




A landscape of checkpoint blockade resistance in cancer: underlying mechanisms and current strategies to overcome resistance

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ABSTRACT

The discovery of immune checkpoints and the development of immune checkpoint inhibitors (ICI) have achieved a durable response in advanced-stage cancer patients. However, there is still a high proportion of patients who do not benefit from ICI therapy due to a lack of response when first treated (primary resistance) or detection of disease progression months after objective response is observed (acquired resistance). Here, we review the current FDA-approved ICI for the treatment of certain solid malignancies, evaluate the contrasting responses to checkpoint blockade in different cancer types, explore the known mechanisms associated with checkpoint blockade resistance (CBR), and assess current strategies in the field that seek to overcome these mechanisms. In order to improve current therapies and develop new ones, the immunotherapy field still has an unmet need in identifying other molecules that act as immune checkpoints, and uncovering other mechanisms that promote CBR.

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1. Introduction

Immunotherapy has revolutionized how advanced-stage cancers are treated by shifting the focus of the treatment from directly killing the tumor cells to activating the host's immune system to target the tumor.¹ One breakthrough in cancer immunotherapy has been possible due to the discovery of immune checkpoints (i.e., cytotoxic T lymphocyte antigen-4 (CTLA-4), program cell-death 1 (PD-1), and program cell-death ligand 1 (PD-L1)).^{2–5} Under homeostasis conditions, the role of immune checkpoints, along with costimulatory signals, is to maintain self-tolerance and regulate the type and magnitude of the immune response.² However, tumor cells can dysregulate the signaling of immune checkpoints to allow the evasion of tumor cells.³ Based on the immense role of checkpoint signaling in cancer, targeting checkpoint molecules is a rational strategy to promote anti-tumor immunity and, indeed, several immune checkpoint inhibitors (ICI) have been developed and proven effective in the clinical setting.^{1,4}

CTLA-4 is an immunoglobulin-related receptor that is upregulated on conventional T cells following activation.^{5,6} As a homolog of CD28, it also binds CD80 and CD86 expressed on antigen-presenting cells (APCs) but at a higher affinity, effectively competing with CD28 for ligand binding. In contrast to CD28, CTLA-4 attenuates T cell responses potentially through various reported mechanisms such as interfering with molecules downstream of CD28 and the T cell receptor (TCR).^{7,8} In addition, CTLA-4 is highly expressed on regulatory T cells (Tregs) and is believed to be important in Treg homeostasis and function.⁹ Ipilimumab, a human monoclonal antibody (mAb) targeting the

immunosuppressor molecule CTLA-4, was the first US Food and Drug Administration (FDA)-approved ICI for the treatment of metastatic, late-stage melanoma patients.^{10–12} The approval of ipilimumab was based on the results of the MDX010–020 clinical trial (NCT00094653) showing that among 676 randomized patients, 20% and 16% of the patients survived ≥ 2 years and ≥ 3 years, respectively.⁴ This was a significant improvement in overall survival (OS) over chemotherapy and cytokine-based therapies, which were used as standard-of-care (SOC) treatment for this patient population.¹³ Currently, ipilimumab in combination with nivolumab is approved for colorectal cancer (CRC), hepatocellular carcinoma (HCC), mesothelioma, and non-small cell lung cancer [NSCLC].^{2,14} For melanoma, ipilimumab is approved as monotherapy or in combination with nivolumab.

The discovery of PD-1—a receptor expressed on activated T cells and its ligand PD-L1, expressed on tumor cells—identified another immunosuppressive mechanism that inactivates TCR and/or CD28 signaling, thereby impairing the normal function of cytotoxic effector T cells (Teff). Nivolumab was the first anti-PD-1 blocking antibody that was approved for treatment of inoperable or metastatic melanoma based on the CheckMate 037 (NCT01721746) clinical trial.¹⁵ The trial demonstrated that a greater proportion of advanced melanoma patients achieved an objective response and fewer toxic effects compared to the alternative chemotherapeutic regimen.¹⁵ Since then, other anti-PD-1 therapies such as pembrolizumab, cemiplimab, and dostarlimab have also been approved.² Targeting PD-L1 is also a viable approach to block the PD-1/PD-L1 inhibition pathway.

