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REVIEW

Immunotherapy resistance in solid tumors: mechanisms and potential solutions

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ABSTRACT

While the emergence of immunotherapies has fundamentally altered the management of solid tumors, cancers exploit many complex biological mechanisms that result in resistance to these agents. These encompass a broad range of cellular activities – from modification of traditional paradigms of immunity via antigen presentation and immunoregulation to metabolic modifications and manipulation of the tumor microenvironment. Intervening on these intricate processes may provide clinical benefit in patients with solid tumors by overcoming resistance to immunotherapies, which is why it has become an area of tremendous research interest with practice-changing implications. This review details the major ways cancers avoid both natural immunity and immunotherapies through primary (innate) and secondary (acquired) mechanisms of resistance, and it considers available and emerging therapeutic approaches to overcoming immunotherapy resistance.

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Introduction

Cancer immunotherapies have captured the attention of the scientific and lay communities alike, yet many obstacles continue to limit their use in solid tumors. Impressive clinical successes of immune checkpoint inhibitors (ICIs) have been notched in immunologically "hot" tumors like melanoma, non-small cell lung cancer (NSCLC), cutaneous squamous cell carcinoma, and renal cell carcinoma. Meanwhile, the efficacy of cancer vaccines and adoptive T cell treatments like tumor infiltrating lymphocytes (TILs) and chimeric antigen receptor (CAR) T cells have been much more tempered, noting the latter has enjoyed particular success in hematologic malignancies.[1] T cell engagers (TCEs), and specifically T cell engaging bispecific antibodies (e.g., BiTE®), have recently become a much more active area of investigation in solid tumors, as well, though the large-scale translation of this technology to clinical practice is limited and is most notably characterized by tabentafusp-tebn for uveal melanoma.[2,3]

The majority of presently available (and investigational) cancer immunotherapies highlight the overarching importance of anti-tumor T cell activity. This is because CD8-expressing cytotoxic T lymphocytes (CTLs) are ultimately responsible for the final step in the process of immune regulation of cancers: killing tumor cells. They perform this task through two main processes. The most well-described is the secretion of the pore-forming protein perforin, which allows co-secreted granzymes to enter the target cell and induce apoptosis.[4] Additionally, CTL-induced Fas signaling results in Fas clustering on target cell membranes and the subsequent recruitment of the Fas-associated death domain (FADD), inducing apoptosis through caspases.[5]

Although accurate, this characterization oversimplifies immunologic tumor death. In reality, the machinations that set the stage for the interaction between tumors and CTLs are carefully coordinated. These are summarized in Figure 1 and explored more in-depth in the following review. Recognition of cancer-related antigens is key to identifying tumors as "non-self" and thus immunogenic, involving antigen-presenting cells (APCs) that express antigenic peptides on major histocompatibility complexes (MHCs). CTLs also rely on supportive and regulatory network of immune cells that signal each other through complex cytokine signaling. Stimulatory and inhibitory immune checkpoints mediate the balance of immune activity to maximize anti-tumor effect without jeopardizing self-tolerance. Other immune cells like T regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSCs) contribute immunosuppressive effects that are important to control excessive inflammatory responses. Tempering of immunity is also mediated by cancer-associated fibroblasts (CAFs) in the tumor microenvironment (TME).

For some tumors, there is primary resistance to immunotherapies owing to baseline characteristics that impact the aforementioned factors and many others. In addition to methods of primary resistance that affect the traditional immune paradigm (i.e., antigen presentation, cytokine signaling, checkpoints, and immunoregulation), these include the presence of driver mutations as well as mutations that confer resistance to immunotherapies, a decreased tendency for immunogenic tumor cell death, metabolic derangements, and individual differences in microbiota. In tumors that are initially sensitive to immune-directed treatments, adaptations to immune surveillance allow them to secondarily resist currently available

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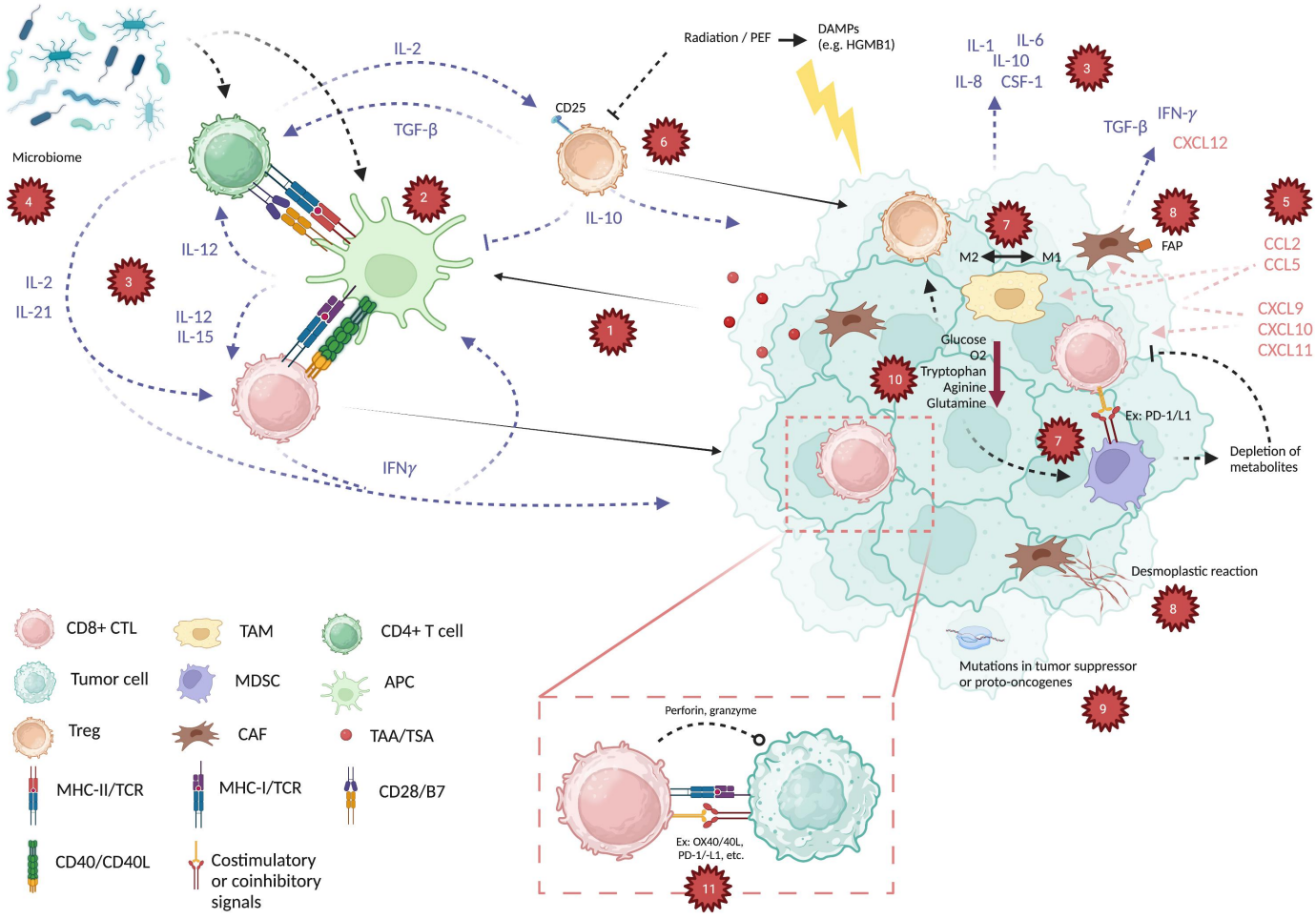

