Resistance to Immunotherapy: Mechanisms and Means for Overcoming

Mohamad A. Salkeni, John Y. Shin, and James L. Gulley

Abstract

Immune checkpoint blockade transformed cancer therapy during the last decade. However, durable responses remain uncommon, early and late relapses occur over the course of treatment, and many patients with PD-L1-expressing tumors do not respond to PD-(L)1 blockade. In addition, while some malignancies exhibit inherent resistance to treatment, others develop adaptations that allow them to evade antitumor immunity after a period of response. It is crucial to understand the pathophysiology of the tumor-immune system interplay and the mechanisms of immune escape in order to circumvent primary and acquired resistance. Here we provide an outline of the most well-defined mechanisms of resistance and shed light on ongoing efforts to reinvigorate immunoreactivity.

Keywords

Malignancy · Immunotherapy · Resistance · Checkpoint · Pathway · Antigen · Effector · Regulatory · Suppressor

Abbreviations

B2M	beta-2 microglobulin
CAF	cancer-associated fibroblast
CAR	chimeric antigen receptor
CCR	chemokine receptor
CR	complete response
CRC	colorectal carcinoma
CSF	colony-stimulating factor
CSF1R	colony-stimulating factor 1 receptor
CTL	cytotoxic T lymphocyte
CTLA-4	cytotoxic T-lymphocyte-associated protein 4
CXCL	CXC chemokine ligand
CXCR	CXC chemokine receptor
DC	dendritic cell
EGFR	epidermal growth factor receptor
FasL	Fas ligand
FcγR	Fcγ receptor
FDA	US Food and Drug Administration
HIF-1	hypoxia-inducible factor 1
ICAM	intercellular adhesion molecule
ICB	immune checkpoint blockade

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ICI	immune checkpoint inhibitor
IDO	indoleamine 2,3-dioxygenase
IFN-γ	interferon-gamma
iRECIST	immune response evaluation criteria in solid tumors
iRs	immune downregulating checkpoints
ITIM	immunoreceptor tyrosine-based inhibitory motif
JAK	Janus kinase
LAG-3	lymphocyte-activation gene 3
LAIR-1	leukocyte-associated immunoglobulin-like receptor 1
mAb	monoclonal antibody
MAPK	mitogen-activated protein kinase
MDSC	myeloid-derived suppressor cell
MHC	major histocompatibility complex
MICA-B	MHC-I-related chain B
M-MDSC	monocytic subtype of myeloid-derived suppressor cell
MMR	mismatch repair
MPR	major pathologic response
MSI-H	microsatellite instability high
NK	natural killer
NSCLC	nonsmall cell lung cancer
OS	overall survival
PBMC	peripheral blood mononuclear cell
PD	progressive disease
PD-1	programmed cell death protein 1
PD-L1	programmed death-ligand 1
PFS	progression-free survival
PI3K	phosphatidylinositol 3-kinase
PR	partial response
PTEN	phosphatase and tensin homolog
RCC	renal cell carcinoma
RECIST	response evaluation criteria in solid tumors
SD	stable disease
STAT	signal transducers and activators of transcription
STING	stimulator of interferon genes
TAM	tumor-associated macrophage
Teff	effector T cell
TGF-β	transforming growth factor beta
Th	T-helper cell
TIGIT	T-cell immunoreceptor with Ig and ITIM domains
TIL	tumor-infiltrating lymphocyte
TIM-3	T-cell immunoglobulin 3
TKI	tyrosine kinase inhibitor
TLR	toll-like receptor
TMB	tumor mutational burden
TME	tumor microenvironment
TNBC	triple-negative breast cancer
TNF-α	tumor necrosis factor alpha
Treg	regulatory T cell
VCAM	vascular cell adhesion molecule
VEGF	vascular endothelial growth factor

1 Introduction and Definitions

Immune checkpoint inhibitors (ICIs) are a class of immunotherapeutics that have scored a remarkable breakthrough across a large spectrum of malignant tumors. Distinct from other modalities, such as chemotherapy and small molecules, which induce temporal apoptosis of tumor cells, immunotherapeutics attempt to re-recruit effector immune cells and create a response that employs immune memory in an effort to produce long-lasting antitumor effects. This class of agents can produce rapid, deep, and, most significantly, durable responses. Still, a large proportion of patients do not respond to treatment, or develop progression of malignancy after a variable period of benefit. Furthermore, since the publication of the first phase III ipilimumab trial, which showed an improvement in overall survival (OS) but not in progression-free survival (PFS), it has been recognized that tumors under the effect of ICI may not always follow the same pattern of response seen in other types of therapy [1].

Several unique issues have emerged since the widespread adoption of ICIs in the treatment of cancer. Unfamiliar patterns of delayed tumor response, initial and late resistance to treatment, oligoprogression, lymph node-only progression, and pseudoprogression have all surfaced. To address these issues and to avoid misinterpretation of tumor response, the Society for Immunotherapy of Cancer assembled a taskforce to create consensus guidelines that would provide a consistent definition for different types of resistance. The recommendations aim to stan-

dardize tumor assessments in patients who are receiving anti-PD-(L)1 (programmed cell death protein 1/programmed death-ligand 1) therapy, and to help investigators in designing clinical trials for drugs being developed in this field. In addition, they identify patients who are unlikely to derive benefit from an initial or more prolonged exposure to anti-PD(L)1, and reduce the chance of mislabeling patients' responses to treatment. In the setting of a clinical trial, these standards are expected to reduce the chance a response is mistakenly attributed to a subsequent line of therapy [2].

The SITC taskforce recognized three different patterns exhibited by tumors progressing in the context of ICI therapy: primary resistance, secondary resistance, and off-treatment progression.

Primary Resistance is applicable to patients experiencing either initial progressive disease (PD), or stable disease (SD) lasting less than 6 months. In addition, to make a reasonably accurate assessment of treatment benefit, a minimum drug exposure of 6 weeks is required. The panel acknowledges that some indolent tumors may need to be evaluated over a longer period of time. In the absence of rapid tumor growth or clinical deterioration, a confirmatory scan, or clinical evaluation for clinically detectable disease (e.g., skin lesions), should be carried out at 4–12-week intervals after first suspicion for PD (Table 1). This would ensure late responders to PD-(L)1 treatment are not removed from therapy inappropriately. Clinical judgment is required in case of a clinical deterioration attributable to PD, as continuing anti-PD-(L)1 therapy in these patients may not be safe.

Secondary/Acquired Resistance Patients receiving PD-(L)1 therapy who demonstrate an initial clinical benefit such as complete response (CR), partial response (PR), or SD for a minimum of 6 months but whose tumors progress while on therapy are classified as having secondary resistance. This was defined with the main goal of aiding in clinical trial design by guiding eligibility and stratification for subsequent analysis. As with primary resistance, a confirmatory evaluation is recommended 4–12 weeks after initial PD, and should demonstrate progression in ≥2 sites in patients with multiple metastases (Table 1). In addition, to be categorized as secondary resistance, lymph node-only progression requires tissue confirmation. Again, patients with disease-related clinical deterioration or rapid disease progression do not require confirmatory radiologic evaluation.

Off-treatment Progression A third scenario is PD after treatment discontinuation due to patient preference, toxicity, or other reasons such as a predetermined finite number of cycles, as in (neo) adjuvant treatment. Mechanisms of resistance in this scenario may or may not resemble those seen in other types of resistance. The taskforce recommends that patients with PD < 12 weeks from the last dose of anti-PD-(L)1 therapy can be considered to have primary resistance (or early relapse). Relapse ≥12 weeks is considered "late relapse", as it is difficult to label this as resistance. A treatment rechallenge is warranted in patients with late relapse, especially if occurring >6 months. In both of these scenarios, a biopsy is required, rather than a confirmatory scan, to confirm progression/recurrence (Table 1).

Noting that macroscopic disease is present in the case of neoadjuvant therapy, and in anticipation of increased utilization of this approach, the definitions of primary and secondary resistance mentioned above can be applied here. However, the unique advantage of having histologic evaluation of residual tumor in this setting allows for further classification based on pathologic response. Patients who achieve a major pathologic response or better (CR, near CR, or major PR) with a subsequent relapse down the road are thought to fit into the secondary resistance category; while those not achieving a major pathologic response fit into the primary resistance category [2]. Notably, some neoadjuvant trials have defined major pathologic response as ≤10% of residual viable tumor [3, 4].

Progression after treatment discontinuation in the metastatic setting can be classified based on attained benefit and interval from last anti-PD-(L)1 treatment. Patients who have not previously achieved PR/CR are considered to have primary resistance; while patients who achieved PR/CR and relapsed after ≤12 weeks are considered to have secondary resistance. Late progression is considered when a patient who achieved PR/CR experiences a relapse >12 weeks from last dose. However, it is difficult to classify this scenario as resistance since these patients have a > 5% chance of responding to rechallenge, regardless of intercurrent treatment.

Caveats These definitions are designed to address anti-PD-(L)1 monotherapy, and may or may not necessarily be applicable to combination ICIs or to chemo-immunotherapy. Indolent tumors that are slowly progressing despite therapy, but not enough to call PD per RECIST, represent a group that may need a longer period of exposure than suggested intervals, and the taskforce urged investigators to use clinical judgment. The definitions are applicable to most but not all solid tumors, especially in cases where conventional response criteria are not commonly used, such as in glioblastoma, hepatocellular carcinoma, and prostate cancer, among others. If feasible, biopsy confirmation should be considered in cases of oligoprogression, especially if involving the lung or lymph nodes. Criteria can generally be applied to patients in clinical trials. In clinical practice, however, local therapy to sites with oligoprogression may be reasonable if deemed appropriate by the treating physician. Finally, it is noteworthy that the taskforce did not reach a unanimous agreement whether to use RECIST 1.1 vs iRECIST for clinical trial eligibility criteria [2, 5, 6] (Table 1).

2 Functional Categorization of Resistance Mechanisms

Multiple classifications of resistance have been suggested, some are based on response phenotype, such as primary and secondary; while others pertain to the type of response exhibited by the immune system, such as innate and acquired. Nevertheless, significant mechanistic overlap exists between tumor resistance to innate immunity and to immunotherapy, and between primary and acquired tumor resistance; therefore, we have elected to propose a functional classification based upon the role of different key players.

2.1 Defective Immune Cell Recognition

2.1.1 Impaired Immunogenicity and Neoantigen Alteration

Neoantigens are novel protein epitopes expressed via major histocompatibility complexes (MHCs) and result from emerging mutations and genomic instability in the tumor genome. The resulting new peptide sequences are immunogenic and are considered cornerstone elements in immune recognition by cytotoxic T lymphocytes (CTLs). There are essentially two types of tumor antigens: tumor-specific antigens (TSA) and tumor-associated antigens (TAA). TSAs are usually present only in tumor cells and are created by two main mechanisms, emerging mutations in tumor genomes, and viral incorporation into cell genomes enforcing the creation of oncoviral neoantigens. TAAs are present both in the tumor and in some other nonmalignant cells to which T cells have developed tolerance [7].

The neoantigen burden is related to the number of mutations present in a specified area of the tumor genome, also known as tumor mutational burden (TMB). Although point mutations are significantly more common, frameshift insertions/deletions, exon skipping, and protein fusions are all events that create proteins which are structurally more altered [8]. This process occurs in a random fashion, and because a large proportion of mutations is not shared among different patients, they can be considered patient-specific [9].