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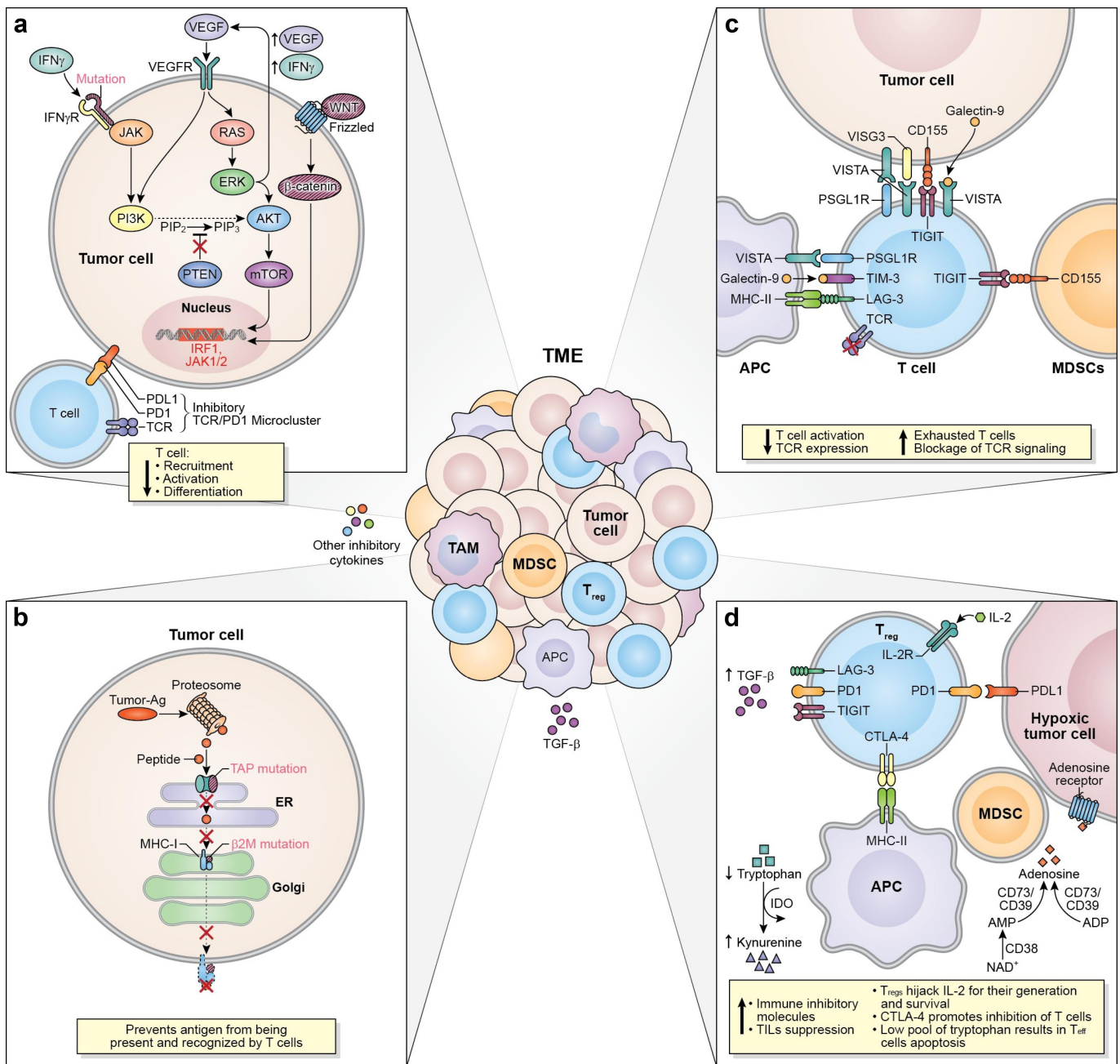


Figure 1. Mechanism associated to ICI resistance. The TME represents a complex interaction between tumor cells, immune cells, and inhibitory cytokines leading to a plethora of mechanisms associated to ICI resistance. (a) Defects in T cells effector function and their exclusion from the TME can occur as a result of dysregulation of MAPK/ERK cell signaling pathways in tumor cells inducing the production of VEGF and IL-8, having a net inhibitory effect on the recruitment of T cells and defects in T-cell activation and differentiation. Due to the crosstalk between the MAPK and the PI3K signaling pathways, an oncogenic mutation in MAPK pathway or a loss of PTEN expression can cause the enhancement of PI3K-AKT signaling as well. Similarly, a mutation or silencing of β -catenin or tumor suppressor wnt protein, results in dysregulation of the WNT/ β -catenin pathway, promoting aberrant signaling of β -catenin contributing to the absence of T cell expression signature. Mutations in the IFN- γ receptor and defects on the IRF1 or JAK1/2 genes can contribute to T cell desensitization and consequently promote acquired resistance to ICIs. Specifically, mutations of PD-L1, PD-L2, and JAK2 genes resulting from the amplification of the locus that contains these genes promote the formation of the PD-1/TCR inhibitory microcluster, which results in the inhibition of T cell activation. (b) Another resistance mechanism is developed after defects in the APM specifically due to mutations of B2M and TAP proteins. These mutations make the antigen unable to reach the tumor cell's surface and be recognized and cleared out by CD8⁺ T cells. (c) The interaction between T cells, tumor cells, APCs, and immunosuppressive cells (MDSCs) throughout immune checkpoints such as VISTA, LAG-3, TIGIT, and TIM-3, triggers and inhibitory signal causing the exhaustion of T cells, blockage of TCR signaling, and decreasing T cell activation and TCR expression. (d) Immunosuppressive signaling also contributes to primary and/or acquired resistance in cancer. Tregs can restrain the effector function of immunocompetent cells by inducing checkpoint-mediated suppression (CTLA-4, PD-1, TIGIT, TIM-3, and LAG-3), competing for IL-2 binding, or by producing anti-inflammatory cytokines. As such, TGF- β is a pleiotropic cytokine involved in tumor evasion and immunotherapy resistance. The upregulation of the metabolic modulator IDO within the TME allows the depletion of tryptophan, resulting in the decrease of T cells. Another metabolic modulator associated with T cell function suppression and ICI resistance is adenosine. Several ectonucleotidases (CD39 and CD73) catalyze the conversion of ADP or AMP to adenosine or NAD⁺ to AMP (CD38) causing the upregulation of adenosine in the tumor milieu. TME: tumor microenvironment; MAPK/ERK: mitogen-activated protein kinase/extracellular signal-regulated kinase; VEGF: vascular endothelial growth factor; IL-8: interleukin-8; PTEN: phosphatase and tensin homolog deleted on chromosome 10; PI3K-AKT: phosphoinositide-3-kinase – protein kinase B/Akt; WNT/ β -catenin: wingless-related integration site/ β -catenin; IRF1: interferon regulatory factor 1; JAK1/2: Janus kinase 1/2; PD-L1/L2: program cell-death ligand 1/ligand 2; PD-1/TCR inhibitory microcluster: program cell-death 1/T cell receptor inhibitory microcluster; APM: antigen presenting machinery; TAP: transporter associated with antigen presentation; APC: antigen presenting cells; MDSCs: myeloid-derived suppressor cells; VISTA: V-domain ig suppressor of T cell activation; LAG-3: lymphocyte activation gene 3; TIGIT: T cell ImmunoGlobulin and ImmunoTyrosine inhibitory motif (ITIM) domain; TIM-3: T cell immunoglobulin and mucin-3; IDO: indoleamine 2,3-dioxygenase 1; CTLA-4: cytotoxic T lymphocyte antigen-4; TGF- β : transforming growth factor beta ; ADP: adenosine triphosphate; AMP: adenosine monophosphate; NAD⁺: nicotinamide adenine dinucleotide.

