T cell-produced transforming growth factor-beta1 controls T cell tolerance and regulates Th1- and Th17-cell differentiation. *Immunity* 26 (5), 579–591. doi:10.1016/j.immuni.2007.03.014

Li, N., Workman, C. J., Martin, S. M., and Vignali, D. A. (2004). Biochemical analysis of the regulatory T cell protein lymphocyte activation gene-3 (LAG-3; CD223). *J. Immunol.* 173 (11), 6806–6812. doi:10.4049/jimmunol.173.11.6806

Liau, L. M., Ashkan, K., Tran, D. D., Campian, J. L., Trusheim, J. E., Cobbs, C. S., et al. (2018). First results on survival from a large Phase 3 clinical trial of an autologous dendritic cell vaccine in newly diagnosed glioblastoma. *J. Transl. Med.* 16 (1), 142. doi:10.1186/s12967-018-1507-6

Lim, S. A., Wei, J., Nguyen, T. M., Shi, H., Su, W., Palacios, G., et al. (2021). Lipid signalling enforces functional specialization of T_{reg} cells in tumours. *Nature* 591 (7849), 306–311. doi:10.1038/s41586-021-03235-6

Lin, R., Zhang, H., Yuan, Y., He, Q., Zhou, J., Li, S., et al. (2020). Fatty acid oxidation controls CD8+ tissue-resident memory T-cell survival in gastric adenocarcinoma. *Cancer Immunol. Res.* 8 (4), 479–492. doi:10.1158/2326-6066.CIR-19-0702

Linsley, P. S., Brady, W., Urnes, M., Grosmaire, L. S., Damle, N. K., and Ledbetter, J. A. (1991). CTLA-4 is a second receptor for the B cell activation antigen B7. *J. Exp. Med.* 174 (3), 561–569. doi:10.1084/jem.174.3.561

Linsley, P. S., Greene, J. L., Brady, W., Bajorath, J., Ledbetter, J. A., and Peach, R. (1994). Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors. *Immunity* 1 (9), 793–801. doi:10.1016/s1074-7613(94)80021-9

Lipson, E. J., Sharfman, W. H., Drake, C. G., Wollner, I., Taube, J. M., Anders, R. A., et al. (2013). Durable cancer regression off-treatment and effective reinduction therapy with an anti-PD-1 antibody. *Clin. Cancer Res.* 19 (2), 462–468. doi:10.1158/1078-0432.CCR-12-2625

Lozano, E., Dominguez-Villar, M., Kuchroo, V., and Hafler, D. A. (2012). The TIGIT/CD226 axis regulates human T cell function. *J. Immunol.* 188 (8), 3869–3875. doi:10.4049/jimmunol.1103627

Lutz, E. R., Wu, A. A., Bigelow, E., Sharma, R., Mo, G., Soares, K., et al. (2014). Immunotherapy converts nonimmunogenic pancreatic tumors into immunogenic foci of immune regulation. *Cancer Immunol. Res.* 2 (7), 616–631. doi:10.1158/2326-6066.CIR-14-0027

MacIver, N. J., Blagih, J., Saucillo, D. C., Tonelli, L., Griss, T., Rathmell, J. C., et al. (2011). The liver kinase B1 is a central regulator of T cell development, activation, and metabolism. *J. Immunol.* 187 (8), 4187–4198. doi:10.4049/jimmunol.1100367

MacIver, N. J., Michalek, R. D., and Rathmell, J. C. (2013). Metabolic regulation of T lymphocytes. *Annu. Rev. Immunol.* 31, 259–283. doi:10.1146/annurev-immunol-032712-095956

Maçon-Lemaître, L., and Triebel, F. (2005). The negative regulatory function of the lymphocyte-activation gene-3 co-receptor (CD223) on human T cells. *Immunology* 115 (2), 170–178. doi:10.1111/j.1365-2567.2005.02145.x

Maksan, S. M., Araib, P. M., Ryschich, E., Gebhard, M. M., and Schmidt, J. (2004). Immune escape mechanism: Defective resting and stimulated leukocyte-endothelium interaction in hepatocellular carcinoma of the rat. *Dig. Dis. Sci.* 49 (5), 859–865. doi:10.1023/b:ddas.0000030100.05979.b7

Maldonado, L., Teague, J. E., Morrow, M. P., Jotova, I., Wu, T. C., Wang, C., et al. (2014). Intramuscular therapeutic vaccination targeting HPV16 induces T cell responses that localize in mucosal lesions. *Sci. Transl. Med.* 6 (221), 221ra13. doi:10.1126/scitranslmed.3007323

Maleki Vareki, S. (2018). High and low mutational burden tumors versus immunologically hot and cold tumors and response to immune checkpoint inhibitors. *J. Immunother. Cancer* 6 (1), 157. doi:10.1186/s40425-018-0479-7

Mao, X., Ou, M. T., Karuppagounder, S. S., Kam, T. I., Yin, X., Xiong, Y., et al. (2016). Pathological α-synuclein transmission initiated by binding lymphocyte-activation gene 3. *Science* 353 (6307), aah3374. doi:10.1126/science.aah3374

Marrack, P., Mitchell, T., Bender, J., Hildeman, D., Kedl, R., Teague, K., et al. (1998). T-cell survival. *Immunol. Rev.* 165, 279–285. doi:10.1111/j.1600-065x.1998.tb01245.x

Martinez, G. J., Pereira, R. M., Äijö, T., Kim, E. Y., Marangoni, F., Pipkin, M. E., et al. (2015). The transcription factor NFAT promotes exhaustion of activated $CD8^+$ T cells. *Immunity* 42 (2), 265–278. doi:10.1016/j.immuni.2015.01.006

Mayes, P. A., Hance, K. W., and Hoos, A. (2018). The promise and challenges of immune agonist antibody development in cancer. *Nat. Rev. Drug Discov.* 17 (7), 509–527. doi:10.1038/nrd.2018.75