Figure 1. Select tumor functions, anti-tumor immune components, and their interactions. (a) tumor-associated and tumor-specific antigens (TAA/TSA) are processed by antigen-presenting cells (APC), which then (b) communicate with both CD4+ and CD8+ T cells (CTL) through major histocompatibility class I and II (MHC-I, MHC-II), along with co-signals CD28/B7 and CD40/CD40L. (c) a complex interplay of cytokines including interleukin-2 (IL-2), IL-12, IL-15, IFNγ, TGF-β that are released by immune cells and from the tumor microenvironment tightly control the activation of the immune system through these steps (blue arrows/text). (d) this is further influenced by an individual's microbiome, influencing immune responses. (e) Chemokines such as CXCL12, CCL5, and others (red arrows/text) mediate recruitment of immune cells to the tumor. Meanwhile, immune regulation is achieved through cellular components such as (f) regulatory T cells (Treg), as well as (g) myeloid-derived suppressor cells (MDSC) and a balance of M1 and M2 tumor-associated macrophages (TAM) in the tumor microenvironment (TME). (h) cancer associated fibroblasts (CAF) express fibroblast associated protein (FAP) and decrease responses to immunotherapies through generation of cytokines and chemokines, as well as causing a desmoplastic reaction in the TME. (i) specific tumor mutations also can provoke pro- or anti-immune functions through various mechanisms. (j) immune evasion is further achieved through depletion of metabolites, decreased glucose, oxygen (O2), tryptophan, arginine, and glutamine. (k) based on these factors, CD8+ CTLs perform direct killing of tumor cells after activation of the T cell receptor (TCR) and a balance of costimulatory (e.g., PD-1, LAG-3) and coinhibitory (e.g., 4-1BB, OX40) signals. Created with BioRender.

treatments. For example, primary (innate) and secondary (acquired) resistance to ICIs can be broken down into categories such as insufficient generation of anti-tumor T cells, inadequate function of those T cells, and impaired T cell memory.[6]

In cases of both primary and secondary resistance, patients may be exposed to more toxic alternatives as a result of the lack of efficacy of immunotherapies for their tumors, driving the need for novel solutions to this problem. Therefore, overcoming this resistance has become an extraordinarily active field of research – with profound clinical implications. Still, the complexity of the interrelated processes involved highlights the need for better understanding of the immune landscape of cancer.This review provides an overview of the major ways that cancers avoid usual immune functions and immune-directed therapies. It also explores how these strategies can be overcome with available and investigational therapeutic approaches, while highlighting existing gaps in research and areas of future interest. Key ongoing clinical trials discussed in the appropriate sections are further summarized in Table 1 for review.

Cancer antigens and antigen presentation

Before carrying out the functions of direct tumor killing, CTLs must first be able to recognize their targets through cancer-related antigens. These come in two primary forms: tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs).[7] TAAs are more weakly immunogenic because they are encoded in germline tissues (and thus "self") but preferentially expressed in tumors. These include familiar examples such as PSA, HER2, CEA, mesothelin, EGFR, and MAGE. Conversely, TSAs are unique neoantigens that arise from oncogenic driver

Table 1. Selected ongoing trials addressing immunotherapy resistance in solid tumors.

Category	Trial	Phase	Mechanism of Experimental Agent	Primary Malignancies	Primary Outcome(s)
Antigen Presentation	NCT04943679	I/II	Pegylated interferon	Hepatocellular carcinoma	AE
	NCT04609579	I	STING agonist	Advanced solid tumors, lymphoma	MTD
					AE
	NCT05139082	I/II	CDK4/6 inhibitor	Gastrointestinal tumors	ORR
Cytokine Signaling/ Production	NCT05472506	I	AhR antagonist	Head and neck squamous cell carcinoma	AE
					ORR
					DCR
					DOR
	NCT04691817	I/II	Anti-IL-6	Non-small cell lung cancer	ORR
	NCT03631199	III	Anti-IL-1β	Non-small cell lung cancer	DLT
					PFS
					OS
Costimulatory Signals	NCT05082610	I	Anti-VISTA	Advanced or metastatic solid tumors	DLT
					MTD
					AE
	NCT04137900	I	Anti-BTLA	Advanced solid tumors, lymphoma	AE
	NCT04080804	II	Anti-LAG-3	Resectable head and neck cancers	AE
Bispecific Antibodies	NCT05263180	I	Anti-PD-L1×OX40	Advanced or metastatic solid tumors	AE
					sAE
					MTD
					DLT
	NCT05442996	I	Anti-EGFRx4-1BB	Advanced or metastatic solid tumors	AE
					DLT
					MTD
	NCT03809624	II	Anti-PDL1×4-1BB	Advanced or metastatic solid tumors	AE
					MTD or RPTD
TAM Polarization	NCT04682431	Ia/Ib	TREM-1 inhibitor	Advanced or metastatic solid tumors	AE
					DLT
	NCT04691375	Ia/Ib	TREM-2 inhibitor	Advanced or metastatic solid tumors	AE
					DLT
Tryptophan Metabolism	NCT03414229	II	IDO1 inhibitor	Advanced or metastatic sarcoma	Best ORR
	NCT03459222	I/II	IDO1 inhibitor	Advanced or metastatic solid tumors	AE
					sAE
					DLT
					ORR
					DCR
					DOR
Fecal Microbiota Modification/ Transplantation	NCT05251389	Ib/IIa	Fecal microbiota from immunotherapy responder	Advanced stage cutaneous melanoma	ORR

AE = adverse events; DCR = disease control rate; DLT = dose-limiting toxicities; DOR = duration of response; MTD = maximum tolerated dose; PFS = progression free survival; ORR = objective response rate; OS = overall survival; RPTD = recommended phase 2 dose; sAE = serious adverse events.

mutations like single nucleotide variants (SNVs), insertion-deletions and fusions, and so they more strongly induce immune responses as they are seen as "non-self." For this reason, though many targeted treatments have targeted on TAAs in the past, it may be prudent to focus on TSAs in the development of immunotherapies.