The JAVELIN Merkel 200 clinical trial (NCT02155647) paved the way for the approval of the PD-L1 blocking antibody, avelumab, for the treatment of metastatic Merkel carcinoma (MCC).^{12,16} In this study, 31.8% (28 out of 88) of MCC patients whose disease had progressed on or after chemotherapy experienced objective responses with avelumab treatment.¹⁶ Avelumab was also later approved for patients with locally advanced or metastatic urothelial carcinoma (mUC).^{12,17} Durvalumab and atezolizumab are among the antibodies blocking PD-L1 that have been FDA-approved for the treatment of patients with locally advanced mUC.^{12,18} Collectively, ICIs targeting the PD-1/PD-L1 axis are therapeutic options for basal cell carcinoma (BCC), cutaneous squamous cell carcinoma (CSCC), NSCLC, CRC, HCC, Hodgkin lymphoma (HL), head and neck squamous cell carcinoma (HNSCC), mesothelioma, renal cell carcinoma (RCC), breast cancer (BC), large B cell lymphoma, and endometrial, esophageal, and gastric carcinomas.²

The blockade of multiple checkpoints as a combinatorial therapy has improved the immunomodulatory ability of the immune system in achieving tumor cell clearance. The combination of ipilimumab plus nivolumab has also been FDA-approved for the treatment of advanced unresectable/metastatic melanoma cancer patients, after showing an improvement in overall response rate (ORR), progression-free survival (PFS), and OS against the SOC chemotherapy (sunitinib).^{12,15,19,20} Furthermore, the combination has resulted in improved PFS when compared to ipilimumab alone in melanoma.²⁰ In fact, except for melanoma, approved indications of ipilimumab are usually in combination with nivolumab.²

Despite the durable response observed with immune checkpoint blockade (ICB) as part of the SOC for the treatment of patients with a broad range of advanced-stage cancers, only a limited population of patients benefit from ICI therapy.^{1,21,22} The lack of response to ICI, also called checkpoint blockade resistance (CBR), can be categorized as primary or acquired.^{1,22,23} Primary CBR is observed in patients who do not respond to initial treatment, while acquired CBR is defined as confirmed early response to the therapy, but with eventual disease progression after prolonged treatment.^{1,22,23} For example, primary CBR is observed in metastatic BC patients,^{24,25} in about 45–70% of melanoma patients,^{19,26} and 7–27% of NSCLC patients.^{27,28}

In conjunction with efforts to understand the mechanisms that result in CBR, methods to overcome CBR are being developed, such as targeting new immune checkpoints and costimulatory molecules or testing new combination therapies.^{29–33} Prevailing strategies are focused on transforming immunological “cold” tumors into “hot” tumors^{30,34,35} by increasing tumoral T cell infiltration, enhancing cytotoxic T cell function,^{30,32,36} repolarizing immunosuppressive population,^{31–33,37} or overcoming tumor-intrinsic resistance mechanisms related to loss of major histocompatibility complex class I (MHC-I), aberrations in antigen processing machinery (APM), and interferon gamma (IFN- γ) pathways.^{33,38–40} In this review, we will discuss the response of ICI in the real world and how this compares to the responses observed in clinical trials, as well as the underlying mechanisms promoting primary and acquired CBR. Finally, we aim to discuss current efforts to suppress these mechanisms that

lead to CBR with the end goal of improving patient response to ICI therapy.