Tumors with germline or somatic deficiencies in DNA repair mechanisms appear to exhibit improved responsiveness to ICIs. Mismatch repair-deficient (dMMR) tumors with high mic-

Table 1 SITC taskforce definitions of resistance [2]

On-treatment progression – advanced/metastatic disease			
Type of resistance	Minimum drug exposure	Best RECIST response	Confirmatory evaluation[a]
Primary resistance	6 weeks	PD SD < 6 months	Required 4–12 weeks after RECIST PD
Secondary resistance	6 months	CR, PR, or SD > 6 months	Required 4–12 weeks after RECIST PD[bc]
Off-treatment progression – Adjuvant settings			
Type of resistance	Last dose of anti-PD-(L)1	Confirmatory biopsy required	Confirmatory evaluation[a]
Primary resistance (early relapse)	< 12 weeks	Yes	Not required
Late relapse[d]	≥ 12 weeks	Yes	Not required
Neoadjuvant settings			
Type of resistance	MPR (defined as CR, near CR, or major PR) achieved?		
Primary resistance	No		
Secondary resistance	Yes		
Off-treatment progression in advanced/metastatic disease			
Type of resistance	End of treatment CR/PR	Time from last dose	Confirmatory evaluation[a]
Primary resistance	No	n/a	Not required
Secondary resistance	Yes	≤ 12 weeks	Required
Late progression	Yes	> 12 weeks	Required

[a]Imaging or clinical for clinically measurable lesions (skin)
[b]Unless clinical deterioration due to PD
[c]Interval depends on tumor biology and rate of growth
[d]Relapse ≥ 6 months may warrant a rechallenge

rosatellite instability (MSI-H) leading to the formation of thousands of neoantigens exhibit a significantly higher response rate to ICIs compared to MMR-proficient tumors across a vast variety of tumors. Thus, in a first tissue-agnostic approval of its kind, the US Food and Drug Administration (FDA) authorized the use of pembrolizumab in dMMR tumors after increased response rates were seen in several different solid tumor types spanning both colorectal cancer (CRC) and non-CRC, with dMMR or MSI-H [10, 11].

Tumor histologies that tend to develop higher TMBs, such as melanoma, nonsmall cell lung cancer (NSCLC), and MSI-H CRC, have shown greater response rates to ICIs, suggesting a predictive role for high TMB as a biomarker of response [10, 12, 13]. This led to another FDA tissue-agnostic approval of pembrolizumab in solid tumors with high TMB, which was ultimately defined as ≥10 mutations/megabase [10].

Some immunologically cold tumors such as pancreatic and breast carcinomas exhibit low response rates to ICB, due in part to the low TMB and low antigen load resulting in poor immunogenicity. These tumors have generally shown disappointing results with ICIs and appear to commonly exhibit patterns of primary resistance [14–16]. On the other hand, different neoantigens exhibit different levels of immunogenicity; hence, a high-quality neoantigen is one that is potently immunogenic. For example, pancreatic ductal carcinomas demonstrate a high level of primary resistance to ICIs due to low neoantigen load and less immunogenic (low-quality) antigens, among other factors [17].

Because a multitude of factors contributes to immunogenicity and immune response, not all TMB-high tumors respond to ICIs. Likewise, some tumors with low TMB respond well to ICIs. Merkel cell carcinomas, for example, respond well to first-line ICIs even when TMB is low.

TMB-low Merkel cell carcinomas were found to be mostly polyomavirus-related, suggesting that viral-associated antigens in tumor cells are highly immunogenic [18]. A similar observation was noted in human papillomavirus-associated head and neck and cervical cancers, which demonstrated higher response rates in virus-positive tumors compared to virus-negative ones [19]. This observation was not universal across all viral-associated malignancies, such as hepatocellular carcinoma, possibly due to different mechanisms of carcinogenesis. Likewise, in renal cell carcinoma (RCC), no association between TMB and clinical benefit from atezolizumab was found in the exploratory molecular analysis of the IMmotion150 randomized phase II trial [20].

Downregulation, epitope modification, loss, and shedding of neoantigens are some examples of how tumors evade ICI therapy. Loss of neoantigens via genomic alteration, commonly deletion, has been shown to play a role in a cohort of NSCLC patients whose disease progressed after initial response [21]. Alternative splicing leading to loss of the CD19 epitope accounts for some relapses after chimeric antigen receptor (CAR) T-cell-based immunotherapy [22]. Whole-exome sequences of paired tumor samples before ICI treatment and after progression revealed a change in the somatic mutation landscape that included both gains and losses. However, several tumor-specific neoantigens were found to have been lost in the resistant clones, compared to the pretreatment tumor, due to genomic alteration as well as elimination of some tumor subclones. This process of therapy-induced immunoediting eliminated antigens that were recognized by circulating T cells.

2.1.2 Dysfunctional Antigen-Processing Machinery

Defective antigen presentation has been described in a study of melanoma patients with tumors that became refractory to ICIs after initial response. The development of a frameshift deletion in the beta-2 microglobulin (B2M) component of MHC-I was noted in one of four patients, and resulted in the loss of outer membrane localization of MHC-I without affecting production, as evidenced by persistent intracellular staining by immunohistochemistry. MHC-I is essential for T-cell recognition, and the loss of surface localization impairs immune destruction in both treatment-naïve and ICI-treated patients [23, 24]. Defective antigen presentation through mutations in B2M was also demonstrated in 29% of metastatic melanoma patients with PD after treatment with ICIs. Threefold enrichment in B2M gene loss of heterozygosity was noted in patients who did not respond to treatment with anti-PD1 and anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) therapy compared to responders [25].

Shedding of surface antigens has long been recognized as a potential resistance mechanism to monoclonal antibodies (mAb) and immunoconjugates [26]. The role of antigen shedding in mediating resistance to ICI remains undefined. However, the combination of anti-PD-(L1) with antibody-drug conjugates has yielded encouraging results in urothelial carcinomas, as an example [27].

Other alterations in MHC-I have been reported. For instance, shedding of natural killer (NK)-activating ligands on MHC-I has been shown to play an important role in tumor immune escape. Proteolytic shedding of MHC-I-related chain A/B (MICA/B), NKG2D activators, is undertaken by tumors to evade cytotoxic destruction [28]. Invigorating the antitumor response through generation of polyclonal anti-MICA antibodies has promising results in preclinical in vivo studies [29].

2.1.3 Immunoediting

Immunoediting is the process through which the immune system both prevents and promotes tumorigenesis through immunogenic "sculpting." Once a tumor cell survives self-correction mechanisms, it is believed to go through three phases of immunoediting: elimination, equilibrium, and escape [30]. Elimination is the phase in which the immune system detects and destroys tumor cells before they become clinically apparent.

Equilibrium is characterized by tumor dormancy. In the escape phase, the immune system fails to restrict tumor growth, resulting in disease progression. This process is described in the pathogenesis of tumor development in treatment-naïve conditions. However, it appears to greatly overlap with primary and acquired resistance to immunotherapy [7] (Fig. 1). Some tumors appear to revert to a state of equilibrium in response to treatment with ICIs, with or without tumor regression. However, later in the course of treatment, less immunogenic clones survive and reenter the escape phase. This phenomenon is usually accompanied by an increase in the number of tolerant immune cells. Interestingly, tumor subclones with immune tolerance-promoting mutations in CDKN2A gene and nearby interferon (IFN)-γ gene were selected for subsequent growth as demonstrated in a cohort of melanoma patients with PD after nivolumab treatment. Therefore, tumor evolutionary selection of less immunogenic clones is considered an important mechanism of resistance following ICI therapy [7, 31].

2.1.4 Tumor Heterogeneity

The degree of PD-L1 expression can differ spatially and temporally within a single patient. This may account, at least in part, for differences in response rates among patients with seemingly similar tumor characteristics [32]. In the same tumor, expression of PD-1 or PD-L1 can vary considerably among different regions. A gene expression signature analysis of 35 tumor regions belonging to 10 NSCLC tumor samples revealed intriguing intertumoral and intratumoral heterogeneity. Furthermore, a heterogeneous tumor microenvironment (TME) was noted using gene expression analysis of stromal and immune cells [33]. Additionally, remarkable differences in PD-L1 expression were observed between primary tumors and metastatic lesions and between coexisting metastatic sites [33, 34]. It should be noted that these differences in expression patterns could be attributed in part to inter-assay variability [35, 36].

3 Barriers to Immune Cell Trafficking into Tumor

Barriers to T-cell trafficking into the tumors have been described as a potential etiology by which tumors escape immunosurveillance. The tumor endothelium establishes a kind of a physical barrier that restricts T-cell infiltration into the tumor nest, possibly established by overexpression of the endothelin B receptor, which limits T-cell adhesion to the endothelium. In ovarian cancer samples, overexpression of endothelin B receptor was found to be strongly associated with lack of tumor-infiltrating lymphocytes (TILs) and with shorter survival [37]. Other proangiogenic growth factors, such as vascular endothelial growth factor A (VEGF-A), also impair T-cell adhesion to endothelium by dysregulating vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) in endothelial cells. VEGF therefore appears to play an important role in impeding effector T-cell (Teff) trafficking into the TME. Furthermore, the VEGF-A gene was found to be downregulated in patients responding to PD-1 blockade, compared to nonresponders, which corresponded to lower VEGF-A levels [38]. These findings provide a rationale for the therapeutic combination of anti-VEGF plus anti-PD-(L)1 agents, which has shown significant improvement in both response rate and PFS in patients with RCC [39].

Fas ligand (FasL, CD95L), a homeostatic mediator of T-cell apoptosis, has been shown to be upregulated by immunosuppressive and pro-angiogenic factors in the TME, and expression of FasL was associated with absence of intratumoral CD8+ T cells [40].

Epigenetic inactivation of the cGAS-STING pathway is believed to be responsible, in part, for decreased immune cell trafficking into the tumor nest. Among other functions, the STING pathway appears to facilitate CTL trafficking and infiltration into tumor tissue. Several tumor types have been found to have defects in the cGAS-STING pathway, including ovarian cancer, colon cancer and melanoma [41, 42]. An intratumoral STING agonist, MK-1454, is being tested in