TAAs and TSAs are taken up by APCs like dendritic cells (DCs), macrophages, and B cells. These cells express MHC class I (MHC-I), which is subsequently loaded with relevant epitopes and prime CD8+ CTLs to kill tumor cells which express those epitopes through their own MHC-I. As such, one strategy employed by cancers to avoid immune killing is the downregulation of MHC-I on tumor cells. This is accomplished by somatic genetic defects (such as those in TAP1/TAP2[8–10] or MHC subunits like β2-microglobulin),[11] transcriptional silencing through epigenetic processes among other methods,[12] and post-transcriptional microRNA (miRNA) silencing.[13–16] Regardless of the cause, a decrease in MHC-I antigen presentation has been associated with resistance to ICI therapy as well as poorer clinical outcomes in solid tumors.[17,18] Tumors may also interfere with the APCs themselves, through methods such as inhibition of DC recruitment, differentiation, and maturation.[7]

Overcoming downregulation of antigen presentation through these means is an active area of research. Conventional chemotherapy has the potential to increase MHC-I expression, as has been shown with gemcitabine in colon, breast, and lung cancer cell lines.[19,20] Similar findings were described by Messaoudene et al. in breast cancer patients treated with neoadjuvant chemotherapy, but differential immune responses were seen in primary tumors *versus* metastatic lymph nodes.[21] There are also indications that radiation similarly increases MHC-I density on tumor cells.[22] Taken together, these findings imply that traditional anti-cancer treatments have been modulating immune responses even without the explicit intention of doing so. It is possible that the intentional use of traditional chemotherapeutics for these effects (and not necessarily pure cytotoxicity) can be combined with immunotherapies for synergistic activity.

However, more targeted mechanisms for increasing MHC-I expression have also been identified. Transcriptional induction of MHC-I expression and subsequent antigen presentation was accomplished pre-clinically

through the modulation of the JAK-STAT pathway (through NF-κ)[23] via administration of interferons (especially IFNγ at low doses),[24,25] reduction in TRAF3,[26] and the use of CDK4/6 inhibitors.[27] Increases in MHC-I have also been seen with other changes in IFNγ and NF-κB pathways, involving application of retinoids, modulating expression of NLRC5, and agonism of Stimulator of Interferon Genes (STING).[13,28] Therapeutic targeting of these mechanisms is under investigation, with multiple trials combining ICIs with treatments such as pegylated interferons (NCT04943679), STING agonists (NCT04609579), and CDK4/6 inhibitors (NCT05139082) in both the upfront and ICI-resistant settings. These trials, along with other active investigations into overcoming immune resistance, are summarized in Table 1. Overall, changes to transcriptional regulation of MHC-I and other methods of increasing its expression may serve a crucial purpose in overcoming both primary and adaptive resistance to immunotherapies.

Meanwhile, adoptive T cell therapies remain a hopeful method of circumventing the need for antigen presentation on MHC-I altogether. CAR T cells and TCEs are not reliant on MHC-I for anti-tumor activity, as they rely on manufactured mechanisms of immune activation. However, tumors present adaptive challenges to these treatments as well. They may either downregulate expression of cell surface antigen- or change-specific immunogenic epitopes, and this has been demonstrated in both hematologic malignancies (such as the therapeutic target CD19) and glioblastoma (IL13Rα2).[29,30] This, along with the antigen heterogeneity and low antigen density in solid tumors, is the reason that there is enthusiasm for developing such therapies to recognize multiple antigens.[2,31]

Cytokines

As previously noted, IFNγ signaling is crucial in the presentation of antigens to CTLs to identify tumors for killing. IFNγ is produced by T cells and NK cells through signaling by interleukin-12 (IL-12) and IL-18, inducing effects on both malignant and immune cells. IFNγ (type II IFN), along with types I (e.g. IFNα) and III (e.g. IFNγ) interferons, mediate upregulation of MHCs, modulation of apoptosis and differentiation, activation of the immune response, and inhibition of tumor growth.[32] However, multiple other cytokines are integral to anti-cancer immunity. IL-2 is produced primarily by CD4+ T cells and controls and supports the expansion of CD8+ CTLs, but it can also contrarily induce immunosuppression by inducing T regulatory cells (Tregs).[33] Cytokines with direct effects on the proliferation, activation, or support of anti-tumor CTLs also include IL-12, IL-15, and IL-21, among others.[34]

Exploitation and hijacking of these interconnected signals by tumors can impair normal immune functions. For instance, it has been shown that persistent IL-2 production in the tumor microenvironment (TME) can surpass CD8+ T cell activation and induce T cell exhaustion by way of a pathway involving STAT5 and aryl hydrocarbon receptor (AhR).[35] In fact, this is one rationale for combining AhR inhibitors with immunotherapies in ICI-resistant tumors, as some clinical trials are now investigating (e.g. NCT05472506). Deletion of the genes encoding IL-2, IL-21, and especially IL-15 in colorectal cancers have also been shown to be associated with higher risk of recurrence, metastatic disease, and poorer patient survival, indicating both the importance of these signals in the TME and possible role for therapeutic (exogenous) administration to overcome this immune avoidance.[36]

Unfortunately, the simple systemic administration of interleukins and interferons at anti-neoplastic doses has historically come with hard-to-tolerate or unacceptable toxicities, and short half-lives can limit their utility.[37] Additionally, because of the paradoxical pro-tumor effects that these signals may induce under different conditions, they do not represent the refined tools necessary to elicit the desired response. Still, modifications to these cytokines may allow their administration, inducing an anti-tumor effect while minimizing on-target, off-tumor toxicities.[38] One such example is a tumor-conditional pro-IL-15, which uses IL-15 Rβ fused to IL-15/IL-15 Rα to overcome ICI resistance, increase intratumoral CD8+ T cells, and improve the toxicity profile in mice.[39] Similarly, when a pro-IL-2 (IL-2 prodrug preferentially activated in tumors) was combined with checkpoint blockade in a model of anti-PD-L1 resistant mice, anti-tumor responses were seen with relatively minimal toxicity.[40] Comparable attempts at overcoming ICI resistance are being undertaken using variations of IL-21.[41] Modifications to interleukins – along with combination strategies – may very well make them viable cancer therapeutics despite their limited roles thus far.