2. Contrasting responses to immune checkpoint blockade in the real-world

The response of patients treated with ICIs in the “real world” occasionally looks different from the responses observed and reported by controlled clinical trials run in different institutions. While there are data showing the consistency between the PFS and objective response rate observed in ICI-treated patients in the real world and the responses reported in the clinical trials,^{41–43} other studies have highlighted a gap between the outcomes of immunotherapy reported by clinical trials (efficacy) and the outcomes observed in real-world (effectiveness) across several cancer types.^{14,44} For example, data support the groundbreaking efficacy and durable response achieved by ICIs for the treatment of advanced-stage cancers, specifically in melanoma,^{45,46} advanced MCC,^{47,48} RCC,⁴⁹ and NSCLC.⁴⁹ Interestingly, these cancers have in common a high density of immune subsets infiltrated in the tumor microenvironment (TME) and the accumulation of pro-inflammatory cytokines that make them classified as “hot” tumors.^{49,50} These characteristics, among other factors that will be discussed later in the review, improve the response rate to ICI therapy in “hot” tumors.⁵⁰ Specifically, in melanoma patients, median survival improved from 6 months when patients were treated with cytotoxic agents such as temozolomide to 6.5 years following treatment with anti-CTLA-4, anti-PD1 or a combination of both ICIs.⁵¹ A study of 60 patients with desmoplastic melanoma treated with antibodies blocking PD-1 or PD-L1 showed an objective response of 70% and 32% of them showed a complete response (CR).⁴⁵ Another study of 230 metastatic melanoma patients showed a longer PFS using a combination of anti-CTLA-4 and anti-PD-1,⁴⁶ highlighting the benefits of blocking different immune checkpoints to improve the immune response. However, it is important to mention that two-thirds of melanoma patients failed to achieve complete response after ICI therapy, hence the challenge of primary and acquired resistance faced by these patients.⁵¹

Blocking PD-1/PD-L1 axis with ICIs for the treatment of advanced MCC has achieved anti-tumor activity in ~30–60% of the patients as well as improvement in PFS and OS compared to patients receiving chemotherapy.^{52–55} Indeed, data from retrospective analyses confirmed the optimistic overview to continue using ICIs for the treatment of metastatic MCC patients.^{52,56} This study evaluated patients from two large academic medical centers who were treated with alternative ICIs after progressive disease (PD). Anti-CTLA-4 alone or in combination with anti-PD-1 revealed an overall response (OR) in 31% of the patients; one patient with MCC refractory – anti-PD-1 and anti-CTLA-4–had tumor regression with anti-PD-L1.⁵² It is worth mentioning that this study is the largest case series to date describing the anti-tumor efficacy of anti-PD-L1 as a second-line treatment for MCC patients with anti-PD-1 refractory disease.