McDonnell, A. M., Prosser, A. C., van Bruggen, I., Robinson, B. W., and Currie, A. J. (2010). CD8alpha+ DC are not the sole subset cross-presenting cell-associated tumor antigens from a solid tumor. *Eur. J. Immunol.* 40 (6), 1617–1627. Research Support, Non-U.S. Gov't. doi:10.1002/eji.200940153

McEver, R. P. S. (2015). Selectins: Initiators of leucocyte adhesion and signalling at the vascular wall. *Cardiovasc Res.* 107 (3), 331–339. doi:10.1093/cvr/cvv154

McMahon, E. J., Bailey, S. L., Castenada, C. V., Waldner, H., and Miller, S. D. (2005). Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis. *Nat. Med.* 11 (3), 335–339. doi:10.1038/nm1202

Melief, C. J., van Hall, T., Arens, R., Ossendorp, F., and van der Burg, S. H. (2015). Therapeutic cancer vaccines. *J. Clin. Invest.* 125 (9), 3401–3412. doi:10.1172/JCI80009

Menetrier-Caux, C., Montmain, G., Dieu, M. C., Bain, C., Favrot, M. C., Caux, C., et al. (1998). Inhibition of the differentiation of dendritic cells from CD34(+) progenitors by tumor cells: Role of interleukin-6 and macrophage colony-stimulating factor. *Blood* 92 (12), 4778–4791. Research Support, Non-U.S. Gov't. doi:10.1182/blood.v92.12.4778

Miao, D., Margolis, C. A., Gao, W., Voss, M. H., Li, W., Martini, D. J., et al. (2018). Genomic correlates of response to immune checkpoint therapies in clear cell renal cell carcinoma. *Science* 359 (6377), 801–806. doi:10.1126/science.aan5951

Michalek, R. D., Gerriets, V. A., Jacobs, S. R., Macintyre, A. N., MacIver, N. J., Mason, E. F., et al. (2011). Cutting edge: Distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. *J. Immunol.* 186 (6), 3299–3303. doi:10.4049/jimmunol.1003613

Mohammed, F., Cobbold, M., Zarling, A. L., Salim, M., Barrett-Wilt, G. A., Shabanowitz, J., et al. (2008). Phosphorylation-dependent interaction between antigenic peptides and MHC class I: A molecular basis for the presentation of transformed self. *Nat. Immunol.* 9 (11), 1236–1243. doi:10.1038/ni.1660

Molon, B., Ugel, S., Del Pozzo, F., Soldani, C., Zilio, S., Avella, D., et al. (2011). Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells. *J. Exp. Med.* 208 (10), 1949–1962. doi:10.1084/jem.20101956

Mora, J. R., and von Andrian, U. H. (2006). T-Cell homing specificity and plasticity: New concepts and future challenges. *Trends Immunol.* 27 (5), 235–243. doi:10.1016/j.it.2006.03.007

Morcrette, G., Hirsch, T. Z., Badour, E., Pilet, J., Caruso, S., Calderaro, J., et al. (2019). APC germline hepatoblastomas demonstrate cisplatin-induced intratumor tertiary lymphoid structures. *Oncoimmunology* 8 (6), e1583547. doi:10.1080/2162402X.2019.1583547

Moyron-Quiroz, J. E., Rangel-Moreno, J., Hartson, L., Kusser, K., Tighe, M. P., Klonowski, K. D., et al. (2006). Persistence and responsiveness of immunologic memory in the absence of secondary lymphoid organs. *Immunity* 25 (4), 643–654. doi:10.1016/j.immuni.2006.08.022

Moyron-Quiroz, J. E., Rangel-Moreno, J., Kusser, K., Hartson, L., Sprague, F., Goodrich, S., et al. (2004). Role of inducible bronchus associated lymphoid tissue (iBALT) in respiratory immunity. *Nat. Med.* 10 (9), 927–934. doi:10.1038/nm1091

Mueller, S. N., Gebhardt, T., Carbone, F. R., and Heath, W. R. (2013). Memory T cell subsets, migration patterns, and tissue residence. *Annu. Rev. Immunol.* 31, 137–161. doi:10.1146/annurev-immunol-032712-095954

Munn, D. H., Sharma, M. D., Hou, D., Baban, B., Lee, J. R., Antonia, S. J., et al. (2004). Expression of indoleamine 2,3-dioxygenase by plasmacytoid dendritic cells in tumor-draining lymph nodes. *J. Clin. investigation* 114 (2), 280–290. Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. doi:10.1172/JCI21583

Murakami, J., Shimizu, Y., Kashii, Y., Kato, T., Minemura, M., Okada, K., et al. (1999). Functional B-cell response in intrahepatic lymphoid follicles in chronic hepatitis C. *Hepatology* 30 (1), 143–150. doi:10.1002/hep.510300107

Murphy, K. M., and Stockinger, B. (2010). Effector T cell plasticity: Flexibility in the face of changing circumstances. *Nat. Immunol.* 11 (8), 674–680. doi:10.1038/ni.1899

Najjar, Y. G., Menk, A. V., Sander, C., Rao, U., Karunamurthy, A., Bhatia, R., et al. (2019). Tumor cell oxidative metabolism as a barrier to PD-1 blockade immunotherapy in melanoma. *JCI Insight* 4 (5), e124989. doi:10.1172/jci.insight.124989

Nakashima, S., Sugita, Y., Miyoshi, H., Arakawa, F., Muta, H., Ishibashi, Y., et al. (2016). Endothelin B receptor expression in malignant gliomas: The perivascular immune escape mechanism of gliomas. *J. Neurooncol* 127 (1), 23–32. doi:10.1007/s11060-015-2017-5