Meanwhile, tumor and TME production of IL-1, IL-6, IL-8, IL-10, colony stimulating factor-1 (CSF-1), and transforming growth factor-beta (TGF-β) can contribute directly to immunosuppression.[42,43] Attempts to combine targets of these cytokines with existing immunotherapies are ongoing. For instance, the anti-IL-6 antibody tocilizumab is currently under investigation in combination with atezolizumab in patients with lung cancer who progressed on ICIs (NCT04691817). Tocilizumab has been shown to decrease the growth of oral squamous cell carcinoma in a murine model,[44] and its combination with anti-PD-L1 in mice is shown to enhance Th1 response and induce a synergistic therapeutic response, even in the setting of ICI resistance.[45–47] Still, clinical applications of IL-6 blockade have been limited to managing immunotherapy toxicities rather than potentiating their anti-cancer effects, as efficacy of this approach has not been established. Significant limitations in efficacy have also been seen with the anti-IL-1β agent canakinumab in lung cancer when combined with pembrolizumab (NCT03631199), but this is another potential strategy for overcoming tumor resistance to immunotherapy. In order to overcome the immunosuppressive aspects of the TME and drive more robust immune responses, co-administration of CAR T cells with low-dose IL-2 has been attempted, while others have modified ("armored") CAR T cells to express a dominant negative receptor designed to inhibit TGF-β signaling.[48,49]

TGF-β is a highly pleiotropic cytokine that plays an important role in wound healing, angiogenesis, immunoregulation, and cancer. There are three isoforms (TGF-β1, TGF-β2, and TGF-β3), all of which signal through the same TGF-β receptor complex. All three isoforms are expressed as inactive protein

complexes, and release of the active growth factor is required to allow signaling via the receptor complex. Signaling of the TGF-β pathway involves binding of TGF-β ligands to a type II receptor on the cell membrane, which recruits and phosphorylates a type I receptor. Phosphorylation of the type I receptor, in turn, phosphorylates the signaling factor Smad2 or Smad3, and trimerizes with Smad4 to enter the nucleus and bind to transcription factors to regulate the expression of specific genes.[50] Recent studies have revealed enhanced TGF-β signaling in some cancer patients who do not respond to ICIs, suggesting immunosuppressive changes in TME due to TGF-β signaling in the stroma that restrains anti-tumor immunity; some studies further indicate there is enhanced anti-tumor activity by ICIs when used in combination with a TGF-β inhibitor.[51–55]

While multiple agents targeting TGF-β have been developed with a focus on the physiological effects in the TME, TGF-β inhibitors that inhibit multiple isoforms have been shown to have serious cardiac toxicity and bleeding in non-clinical studies.[56–58] In the clinical development of a pan-TGF-β inhibitor, a variety of on-target toxicities have been observed related to disruption of physiologic TGF-β signaling, including clinically significant bleeding.[59–62] Mouse and human genetic data suggest that some of the toxicity, including cardiotoxicity, may be related to inhibition of TGF-β isoforms 2 and 3.[63,64] Recent analysis of RNA sequencing data from The Cancer Genome Atlas identified TGF-β1 as the most prevalent TGF-β isoform in solid tumors, which suggest TGF-β1 may be important in tumors.[54] Therefore, selectively inhibiting TGF-β1, while sparing isoforms 2 and 3, may lead to a potentially safer and more effective approach to targeting the TGF-β pathway in tumors and may result in potentiation of ICIs, and this approach is currently under investigation.

Finally, a subset of cytokines called chemokines can affect both pro- and anti-tumor responses based on their roles in chemotaxis and cell adhesion. The entire cohort of these signals is outside the scope of this review but is explored extensively elsewhere.[65] However, it is important to note that tumors sensitive to immune checkpoint blockade are generally rich in CXCL9, CXCL10, and CXCL11. Meanwhile, tumor and stromal cell production of CCL2 is associated with immunosuppression through recruitment of MDSCs and monocytes, and inhibition of its interaction with CCR2 has the potential to sensitize tumors to anti-PD-1 treatment *in vitro*.[66] Cancers that exhibit loss of the phosphatase and tensin homolog gene (*PTEN*) may be particularly effective at immune evasion through this mechanism, as they exhibit significant upregulation of CCL2.[67] Meanwhile, co-targeting of CCL5 with maraviroc and PD-1 with pembrolizumab has been investigated in mismatch repair-proficient colorectal cancer in the phase I PICASSO trial.[68] Although objective responses were underwhelming, the patient population had a longer survival than would be expected. Finally, developers of CAR T cells have also sought to more accurately target tumors by incorporating chemokine receptors into their constructs, including CXCR1, CXCR2, CCR4, CCR2b.[69]

In summary, although cytokines have so far had limited success in cancer due to both middling effectiveness as monotherapies and unacceptable toxicity profiles, recent advancements may allow them to be incorporated into cancer care. They have shown most notable promise in boosting the effects of existing checkpoint inhibitors and CAR T cell therapies, and these parallel immunotherapy approaches may prove to be how cytokine treatments ultimately reach patients.

Costimulatory signals and immune checkpoints

Once T cells have been primed for antigen recognition, have been activated, and are recruited to the proximity of tumor cells by chemokines, they rely on a balance of stimulatory and inhibitory signals to direct their killing. This is accomplished through what are called immune checkpoints, the arbiters of these signals, beyond the interaction between MHC and the T cell receptor (TCR). In general, cancer therapeutics either block inhibitory checkpoint signaling (including PD-1, CTLA-4, LAG-3, TIM-3, TIGIT, BTLA, and VISTA) or induce stimulatory checkpoints (including CD27, CD28, 4-1BB, CD40, OX40, GITR, and ICOS) in order to generate anti-tumor responses.[70]

The first checkpoint inhibitor was approved by the FDA in 2011, and since then the dominant immunotherapies both in clinical use and under active scientific investigation have been ICIs. Unfortunately, well-established monoclonal antibodies directed against CTLA-4 and PD-1/PD-L1 have shown activity only in certain solid tumors and limited to certain conditions such as high tumor mutational burden and microsatellite instability-high (MSI-H). Moreover, while responses can be durable in some cases of advanced/metastatic disease, resistance ultimately develops in the majority.

The mechanisms by which tumors develop resistance to ICIs are manifold. Resistance may be primary or acquired, and it generally occurs because of insufficient generation of effector T cells, dysfunction of those T cells, or lack of memory T cell development.[6] While these steps involve the aforementioned biological machinery (i.e. antigen presentation, cytokines, and chemokines), there is ongoing interest in determining what modifies surface expression of PD-1/-L1 and other checkpoints, as this dictates responses to ICIs (albeit not always reliably).[71–74] After transcription and translation, regulation of surface inhibitory immune checkpoints relies on some combination of delivery to the lipid bilayer of cells, glycosylation, internalization, and recycling or ubiquitination.[70] However, these are yet untargetable by clinically available drugs. Therefore, overcoming inhibitory checkpoint resistance largely relies on increasing antigen presentation/recognition, modulating the balance of cytokines, targeting multiple checkpoints simultaneously, and reducing the effect of immunosuppressive tumor-associated macrophages (TAMs) and MDSCs.