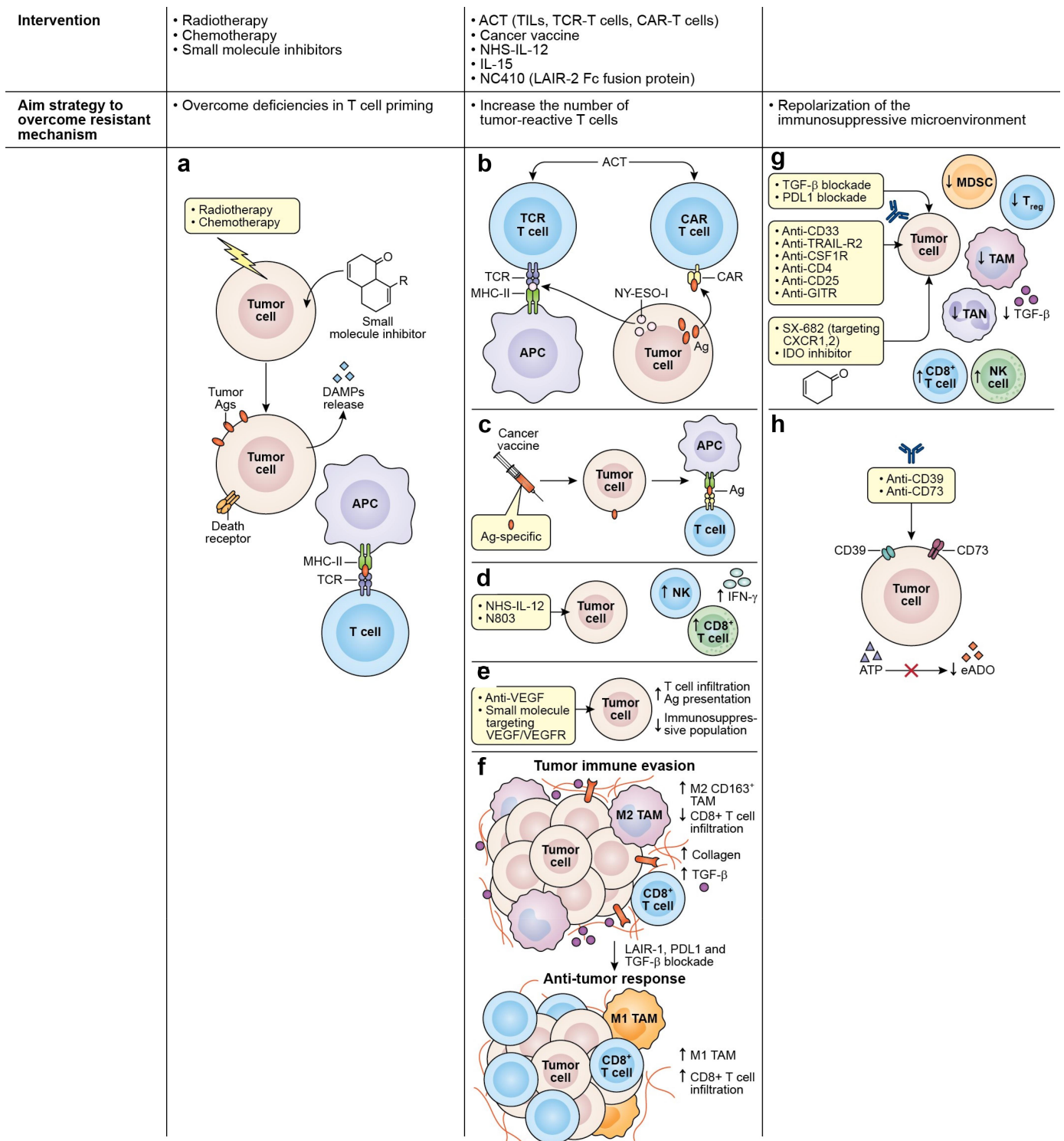


Figure 2. Strategies to overcome associated mechanisms contributing to immunotherapy resistance. (a) Chemotherapy, radiotherapy, and small molecule inhibitors are among the strategies used to overcome deficiencies in T-cell priming. These agents can induce ICD in tumor cells causing the release of antigens and DAMPs, allowing the recruitment and maturation of APCs and the presentation of targetable antigens to effector T cells. Other interventions are used to increase the number of tumor-reactive T cells interacting in the TME, such as (b) ACT using TCR T cell or CAR T cell. TCR T directed against specific cancer antigens (testis antigen, NY-ESO-1) can recognize the antigen through MCH molecules and CAR T cells act in an MHC-independent manner targeting cell surface antigens. (c) the use of cancer vaccines is also used to expand tumor-specific T cell populations, broaden the T cell repertoire, and promote the transport of T cells to tumor lesions. Additionally, the use of (d) immunostimulatory cytokines such as NHS-IL-12 or N803 can promote the enhancement of effector cell recruitment and boost CD8⁺ T cell and NK cell cytolytic functions. (e) physical barriers also prevent the infiltration of effector T cells in the TME, and tumor vasculature represents one of these barriers. Therefore, VEGF targeting using small molecule inhibitors or mAbs represents an effective strategy to disrupt angiogenic pathways that are fostering the aberrant vasculature. (f) another physical barrier is the extracellular matrix that is composed mainly of collagen. Collagen is produced by TAMs, CAFs, and tumor cells and can impair immune activity through interactions with LAIR-1. Blockade of LAIR-1 combined with PD-1, and TGF-β blockade can increase M1 TAMs population, CD8⁺ T cell infiltration, while decreasing TGF-β and collagen. Figure adapted from *J Clin Invest.* 2022;132⁸: e155148. <https://doi.org/10.1172/JCI155148>. lastly, repolarization of the immunosuppressive microenvironment as an alternative strategy to overcome certain resistant mechanisms can be achieved by (g) the use of mAbs targeting CD33 and TRAIL-R2 receptors expressed in tumor cells were demonstrated to eliminate MDSCs. The use of anti-CFR-1 were demonstrated to eliminate M2 macrophages. Likewise, the depletion of tregs using anti-CD4, anti-CD25 or anti-GITR in combination with ICI have shown to improve CD8⁺ T cell activity. Another approach uses to decrease the

immunosuppressive population that abrogates the effect of ICIs, is the use of small molecule inhibitors targeting CXCR1/2 which are receptors to chemokines essential for the recruitment of MDSCs and TANs. Growing evidence have shown that blocking TGF- β and PD-L1 simultaneously can decrease immunosuppressive population in the TME. (h) the use of inhibitors against IDO enzyme, halt the conversion of tryptophan to kynurenine allowing the increase of NK cells and CD8⁺ T cell and the decrease of Tregs. ICD: immunogenic cell death; DAMPs: damage-associated molecular patterns; APC: antigen presenting cells; TME: tumor microenvironment; ACT: adoptive cell transfer; TCR T: T cell receptor-engineered T cells; CAR T cells: chimeric antigen receptor T cells; MCH: major histocompatibility complex; VEGF: vascular endothelial growth factor; TAMs: tumor-associated macrophages; LAIR-1: leukocyte-associated immunoglobulin-like receptor-1; CAFs: cancer-associated fibroblasts; TANs: tumor-associated neutrophils; PD-1: program cell-death 1; TGF- β :transforming growth factor beta.

NSCLC, as one of the most common and deadliest malignancies worldwide, also benefits from blocking the immune checkpoints PD-1, PD-L1, and CTLA-4 alone or in combination with SOC.²⁹ Approximately 20–30% of NSCLC patients respond to ICI treatment and several studies continue to evaluate combination therapies to improve the response rate.²⁹ Despite the progress observed, response to ICI is not always compared equally to the response reported by clinical trials. For example, a retrospective analysis of stage-IV NSCLC patients treated with pembrolizumab as a first-line treatment and nivolumab as a second-line treatment showed that the OS in real world was significantly shorter in patients receiving pembrolizumab as a first-line treatment compared to ORR as reported in clinical trials.¹⁴ The PFS of patients in pembrolizumab or nivolumab cohorts was comparable between real-world and trials.¹⁴ Likewise, another retrospective study with a cohort of 19,529 Medicare patients with NSCLC, ranging from 66 to 89 years old, showed an unadjusted median survival of 11.4 months among patients receiving single-agent pembrolizumab, which was 15 months shorter than the median survival of pembrolizumab-treated participants in the KEYNOTE-024 trial.^{44,57} In many studies, the use of chemoimmunotherapy for cancer patients with advanced stages has been incorporated into their SOC treatment regimen^{58–61}; however, this study also showed that the unadjusted median survival for patients receiving chemoimmunotherapy (platinum/pemetrexed/pembrolizumab) was 12.9 months, approximately 10 months shorter than for participants in the KEYNOTE-189 trial who received the same regimen.^{44,62}