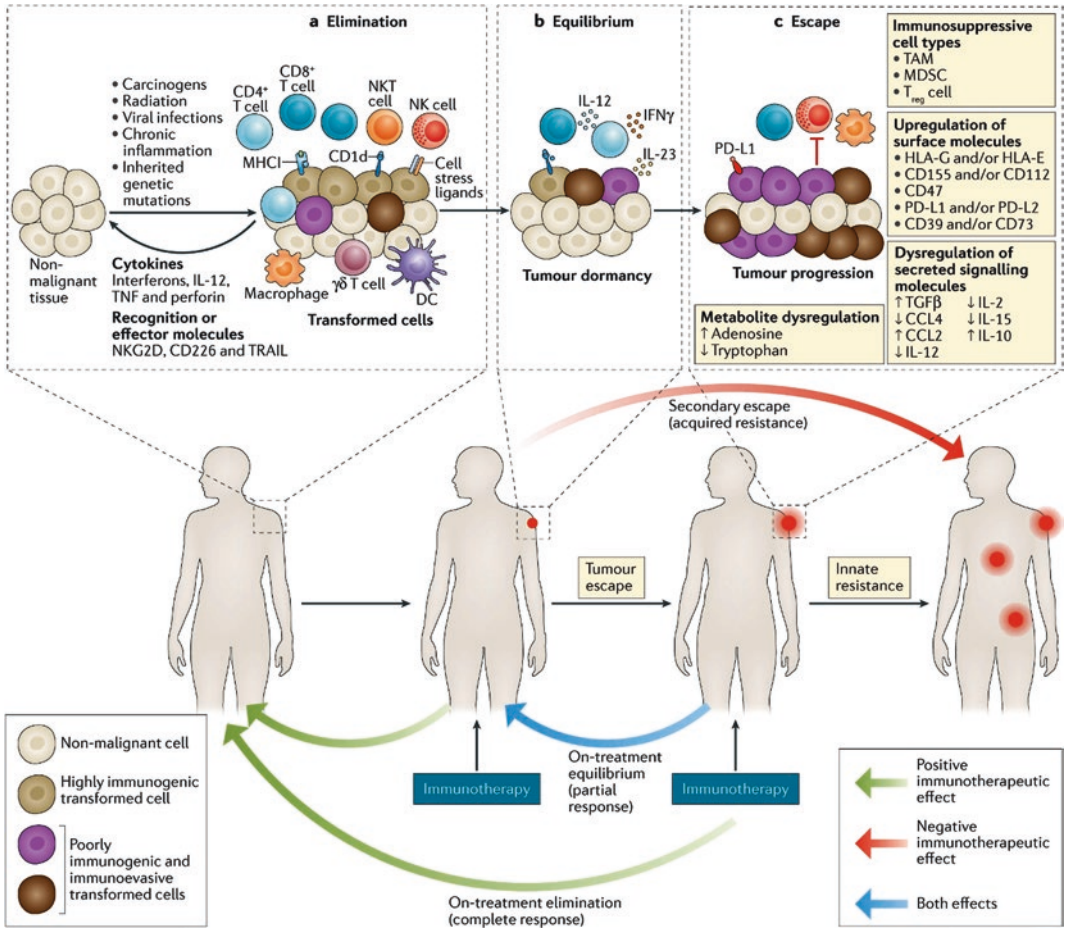

Fig. 1 Cancer immunoediting phases. a. Elimination: transformed cells that have escaped tumor suppressors are recognized and eliminated by innate and acquired immunity. b. Equilibrium: surviving cells enter a state of quiescence or limited growth where their immunogenicity is edited by the adaptive immunity. c. Escape: activation of immunosuppressive pathways allows unrestrained growth of tumors. Complete response occurs when immunotherapy is successful in overcoming immunosuppressive mechanisms and restoring anti-tumor immunity, i.e., reverting tumors to elimination phase. Incomplete reversal of tumor-induced immunosuppression results in tumors reverting to a state of on-treatment equilibrium that lasts until tumor subclones become capable of restoring immunosuppression and regrow resulting tumor progression and acquired resistance. Innate tumor resistance occurs as a result of immunotherapy failure to significantly restore anti-tumor immunity. Abbreviations: DC, dendritic cell; MDSC, myeloid-derived suppressor cell; MHCI, MHC class I; NK cell, natural killer cell; NKT cell, natural killer T cell; PD-L1, programmed cell death 1 ligand 1; TAM, tumor-associated macrophage; Treg cell, regulatory T cell. Adopted with permission from O'Donnell et al, Nat Rev Clin Oncol. 2019;16(3):151–67. [7]

combination with an anti-PD-1 agent in clinical trials (NCT04220866, NCT03010176).

Intratumoral injection of various immunotherapeutics has shown promising synergistic efficacy with PD-(L)1 blockade, inducing abscopal responses in noninjected tumors. Oncolytic and non-oncolytic viruses, myeloid dendritic cells (DCs), encapsulated mRNA (mRNA-2752), bifunctional fusion protein targeting CD47 checkpoints (SL-172154, TTI-621), cell-based inflammatory DCs (ilixadencel, immune primer), STING-activating agonist (MIW815), and others are being tested in combination with ICIs to enhance T-cell trafficking into the tumor bed and

to enhance antitumor activity by bypassing both physical and chemokine barriers [43–46] (NCT04502888).

Lastly, an interesting preclinical study of the intratumoral administration of seasonal flu vaccine in mice was successful in converting immunologically inert tumors into hot tumors and in increasing T-cells and DCs infiltration into tumors. In addition, this treatment enhanced the effect of PD-L1 blockade and re-sensitized resistant tumors to such therapy [47].

4 Dysfunctional Effector Immune Cells within the TME

Teffs are produced from naïve T cells upon acute antigen exposure. Once the antigen is cleared, the majority of Teffs undergo apoptosis while a minority change into memory T cells that are normally present in small numbers that can sharply increase upon antigen re-exposure. However, in cases of prolonged and/or repetitive exposure to the involved antigen, such as in chronic infections and in cancer, an immune-tolerant state ensues as T cells undergo transcriptional and epigenetic changes under the effects of inhibitory cytokines rendering them less functional and less reactive to the antigen in question. Upregulation of the inhibitory checkpoint PD-1 on T cells has been shown to occur as a result of chronic exposure to an antigen [48]. Dysfunctional T cells have low proliferative activity and are believed to exist in three forms: anergic, senescent and exhausted. Anergic T cells form in response to suboptimal stimulation and inadequate antigen exposure, and have low or no effector function. Senescent T cells arise from repetitive stimulation and have good effector functions but low proliferative properties. Exhausted T cells arise due to persistent overstimulation, have a high expression of inhibitory receptors, and are believed to have a mechanism of evolution in cancers that is distinct from that in chronic infections [49]. Several factors contribute to the development of dysfunctional T cells, including upregulation of inhibitory receptors, production of suppressive cytokines in an immunosuppressive TME, as well as the epigenetic and transcriptional dysregulation of T cells [49] (Fig. 2). Moreover, deficient immunologic memory is a hallmark of T-cell exhaustion resulting from chronic antigen exposure [50]. PD-(L)1 blockade, despite its ability to reinvigorate T cells, frequently falls short of efficiently restoring long-lasting memory, especially with continued high antigen exposure [51].

4.1 Co-Expression of Inhibitory Receptors on T Cells

Dysfunctional T cells are characterized by increased expression of multiple immune down-regulating checkpoint receptors (iRs) such as PD-1, CTLA-4, TIM-3, LAG-3, TIGIT, LAIR-1, and others (Fig. 3). In general, the more iRs expressed, the more significant the dysfunction.

Immunotherapy-induced upregulation of alternative checkpoints with Teff-repressive functions is now well-described in several tumor types (Table 2). Thirty-two NSCLC tumors were analyzed for iRs expression. Compared to circulating T cells from healthy donors, which had virtually no expression, TILs from patient samples were found to express PD-1 (43.5%), CTLA-4 (~25%), and LAG-3 (~12%). The study also demonstrated that the expression of checkpoints increased with tumor progression, providing an important proof of concept for the dynamicity of T-cell dysfunction as a progressive process. Treatment with PD-1 blockade restored Teff functions, as evidenced by increased IL-2, IFN-γ, and tumor necrosis factor (TNF)-α production in some, but not all, tumor samples. Failure of PD-1 blockade to restore effector function correlated with high PD-1 expression, and was also associated with upregulation of TIM-3, CTLA-4, and LAG-3 [52]. This observation was also reported in other tumor types where blocking a single checkpoint such as PD-1, LAG-3, or CTLA-4 in a murine model of ovarian cancer produced a compensatory upregulation of the other iRs. In this study, combination checkpoint blockade elicited superior tumor control compared to monotherapy inhibition [54].

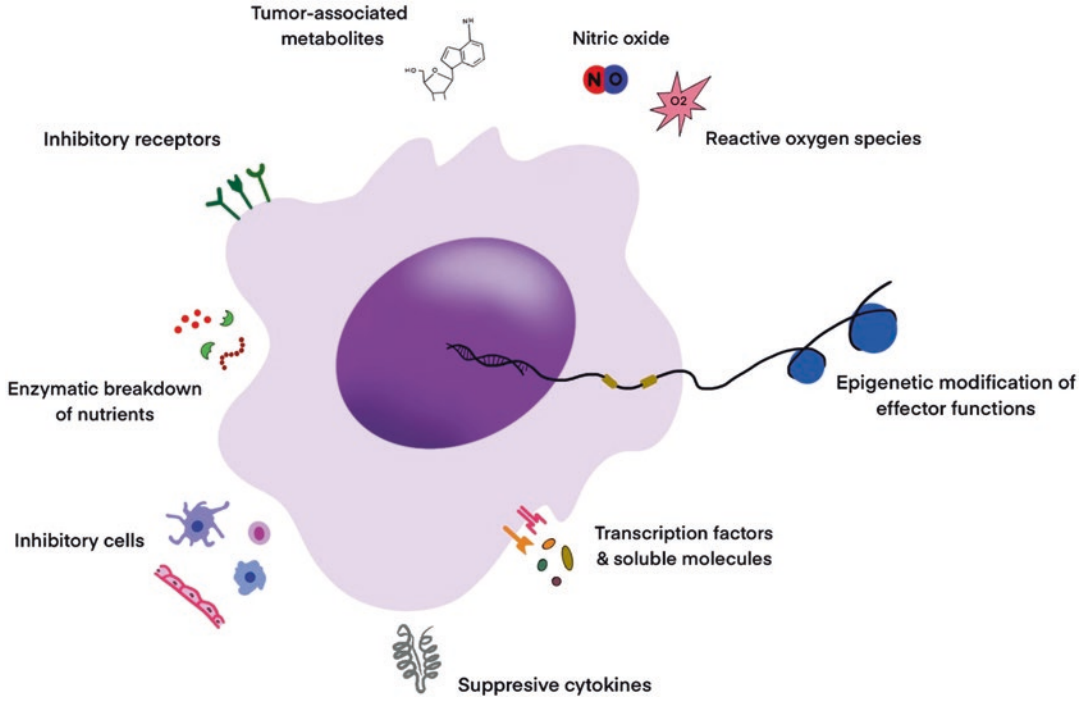

Fig. 2 Illustration of factors in the TME that are implicated in T-cell dysfunction. For instance, the upregulation of inhibitory receptors on immune cells, the production of suppressive cytokines and transcription factors by inhibitory cells, the generation of tumor-associated metabolites, NO and ROS, and the epigenetic dysregulation of inflammatory cells and cytokines are all elements that contribute to dysregulation of effector T-cell functions [49, 52, 53]

From a therapeutic standpoint, reversing/overcoming T-cell dysfunction can be achieved by either combining multiple ICIs that target different checkpoints or combining an ICI with a T-cell costimulatory agonist. The former has been successfully applied in the clinical setting as dual inhibition of PD-1 and CTLA-4 has shown enhanced efficacy in tumors like melanoma, NSCLC, and malignant pleural mesothelioma, albeit with increased immune-related adverse events [59–61]. Results of other ICI combinations such as anti-TIGIT mAb are starting to be reported [62, 63].

LAIR-1 is an inhibitory receptor expressed by a wide variety of immune cells, including NK cells, monocytes, DCs, and T and B cells, among others. LAIR-1 can inhibit NK and CTL cytotoxicity by binding to its ligands, collagen, C1q complement component and surfactant protein D, or by cross-linking with monoclonal antibodies . The LAIR-2 protein is highly homologous to the extracellular component of LAIR-1 and, when binding to the common ligands, can antagonize LAIR-1's inhibitory function [57]. The experimental drug NC410, a dimeric LAIR-2 bound to an Fc receptor, can serve as a decoy for the LAIR-1 ligands, thereby it helps decrease the inhibitory signal. It is currently being tested in a clinical trial in advanced solid malignancies (NCT04408599).

Potentiating T-cell function by using an agonist mAb to costimulatory receptors is another method of restoring function of exhausted T cells (Table 3). Utomilumab is an agonist of the costimulatory receptor 4-1BB (CD137), and has shown clinical activity as a single agent and in various combinations with anti-PD-1 and anti-chemokine receptor-4 (CCR4) agents [64–66]. Other costimulatory receptors, such as OX40, CD40, GITR, and ICOS may also become potential targets of agonist-based therapeutic interventions [67].