Nielsen, J. S., and Nelson, B. H. (2012). Tumor-infiltrating B cells and T cells: Working together to promote patient survival. *Oncoimmunology* 1 (9), 1623–1625. doi:10.4161/onci.21650

Nishikawa, H., and Koyama, S. (2021). Mechanisms of regulatory T cell infiltration in tumors: Implications for innovative immune precision therapies. *J. Immunother. Cancer* 9 (7), e002591. doi:10.1136/jitc-2021-002591

Noman, M. Z., Hasmim, M., Messai, Y., Terry, S., Kieda, C., Janji, B., et al. (2015). Hypoxia: A key player in antitumor immune response. A review in the theme: Cellular responses to hypoxia. *Am. J. Physiol. Cell Physiol.* 309 (9), C569–C579. doi:10.1152/ajpcell.00207.2015

Ott, P. A., Hu, Z., Keskin, D. B., Shukla, S. A., Sun, J., Bozym, D. J., et al. (2017). An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature* 547 (7662), 217–221. doi:10.1038/nature22991

Ott, P. A., Hu-Lieskovan, S., Chmielowski, B., Govindan, R., Naing, A., Bhardwaj, N., et al. (2020). A phase ib trial of personalized neoantigen therapy plus anti-PD-1 in patients with advanced melanoma, non-small cell lung cancer, or bladder cancer. *Cell* 183 (2), 347–362. doi:10.1016/j.cell.2020.08.053

Pan, Y., Tian, T., Park, C. O., Lofftus, S. Y., Mei, S., Liu, X., et al. (2017). Survival of tissue-resident memory T cells requires exogenous lipid uptake and metabolism. *Nature* 543 (7644), 252–256. doi:10.1038/nature21379

Pao, W., Ooi, C. H., Birzele, F., Ruefli-Brasse, A., Cannarile, M. A., Reis, B., et al. (2018). Tissue-specific immunoregulation: A call for better understanding of the "immunostat" in the context of cancer. *Cancer Discov.* 8 (4), 395–402. doi:10.1158/2159-8290.CD-17-1320

Parry, R. V., Chemnitz, J. M., Frauwirth, K. A., Lanfranco, A. R., Braunstein, I., Kobayashi, S. V., et al. (2005). CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. *Mol. Cell Biol.* 25 (21), 9543–9553. doi:10.1128/MCB.25.21.9543-9553.2005

Patsoukis, N., Bardhan, K., Chatterjee, P., Sari, D., Liu, B., Bell, L. N., et al. (2015). PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. *Nat. Commun.* 6, 6692. doi:10.1038/ncomms7692

Pauken, K. E., Sammons, M. A., Odorizzi, P. M., Manne, S., Godec, J., Khan, O., et al. (2016). Epigenetic stability of exhausted T cells limits durability of

reinvigoration by PD-1 blockade. *Science* 354 (6316), 1160–1165. doi:10.1126/science.aaf2807

Pavlick, A., Blazquez, A. B., Meseck, M., Lattanzi, M., Ott, P. A., Marron, T. U., et al. (2020). Combined vaccination with NY-ESO-1 protein, poly-ICLC, and montanide improves humoral and cellular immune responses in patients with high-risk melanoma. *Cancer Immunol. Res.* 8 (1), 70–80. doi:10.1158/2326-6066.CIR-19-0545

Peggs, K. S., Quezada, S. A., Chambers, C. A., Korman, A. J., and Allison, J. P. (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 (8), 1717–1725. doi:10.1084/jem.20082492

Perez, V. L., Lederer, J. A., Lichtman, A. H., and Abbas, A. K. (1995). Stability of Th1 and Th2 populations. *Int. Immunol.* 7 (5), 869–875. doi:10.1093/intimm/7.5.869

Peske, J. D., Woods, A. B., and Engelhard, V. H. (2015). Control of CD8 T-cell infiltration into tumors by vasculature and microenvironment. *Adv. Cancer Res.* 128, 263–307. doi:10.1016/bs.acr.2015.05.001

Petitprez, F., de Reyniès, A., Keung, E. Z., Chen, T. W., Sun, C. M., Calderaro, J., et al. (2020). B cells are associated with survival and immunotherapy response in sarcoma. *Nature* 577 (7791), 556–560. doi:10.1038/s41586-019-1906-8

Pfeifer, J. D., Wick, M. J., Roberts, R. L., Findlay, K., Normark, S. J., and Harding, C. V. (1993). Phagocytic processing of bacterial antigens for class I MHC presentation to T cells. *Nature* 361 (6410), 359–362. doi:10.1038/361359a0

Philip, M., Fairchild, L., Sun, L., Horste, E. L., Camara, S., Shakiba, M., et al. (2017). Chromatin states define tumour-specific T cell dysfunction and reprogramming. *Nature* 545 (7655), 452–456. doi:10.1038/nature22367

Piali, L., Fichtel, A., Terpe, H. J., Imhof, B. A., and Gisler, R. H. (1995). Endothelial vascular cell adhesion molecule 1 expression is suppressed by melanoma and carcinoma. *J. Exp. Med.* 181 (2), 811–816. doi:10.1084/jem.181.2.811

Pieper, R., Christian, R. E., Gonzales, M. I., Nishimura, M. I., Gupta, G., Settlage, R. E., et al. (1999). Biochemical identification of a mutated human melanoma antigen recognized by CD4(+) T cells. *J. Exp. Med.* 189 (5), 757–766. doi:10.1084/jem.189.5.757

Pilon-Thomas, S., Kodumudi, K. N., El-Kenawi, A. E., Russell, S., Weber, A. M., Luddy, K., et al. (2016). Neutralization of tumor acidity improves antitumor responses to immunotherapy. *Cancer Res.* 76 (6), 1381–1390. doi:10.1158/0008-5472.CAN-15-1743