The efficacy of co-blockade of inhibitory checkpoints was first demonstrated through the use of ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1) in melanoma, and this combination has been expanded to other tumors including NSCLC and renal cell carcinoma.[75–77] More recently, relatlimab (anti-LAG-3) plus nivolumab was shown to have improved clinical efficacy compared with PD-1 blockade alone in patients with advanced melanoma with a 12-month progression-free survival (PFS) 47.7% vs. 36%.[78] This is a logical approach to

overcoming the resistance to single checkpoint blockade because of the compensatory surface upregulation of other inhibitory checkpoints.[79] Attendant to these findings, combinations of anti-PD-1/-L1 treatments with ICIs targeting other inhibitory checkpoints have been under investigation in both pre-clinical and clinical trials, including those that target VISTA (e.g. NCT05082610),[80] TIM-3 (e.g. NCT02608268),[81] BTLA (e.g. NCT04137900),[82] and LAG-3 (e.g. NCT04080804).[83] Bispecific antibodies targeting multiple inhibitory checkpoints may also provide the opportunity to administer one agent with effects on two checkpoint targets such as TIM-3 and PD-1.[84,85] Finally, some CAR T cell products have begun to incorporate dominant negative receptors (DNRs). A PD-1 DNR, for instance, provides a nonfunctional "decoy" receptor for tumors expressing PD-L1, allowing T cells to achieve better cytotoxicity.[86] Although the most common of these approaches has focused on the PD-1/PD-L1 checkpoint, others have incorporated DNRs against Fas and TGF-β.[87,88]

Targeting stimulatory checkpoints is also a viable way to overcome resistance to existing ICIs and adoptive T cell therapies. In fact, the rescue of T cell exhaustion during PD-1 therapy may be dependent on stimulatory factors such as CD28.[89] CD28 and 4-1BB are used as costimulatory domains in CAR T cell products, with neither demonstrating convincing benefit over the other.[90] Although agonists of such checkpoints are biologically promising as immune activators, they have historically been limited by toxicity in the form of cytokine release syndrome.[91] However, there are still models of combined 4-1BB stimulation and PD-1 inhibition that have some promise due to anti-tumor effect.[92] Similar models of CD27 checkpoint stimulation with PD-1 blockade have been established, and this includes synergism with adoptive T cell therapy.[93] In fact, many recent investigations have focused on multi-targeting through the use of bispecific antibodies. These new approaches may be able to potentiate anti-PD-1/-L1 therapies when given together at the outset, or else allow for improved and continued efficacy of established ICIs even after progression on those agents. Currently, they are being investigated in trials such as NCT05263180 (anti-PD-L1×OX40), NCT05442996 (anti-EGFRx4-1BB plus anti-PD-1), and NCT03809624 (anti-PD-L1×4-1BB). As compared with blocking inhibitory checkpoints, the stimulatory checkpoint approach has not advanced as quickly to clinical uses despite viability in the lab setting. These investigations provide hope that there is still a role for it, as long as their anti-tumor activity can be balanced against inflammatory adverse effects.

Cell-dependent immunosuppressive processes

Several immunosuppressive processes exist in order to keep the immune system from becoming so activated that it damages its host. Some of these have been reviewed already, as they are natural processes – inhibitory checkpoints, for instance – that are co-opted by cancers and their microenvironments. However, certain cells that mediate the dampening of an immune response deserve mention, as reducing their function may reinvigorate immune responses in tumors that have become resistant to immunotherapies. These include myeloid derived suppressor cells (MDSCs), tumor associated macrophages (TAMs), tumor associated neutrophils (TANs), and regulatory T cells (Tregs).

Historically, both characterization of MDSCs and their distinction from the myeloid lineage TAMs/TANs have been difficult.[94] Broadly, it is held that MDSCs have a pro-tumor effect whereas TAMs and TANs can either aid or inhibit cancer growth. Further, MDSCs represent a spectrum of cells and can be divided into polymorphonuclear (PMN-MDSC) and monocytic (M-MDSC) phenotypes. They support other immunosuppressive elements of the tumor and immune system like Tregs through arginase and indoleamine 1,2-dioxygenase (IDO)-dependent pathways; directly interfere with T cell functioning by producing reactive oxygen species, nitric oxide, and cytokines; modulate checkpoint expression; act as a source of chemokines; and mediate carcinogenesis, cancer progression, and metastasis.[95] Meanwhile, TAMs and TANs also have roles in tumor initiation, angiogenesis, and metastasis, inspiring treatments focus on these myeloid lineage cells as a strategy to induce or reinvigorate anti-tumor immune responses.[96]

Since MDSCs are regulators of immunosuppression, they have been targeted as ways to overcome both primary and acquired resistance to immunotherapies. Platinum drugs have shown some activity against MDSCs, with evidence that cisplatin reduces PMN-MDSCs and oxaliplatin reduces M-MDSCs *in vitro*, indicating that different cytotoxic therapies may be able to target specific populations.[97–99] Bevacizumab has also demonstrated an ability to decrease PMN-MDSCs in lung cancer because of the reliance of these cells on VEGF signaling.[100] Other attempts at decreasing the number and/or function of MDSCs in tumors – or else forcing their differentiation into more mature cells – have used agents such as those targeting calgranulin A and B, anti-IL-1γ drugs, all-trans retinoic acid (ATRA), cytokine signaling inhibitors (CCL2/CCR2, CXCR1/2), histone deacetylase (HDAC) inhibitors, COX2 inhibitors, PDE5 inhibitors, and toll-like receptor (TLR) agonists.[101] Much of the evidence is currently preclinical, but some early phase data has been promising. For instance, adding an HDAC inhibitor (entinostat) to pembrolizumab after progression on ICI was associated with an overall response rate of 9.2% and median duration of response of 10.1 months in patients with lung cancer, and more benefit was seen in patients with high levels of circulating monocytes.[102]

Meanwhile, targeting of TAMs mainly has focused on the tendency toward M2 polarization, which establishes an overall immunosuppressive state – though this characterization is an oversimplification of dynamic processes. The balance of M2 over M1 polarization may be at least partially related to hypoxia in the TME that occurs along with abnormal angiogenesis; this hypoxia then induces the triggering receptor expressed on myeloid cells-1 (TREM-1).[103,104] In fact, a clinical trial is investigating a TREM-1 inhibitor in combination with pembrolizumab in patients with advanced cancers, including those who progressed on ICI (NCT04682431). TREM-2 is being targeted for similar reasons (NCT04691375). Furthermore, signaling between CSF-1 and its receptor (CSF-1 R), expressed on macrophages, aids in the survival and proliferation of TAMs.[105] Unfortunately, a phase

I trial of pexidartinib (a CSF-1 R inhibitor) with durvalumab did not have significant clinical activity in patients with advanced pancreatic and colorectal cancers.[106] Zoledronic acid (ZA) may be another way to inhibit M2 polarization of TAMs, and a small study of ZA + ICI in patients with lung cancer showed improved clinical responses when compared with ICI therapy alone.[107] Trials of interventions targeting TANs (i.e. with PDE5 inhibitors, COX2 inhibitors, etc.) are even more limited,[96] especially in the setting of immunotherapy resistance, although there is active interest in better characterizing *in vivo* effects of these cells, as is being investigated in bone sarcomas (NCT04867421).