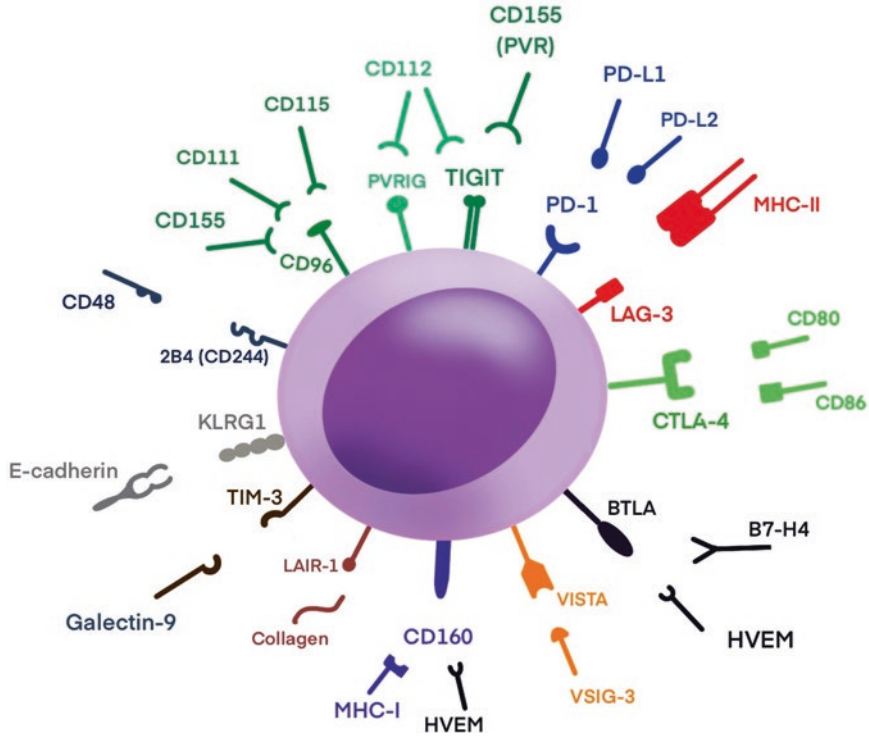

Fig. 3 Illustration of known inhibitory receptors and checkpoints and their ligands on T cells
Abbreviations: *BTLA* B- and T-lymphocyte attenuator, *CTLA-4* cytotoxic T-lymphocyte-associated protein 4, *HVEM* herpes virus entry mediator, *ITIM* immunoreceptor tyrosine-based inhibitory motif, *KLRG1* killer cell lectin-like receptor G1, *LAG-3* lymphocyte-activation gene 3, *LAIR-1* leukocyte-associated immunoglobulin-like receptor 1, *MHC* major histocompatibility complex, *PD-1* programmed cell death-1, *PD-L1* programmed cell death-ligand 1, *PVR* poliovirus receptor, *PVRIG* PVR-related immunoglobulin domain containing, *TIGIT* T-cell immunoreceptor with Ig and ITIM domains, *TIM-3* T-cell immunoglobulin 3, *VISTA* V-domain immunoglobulin suppressor of T-cell activation, *VSIG-3* V-set and immunoglobulin domain containing 3 [54–58]

Table 2 Illustration of T-cell inhibitory receptors with examples of targeting drugs

Inhibitory receptors on T cell [54]	Targeting drugs
PD-1	Pembrolizumab, nivolumab, pidilizumab [68], cemiplimab [69]
CTLA-4	Ipilimumab, tremelimumab [70]
TIGIT	Tiragolumab (NCT04300647, NCT04294810, NCT04513925)
LAG-3	Relatlimab (NCT04552223, NCT04095208, NCT04080804)
TIM-3	TSR-022, MBG453, LY3321367, Sym023 [71]
BTLA	JS004, TAB004 (NCT04278859, NCT04137900)
CD160	ELB01101 [72]
LAIR-1	NC410 (NCT04408599)

Table 3 Examples of T-cell stimulatory receptors with potential targeting drugs

Stimulatory receptors on T cell	Drugs
OX40	Pogalizumab, IBI101 [73], PF-04518600 (NCT03092856), BMS-986178 (NCT03831295), MEDI6469 (NCT02205333)
CD40	Selicrelumab, APX005M, ChiLob7/4, JNJ-64457107, SEA-CD40, CDX-1140H, ABBV-428, dacetuzumab [74], LVGN7409 (NCT04635995)
GITR	BMS-986156 [75], INCAGN01876 (NCT03277352), ASP1951 (NCT03799003)
ICOS	GSK3359609 (NCT04128696), MEDI-570 (NCT02520791)
4-1BB (CD137)	Utomilumab [64]

4.2 Immunosuppressive Cells in the TME

The TME is a complex interactive tumor cell-extrinsic system of cellular components, paracrine and autocrine factors, soluble molecules in the extracellular matrix, and vasculature. In some tumors, the TME cell composition can be a hostile milieu for Teffs, resulting in various degrees of dysfunction. Inhibitory cells interact with Teffs by several mechanisms, the most important of which is activation of iRs and secretion of inhibitory cytokines. Regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), cancer-associated macrophages, cancer-associated fibroblasts (CAFs), adipocytes and endothelial cells have all been shown to have an important role in fostering T-cell exhaustion [49].

4.2.1 Regulatory T Cells

The FoxP3+ CD4+ subgroup of infiltrating T cells, Termed Tregs, are the main inflammatory downregulators in the TME. Tregs play an important role in promoting immune tolerance, and are found in abundance in many tumors. Their abundance has been linked to shorter OS in several tumor types including melanoma, hepatocellular carcinoma, RCC, gastric cancer and breast carcinomas, among others [76]. Tregs are chemotaxed into the TME via complex processes, most notably through chronic antigen exposure and the subsequent production of multiple Treg-upregulating cytokines by other immunosuppressive cells. The role of Tregs is important in mediating tumor resistance to both innate immunity and immunotherapeutics. Treatment with ICIs has been shown to increase the Teff/Treg ratio. However, it has also been shown that, in some cases of treatment refractoriness, ICI treatment may lead to further recruitment of Tregs to the TME, which plays a role in mediating resistance. This was shown to be the case in a murine model of claudin-low breast cancer that is generally known to be resistant to ICB [77]. In melanoma murine models, tumors with a higher Teff/Treg ratio were shown to be more responsive to ICB, which further highlights the role played by Tregs in mediating resistance to therapy [78, 79].

4.2.2 Myeloid-Derived Suppressor Cells

Treg proliferation and attraction to the TME is orchestrated by a network of immune and stromal cells that produce immunomodulatory cytokines and soluble molecules. MDSCs are increasingly recognized as a major player in the tumor evasion of innate immunity and also in mediating resistance to ICB. MDSC expansion and activation are controlled by various soluble factors such as IL-6, colony-stimulating factors, IL-10, VEGF, and toll-like receptors (TLRs) [80]. In addition, preclinical models suggest a role for CCL2 and CCL5 in their migration to the tumor niche through binding to receptors such as CCR2, CCR4, and CCR5 [80, 81]. Other molecules such as CXC chemokine ligand (CXCL)3 appear to also play a role in MDSC recruitment to the tumor bed by binding CXC chemokine receptor (CXCR)2 on MDSCs [80, 82]. IL-8 has also been shown to play a role in recruiting MDSCs to the TME [83]. The monocytic subtype of MDSC (M-MDSC) contributes to T-cell dysfunction via antigen-specific and antigen-nonspecific mechanisms; these include the production of reactive oxygen species and nitric oxide, the production of immunosuppressive transcription factors and cytokines such as transforming growth factor (TGFβ) and IL10, the production of arginase and other enzymes that degrade nutritionally important amino acids, and the production of ADAM17 which disrupts the ability of T cells to home to activation sites [81, 84]. Further evidence suggests that accumulating MDSCs within the tumor bed limits the efficacy of ICIs [85]. Clinical response to CTLA-4 blockade in melanoma patients was associated with lower frequencies of M-MDSCs by flow cytometry of circulating peripheral blood mononuclear cells (PBMCs) [86]. In addition to this predictive biomarker role, MDSCs' role in resistance is also suggested by the finding that higher circulating M-MDSCs frequency was associated with reduced tumor-specific T-cell activation and expansion and was independently associated with inferior survival in

a cohort of melanoma patients [87]. Overcoming MDSCs' effects and restoring sensitivity to ICIs can be achieved through several mechanisms, including decreasing frequency, blocking recruitment, and even directly neutralizing MDSCs [80].

4.2.3 Tumor-Associated Macrophages

M-MDSCs give rise to another type of regulatory cells, the TAMs, which are the most abundant immune cells in the TME. Although not completely understood, the differentiation of MDSCs to M2-phenotype TAMs appears to be promoted through hypoxia-induced production of HIF1α which leads to pSTAT3 downregulation. Therefore, hypoxic conditions within the tumor milieu appear to shift MDSC differentiation toward the immunosuppressive phenotype M2-TAM, rather than the effector phenotype M1-TAM [81, 88, 89]. The M1/M2 subtypes represent a continuum of phenotypes determined by upregulation/downregulation of stimulatory and inhibitory chemokines and receptors; polarization of TAMs toward M2 has been shown to be an important mechanism of resistance to therapy [90]. TAMs interact directly with naïve T cells by inhibiting their proliferation and function, and indirectly by preventing T-cell interaction with MHC, with consequential tumor progression [91]. TAMs can express several immune checkpoint ligands, including PD-L1 and the co-inhibitory receptor B7-H4, which plays a role in inhibiting the antitumor response of T cells. Production of IL-10 and other suppressors of CD8+ T-cell activation is another important role of M2-TAMs [90]. Using in vivo imaging, Arlauckas and colleagues demonstrated that anti-PD-1 mAbs are swiftly captured from the T-cell surface by PD-1-negative TAMs minutes after administration [92]. The role of TAMs in mediating resistance to anti-PD-1 therapy is also suggested by the finding of increased TAMs relative to CTLs in the pretreatment tumor samples of nonresponding melanoma patients, whereas responders were found to have an abundance of CTLs relative to TAMs which correlated with improved survival. Co-inhibition of colony-stimulating factor 1 receptor (CSF1R) and PD-1 induced complete regression of all BRAF-mutant cell-line tumors via effective elimination of TAMs [93]. Likewise, targeting TAMs via CSF1R blockade appears to be a promising strategy by which resistance to ICIs may be overcome. In a preclinical mouse model of pancreatic cancer, the combination of PD-1 or CTLA-4 inhibition with CSF1R blockade greatly enhanced antitumor effects compared to monotherapy with either ICI [94]. ARRY-382 is a CSF1R inhibitor that is currently being tested in solid tumor clinical trials as monotherapy and in combination with a PD-1 inhibitor (NCT02880371). B7-H4 is a co-inhibitory receptor upregulated by IL-6 and IL-10 that is expressed on TAMs as well as various tumors and plays an important role in T-cell inhibition [95]. FPA150 is an anti-B7-H4 mAb that is currently being tested in trials in combination with anti-PD-1 therapy (NCT03514121). Inhibition of phosphatidylinositol 3-kinase (PI3K)-γ, which is highly expressed on myeloid cells, including both M-MDSCs and TAMs, has been shown to inhibit the immunosuppressive phenotype polarization of TAMs from M1 toward M2 and promote CTL-mediated tumor killing, thus reversing myeloid-mediated ICI resistance [96]. An ongoing phase 1 clinical trial is currently evaluating the combination of nivolumab with IPI-549 (eganelisib) in solid tumors (NCT02637531).