Pitzalis, C., Jones, G. W., Bombardieri, M., and Jones, S. A. (2014). Ectopic lymphoid-like structures in infection, cancer and autoimmunity. *Nat. Rev. Immunol.* 14 (7), 447–462. doi:10.1038/nri3700

Pollizzi, K. N., Sun, I. H., Patel, C. H., Lo, Y. C., Oh, M. H., Waickman, A. T., et al. (2016). Asymmetric inheritance of mTORC1 kinase activity during division dictates CD8(+) T cell differentiation. *Nat. Immunol.* 17 (6), 704–711. doi:10.1038/ni.3438

Qureshi, O. S., Zheng, Y., Nakamura, K., Attridge, K., Manzotti, C., Schmidt, E. M., et al. (2011). Trans-endocytosis of CD80 and CD86: A molecular basis for the cell-extrinsic function of CTLA-4. *Science* 332 (6029), 600–603. doi:10.1126/science.1202947

Ramanjulu, J. M., Pesiridis, G. S., Yang, J., Concha, N., Singhaus, R., Zhang, S. Y., et al. (2018). Design of amidobenzimidazole STING receptor agonists with systemic activity. *Nature* 564 (7736), 439–443. doi:10.1038/s41586-018-0705-y

Rangel-Moreno, J., Moyron-Quiroz, J. E., Hartson, L., Kusser, K., and Randall, T. D. (2007). Pulmonary expression of CXC chemokine ligand 13, CC chemokine ligand 19, and CC chemokine ligand 21 is essential for local immunity to influenza. *Proc. Natl. Acad. Sci. U. S. A.* 104 (25), 10577–10582. doi:10.1073/pnas.0700591104

Remark, R., Lupo, A., Alifano, M., Biton, J., Ouakrim, H., Stefani, A., et al. (2016). Immune contexture and histological response after neoadjuvant chemotherapy predict clinical outcome of lung cancer patients. *Oncoimmunology* 5 (12), e1255394. doi:10.1080/2162402X.2016.1255394

Ren, L., Yu, Y., Wang, L., Zhu, Z., Lu, R., and Yao, Z. (2016). Hypoxia-induced CCL28 promotes recruitment of regulatory T cells and tumor growth in liver cancer. *Oncotarget* 7 (46), 75763–75773. doi:10.18632/oncotarget.12409

Renkvist, N., Castelli, C., Robbins, P. F., and Parmiani, G. (2001). A listing of human tumor antigens recognized by T cells. *Cancer Immunol. Immunother. CII* 50 (1), 3–15. Research Support, Non-U.S. Gov't Review. doi:10.1007/s002620000169

Riley, J. L., Mao, M., Kobayashi, S., Biery, M., Burchard, J., Cavet, G., et al. (2002). Modulation of TCR-induced transcriptional profiles by ligation of CD28, ICOS, and CTLA-4 receptors. *Proc. Natl. Acad. Sci. U. S. A.* 99 (18), 11790–11795. doi:10.1073/pnas.162359999

Robbins, P. F., El-Gamil, M., Li, Y. F., Kawakami, Y., Loftus, D., Appella, E., et al. (1996). A mutated beta-catenin gene encodes a melanoma-specific antigen recognized by tumor infiltrating lymphocytes. *J. Exp. Med.* 183 (3), 1185–1192. doi:10.1084/jem.183.3.1185

Robbins, P. F., Morgan, R. A., Feldman, S. A., Yang, J. C., Sherry, R. M., Dudley, M. E., et al. (2011). Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. *J. Clin. Oncol.* 29 (7), 917–924. doi:10.1200/JCO.2010.32.2537

Rock, K. L., Rothstein, L., Gamble, S., and Fleischacker, C. (1993). Characterization of antigen-presenting cells that present exogenous antigens in association with class I MHC molecules. *J. Immunol.* 150 (2), 438–446. Research Support, U.S. Gov't, P.H.S. doi:10.4049/jimmunol.150.2.438

Rodriguez, A. B., Peske, J. D., Woods, A. N., Leick, K. M., Mauldin, I. S., Meneveau, M. O., et al. (2021). Immune mechanisms orchestrate tertiary lymphoid structures in tumors via cancer-associated fibroblasts. *Cell Rep.* 36 (3), 109422. doi:10.1016/j.celrep.2021.109422

Rodriguez, P. C., Zea, A. H., DeSalvo, J., Culotta, K. S., Zabaleta, J., Quiceno, D. G., et al. (2003). L-arginine consumption by macrophages modulates the expression of CD3 zeta chain in T lymphocytes. *J. Immunol.* 171 (3), 1232–1239. doi:10.4049/jimmunol.171.3.1232

Ruddle, N. H. (1999). Lymphoid neo-organogenesis: lymphotoxin's role in inflammation and development. *Immunol. Res.* 19 (2-3), 119–125. doi:10.1007/bf02786481

Sahin, U., Derhovanessian, E., Miller, M., Kloke, B. P., Simon, P., Löwer, M., et al. (2017). Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature* 547 (7662), 222–226. doi:10.1038/nature23003

Sasidharan Nair, V., Saleh, R., Toor, S. M., Cyprian, F. S., and Elkord, E. (2021). Metabolic reprogramming of T regulatory cells in the hypoxic tumor microenvironment. *Cancer Immunol. Immunother.* 70 (8), 2103–2121. doi:10.1007/s00262-020-02842-y

Sato, M., Hirayama, S., Hwang, D. M., Lara-Guerra, H., Wagnetz, D., Waddell, T. K., et al. (2009). The role of intrapulmonary de novo lymphoid tissue in obliterative bronchiolitis after lung transplantation. *J. Immunol.* 182 (11), 7307–7316. doi:10.4049/jimmunol.0803606