In total contrast to the response observed in “hot” tumors toward ICIs, the response documented in “cold” tumors is poor.⁴⁹ “Cold” tumors such as prostate, pancreatic, and most colorectal cancers are characterized for being non-T cell infiltrated (non-inflamed) and largely resistant to ICI therapy.^{49,50} As of today, the only ICI FDA-approved treatment for prostate cancer patients is pembrolizumab (anti-PD-1), but only for metastatic castration-resistant prostate cancer (mCRPC) patients with high tumor mutational burden (TMB-H), high microsatellite instability (MSI-H), or mismatch repair deficiency (MMR-D).⁶³ However, several clinical trials continue to show the limited response of mCRPC patients to single-agent ICI therapy, including pembrolizumab and other ICIs such as atezolizumab (anti-PD-L1) and ipilimumab (anti-CTLA-4).⁶³ Besides poor infiltration in “cold tumors,” these tumors rarely express PD-L1 (immunologically ignorant) and show low expression of neoantigens

and immunosuppressive TME, all contributing factors to their unresponsiveness to checkpoint blockade.⁴⁹

3. Checkpoint blockade resistance

CBR needs to be discussed not only in terms of the biological concepts, but also in the complex scenario of the clinical setting, making it challenging to convey a uniform clinical definition of resistance to ICB for advanced diseases.²³ To bring consensus to defining CBR, several principles and guidelines have to be considered and established, including the underlying mechanisms driving primary or acquired resistance,^{64–66} treatment duration criteria to determine the cutoff to distinguish primary resistance,^{23,66} and treatment discontinuation criteria to determine if acquired resistance is considered even after cessation of the ICI.^{23,66} In response to this heterogenous scenario, the Society for Immunotherapy of Cancer (SITC) taskforce agreed to take into consideration the duration of drug exposure, scan requirements, and response evaluation to define both primary and acquired resistance.

First, the taskforce stated that it was critical to define a minimal exposure timeframe for patients treated with an FDA-approved PD-1 or PD-L1 inhibitor to derive any possible clinical benefit.²³ The taskforce established a required exposure to ICI therapy of at least 6 weeks but not more than 6 months. For patients with indolent tumor types, however, this timeframe needs to be redefined.^{23,67} To determine whether a treated patient is showing immune checkpoint primary resistance, a confirmatory scan needs to be performed to validate PD.²³ On the other hand, the taskforce disagreed on which response evaluation criteria – Response Evaluation Criteria in Solid Tumors⁶⁸ or RECIST 1.1—was to be employed and agreed that immune-based Response Evaluation Criteria in Solid Tumors (iRECIST) could not be used as a sole criterion to determine ICI efficacy. Although iRECIST was created to address cases of mixed responses and pseudoprogression, the taskforce stated that further validation is needed.^{23,68} However, the group agreed that the use of a fluorodeoxyglucose (FDG)/positron emission tomography (PET) scan combined with metabolically active immune infiltrates may be used as an indicator of response to ICI therapy.²³

The taskforce defined acquired resistance in advanced disease settings, otherwise known as secondary resistance, when a patient is treated for 6 months or longer, has a CR, partial response (PR), or prolonged stable disease (SD) – confirmed by scan – for more than 6 months and then presents PD in the setting of ongoing treatment.²³ Members of the taskforce did not reach a consensus regarding the requirement of confirmatory imaging for validating secondary resistance. Two of the

three groups recommended a scan within 4–12 weeks after evidence of PD, including verification of ≥ 2 metastatic sites/lesions for patients with multiple metastases, for acquired resistance confirmation.²³

Uncommon response patterns represent an additional challenge in defining clinical resistance to ICIs.^{23,69} One of these patterns is known as pseudoprogression and describes patients who appear to have PD after radiographic confirmation, but experienced tumor shrinkage months after treatment cessation.^{23,69,70} This type of response was first observed in a phase II clinical trial that evaluated the efficacy of ipilimumab in metastatic melanoma patients.⁷¹ In this study, Wolchok et al. showed that 9.7% (22 out of 227) of the treated patients were characterized with PD – following World Health Organization (WHO) and iRECIST guidelines – in spite of having clinical responses (PR and SD).^{70,71} Clinical trial protocols did not require patient follow-up after PD was observed, thus limiting data collection for those showing a response after leaving the clinical trial. Accordingly, the number of patients with pseudoprogression may have been underestimated.⁷¹ Current data support that pseudoprogression occurs in approximately 5–10% of the patients receiving anti-CTLA-4 or anti-PD-L1 therapy, across several solid tumor types.^{23,70,72} Building on these findings, a novel benchmark designated as immune-related response criteria (irRC) is currently used to differentiate pseudoprogression from true progression and may provide a more comprehensive evaluation of the response toward the immuno-oncology (IO) agent.^{71,72} As such, a study evaluating irRC and RECIST criteria in advanced melanoma patients treated with pembrolizumab showed that the sole use of RECIST criteria may have underestimated the benefit of the checkpoint in approximately 15% of the patients.⁷³