4.2.4 Gamma-Delta (γδ) T Cells

γδ T cells represent a small proportion of tissue-dwelling lymphocytes and less than 5% of circulating lymphocytes [97, 98]. This MHC-nonrestricted subset of lymphocytes play an important role in innate immunity against both infections and tumors directly through the swift production of soluble cytotoxic molecules such as granzymes and perforin, as well as indirectly through the production of inflammatory cytokines such as TNF-α and IFN-γ; hence, these cells contribute to innate and adaptive immunity, and are not typically considered inhibitory cells. However, a small subset of γδ T cells has been shown to play an immunosuppressive and pro-tumorigenic role. IL-17-producing γδ T cells

enhance the recruitment of MDSCs and immunosuppressive neutrophils, restrain αβ T-cell activation, promote angiogenesis, and may directly induce apoptosis of effector immune cells [98–102].

While conventional CAR αβ T-cell therapy has proven effective in the treatment of B-cell hematologic malignancies, its efficacy against solid tumors remains very limited [103]. Taking advantage of their natural residence in the TME of solid tumors and their antigen-presenting properties, CAR-transduced γδ T cells, particularly the Vδ1 and Vδ2 subsets, appear to be an appealing therapeutic approach with enhanced antitumor efficacy [104].

4.2.5 Cancer-Associated Fibroblasts

CAFs are another type of TME regulatory cell that plays an important role in T-cell dysfunction, in addition to desmoplasia promotion. Considered one of the most abundant cells in the stroma of most tumors, CAFs play a bidirectional signaling role between tumor cells and other immune cells, including TILs and TAMs [105]. In addition to altering the extracellular matrix, CAFs produce angiogenic factors like VEGF that contribute to metastasis and neoangiogenesis. They also cross talk with tumor cells via the production of amino acid metabolites that act as a diverse fuel source promoting proliferation and aggressiveness [106]. However, like TAMs, there appears to be phenotypic heterogeneity in CAFs, as some types appear to impede tumor progression [107]. More recently, CAFs have emerged as a major mediator in the immunosuppressive TME. CAFs contribute to T-cell dysfunction through multiple mechanisms, most importantly by impairing T-cell trafficking and recruitment to the tumor milieu, and secondly by repressing the cytotoxic function of CD8+ T cells [108]. These effects are mediated through the production of several CAF-derived molecules and ligands, including TGF-β, CXCL12, CXCL5, IL-6, collagen, and fibronectin, and through the upregulated expression of immune checkpoint ligands including PD-L1, PD-L2, and FasL. The production of collagen by CAFs traps immune cells and creates high interstitial pressure within the tumor, which promotes progression of metastases [109]. CAFs also promote a DC phenotype that is unable to interact with and present antigens to CTLs [105]. Furthermore, CAF-mediated CXCL-1 and CXCL-2 have been shown to promote the growth and recruitment of MDSCs and Tregs to tumor stroma, as well as polarize TAMs toward the M2 phenotype [110–112]. Chakravarthy et al. identified a poor prognosis phenotype of CAFs that is upregulated in many cancer types and is driven mainly by TGF-β signaling. More importantly, this phenotype was associated with resistance to PD-1 blockade in melanoma and bladder tumor samples [113]. The combined inhibition of TGF-β and PD-L1 using a bidirectional fusion protein has shown enhanced antitumor activity in preclinical mouse models [114]. In mouse models of hepatocellular carcinoma, increased infiltration of CAFs was associated with resistance to PD-1 blockade. More interestingly, inhibiting activated CAFs rescued the antitumor effects of anti-PD-1 treatment in orthotopic immune competent models [115]. Galunisertib, a novel TGF-β inhibitor, in combination with nivolumab, is currently being investigated in an early-phase clinical trial in solid tumors with focus on NSCLC and hepatocellular carcinoma (NCT02423343).

4.2.6 Dendritic Cells

Through antigen presentation and T-cell priming, DCs are frequently the initial inducers of inflammatory response, and they conceivably play a pivotal role in the tumor-immunity cycle. Although several phenotypes have been identified, the function of DCs is largely context-dependent in that it can be skewed toward a stimulatory or an inhibitory phenotype. The conventional DC1 subtype is the principal primer of T cells after antigen exposure, consequently promoting effector function [116]. DC1s produce stimulatory cytokines like CXCL9/CXCL10 which help recruit and locally activate CD8+ T cells in the TME [117, 118]. The type 2 conventional DCs (DC2) interact with CD4+ T cells, while the plasmacytoid DCs produce IFN. Monocyte-derived DCs are effective in antigen uptake but less efficient in activation of T cells [116]. DC functions are context-dependent and therefore can be skewed toward an inhibitory

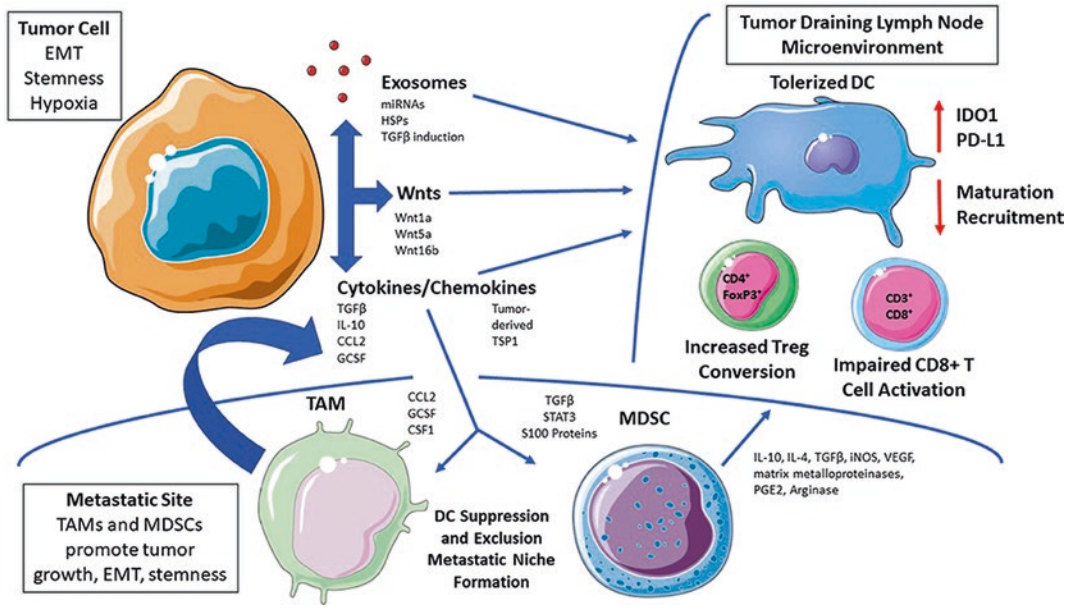

Fig. 4 Mechanisms of DC Tolerization. DCs residing within the tumor are functionally tolerized in the TME by immunosuppressive cells, inhibitory cytokines, and tumor exosomes. Tolerized DCs suppress T cell effector functions and enhances Treg differentiation, thus promoting tumor growth and metastasis. Abbreviations: EMT, epithelial-mesenchymal transition. TAM, tumor-associated macrophage; MDSC, myeloid-derived suppressor cell; IDO, indoleamine 2,3-dioxygenase; RA, retinoic acid; Arg, arginase; TSP1, thrombospondin-1. Adopted with permission from DeVito et al, Front Immunol. 2019;10:2876 [120]

phenotype upon tumor progression through a mechanism which is not fully defined [119]. IFN-γ produced by activated T cells in turn upregulates PD-L1 expression on DC1s, which plays a key role in limiting T-cell activation. Upregulation of PD-L1 on DCs occurs after antigen uptake as a mechanism to shield DCs from the cytotoxicity of activated T cells. However, this also suppresses tumor-directed immunity by contributing to T-cell dysfunction [119]. Furthermore, it has been shown that tumors subvert DCs by promoting a tolerization phenotype. This occurs via multiple mechanisms, including tumor-derived soluble molecules (IL-10, TGF-β, VEGF), tumor-derived exosomes (promoting a pre-metastatic niche), and the recruitment of other inhibitory cells in the TME (MDSCs, TAMs, Tregs) [120] (Fig. 4). PD-L1 expression on DCs appears to be indispensable for the efficacy of PD-L1 blockade therapy as the antitumor effect is completely lost in DC/PD-L1 knockout mice [119]. Targeting DCs is appealing and has been achieved through several novel mechanisms with variable success. The first DC-based vaccine, sipuleucel-T, was FDA-approved in 2010 and relies on ex vivo activation of and antigen delivery to DCs. Single-agent use of this form of immunotherapy yielded limited antitumor activity [121]. However, the combination of CTLA-4 blockade with sipuleucel-T resulted in remarkable activity in a small trial in patients with castration-resistant prostate cancer, and is currently being tested in a larger cohort with anti-CTLA-4 and anti-PD-L1 agents [122, 123] (NCT01804465).

Nanovaccines represent another modality that can target TLR signaling on DCs using insoluble nanoparticles that directly deliver peptide antigens to DCs with promising preclinical efficacy in vivo [124]. Ex vivo culture, activation, and antigen-loading of autologous myeloid-derived DCs followed by administration in patients' lymph nodes is another DC-based immunotherapeutic strategy with promising clinical activity in small cohorts of patients with melanoma [125, 126].

Toll-like Receptors

TLRs are receptors that play a role in innate and acquired immunity, and in antitumor immune response. They are either expressed on the cell surface and bind proteins and lipids (TLR1, TLR2, TLR4, TLR5, TLR6), or are expressed intracellularly on the endosomal membrane and bind nucleic acid (TLR3, TLR7, TLR8, TLR9). They can be expressed by several immune cells, particularly antigen-presenting cells including DCs and macrophages, and several types of tumors [127, 128]. Pathogen- and damage-associated molecular patterns bind to TLRs on DCs and other antigen-presenting cells inducing their maturation and initiating the immune response cycle. Foreign antigens, including cancer neoantigens, are then presented to T cells, leading to their activation [127].

TLR targeting has gained considerable interest over the past decade, as TLR agonists were found to exert an antitumor effect when administered locally. Single-agent use of TLR agonists has been implemented in different scenarios (e.g., bacillus Calmette-Guerin vaccine binding TLR2/TLR4 approved for superficial bladder cancer and topically applied imiquimod for actinic keratosis), but efficacy has been modest at best. The use of TLR agonists as an adjunct to DC-based vaccines has yielded promising results by enhancing immunogenicity in a cohort of patients with melanoma, the majority of whom had high-risk nonmetastatic disease [129]. Intratumoral TLR9 agonists are currently being tested in advanced stages of clinical development, after earlier phase trials showed promising activity in both injected and noninjected tumors. Injection with tilsotolimod, a TLR9 agonist, in combination with ipilimumab yielded a 38% response rate and a 71% disease control rate in a cohort of patients with anti-PD-1-refractory melanoma [130]. In another phase Ib trial, the combination of intratumoral TLR9 agonist CMP-001 with pembrolizumab yielded clinical responses in anti-PD-1-refractory patients, serving as a proof of concept of the ability to reverse resistance to ICI [131]. Other intratumoral TLR agonists are being tested in various clinical trials, such as the TLR4 agonist GLA-SE in CRC (NCT03982121), the TLR7 agonist imiquimod in breast cancer (NCT01421017), the TLR7 agonist DSP-0509 in combination with pembrolizumab for advanced solid tumors (NCT03416335), and MEL60 in combination with long-peptide vaccine in resected melanoma (NCT02126579).