Saxena, M., van der Burg, S. H., Melief, C. J. M., and Bhardwaj, N. (2021). Therapeutic cancer vaccines. *Nat. Rev. Cancer* 21 (6), 360–378. doi:10.1038/s41568-021-00346-0

Scharping, N. E., Menk, A. V., Moreci, R. S., Whetstone, R. D., Dadey, R. E., Watkins, S. C., et al. (2016). The tumor microenvironment represses T cell mitochondrial biogenesis to drive intratumoral T cell metabolic insufficiency and dysfunction. *Immunity* 45 (2), 374–388. doi:10.1016/j.immuni.2016.07.009

Schenkel, J. M., and Masopust, D. (2014). Tissue-resident memory T cells. *Immunity* 41 (6), 886–897. doi:10.1016/j.immuni.2014.12.007

Schietinger, A., Arina, A., Liu, R. B., Wells, S., Huang, J., Engels, B., et al. (2013). Longitudinal confocal microscopy imaging of solid tumor destruction following adoptive T cell transfer. *Oncoimmunology* 2 (11), e26677. doi:10.4161/onci.26677

Schneider, H., Downey, J., Smith, A., Zinselmeyer, B. H., Rush, C., Brewer, J. M., et al. (2006). Reversal of the TCR stop signal by CTLA-4. *Science* 313 (5795), 1972–1975. doi:10.1126/science.1131078

Schneider, H., Mandelbrot, D. A., Greenwald, R. J., Ng, F., Lechler, R., Sharpe, A. H., et al. (2002). Cutting edge: CTLA-4 (CD152) differentially regulates mitogen-activated protein kinases (extracellular signal-regulated kinase and c-jun N-terminal kinase) in CD4+ T cells from receptor/ligand-deficient mice. *J. Immunol.* 169 (7), 3475–3479. doi:10.4049/jimmunol.169.7.3475

Schrama, D., thor Straten, P., Fischer, W. H., McLellan, A. D., Bröcker, E. B., Reisfeld, R. A., et al. (2001). Targeting of lymphotoxin-alpha to the tumor elicits an efficient immune response associated with induction of peripheral lymphoid-like tissue. *Immunity* 14 (2), 111–121. doi:10.1016/s1074-7613(01)00094-2

Schrama, D., Voigt, H., Eggert, A. O., Xiang, R., Zhou, H., Schumacher, T. N., et al. (2008). Immunological tumor destruction in a murine melanoma model by targeted LTalpha independent of secondary lymphoid tissue. *Cancer Immunol. Immunother.* 57 (1), 85–95. doi:10.1007/s00262-007-0352-x

Schumacher, T. N., and Thommen, D. S. (2022). Tertiary lymphoid structures in cancer. *Science* 375 (6576), eabf9419. doi:10.1126/science.abf9419

Scott, A. C., Dündar, F., Zumbo, P., Chandran, S. S., Klebanoff, C. A., Shakiba, M., et al. (2019). TOX is a critical regulator of tumour-specific T cell differentiation. *Nature* 571 (7764), 270–274. doi:10.1038/s41586-019-1324-y

Seo, H., Chen, J., González-Avalos, E., Samaniego-Castruita, D., Das, A., Wang, Y. H., et al. (2019). TOX and TOX2 transcription factors cooperate with NR4A transcription factors to impose CD8+ T cell exhaustion. *Proc. Natl. Acad. Sci. U. S. A.* 116 (25), 12410–12415. doi:10.1073/pnas.1905675116

Shankaran, V., Ikeda, H., Bruce, A. T., White, J. M., Swanson, P. E., Old, L. J., et al. (2001). IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* 410 (6832), 1107–1111. doi:10.1038/35074122

Shrimali, R. K., Yu, Z., Theoret, M. R., Chinnasamy, D., Restifo, N. P., and Rosenberg, S. A. (2010). Antiangiogenic agents can increase lymphocyte infiltration into tumor and enhance the effectiveness of adoptive immunotherapy of cancer. *Cancer Res.* 70 (15), 6171–6180. doi:10.1158/0008-5472.CAN-10-0153

Sims, G. P., Shiono, H., Willcox, N., and Stott, D. I. (2001). Somatic hypermutation and selection of B cells in thymic germinal centers responding to acetylcholine receptor in myasthenia gravis. *J. Immunol.* 167 (4), 1935–1944. doi:10.4049/jimmunol.167.4.1935

Sinclair, L. V., Rolf, J., Emslie, E., Shi, Y. B., Taylor, P. M., and Cantrell, D. A. (2013). Control of amino-acid transport by antigen receptors coordinates the metabolic reprogramming essential for T cell differentiation. *Nat. Immunol.* 14 (5), 500–508. doi:10.1038/ni.2556

Singer, G. G., and Abbas, A. K. (1994). The fas antigen is involved in peripheral but not thymic deletion of T lymphocytes in T cell receptor transgenic mice. *Immunity* 1 (5), 365–371. doi:10.1016/1074-7613(94)90067-1

Siska, P. J., Beckermann, K. E., Mason, F. M., Andrejeva, G., Greenplate, A. R., Sendor, A. B., et al. (2017). Mitochondrial dysregulation and glycolytic insufficiency functionally impair CD8 T cells infiltrating human renal cell carcinoma. *JCI Insight* 2 (12), e93411. doi:10.1172/jci.insight.93411

Slaney, C. Y., Kershaw, M. H., and Darcy, P. K. (2014). Trafficking of T cells into tumors. *Cancer Res.* 74 (24), 7168–7174. doi:10.1158/0008-5472.CAN-14-2458

Slingluff, C. L., Jr., Colella, T. A., Thompson, L., Graham, D. D., Skipper, J. C., Caldwell, J., et al. (2000). Melanomas with concordant loss of multiple melanocytic differentiation proteins: Immune escape that may be overcome by targeting unique or undefined antigens. *Cancer Immunol. Immunother.* 48 (12), 661–672. doi:10.1007/s002620050015