Regulatory T cells (Tregs) are a subset of CD4+ T cells that aid in immunosuppression through a number of mechanisms, and their activity is especially increased in the setting of PD-L1 targeting by ICIs.[108] Tregs have been difficult to specifically target with treatments, as anti-Treg therapies can have more broad effects on other immune cells populations, as well. However, Treg depletion with anti-CD25 agents like daclizumab may be able to selectively reduce the effects of Tregs in the TME, and this approach has been used to augment the effects of a dendritic cell vaccine in a phase I/II trial in patients with melanoma.[109] Inhibiting CCR4 with mogamulizumab, in combination with nivolumab, has also been shown to reduce populations of Treg cells while simultaneously increasing tumor infiltrating lymphocytes (TILs).[110] However, while a similar approach with mogamulizumab plus other ICIs (durvalumab or tremelimumab) also showed decreases in peripheral and tumoral Tregs, this did not correlate with clinical outcomes.[111] Similar to other approaches mentioned above, cytotoxic chemotherapy (especially cyclophosphamide),[112] checkpoint blockade, use of small molecules, and agents acting on angiogenesis have demonstrated effects on Tregs as they have with various other immune cells. However, narrowing the effects of these therapies to a small subpopulation of T cells remains a challenge in the clinical setting.

Cancer associated fibroblasts

Cancer-associated fibroblasts (CAFs) are a functional part of the TME that are derived from mesenchymal tissues. They have a number of roles that are distinct from normal fibroblasts, and some of these relate to the immune nature of tumors.[113] CAFs participate in complex interactions with T cells, MDSCs, TAMs, and TANs, and they provide a primary source of pro-tumor cytokines and chemokines (most notably TGF-β, IFNγ, and CXCL12).[114] Among other mechanisms, CAFs have also been shown to cause resistance to traditional cancer treatments like chemotherapy and radiation by mediating the desmoplastic reaction, as is seen in gastrointestinal cancers – most notably in pancreatic cancer, a quintessential example of an immunologically "cold" tumor.[115] Therefore, targeting CAFs has more recently garnered interest as a method to improve the efficacy of cancer therapeutics, especially with regards to immune processes.

One strategy to decrease the immunosuppressive effects of CAFs in tumors has been to prevent differentiation of resident fibroblasts into CAFs by blocking signaling of the TGF-β family. In a model of gastrointestinal stromal tumor (GIST), administering an anti-TGF-β1 antibody inhibited the transition of resident fibroblasts to CAFs.[116] Approached a different way, agonism of liver X receptors (LXR) inhibits signaling of TGF- and thus CAF differentiation.[117] A unique approach was taken by Freedman et al., who used an oncolytic virus that expressed a TCE targeting fibroblast activation protein (FAP) on CAFs. This caused T cell dependent killing of CAFs with an attendant increase in gene expression for checkpoint markers like CTLA-4 and LAG-3, decreases in fibroblast-associated gene expression, and repolarization of M2 macrophages.[118] Although these approaches have promise in reducing the immunosuppressive effects of CAFs, it remains to be seen whether they can aid in overcoming adaptive resistance to immunotherapies like ICIs and adoptive T cell therapies in the clinic. However, there is at least some pre-clinical evidence that interfering with functions of CAFs – such as blocking the interaction of CXCL12-CXCR4 and inhibiting the NOX4 enzyme – can enhance or restore responses to immunotherapies.[119,120] Still, targeting CAFs is a more nascent area of research, meaning that any understanding of their roles is incomplete. Since they clearly affect the immune TME they remain a potential target for novel drug development – especially for tumors with well-defined desmoplastic reactions like those of the pancreas and breast.

Specific tumoral mutations with immunologic implications

Tumor-specific mutations play a critical role in the regulation of the TME, often generating a hostile local environment and characteristic chemical changes. This provides insight into how these mutations impart resistance to immunotherapies. Some of the most important specific mutations that affect tumor immunityacross solid malignanciesare those in tumor suppressor genes, proto-oncogenes, and primary driver mutations.

Several tumor suppressor genes have been characterized for their role in oncogenesis and their effect on immunotherapy response. *PTEN* mutations are implicated in the proliferation of numerous cancers and result in enhanced MAPK signaling, increased spatial clustering of tumor cells, and decreased CD8 + CTL tumor infiltration.[121] The release of immunosuppressive cytokines and modulation of the TME is correlated with decreased response to anti-PD-1 therapy in *PTEN*-mutated melanoma and glioblastoma patients, suggesting that combined MAPK inhibition with anti-PD-1 therapy may present an opportunity for future therapies and an ongoing area of needed research.[67,121] Additionally, F-box/WD repeat-containing protein 7 (FBXW7) acts upon cellular proliferation targets including NOTCH1 and c-MYC, and mutations can be seen in a variety of malignancies including breast, colorectal, gastric, hepatocellular, and NSCLC.[122] Loss of FBXW7 impairs cytokine signaling, decreases CD8+ CTL infiltration, and decreases dsRNA sensing, corresponding with a diminished response to anti-PD-1 therapy. As such, restoration of dsRNA sensing results in a restored response.[123] Additionally, serine/threonine kinase 11 (STK11), also known as liver kinase B1 (LKB1), activates AMPK to inhibit growth via the mTOR pathway, and inactivation or mutation of *STK11* has

a profound effect on the TME via increased neutrophil recruitment through cytokine (IL-1) and chemokine (CXCL7, G-CSF, STAT3) production, increased IL-6 expression, and decreased CD8+ CTL infiltration.[124,125] In fact, *STK11*-mutated NSCLC displays decreased PD-L1 expression and poor response to anti-PD-1 therapy, though this response was reversed with the administration of IL-6 blockade.[124]