4.2.7 Endothelial Cells

Transmigration of circulating T cells into the tumor nest is mediated through chemotactic cytokines and the upregulated expression of adhesion molecules and ligands on activated endothelial cells. However, constitutive activation of the tumor vasculature by proangiogenic factors in the TME can paradoxically lead to dysfunctional endothelial cells that impair leukocyte adhesion and transendothelial migration [132]. Dysfunctional endothelial cells express ligands that greatly reduce immune cell permeability. The FAS antigen ligand (FasL), under the effect of IL-10 and prostaglandin E, can induce apoptosis of CTLs but not Tregs [40]. Dysfunctional tumor vasculature is known to represent an efficient barrier for recruitment of Teffs and thus pose a challenge toward effective immune checkpoint blockade (ICB) [133]. Suppression of VEGF-A has been shown to increase CD8+ T-cell influx into tumors [40]. Treatment strategies that harness the crosstalk between tumor angiogenesis and the immune system can restore the antitumor effects of ICIs. Several proangiogenic molecules have been found to effectively contribute to immunosuppression. VEGF has been shown to impair DC functional maturation; thus, anti-VEGF treatment was successful in restoring the differentiation of monocytes into DCs [134, 135]. In addition, VEGF contributes to T cell exhaustion by enhancing PD-L1 expression on DCs and suppressing antigen presentation [136]. Direct VEGF binding to the VEGFR2 receptor on Teffs suppresses proliferation and upregulates PD-1 expression, while binding to the same receptor on Tregs and MDSCs enhances their infiltration into the tumor milieu [137]. VEGF-mediated modulation of VCAM-1 and ICAM-1 adhesion molecules creates a barrier that is impermeable

to effector immune cells, precluding homing of T cells to tumors [138]. Consequently, it is postulated that vascular normalization via the use of VEGF inhibitors has the potential to augment anti-PD-(L)1 therapy and enhance antitumor response. Moreover, treatment with VEGF/VEGFR inhibitors has been shown to upregulate PD-L1 on tumor cells, and the combined blockade of PD-L1 and VEGF showed synergistic antitumor effect in pancreatic neuroendocrine and breast cancer mouse models [139]. This combination has demonstrated clinical efficacy across a variety of tumor types in phase III trials and is already FDA-approved in RCC, NSCLC, hepatocellular carcinoma, and endometrial carcinoma [140] (Fig. 5).

4.2.8 Tumor-Derived Pericytes

Pericytes are perivascular cells that play an important role in vascular structure and integrity. However, in tumor beds, pericytes may frequently lose close attachment to endothelial cells in the tortuous, erratic tumor vessels, causing aberrant permeability and dysfunctional, leaky flow [141]. Besides their role in tumor angiogenesis, the type 2 pericytes appear to play an important interactive role with other cells and chemokines in the TME. In vitro studies have shown that pericytes may contribute to some immunological functions like phagocytosis and antigen presentation [142]. Pericytes can produce several types of cytokines, growth factors, and adhesion molecules, and are considered an important component of the immunologic shield [141, 143]. Promoting pericyte maturation has been show to restore vasculature function and improve CD8+ T-cell transmigration into the tumor niche which resulted in improved antitumor immunity in mouse models [144]. Tumor-derived pericytes express PD-L1, which has a known role in CD8+ T-cell dysfunction, and Rgs5, which prompts anergy of CD4+ T cells. These effects contribute to shielding of tumor cells from immune-mediated destruction, a finding that suggest pericytes may be an appealing target for immunomodulation. Needless to say that therapeutic approaches should focus on normalizing pericyte functionality rather than elimination [145].

4.3 Cytokines and Other Soluble Molecules in T-Cell Dysfunction

As a critical component of autocrine and paracrine signaling, cytokines are involved in all pathways leading activation and trafficking, as well as to the dysfunction and exhaustion, of T cells. Many cytokines are receptor-pluripotent in that they can bind several receptors on a cell surface. Receptors, likewise, may bind different types of ligands. Manipulating cytokine production, or receptor binding, can potentiate the effectiveness of ICB by preventing the development of resistance [146]. In this context, it is noteworthy that the use of cytokines such as IL-2 for RCC and melanoma, and IFN-γ for myeloproliferative neoplasms, was one of the earlier forms of immunotherapy implemented in clinic, albeit with limited success [147]. Among the cytokines that seem to have a great impact on T cell functions are the C-X-C motif ligands 9 and 10 (CXCL9 and CXCL10). CD8+ Teffs, NK cells, and type 1 helper T cells (Th1) all express CXCR3, which binds ligands CXCL9 and CXCL10 produced by Th1. This binding results in the chemotaxis and infiltration of effector cells into tumors, which in turn is correlated with improved clinical outcomes in response to PD-(L)1 blockade [148, 149]. Epigenetic silencing of CXCL9 and CXCL10 leads to poor Tcell infiltration into tumors; and treatment of colon cell lines with a histone methylation inhibitor leads to higher CXCL9 and CXCL10 expression and more efficient T-cell migration toward tumors [150]. The reversal of epigenetic silencing of CXCL9 and CXCL10 was also synergistic with PD-L1 blockade therapy in ovarian cancer xenografts [151]. This has raised interest in epigenetic reprogramming as a method to improve T-cell trafficking to the TME and therefore improve response to ICIs. Combination therapies of anti-PD-(L)1 with a hypomethylating agent are currently being evaluated in clinical trials in a variety of liquid and

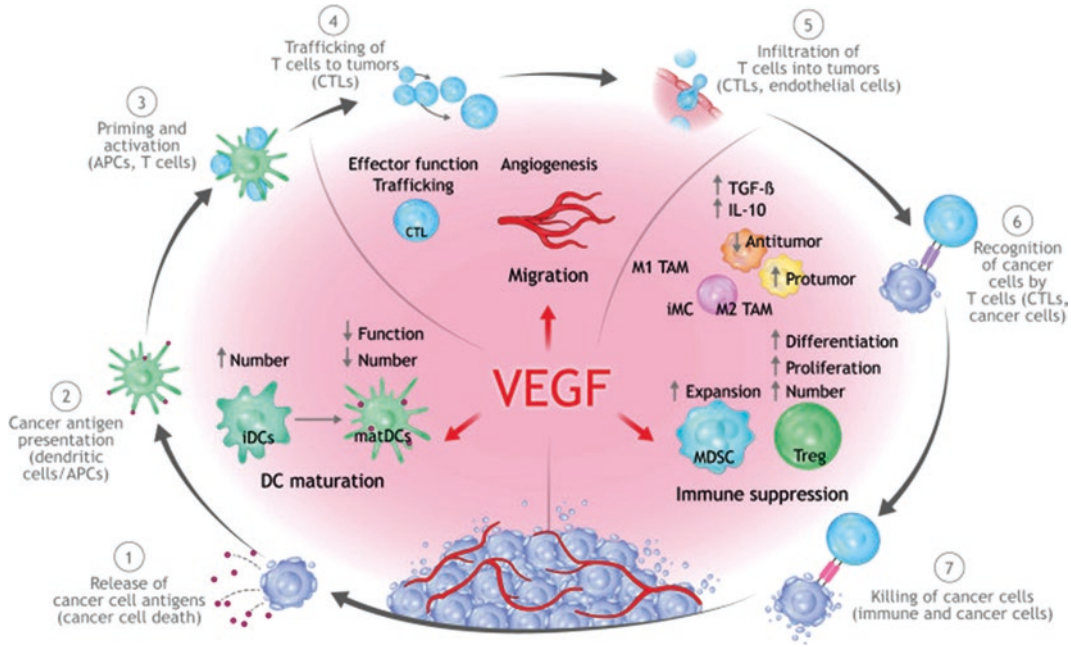

Fig. 5 VEGF-mediated immunosuppression in the TME. VEGF-induced constitutive activation of tumor vasculature leads to endothelial cell dysfunction and vascular aberration. VEGF is also implicated in reduced T cell permeability, increased inhibitory cytokines and regulatory cells, and impaired DC maturation. Abbreviations: APC, antigen-presenting cells; CTLA, cytotoxic T lymphocyte associated; DC, dendritic cell; MHC, major histocompatibility complex; PD-1, programmed cell death 1 protein; PD-L1, programed cell death ligand 1; PlGF, placental growth factor; TME, tumor microenvironment; TCR, T-cell receptor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor. Reproduced with permission from Hack et al, Front Immunol. 2020;11:598877 [140]

solid malignancies (NCT03233724); and some trials started to report outcomes [152].

In contrast, the interaction of CXCL12, a cytokine produced by stromal cells particularly CAFs, with its receptor on T cells, CXCR4, has been shown to play a role in recruiting and retaining FoxP3 + CD4+ Tregs in tumors like basal-like breast cancer and epithelial ovarian carcinoma [153, 154]. Moreover, high CXCR4 expression predicts a more advanced stage and lower survival in other tumors like gastric cancer [155]. CXCL12/CXCR4 blockade by a CXCR4 antagonist or by oncolytic virotherapy has been shown to reduce tumor growth and improve survival in immunocompetent murine models of ovarian cancer [154, 156]. Dual blockade of PD-(L)1 and CXCL12-CXCR4 has been shown to be synergistic in thwarting immunosuppression in the TME and enhancing antitumor immunity in preclinical models [157]. This combination is being tested in early-phase trials (NCT04177810).

The monocyte chemoattractant protein 1 (CCL2) is produced by immune cells and implicated in the migration of monocytes. In addition, it is also produced by some tumors and implicated in the migration of other cells like Tregs and endothelial cells to sites of inflammation [158]. Other cytokines, including CCL3 and CCL5, are also involved in immune cell migration to the TME, particularly neutrophils and macrophages. Inhibition of these cytokines has been shown to reduce invasive potential and neoangiogenesis in preclinical models of breast and ovarian cancers [159, 160]. Blocking CCL2 reduces immunosuppression and enhances the antitumor activity of an adenoviral vector expressing IFN-α [158].

As a major stimulator of the T-cell adaptive response, DCs produce a wide array of cytokines that are involved in immune response. Among these, CCL17 and CCL22 produced particularly by monocyte-derived DCs, among other immune cells, as well as by some tumors, appear to play an important role in Treg recruitment to tumors. Blocking CCL17 and CCL22 in monocyte-derived DCs using RNA interference reduces the frequency of Treg recruitment and increases CD8+ T cells in human breast cancer xenografts [161, 162]. Moreover, the CCL17/CCL22 receptor, CCR4, is expressed by Th2 cells and by some of the most terminally differentiated and immunosuppressive tumor-infiltrating FoxP3-high Tregs [163]. CCR4 expression has been found in several tumors, especially T-cell malignancies. In addition to its efficacy related to antibody-dependent cytotoxicity in T-cell neoplasms, anti-CCR4 mAb was effective in inducing FoxP3-high Treg depletion [162, 164]. Signaling of CCL17/CCL22-CCR4 is implicated in tumor resistance to ICIs, as upregulation of both ligands has been shown to occur as a result of ICI therapy in vivo. More interestingly, CCR4 inhibition had a synergistic antitumor effect with anti-CTLA-4 therapy [165].