Smith-Garvin, J. E., Koretzky, G. A., and Jordan, M. S. (2009). T cell activation. *Annu. Rev. Immunol.* 27, 591–619. doi:10.1146/annurev.immunol.021908.132706

Swain, S. L., Weinberg, A. D., English, M., and Huston, G. (1990). IL-4 directs the development of Th2-like helper effectors. *J. Immunol.* 145 (11), 3796–3806. doi:10.4049/jimmunol.145.11.3796

Szabo, P. A., Miron, M., and Farber, D. L. (2019). Location, location, location: Tissue resident memory T cells in mice and humans. *Sci. Immunol.* 4 (34), eaas9673. doi:10.1126/sciimmunol.aas9673

Takeuchi, Y., and Nishikawa, H. (2016). Roles of regulatory T cells in cancer immunity. *Int. Immunol.* 28 (8), 401–409. doi:10.1093/intimm/dxw025

Tanaka, T., Sho, M., Takayama, T., Wakatsuki, K., Matsumoto, S., Migita, K., et al. (2014). Endothelin B receptor expression correlates with tumour angiogenesis and prognosis in oesophageal squamous cell carcinoma. *Br. J. Cancer* 110 (4), 1027–1033. doi:10.1038/bjc.2013.784

Teague, T. K., Marrack, P., Kappler, J. W., and Vella, A. T. (1997). IL-6 rescues resting mouse T cells from apoptosis. *J. Immunol.* 158 (12), 5791–5796. doi:10.4049/jimmunol.158.12.5791

Thompson, E. D., Enriquez, H. L., Fu, Y. X., and Engelhard, V. H. (2010). Tumor masses support naive T cell infiltration, activation, and differentiation into effectors. *J. Exp. Med.* 207 (8), 1791–1804. doi:10.1084/jem.20092454

Tomaić, V. (2016). Functional roles of E6 and E7 oncoproteins in HPV-induced malignancies at diverse anatomical sites. *Cancers (Basel)* 8 (10), 95. doi:10.3390/cancers8100095

Topalian, S. L., Hodi, F. S., Brahmer, J. R., Gettinger, S. N., Smith, D. C., McDermott, D. F., et al. (2012). Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N. Engl. J. Med.* 366 (26), 2443–2454. doi:10.1056/NEJMoa1200690

Topalian, S. L., Rivoltini, L., Mancini, M., Ng, J., Hartzman, R. J., and Rosenberg, S. A. (1994). Melanoma-specific CD4+ T lymphocytes recognize human melanoma antigens processed and presented by Epstein-Barr virus-transformed B cells. *Int. J. cancer. J. Int. du cancer* 58 (1), 69–79. Comparative Study. doi:10.1002/ijc.2910580113

Townsend, A. R., Rothbard, J., Gotch, F. M., Bahadur, G., Wraith, D., and McMichael, A. J. (1986). The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. *Cell* 44 (6), 959–968. Research Support, Non-U.S. Gov't. doi:10.1016/0092-8674(86)90019-x

Triebel, F., Jitsukawa, S., Baixeras, E., Roman-Roman, S., Genevee, C., Viegas-Pequignot, E., et al. (1990). LAG-3, a novel lymphocyte activation gene closely related to CD4. *J. Exp. Med.* 171 (5), 1393–1405. doi:10.1084/jem.171.5.1393

Tromp, S. C., oude Egbrink, M. G., Dings, R. P., van Velzen, S., Slaaf, D. W., Hillen, H. F., et al. (2000). Tumor angiogenesis factors reduce leukocyte adhesion *in vivo*. *Int. Immunol.* 12 (5), 671–676. doi:10.1093/intimm/12.5.671

Tsukahara, H., Ende, H., Magazine, H. I., Bahou, W. F., and Goligorsky, M. S. (1994). Molecular and functional characterization of the non-isopeptide-selective ETB receptor in endothelial cells. Receptor coupling to nitric oxide synthase. *J. Biol. Chem.* 269 (34), 21778–21785. doi:10.1016/s0021-9258(17)31872-0

Tvinnereim, A. R., Hamilton, S. E., and Harty, J. T. (2004). Neutrophil involvement in cross-priming CD8+ T cell responses to bacterial antigens. *J. Immunol.* 173 (3), 1994–2002. Comparative Study Research Support, U.S. Gov't, P.H.S. doi:10.4049/jimmunol.173.3.1994

Twomey, J. D., and Zhang, B. (2021). Cancer immunotherapy update: FDA-approved checkpoint inhibitors and companion diagnostics. *AAPS J.* 23 (2), 39. doi:10.1208/s12248-021-00574-0

Urban, J. L., Kripke, M. L., and Schreiber, H. (1986). Stepwise immunologic selection of antigenic variants during tumor growth. *J. Immunol.* 137 (9), 3036–3041. Research Support, U.S. Gov't, P.H.S. doi:10.4049/jimmunol.137.9.3036

Utzschneider, D. T., Charmoy, M., Chennupati, V., Pousse, L., Ferreira, D. P., Calderon-Copete, S., et al. (2016). T cell factor 1-expressing memory-like CD8(+) T cells sustain the immune response to chronic viral infections. *Immunity* 45 (2), 415–427. doi:10.1016/j.immuni.2016.07.021

Utzschneider, D. T., Legat, A., Fuertes Marraco, S. A., Carrié, L., Luescher, I., Speiser, D. E., et al. (2013). T cells maintain an exhausted phenotype after antigen withdrawal and population reexpansion. *Nat. Immunol.* 14 (6), 603–610. doi:10.1038/ni.2606

Vaddepally, R. K., Kharel, P., Pandey, R., Garje, R., and Chandra, A. B. (2020). Review of indications of FDA-approved immune checkpoint inhibitors per NCCN guidelines with the level of evidence. *Cancers* 12 (3), 738–767. doi:10.3390/cancers12030738