4. Underlying mechanism associated to ICI resistance

4.1 T cells exclusion and dysfunction in the TME

The density of preexisting T cells in the TME as well as their functionality are two of the factors used to determine the outcome of ICI therapy.¹ Immune-excluded tumors – “cold tumors” – lack infiltrating effector T cells and are less prone to respond to ICI therapy. This lack of response may be due to primary resistance conferred by mutations and dysregulation of cell signaling pathways in tumor cells, resulting in poor T cell recruitment and T cell dysfunction.^{1,39} For example, a study in highly aggressive HCC determined that low T cell numbers may be associated with deletions in the MAX/TP53 genes, which in turn resulted in the downregulation of TP53-related chemokines that are pivotal in T cell recruitment.^{74,75}

Multiple oncogenic signaling pathways affecting primary resistance have been identified, including mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase⁷⁶ signaling pathways and/or loss of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) expression.^{1,74,77} Dysregulated MAPK/ERK pathways induce the production of vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8), resulting in an inhibitory effect on the recruitment of T cells and defects in T-cell activation and differentiation (Figure 1a).^{74,78}

Different cancer types harbor somatic mutations related to the MAPK pathways, many of which had been demonstrated to be oncogenic.⁷⁹ For example, approximately 18% of HNSCC patients harbor MAPK pathway mutations and half of these mutations are oncogenic in nature.^{79,80} As a result of the crosstalk between the MAPK and the phosphoinositide-3-kinase – protein kinase B/Akt (PI3K-AKT) signaling pathways, an oncogenic mutation in MAPK pathway – or loss of PTEN expression – will cause the enhancement of PI3K-AKT signaling, associated with resistance to checkpoint blockade resistance (Figure 1a).^{1,77,81} A study of melanoma cancer patients correlated the constitutive activation of PI3K-AKT signaling, due to PTEN loss, with low numbers and impaired function of the tumor-infiltrated lymphocytes (TILs), as well as poor outcomes after PD-1 inhibitor treatment.⁸¹

Another common mutation in cancer occurs in the WNT/ β -catenin signaling pathway, particularly due to mutation or silencing of the Wnt protein, which acts as a tumor suppressor (Figure 1a).⁸² In melanoma cancer patients, dysregulation of the WNT/ β -catenin pathway has been correlated with unresponsiveness to ICI therapy.⁸² This was corroborated in a murine melanoma model wherein continuous β -catenin signaling in the TME contributed to the absence of T cell expression signature and, consequently, resistance to anti-PD-L1 and anti-CTLA-4 therapy.⁸² The growing evidence of the relationship between the activation of WNT/ β -catenin signaling and ICI resistance has given the rationale to target β -catenin pathway to enhance the efficacy of ICIs.⁸³

Cancer-associated resistance also arises as a result of mutations in the IFN- γ signaling pathway (Figure 1a).^{1,77} IFN- γ is a pleiotropic cytokine with antitumor and immunomodulatory functions; thus, it plays an important role in both innate and adaptive immune responses.⁸⁴ IFN- γ activates its receptor (IFNG1/2) which is intracellularly associated with kinases from the Janus kinase (JAK) family (JAK1 and JAK2), inducing the expression of genes involved in cell cycle regulation, apoptosis, growth inhibition, and tumor suppression.⁸⁴ The growing data support the idea that defects in the IFN- γ signaling pathway cause T cell desensitization, allowing acquired resistance to checkpoint blockade.^{1,77,85,86} Recent data showed that biopsies from melanoma patients who did not respond to anti-CTLA-4 therapy have mutations in IFNG1/2, JAK2, and interferon regulatory factor 1 (IRF1) genes.⁸⁵ In a separate study, two out of four metastatic melanoma patients who had progressive disease after initial objective tumor regression with pembrolizumab had loss-of-function mutations in JAK1 or JAK2 genes.⁸⁶ Mutations in the IFN- γ signaling pathway can also foster primary or acquired resistance via constitutive PD-L1 expression or the loss of PD-L1 expression in cancer cells.^{1,77} The mutations in the first scenario arise after the amplification of the locus in chromosome 9 that contains the genes for PD-L1, PD-L2, and JAK2.¹ The interaction of the PD-1 receptor with its ligand, PD-L1, now overexpressed in tumor cells due to the mutation, forms the PD-1/TCR inhibitory microcluster, resulting in the inhibition of T cell activation (Figure 1a).¹ In

the second scenario, in which PD-L1 expression is no longer inducible by IFN- γ , anti-PD-1/PD-L1 antibodies would not be effective, and patients would manifest primary resistance to ICI.^{1,87}

4.2 Defects in antigen processing machinery and lack of tumor-associated antigens

The antigen processing and presentation pathway are critical in the immunosurveillance of cancer.^{88,89} Tumor antigen recognition by CD8⁺ T cells requires that for an effector CD8⁺ T cell to recognize an antigen from a tumor cell, the antigen must be expressed in the surface of the tumor cells and the T cell must be able to recognize that antigen presented by an MHC class I molecule (MHCI).⁸⁹ In humans, MHCI molecules are heterodimers composed of heavy chains encoded by Human Leukocyte Antigen (HLA) genes (*HLA-A*, *HLA-B*, and *HLA-C*) and a light chain β_2 -microglobulin (B2M).^{88,89} Antigen processing is a multi-step procedure that includes antigen fragmentation by the proteasome, relocation of the antigenic peptide to the endoplasmic reticulum (ER) by specific transporter proteins, docking of the peptide on an MHC-I molecule, and the transport of the MHC-I-peptide complex to the cell surface.⁸⁹