The transmigration of MDSCs into the TME is mediated through the CXCR2 receptor, which binds CXCL1, CXCL8, CXCL5, and CXCL7, among others. Elevated levels of CXCR2 ligands, CXCL1 and CXCL8, was detected in pediatric sarcoma patients, and appear to confer worse prognosis. Mice reconstituted with CXCR2-negative hematopoietic cells showed enhanced antitumor activity when exposed to PD-1 blockade [166]. In addition to its role in promoting angiogenesis and epithelial-to-mesenchymal transition, CXCL8 (IL-8) plays an immunosuppressive role in the TME. Produced by many tumors, CXCL8 recruits both types of MDSCs. Furthermore, high CXCL8 levels were found to predict poor outcome in patients treated with immunotherapy [83]. Anti-IL-8 mAbs can abolish signaling through both receptors, CXCR1 and CXCR2. Preclinical studies in claudin-low breast cancer showed this strategy to be highly effective in reducing MDSCs and increasing immune-mediated cytotoxicity [167]. Early reduction of IL-8 levels was shown to be strongly correlated with tumor response to anti-PD-1 therapy in two cohorts of NSCLC and melanoma patients [168]. Single-agent anti-IL-8 mAb therapy yielded modest antitumor activity in pretreated patients with a variety of solid tumors [169]. Studies with combined PD-1/IL-8 blockade are underway to evaluate clinical activity (NCT03400332, NCT03689699, NCT04050462).

In addition to its role in cell growth, proliferation, differentiation, and cell matrix formation, TGF-β appears to play a key role in driving immune evasion. In patients with CRC, elevated TGF-β levels was associated with lack of T-cell infiltration, low Th1 activity, reduced cytotoxicity, and poor clinical outcome. In genetically reconstituted low TMB, MS-stable, T-cell-excluded colon cancer metastases, PD-(L)1 inhibition produced limited antitumor efficacy, as would be expected; however, the subsequent blocking TGF-β signaling produced a potent cytotoxic T-cell response and restored sensitivity to anti-PD-(L)1 therapy. This suggests an important role for TGF-β in promoting T-cell exclusion and blocking the Th1 effector phenotype in the TME [170]. Likewise, TGF-β signaling was found to be one of the main determinants of clinical outcome in a cohort of patients with urothelial carcinoma. Lack of response to anti-PD-L1 therapy was associated with a TGF-β signaling signature in fibroblasts. Furthermore, co-blockade of PD-L1 and TGF-β enhanced T-cell trafficking into tumors and produced a more profound antitumor effect [171]. Consistent with these findings, an elevated plasma level of TGF-β was also found to be a significant predictor for poor treatment outcome in a cohort of patients with hepatocellular carcinoma treated with anti-PD-1 therapy [172]. Several TGF-β inhibitors have been developed, including small molecule inhibitors and mAbs. Some of these agents have shown activity as monotherapy or in combination in early-phase trials [173–175]. Trials evaluating the combined inhibition of PD-(L)1 and TGF-β in a variety of solid tumors are underway (NCT02423343, NCT04390763).

Bintrafusp alfa is bifunctional fusion protein composed of the extracellular domain of TGF-β receptor 2, linked to the heavy chain segment of the anti-PD-L1 antibody. Bintrafusp alfa functions as a trap to all isoforms of TGF-β while simultaneously mitigating immunosuppression. Preclinical data have demonstrated the ability of bintrafusp alfa to increase T-cell trafficking and cytotoxicity in cell lines and mouse models [114, 176]. In addition, PD-L1 binding allows for concentration within a PD-L1-positive tumor; and preclinical studies showed that up to 27% of the injected dose concentrate in the tumor with a peak tumor/blood ratio of 58:1 [177]. Promising clinical activity have been noted in a cohort of patients with heavily pretreated advanced solid tumors in a phase 1 trial [178] and in several solid tumor indications [179–181].

IL-10, previously termed "cytokine inhibitory factor," is one of the first inhibitory factors to be identified. IL-10's immunosuppressive role is considered a key component in limiting excessive inflammatory response. IL-10 is produced by many immune cells including CD4+ and CD8+ T cells, TAMs, and DCs, as well as tumor cells. It plays a role in the downregulation of Th1 inflammatory cytokines, namely, IL-2, TNF-α, and IFN-γ, and inhibits MHC-II expression on activated monocytes. Nevertheless, it is currently believed that IL-10 may in fact possess a bifunctional role, as it has also been shown to have an immune-stimulatory role by inducing tumor-dwelling CD+ T-cell activation and expansion [182–184]. An elevated level of IL-10 has been identified as an adverse prognostic indicator in several tumor types, including both hematological and solid malignancies [185]. In vivo inhibition of IL-10 enhances cytotoxic T-cell function and the antitumor activity of PD-L1 blockade [186]. In contrast, pegilodecakin, a pegylated recombinant IL-10, has been tested in clinical trials and demonstrated activity in pretreated advanced RCC (NCT02009449).

As discussed above, VEGF is another important mediator of immunotherapy resistance. In addition to its role in disrupting normal vasculature, VEGF impairs CTL proliferation and trafficking, and inhibits DC maturation and antigen processing [140].

IFN-γ is believed to play a role in innate antitumor immunity by enhancing antigen presentation through upregulation of MHC-I. However, it can also promote an immunosuppressive TME through activation of the JAK/STAT pathway, resulting in increased expression of PD-L1 in what represents a negative feedback loop [187]. The efficacy of combining IFN-γ and ICIs is being evaluated in early-phase trials in a variety of solid and liquid tumors (NCT02614456, NCT03063632).

IFN-α is a pleiotropic cytokine with antineoplastic properties and has been in clinical use for adjuvant therapy of high-risk melanoma. The immunomodulatory effects of IFN-α include stimulating CXCL10 secretion, which in turn enhances CD8+ T-cell trafficking and effector activity within the TME [188]. In vivo IFN-α treatment of a murine colon cancer cell line increased PD-1 expression on TILs. Co-inhibition of PD-1 and IFN-α increased CD4+ and CD8+ TILs and reduced tumor growth more than IFN-α alone [189]. Several studies are underway evaluating this combination in humans in metastatic and adjuvant settings (NCT02506153, NCT02174172).

Another cytokine implicated in immunomodulation is the IL-6, which is produced by tumor cells and tumor-infiltrating immune cells. Elevated circulating IL-6 levels was noted in several tumor types, and correlated with advanced tumor stage and reduced response to therapy [190, 191]. Both IL-6 and IFN-γ were shown to upregulate PD-L1 expression on antigen-presenting cells; and this process appears to be mediated by activation of the Janus kinase/signal transducers and activators of transcription 3 (JAK/STAT3) signaling pathway [187, 191]. A positive autocrine feedback loop then forms as STAT3 enhances IL-6 gene expression, which contributes to the development of an immunosuppressive TME in epidermal growth factor receptor (EGFR)-mutant NSCLC. In addition, STAT3 hyperactivation in immune cells in the TME has been shown to upregulate both MDSCs and Tregs [191, 192]. In vivo silencing of the

STAT3 pathway led to downregulation of PD-L1 expression and reduced metastatic potential in a murine mouse model of breast cancer [193]. Single targeting of either IL-6 or STAT3 has generally yielded dismal results and limited antitumor activity in early-phase trials [194–196]. However, the use of a combination strategy with ICB may prove more promising, and is being investigated in a variety of solid tumors (NCT04191421, NCT04691817).

5 Oncogenic Signaling Pathways

Oncogenic alterations in the tumor cell genome, both gain- and loss-of-function mutations, have been implicated in promoting an immunosuppressive TME. Oncogene addiction is not an exclusive cell-intrinsic process; rather, it is greatly influenced by crosstalk with an immune-permissive TME composition [197]. Advances in molecular technologies have shed light on the interaction between the immune system and the tumor's driver mutations; consequently, several aberrations have been identified as potential mechanisms for tumor resistance to innate immunity and immunotherapy.

5.1 JAK/STAT Mutations

Inactivation of the IFN-JAK1/JAK2 pathway resulting from an emerging loss-of-funcion mutation has been described in melanoma patients who developed secondary resistance to ICIs. Tumor cells appear to resort to abrogation of IFN-mediated signaling as a potential way to evade treatment with anti-PD-1 therapy. As discussed above, IFN signaling leads to an adaptive increase in PD-L1 expression. Eliminating this pathway is postulated to decrease therapeutic target receptors, rendering treatment ineffective [23]. Activation of the JAK/STAT pathway through the amplification of chromosome 9p24.1 region, which encodes for JAK2 and PD-L1/L2, has been described in a subset of triple-negative breast cancers (TNBC) and was linked to poor prognosis. This PDJ amplicon leads to an IFN-induced increase in PD-L1 expression by 5- to 38-fold. Subseqently, JAK2 knockdown in TNBC cell lines completely blocked inducible PD-L1 expression [198]. An ongoing phase I trial is evaluating the combination of JAK2 inhibitor with ICIs in TNBC patients [199].

5.2 Mutations in the Ras-Mitogen-Activated Protein Kinase (Ras-MAPK) Pathway

Activation of the Ras-MAPK pathway has been shown to correlate with reduced TIL in a subset of TNBC patients who failed to achieve pathologic CR after neoadjuvant therapy. In addition, activation of this pathway may suppresses MHC expression and upregulates PD-L1, an effect possibly mediated through IFN-γ signaling. A similar finding was reported in human melanoma cell lines. The process is believed to play an important role in tumor evasion of innate immunity, as well as in MAPK-activated tumor resistance to ICB [200–202]. The synergy of MEK inhibitors and PD-(L)1 blockers was demonstrated in syngeneic murine models of triple-negative and HER2-positive breast cancer [200, 201]. In early-phase trials, the combination of dual MAPK pathway inhibitors with an anti-PD-(L)1 agent led to increased immune infiltration into tumors and yielded promising activity [203, 204].

5.3 Loss of Phosphate and Tensin Homolog (PTEN) Tumor Suppressor

Loss of PTEN, with subsequent PI3K-AKT-mTOR signaling activation, is not only oncogenic but is also implicated in mediating resistance to immunotherapy. PTEN loss induces VEGF and immunosuppressive cytokines production, reduces T-cell trafficking and cytotoxicity, and promotes MDSCs in the TME [96, 205, 206]. For instance, acquired PTEN loss was shown to confer primary resistance to anti-PD-1

therapy in patients with uterine leiomyosarcoma [205]. Treatment with PI3K inhibitors improved the antitumor efficacy of ICIs in murine models [96, 206]. A phase I trial of a PI3K-γ inhibitor in combination with an anti-PD-1 agent reported favorable outcomes and early signs of clinical activity [207]. Several trials are underway evaluating the safety and efficacy of combined inhibition of PI3K and PD-1 (NCT04193293, NCT03711058).

5.4 Activation of the Wnt/β--Catenin Signaling Pathway

The role of Wnt signaling in oncogenesis and tumor propagation has been documented in several tumor types, including CRC, mammary carcinoma, hematologic malignancies, and melanoma, among others. The effect of aberrant Wnt/β-catenin signaling extends beyond tumor cells to include the TME [208]. For example, in metastatic melanoma, activation of the Wnt/β--catenin pathway impaired T-cell priming and activation by tolerizing DCs, and was correlated with reduced TILs [120, 209, 210]. A novel β-catenin inhibitor is being combined with an anti-PD-1 agent in a phase I clinical trial in solid tumors (NCT02521844).

5.5 KRAS Mutation

KRAS is one of the most altered genes in human malignancies, and is known to play several critical roles in the immune composition of the TME. KRAS mutation in NSCLC appears to be associated with increased tumor C8+ T-cell infiltration, inflammed TME phenotype, and increased responsiveness to ICB [211, 212]. In contrast, KRAS-mutated CRC and pancreatic adenocarcinoma exhibit an immunosuppressive TME, which was also associated with lower response rate to ICB [82, 213, 214].