Van den Eynde, B., Peeters, O., De Backer, O., Gaugler, B., Lucas, S., and Boon, T. (1995). A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. *J. Exp. Med.* 182 (3), 689–698. Comparative Study Research Support, Non-U.S. Gov't. doi:10.1084/jem.182.3.689

van der Bruggen, P., Traversari, C., Chomez, P., Lurquin, C., De Plaen, E., Van den Eynde, B., et al. (1991). A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science* 254 (5038), 1643–1647. Research Support, Non-U.S. Gov't. doi:10.1126/science.1840703

van der Burg, S. H., Arens, R., Ossendorp, F., van Hall, T., and Melief, C. J. (2016). Vaccines for established cancer: Overcoming the challenges posed by immune evasion. *Nat. Rev. Cancer* 16 (4), 219–233. doi:10.1038/nrc.2016.16

van Dijk, N., Gil-Jimenez, A., Silina, K., Hendricksen, K., Smit, L. A., de Feijter, J. M., et al. (2020). Preoperative ipilimumab plus nivolumab in locoregionally advanced urothelial cancer: The NABUCCO trial. *Nat. Med.* 26 (12), 1839–1844. doi:10.1038/s41591-020-1085-z

Veldhoen, M., Hocking, R. J., Atkins, C. J., Locksley, R. M., and Stockinger, B. (2006). TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 24 (2), 179–189. doi:10.1016/j.immuni.2006.01.001

Vella, A. T., Dow, S., Potter, T. A., Kappler, J., and Marrack, P. (1998). Cytokine-induced survival of activated T cells *in vitro* and *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* 95 (7), 3810–3815. doi:10.1073/pnas.95.7.3810

Verbist, K. C., Guy, C. S., Milasta, S., Liedmann, S., Kamiński, M. M., Wang, R., et al. (2016). Metabolic maintenance of cell asymmetry following division in activated T lymphocytes. *Nature* 532 (7599), 389–393. doi:10.1038/nature17442

Virgin, H. W., Wherry, E. J., and Ahmed, R. (2009). Redefining chronic viral infection. *Cell* 138 (1), 30–50. doi:10.1016/j.cell.2009.06.036

Waldmann, T. A. (2018). Cytokines in cancer immunotherapy. *Cold Spring Harb. Perspect. Biol.* 10 (12), a028472. doi:10.1101/cshperspect.a028472

Wang, H., Franco, F., Tsui, Y. C., Xie, X., Trefny, M. P., Zappasodi, R., et al. (2020). CD36-mediated metabolic adaptation supports regulatory T cell survival and function in tumors. *Nat. Immunol.* 21 (3), 298–308. doi:10.1038/s41590-019-0589-5

Wang, J., Sanmamed, M. F., Datar, I., Su, T. T., Ji, L., Sun, J., et al. (2019). Fibrinogen-like protein 1 is a major immune inhibitory ligand of LAG-3. *Cell* 176 (1-2), 334–347. doi:10.1016/j.cell.2018.11.010

Wang, R., Dillon, C. P., Shi, L. Z., Milasta, S., Carter, R., Finkelstein, D., et al. (2011). The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity* 35 (6), 871–882. doi:10.1016/j.immuni.2011.09.021

Weishaupt, C., Munoz, K. N., Buzney, E., Kupper, T. S., and Fuhlbrigge, R. C. (2007). T-cell distribution and adhesion receptor expression in metastatic melanoma. *Clin. Cancer Res.* 13 (9), 2549–2556. doi:10.1158/1078-0432.CCR-06-2450

Weng, N. P., Levine, B. L., June, C. H., and Hodes, R. J. (1995). Human naive and memory T lymphocytes differ in telomeric length and replicative potential. *Proc. Natl. Acad. Sci. U. S. A.* 92 (24), 11091–11094. doi:10.1073/pnas.92.24.11091

Wherry, E. J. (2011). T cell exhaustion. *Nat. Immunol.* 12 (6), 492–499. doi:10.1038/ni.2035

Wing, K., Onishi, Y., Prieto-Martin, P., Yamaguchi, T., Miyara, M., Fehervari, Z., et al. (2008). CTLA-4 control over Foxp3+ regulatory T cell function. *Science* 322 (5899), 271–275. doi:10.1126/science.1160062

Wolf, Y., Anderson, A. C., and Kuchroo, V. K. (2020). TIM3 comes of age as an inhibitory receptor. *Nat. Rev. Immunol.* 20 (3), 173–185. doi:10.1038/s41577-019-0224-6

Wolkers, M. C., Stoetter, G., Vyth-Dreese, F. A., and Schumacher, T. N. (2001). Redundancy of direct priming and cross-priming in tumor-specific CD8+ T cell responses. *J. Immunol.* 167 (7), 3577–3584. doi:10.4049/jimmunol.167.7.3577

Woo, S. R., Fuertes, M. B., Corrales, L., Spranger, S., Furdyna, M. J., Leung, M. Y., et al. (2014). STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. *Immunity* 41 (5), 830–842. doi:10.1016/j.immuni.2014.10.017

Workel, H. H., Lubbers, J. M., Arnold, R., Prins, T. M., van der Vlies, P., de Lange, K., et al. (2019). A transcriptionally distinct CXCL13+CD103+CD8+ T-cell population is associated with B-cell recruitment and neoantigen load in human cancer. *Cancer Immunol. Res.* 7 (5), 784–796. doi:10.1158/2326-6066.CIR-18-0517

Xu, F., Liu, J., Liu, D., Liu, B., Wang, M., Hu, Z., et al. (2014). LSECtin expressed on melanoma cells promotes tumor progression by inhibiting antitumor T-cell responses. *Cancer Res.* 74 (13), 3418–3428. doi:10.1158/0008-5472.CAN-13-2690