Proto-oncogenes have also been shown to modulate the TME, with both beneficial and adverse effects noted from an immunotherapeutic perspective. One notable proto-oncogene with pro-inflammatory TME modulation and induction of immunotherapy resistance is pescadillo ribosomal biogenesis factor 1 (PES1). PES1 is over-expressed in a variety of tumors and regulates the PI3K cell signaling pathway, enhancing malignant cellular proliferation.[126] PES1 expression also decreases IL-15 production, reduces CD8+ CTL esophageal tumor cell infiltration, and diminishes response to anti-PD-1 therapy; murine models suggest targeted knockdown of PES1 with co-administration of anti-PD-1 treatment resulted in improved CTL infiltration and increased pathologic response rates, and investigation of combined treatment with anti-PD-1 therapy and PES1 inhibition with CDK inhibitors represents an exciting area of future clinical inquiry.[127–129] Gain of function mutations and overexpression of TP53 are shown to correlate with overexpression of cytokines promoting angiogenesis, inflammation and cellular migration; this includes CCL2, which is associated with IL-6 upregulation and may confer resistance to immunotherapy through increased recruitment of Tregs.[130] On the other hand, other TP53 mutations are associated with increased tumor mutational burden (TMB), increased PD-1 expression, and increased CD8+ CTL tumor infiltration, which conversely may enhance the efficacy of immunotherapy; in fact, preclinical murine models have suggested targeted therapies to TP53 given in combination with anti-PD-1 therapies may enhance immunotherapy response, though this treatment approach requires further clinical trial investigation.[131–133]

Finally, driver mutations have reshaped the classification and treatment of numerous malignancies, further supported by the emergence of targeted therapies. One such cancer is NSCLC, for which medical management is greatly influenced by the presence of *EGFR*, *ALK*, and other aberrations, as available targeted treatments have become first-line options. The presence of these mutations is shown to have a significant impact on the TME, as oncogene-associated lung adenocarcinoma has been shown to display low TMB, decreased expression of PD-1, and decreased CD8+ CTL tumor infiltration, which may contribute to their immunotherapy resistance.[134–136] Although many NSCLC immunotherapy clinical trials have excluded patients with *EGFR* or *ALK* mutations, several large trials included patients with these mutations which were then assessed via subgroup analyses. EGFR-mutated patients in CheckMate-057 (nivolumab versus docetaxel), CheckMate-010 (pembrolizumab versus docetaxel), and the OAK trial (atezolizumab versus docetaxel) did not experience improved OS with immunotherapy, and those in the BIRCH trial (atezolizumab) showed a 19% response rate in the first line setting (versus 23% in EGFR-wild type patients) and almost no response in the second line and beyond.[137–140] Conversely, *EGFR*- and *ALK*-mutated patients in the IMpower150 trial demonstrated an improvement in PFS with the addition of atezolizumab to bevacizumab and chemotherapy.[141] In the ATLANTIC trial, 12.2% of pre-treated patients with either mutation and PD-L1 expression more than 25% had encouraging responses to durvalumab, but this was less than counterparts who were *EGFR*- or *ALK*-wild type.[142]

Nonetheless, attempts have been made to combine immunotherapy with mutation-targeted therapy or chemotherapy to overcome resistance, but these have encountered significant setbacks.[143] The TATTON trial, a phase Ib trial of osimertinib with durvalumab in *EGFR*-mutated NSCLC, found the combination not feasible due to increased incidence of interstitial lung disease (22%); this finding also resulted in the early closure of the phase III CAURAL trial, in which 1/12 patients reported development of interstitial lung disease.[144,145] KEYNOTE-789, a phase III trial of platinum chemotherapy and pemetrexed with or without pembrolizumab in *EGFR*-mutated NSCLC patients who previously progressed on EGFR-targeted therapy, was closed early after no difference in OS was observed compared to placebo.[146] Similarly, disappointing results were noted in CheckMate-722, a phase III randomized trial which failed to show difference in PFS with the addition of nivolumab to platinum chemotherapy and pemetrexed in *EGFR*-mutated NSCLC patients who previously progressed on EGFR-targeted therapy.[147]

Metabolic characteristics of tumors

Since the description of the Warburg effect in the 1920s, there has been considerable attention to the unique metabolic signature of cancer cells and how these mechanisms can be leveraged to improve cancer therapies. Enhanced glycolysis and lactic acid formation outside the mitochondria by tumor cells – a major source of energy production for increased cellular proliferation – results in glucose deprivation. This subsequently suppresses tumor-infiltrative CD4+ and CD8+ T cells, decreases CTL tumor infiltration, and profoundly alters the TME.[148–150] Acidification due to increased production of lactic acid directly decreases the cytotoxic activity of T cells while increasing recruitment of MDSCs to promote an immunosuppressive environment.[151] Nutrient depletion also results in a hypoxic environment with increased expression of hypoxia inducible factors (HIFs), which increase recruitment of MDSCs and Tregs while upregulating tumor expression of PD-L1.[152] Normalization of pH has been shown to increase CTL infiltration into tumors and improve response to immunotherapy, and numerous therapeutic strategies to mitigate TME acidification are currently being evaluated including proton transport inhibitors and alkalinizing agents.[153,154]

Critical attention has also been given to the utilization of specific ammino acids in TME modification. Tryptophan, whose degradation is mediated by the enzyme indoleamine 2,3-dioxygenase (IDO), is critical for T cell activation and proliferation.[155] IDO expression is a key feature of many tumors and serves as an intrinsic mechanism for immune resistance, further supported by IDO-mediated increased T cell PD-1 expression.[156,157] While preclinical models have shown the potential of IDO1 inhibitors in combination with

immunotherapy, the phase III trial KEYNOTE-252/ECHO-301 failed to show efficacy of epacadostat (an IDO1 inhibitor) with pembrolizumab compared to pembrolizumab alone in metastatic melanoma; nonetheless, epacadostat and alternative agents targeting tryptophan degradation, paired with ICIs, have been assessed via clinical trial in the treatment of various solid tumors (e.g. NCT03414229, NCT03459222).[158,159]

Arginine and glutamine have also been implicated in TME modification and immunotherapy resistance. Arginine depletion occurs in cancer cells through a variety of mechanisms, including decreased arginine synthesis via downregulation or silencing of arginosuccinate synthetase (ASS1) and increased arginine metabolism via HIF-mediated activation of arginase 1 (Arg1).[144,152] Elevated arginase levels are associated with quiescence of CD8+ CTLs and Treg survival, promoting an immunosuppressive environment.[160,161] Additionally, Arg1-mediated arginine metabolism produces urea and ornithine, the latter of which is used in the synthesis of polyamines which are necessary for cellular proliferation.[162]

Glutamine is a critical amino acid for rapid cellular division, and many tumor cells express glutamine-uptake transporters to competitively harvest glutamine from the local environment to support their glutamine addiction.[163] Glutamine-deficient tumor-infiltrative CTLs have a diminished anti-tumor immune response, and glutamine restriction is associated with increased CD4+ Treg proliferation.[164,165] Studies of both arginase inhibitors and glutaminase inhibitors with immunotherapy are ongoing, but none have achieved FDA approval yet, with studies such as the CANTANA trial (telanglenastat, glutaminase inhibitor) failing to show clinical benefit.[166]

Nonetheless, manipulation of amino acid metabolism in cancer therapy remains both an area of needed research and potentially exciting future therapeutic option.