KRAS mutations cause upregulation of PD-L1 through activation of the PI3K/AKT/mTOR pathway [215]. In addition, MAPK/ERK signaling was shown to contribute to stabilization of PD-L1 mRNA [216, 217]. Activation of the MEK-ERK pathway in KRAS-mutated lung cancer can modulate the TME through increased secretion of IL-10 and TGF-β. Further, in vivo inhibition of KRAS in a KRAS-driven lung tumorigenesis model significantly reduced Treg infiltration, which provides a proof of concept of the cell-extrinsic activity of this mutation [218]. Furthermore, through the suppression of interferon regulatory factor 2, KRAS leads to increased CXCL3 expression and binding to CXCR2 on MDSCs prompting their migration to the TME [82]. Lastly, the occurrence of STK11/LKB1 co-mutation in KRAS-mutated NSCLC has been shown to significantly reduce response rate to PD-1 inhibition [214, 219].

Inhibition of the KRAS downstream pathway through MEK inhibitors, or through a KRAS mRNA vaccine, in combination with anti-PD-(L)1 therapy is currently being studied in early-phase trials (NCT03948763, NCT03681483, NCT03299088).

5.6 Epidermal Growth Factor Receptor Mutation

An immune-tolerant TME is a hallmark of EGFR-mutant NSCLC, which is known to exhibit reduced responsiveness to anti-PD-(L)1 therapy [220–222]. Despite some controversy, it appears that both lower PD-L1 expression and lower TMB in these tumors lead to the relative refractoriness to ICB [223, 224]. The EGFR signaling pathway promotes an uninflamed TME through enhanced Treg migration and through skewing DCs toward a tolerant phenotype [223, 225]. Phosphorylation of STAT3, a downstream signaling transducer of EGFR, increases expression of indoleamine 2,3-dioxygenase (IDO) which in turn promotes the expansion of MDSCs and enhances their immunosuppressive effect [226]. In an interesting study by Huang and colleagues, most exosomes purified from biopsies of lung tumors were found to contain large quantities of EGFR protein. When captured by DCs, these EGFR-laden exosomes promote DC to differentiate to a tolerogenic phenotype

that promotes Tregs and suppresses tumor-specific CD8+ T cells [225]. Tyrosine kinase inhibitor (TKI) therapy with EGFR inhibitors has been shown to revive some of the inflammatory aspects of the TME and increase CD8+ T-cell infiltration [227]. The combination of anti-PD-L1 and EGFR-TKI yielded a response rate of 43% in a cohort of patients previously resistant to TKI monotherapy, albeit with increased incidence of interstitial lung disease [228]. There are several ongoing trials evaluating different TKI-ICI combinations (NCT02364609, NCT03082534, NCT04017650).

BCA101 is a first-in-class bifunctional antibody that targets both TGF-β and EGFR. It is being tested in combination with anti-PD1 therapy in EGFR-driven tumors in a phase I trial (NCT04429542).

6 Tumor-Associated Enzymatic Activity and Metabolites

Enzymatic activity in the TME impacts the innate and adaptive immune response by catabolizing important immune cell amino acid nutrients, creating inhibitory metabolic byproducts, and playing a role in intracellular signaling pathways.

6.1 Indoleamine 2,3-Dioxygenase-1 (IDO-1)

IDO-1 is a versatile enzyme, mainly induced by IFN-γ, that has been shown to regulate immune response by reducing uncontrolled activation in inflammatory conditions. It has gained attention due to its notable role in modifying antitumor immune response and the potential for targeting in clinic. In response to immune activation, IDO catalyzes the metabolism of tryptophan, thus depleting an essential element for effector T cell function. The metabolic product of this process is kynurenine, which is the ligand for the aryl hydrocarbon receptor. Kynurenine promotes the differentiation of FoxP3+ Tregs and enhances their immunosuppressive effects [229, 230]. More interestingly, a distinct intracellular signaling role of IDO-1 was identified as it was found to promote a regulatory phenotype in plasmacytoid DCs under the effect of TGF-β [231]. Upregulation of IDO-1 has been shown to occur in some tumors as a response to ICI therapy. A phase I/II trial revealed an encouraging response rate for the combination of pembrolizumab and IDO-1 inhibitor, epacadostat, in a variety of tumor types [232]. However, in a larger cohort of patients, this combination failed to produce significant benefit over single-agent pembrolizumab in a randomized double-blind phase III trial [233].

6.2 Adenosine

CD73 (ecto-5′-nucleotidase) is a cell surface enzyme implicated in purinergic signaling by mediating the breakdown of adenosine monophosphate to adenosine. CD73 is upregulated by many tumor types and has key functions in regulating tumor proliferation, invasiveness, angiogenesis, and immune-evasion. The metabolic product, adenosine, promotes cancer cell survival and progression, and plays an important immunosuppressive role in the TME [53, 234, 235]. CD73 can be expressed on neoplastic cells of several tumor types, as well as on Tregs, MDSCs, and endothelial cells. TGF-β plays an important role in sustaining CD73 expression on CD8+ T cells. Adenosine binds to receptors A2AR/A2BR on lymphocytes, suppressing their effector function and downregulating the inflammatory response. Moreover, adenosine has been shown to inhibit DC maturation, thus impairing antigen presentation. The adenosinergic immunosuppressive role of CD73 is an appealing target to revive antitumor immunity [234, 236–238]. In addition to conferring an adverse prognosis, CD73 expression is associated with reduced ICI efficacy [239, 240]. Targeting CD73 has been achieved through direct antibody blockade or by blocking the adenosine receptor. The anti-CD73 mAb MEDI9447 in combination with durvalumab demonstrated some clinical activity in the treatment of refractory CRC and pancreatic carcinoma [241]. AZD4635, a small molecule inhibitor of A2AR, rescued antitumor immunity

in DCs in vitro, and inhibited tumor growth in syngeneic mouse models [238]. AZD4635 yielded notable antitumor activity as a single agent and in combination with durvalumab in a phase I trial [242].

7 Impact of Anatomical Site

While immunotherapy achieved remarkable milestones in malignancies like melanoma and NSCLC, it yielded disappointing results in other tumors like luminal-type breast cancer and pancreatic adenocarcinoma. Tissue-specific differences in immune infiltrate composition and function are plausibly implicated in these differences, especially the tissue-dwelling myeloid cells and DCs. Zagorulya and colleagues proposed a phenotypic classification of DCs that infiltrate different anatomic sites and correlated this with the likelihood of successful ICB. For instance, lung tissue appears to skew DCs toward a stimulatory phenotype that is efficient in antigen presentation and T-cell activation, leading to a more inflammatory TME and higher ICB success rate. This is in contrast to immune-desert tumors, like pancreatic ductal carcinomas, which are infiltrated with rare DCs that are skewed toward an inhibitory phenotype [116]. On the other hand, some metastatic sites appear to be particularly less responsive to ICB. For example, liver metastases exhibit lower response rates to ICB even if they originate from primary tumors known to respond to such therapy [243]. Several mechanisms have been found to account for the immune-tolerant TME in liver tissue. Tolerogenic DCs with a weak antigen-presenting phenotype predominate in the liver and produce IL-10 and TGF-β, resulting in Treg induction and Teff inhibition. In addition, Kupffer cells in the liver appear to display an immunosuppressive macrophage phenotype. Despite their ability to prime CD8+ T cells, the resulting cells are largely dysfunctional in that they produce low levels of IFN-γ and have poor effector capabilities [116, 244].

8 Hyperprogression Phenomenon

In discussing mechanisms of immune evasion, one cannot overlook the few instances where ICI therapy may paradoxically enhance tumor growth and cause accelerated progression. Hyperprogression is a distinct entity that has been noted to occur in several tumor types in response to treatment with ICIs. Depending on the criteria used to define it, the estimated incidence ranges between 4% and 29% of treated patients [245, 246]. A definition for hyperprogression has not been unanimously agreed upon, but some authors suggest using the combined findings of RECIST progression on first evaluation scan plus a twofold volumetric tumor growth rate, where volume is calculated as $V = 4\,\pi\,R^3/3$, R being the radius is half the sum of maximum dimensions of target lesions, assuming a spherical tumor shape [247]. Others have proposed using a more than 50% increase in monthly tumor growth rate, or a twofold increase in tumor growth rate between the pretreatment and first evaluation scans [248, 249]. Lastly, Lo Russo and colleagues suggested criteria that take into consideration clinical deterioration and shortened time to treatment discontinuation [250]. The biochemical and molecular basis of hyperprogression is not fully understood, but resistance mechanisms discussed earlier are plausibly implicated. More interestingly, however, a role for the anti-PD-(L)1 Fc region interaction with the Fc receptor (FcγR) on TAMs has been suggested. This Fc-FcγR interaction was shown in human lung cancer-derived xenografts to cause significant tumor growth in mice treated with nivolumab. Using an anti-PD-1 agent that lacks the Fc region [F(ab)2] did not lead to tumor growth. This paradoxical tumor growth in response to ICI treatment occurs as a result of macrophages reprogramming toward a tumor-promoting M2 phenotype in response to the Fc-FcγR binding [250]. Immune-mediated dedifferentiation of breast cancer models was described by Stein and colleagues who demon-

strated how tumor cells' interaction with nonlytic CD8+ T cells induced a stem cell-like phenotype in the tumor [251]. Another group compared pretreatment and posttreatment gastric cancer tissue samples from a patient with hyperprogression and showed that anti-PD-1 therapy may have caused a significant increase in proliferation and activation of PD-1+ tumor-infiltrating effector Tregs, a finding that was not seen in patients without hyperprogression. Treg suppression, e.g., by targeting OX40, could prove critical in preventing hyperprogression for at-risk patients [246].

9 Conclusion

ICI therapy fundamentally altered the way we treat many solid tumors due to the rapid, deep, and durable responses seen. While this promise has led to functional cures in some patients, only a proportion of patients with solid tumors treated with immunotherapy have a sustained response, with the majority manifesting primary resistance. Patients who initially respond and subsequently progress may have completely different underlying biology of their tumors than those with primary resistance. Thus, subsequent trials of immunotherapy approaches in these patients should take this into account. For instance, if the patient initially had a response to PD-1 inhibition, it is likely that there are tumor-directed T cells that could be further induced by effectively addressing other negative regulatory influences in the tumor. However, in a patient with a TMB-low cancer, with no viral antigens that have primary resistance, a strategy that includes generating a T-cell response (such as a vaccine, oncolytic virus, or tumor-targeted cytokine) or delivering a T-cell response (CAR-T, bispecific antibody, or T-cell receptor-engineered cells) would be a rational approach. Thus, understanding the immune-relevant biology of the tumor is important when considering immunotherapy, especially in tumors resistant to front-line single-agent immunotherapy.

Combination immunotherapy approaches for patients with common underlying deficiencies in the tumor immunity cycle offer the best way to move the field forward to better therapeutic options. These approaches include addressing the need to generate tumor-targeting effector cells, to expand their numbers, and to allow them to be functional in the often hostile TME. Immunotherapeutic drugs that can address multiple mechanisms with one agent could prove critical in these strategies, especially if they have a targeting component to enrich the agent in the TME.

The explosion of omics approaches (including single-cell RNA-Seq) and the added context gained with multiplexed multispectral imaging and spatial transcriptomics offer many opportunities to better understand the underlying biology of the tumor and to gain insights into rational combination approaches as we seek to make functional cures a reality for people with solid tumors.

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