Xu, X., Araki, K., Li, S., Han, J. H., Ye, L., Tan, W. G., et al. (2014). Autophagy is essential for effector CD8(+) T cell survival and memory formation. *Nat. Immunol.* 15 (12), 1152–1161. doi:10.1038/ni.3025

Yamshchikov, G. V., Mullins, D. W., Chang, C. C., Ogino, T., Thompson, L., Presley, J., et al. (2005). Sequential immune escape and shifting of T cell responses in a long-term survivor of melanoma. *J. Immunol.* 174 (11), 6863–6871. Case Reports Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. doi:10.4049/jimmunol.174.11.6863

Yan, M., Jene, N., Byrne, D., Millar, E. K., O'Toole, S. A., McNeil, C. M., et al. (2011). Recruitment of regulatory T cells is correlated with hypoxia-induced CXCR4 expression, and is associated with poor prognosis in basal-like breast cancers. *Breast Cancer Res.* 13 (2), R47. doi:10.1186/bcr2869

Yao, C., Sun, H. W., Lacey, N. E., Ji, Y., Moseman, E. A., Shih, H. Y., et al. (2019). Single-cell RNA-seq reveals TOX as a key regulator of CD8+ T cell persistence in chronic infection. *Nat. Immunol.* 20 (7), 890–901. doi:10.1038/s41590-019-0403-4

Yu, P., Lee, Y., Liu, W., Chin, R. K., Wang, J., Wang, Y., et al. (2004). Priming of naive T cells inside tumors leads to eradication of established tumors. *Nat. Immunol.* 5 (2), 141–149. doi:10.1038/ni1029

Yu, X., Harden, K., Gonzalez, L. C., Francesco, M., Chiang, E., Irving, B., et al. (2009). The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. *Nat. Immunol.* 10 (1), 48–57. doi:10.1038/ni.1674

Zarling, A. L., Ficarro, S. B., White, F. M., Shabanowitz, J., Hunt, D. F., and Engelhard, V. H. (2000). Phosphorylated peptides are naturally processed and presented by major histocompatibility complex class I molecules *in vivo*. *J. Exp. Med.* 192 (12), 1755–1762. doi:10.1084/jem.192.12.1755

Zarling, A. L., Obeng, R. C., Desch, A. N., Pinczewski, J., Cummings, K. L., Deacon, D. H., et al. (2014). MHC-restricted phosphopeptides from insulin receptor substrate-2 and CDC25b offer broad-based immunotherapeutic agents for cancer. *Cancer Res.* 74 (23), 6784–6795. doi:10.1158/0008-5472.CAN-14-0043

Zarling, A. L., Polefrone, J. M., Evans, A. M., Mikesh, L. M., Shabanowitz, J., Lewis, S. T., et al. (2006). Identification of class I MHC-associated phosphopeptides as targets for cancer immunotherapy. *Proc. Natl. Acad. Sci. U. S. A.* 103 (40), 14889–14894. doi:10.1073/pnas.0604045103

Zeh, H. J., III, Perry-Lalley, D., Dudley, M. E., Rosenberg, S. A., and Yang, J. C. (1999). High avidity CTLs for two self-antigens demonstrate superior *in vitro* and *in vivo* antitumor efficacy. *J. Immunol.* 162 (2), 989–994. doi:10.4049/jimmunol.162.2.989

Zehn, D., Thimme, R., Lugli, E., de Almeida, G. P., and Oxenius, A. (2022). 'Stem-like' precursors are the fount to sustain persistent CD8$^+$ T cell responses. *Nat. Immunol.* 23 (6), 836–847. doi:10.1038/s41590-022-01219-w

Zhai, L., Ladomersky, E., Lenzen, A., Nguyen, B., Patel, R., Lauing, K. L., et al. (2018). Ido1 in cancer: A gemini of immune checkpoints. *Cell Mol. Immunol.* 15 (5), 447–457. doi:10.1038/cmi.2017.143

Zhang, H., and Issekutz, A. C. (2002). Down-modulation of monocyte transendothelial migration and endothelial adhesion molecule expression by fibroblast growth factor: Reversal by the anti-angiogenic agent SU6668. *Am. J. Pathol.* 160 (6), 2219–2230. doi:10.1016/S0002-9440(10)61169-8

Zhang, X., Sun, S., Hwang, I., Tough, D. F., and Sprent, J. (1998). Potent and selective stimulation of memory-phenotype CD8+ T cells *in vivo* by IL-15. *Immunity* 8 (5), 591–599. doi:10.1016/s1074-7613(00)80564-6

Zhang, Y., Cai, P., Liang, T., Wang, L., and Hu, L. (2017). TIM-3 is a potential prognostic marker for patients with solid tumors: A systematic review and meta-analysis. *Oncotarget* 8 (19), 31705–31713. doi:10.18632/oncotarget.15954

Zheng, L., Fisher, G., Miller, R. E., Peschon, J., Lynch, D. H., and Lenardo, M. J. (1995). Induction of apoptosis in mature T cells by tumour necrosis factor. *Nature* 377 (6547), 348–351. doi:10.1038/377348a0

Zhou, L., Ivanov, I. I., Spolski, R., Min, R., Shenderov, K., Egawa, T., et al. (2007). IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat. Immunol.* 8 (9), 967–974. doi:10.1038/ni1488

Zhu, C., Anderson, A. C., Schubart, A., Xiong, H., Imitola, J., Khoury, S. J., et al. (2005). The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. *Nat. Immunol.* 6 (12), 1245–1252. doi:10.1038/ni1271

Zhu, J. T. (2018). T helper cell differentiation, heterogeneity, and plasticity. *Cold Spring Harb. Perspect. Biol.* 10 (10), a030338. doi:10.1101/cshperspect.a030338

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