Cancer cell death

Programmed cell death (PCD), and the ways cancer cells evade and resist this process, is one of the hallmarks of cancer.[167] PCD via apoptosis, efferocytosis, and necroptosis result in a complex cascade of biochemical signals including chemokines, mitogens, and extracellular vesicles that promote chemotaxis and cellular proliferation in the TME.[168] The release of danger-associated molecular patterns (DAMPs) such as calreticulin (CRT), heat shock proteins (HSPs), and high mobility group box protein 1 (HMGB1) after PCD promote a pro-inflammatory state and increased activity of dendritic cells, NK cells, and CTLs.[169,170] Through the process of immunoediting, immunogenic tumor cells associated with the aforementioned processes are selectively lost during tumor proliferation. Combined with a host of alterations to antigen presentation and lymphocyte proliferation and activation, this may serve as a significant mechanism of immunotherapy resistance.[171,172]

A variety of novel treatments are under active investigation to induce immunogenic cell death and enhance immunotherapy efficacy. Vaccine therapies promote tumor antigen uptake with APC activation and CTL tumor infiltration, which works synergistically with conventional immunotherapy.[173,174] Vaccines utilizing both tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs) have been studied in an array of primary tumor types including leukemia, NSCLC, gynecologic, genitourinary, and neurologic cancers.[174,175] Radiation promotes immunogenic cell death via release of DAMPs such as HMGB1 which subsequently increase antigen presentation and CD8+ CTL proliferation.[176] Combination radiotherapy and immunotherapy has been utilized successfully across a variety of primary tumors, and the addition of radiotherapy may both stimulate immunogenically "hot" tumors and convert immunogenically "cold" tumors.[177–179] Meanwhile, utilization of pulsed electric fields (PEFs) is associated with the release of DAMPs such as HMGB1 and CRT which modulate the TME, resulting in decreased Tregs and MDSCs with increased CD8+ CTLs.[180–182] Preclinical data with combination PEF and immunotherapy shows elevated HMGB1, increased CD8+ CTL tumor infiltration, and improved survival compared to immunotherapy alone.[183–185]

Microbiota

The role of the microbiome has been explored in a wide spectrum of diseases, and recent attention has been given to its contribution to cancer pathogenesis and immunotherapy responsiveness. Bacteria and bacterial metabolites act upon a variety of cells including DCs, stimulating T cell activation and cytokine release resulting in profound modulation of the local inflammatory environment; specific genera including *Bifidobacterium* have been shown to increase lymphocyte recruitment and T cell activation, while others including *Enterococcus* and *Akkermansia* increase central memory CD4 + T cells.[186,187] Higher gut microbiome diversity is associated with an increased response to anti-PD-1 therapy in melanoma patients, and certain bacterial species including *Bifidobacterium longum*, *Enterococcus faecium*, and *Collinsella aerofaciens* were found in higher quantities in patients with metastatic melanoma who had clinical responses to anti-PD-1 therapy.[188,189] Conversely, relatively low levels of these bacteria have been correlated with non-response to anti-PD-1 therapy, as with *Akkermansia muciniphila* in non-responders to anti-PD1 therapy with lung and kidney cancers.[190] As demonstrated by Routy et al., fecal microbiota transplantation (FMT) from non-responders to anti-PD-1 therapy to antibiotic-treated mice failed to improve anti-PD -1 response, but oral supplementation with *Akkermansia muciniphila* restored responsiveness to anti-PD1 therapy.[190] This observation has been further explored by evaluating cancer patients with suspected dysbiosis related to antibiotic use. A recent meta-analysis included 12 studies enrolling 6,010 cancer patients and analyzed concurrent use of antibiotic with ICIs. Results indicated significantly worse PFS and OS with concurrent use of ICIs and antibiotics compared to those without antibiotic use.[191] This observation provides further credence to the role of microbiota in cancer-immunity regulation and opens potential avenues for future research.

From these observations, the safety and efficacy of FMT as a strategy to overcome immunotherapy resistance has gained early clinical trial support. A phase I trial of 10 patients with anti-PD-1 refractory metastatic melanoma

received FMT from 2 donors with documented complete response (CR) to anti-PD-1 therapy. Two patients achieved partial response (PR) at 90 days, while 1 achieved CR and none demonstrated grade 2–4 adverse events from FMT.[192] Similarly, a phase II trial of 16 patients with anti-PD-1 refractory melanoma received FMT from 7 donors with documented CR or PR to anti-PD-1 therapy, and 6 of 15 assessed patients experiencing clinical benefit.[193] Notably, response to anti-PD1 therapy after FMT was associated with increased CD8+ CTL activation, decreased IL-8 expression, increased TNF expression, and durable modification of microbiome composition with increased expression of bacteria such as *Akkermansia muciniphilia*. Ongoing studies seek to build upon these foundational findings, including a randomized double-blinded phase Ib/IIa trial of 24 metastatic melanoma patients to receive FMT from either anti-PD-1 responders or non-responders (NCT05251389).[194]

Conclusion

The natural immune response to solid tumors is a carefully coordinated one. Intrinsically, it involves a host of cell–cell interactions through antigen presentation and immune checkpoints, direct cell killing, inflammatory signaling via cytokines and chemokines, and a balance with immunosuppressive components at each of these steps. However, it is also extrinsically influenced by other elements in the TME such as CAFs, driver and immunity-influencing mutations, methods of cancer cell death, and the host gut microbiome. Because of these observations, cancer immunity dysregulation has increasingly become recognized not only as a harbinger of carcinogenesis but also as a target for novel therapeutics. This review is unable to address every method by which tumors exhibit either primary or adaptive resistance to immunotherapies, especially as more mechanisms are being discovered through continued investigation.

However, with each one of these methods of resistance – both intrinsic and extrinsic – comes a possible way to overcome it. A preponderance of strategies is still being investigated in the pre-clinical setting, and some have made it into clinical trials with varying successes. These have mainly focused on how oncologists can overcome the resistance that tumors develop to ICIs, as these immunotherapies have enjoyed first-mover advantage over others. However, this limitation should not bar the investigation into other immunotherapies such as CAR T cell products, vaccines, and T cell engagers, especially because the immunologic methods by which tumors evade these treatments may differ significantly from the methods by which they resist ICIs.

In summary, understanding and overcoming resistance to immunotherapies is a nascent, but rich field of research. The community has much to learn, but small advances in the fundamental science and clinical trial settings give hope that more leaps in effective treatments are on the horizon.

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