Evidence indicates that mutations and epigenetic modifications in the cancer DNA that alter the antigen processing and presentation machinery can contribute to checkpoint immunotherapy resistance.^{1,88} Specifically, tumor cells can develop acquired resistance through loss of surface expression of MHC-I molecules via mutations in the B2M light chain (Figure 1b).^{1,88} Without B2M, the MHC-I molecules cannot be folded and transported to the surface of the cells in order to present the antigen; thus, CD8⁺ T cells will not be able to recognize the antigen and clear out the tumor cell.⁸⁸ In addition, mutations in genes encoding transporter proteins, such as transporters associated with antigen presentation 1/2 (TAP) 1/2, which are involved in the process of importing antigen peptides into the endoplasmic reticulum, inhibit the formation of MHC-I-peptide complexes, thereby preventing the antigen processing and presentation machinery to proceed (Figure 1b).⁸⁸

Logically, the presence of targetable tumor antigens is a critical factor contributing to the ability of T cells to mount an immune response and to the ability to gain clinical benefits with ICI therapy.⁸⁹ These tumor antigens can be generated through genetic diversity, missense, and silent mutations, insertions, deletions, as well as copy number gains and losses.⁸⁹ For example, cancers with MMR-D as a result of loss of function of certain genes (*MLH-1*, *PMS-2*, *MSH-2*, and *MSH-6*) involved in the DNA repair pathway and those that have MSI-H are examples of cancers with TMB-H.⁴⁰ Both MSI-H and MMR-D induce frameshift mutations allowing for neoantigen formation.^{38,40}

The number of all the somatic mutations per megabase (Mb) in the genome of a tumor cell, also known as TMB, seems to be the driver generating the immunogenic neoantigens presented by the MHC complex.^{89,90} Cancers with a high TMB, which is broadly defined as cancers with ≤ 20 mutations per Mb, have a higher objective response rate to ICI

therapy.^{78,91–93} As such, melanoma and NSCLC are among the malignancies with TMB-H and higher OR to anti-PD-1 therapy.^{38,94} Nonetheless, only 45% of the patients with TMB-H responded to ICI therapy, indicating the existence of other mechanisms promoting primary resistance in these cases.^{91,95} Whereas high ORR and OS are associated with tumors with TMB-H, cancers categorized as low TMB (TMB-L), defined as ≤ 5 mutations per Mb,^{38,91} such as pancreatic cancer and triple-negative breast cancer (TNBC), have a low probability of response to ICIs and manifest as primary or acquired resistance.^{38,91,96,97} These observations were highlighted in a study that analyzed patients across several solid tumors, excluding NSCLC and melanoma, who had TMB assessment and received ICI therapy. The study revealed that only a low percentage of patients (9%) harboring TMB-L tumors had CR/PR; conversely, 47% of the patients with TMB-H had CR/PR.⁹⁵

4.3 Presence of alternative inhibitory immune checkpoints

V-domain Ig suppressor of T cell activation (VISTA), T cell immunoglobulin and mucin-3 (TIM-3), lymphocyte activation gene 3 (LAG-3), and T cell ImmunoGlobulin and ImmunoTyrosine inhibitory motif (ITIM) domain (TIGIT) are examples of alternative checkpoint molecules that elicit immune inhibitory signals that may contribute to primary and acquired resistance (Figure 1c).^{1,98–100} VISTA is a negative regulator of T cell activation and is expressed by neutrophils, monocytes, macrophages, dendritic cells, TILs, and by some human cancer cells (ovarian cancer, endometrial cancer, and CRC).^{98,100–102} Although the specific mechanisms through which VISTA exerts immune inhibition are still unclear, its role in acquired resistance is being elucidated. A study of metastatic melanoma patients treated with ipilimumab found that the majority of patients (12/18) had biopsies with significantly higher densities of VISTA⁺ lymphocytes during disease progression compared to pre-treatment.^{98,103} Interestingly, another study that analyzed tumor tissue biopsies from prostate cancer patients found that suppressive macrophages express either VISTA (26.5%) or PD-L1 (29.4%), but rarely both markers (2%).^{85,98} The presence of distinct macrophages suggests that these subsets may compensate for each other during ICI therapy.^{85,98} Overall, there is evidence that VISTA may be a compensatory inhibitory pathway that results in acquired resistance to currently available ICI therapy.¹⁰³

LAG-3 is expressed on activated T cells, natural killer (NK) cells, B cells, and dendritic cells (DCs), and currently, the only known ligand for LAG-3 is the MHC-II molecule.¹⁰⁰ LAG-3/MHC-II high-affinity interaction blocks proper TCR signaling, resulting in hampered T cell functions.¹⁰⁴ Data showed that MHC-II expression in tumor cells is associated with increased response to anti-PD-1 therapy.¹⁰⁵ A follow-up study demonstrated that MHC-II expression in tumors is associated with an increased infiltration of LAG-3⁺ TILs.^{100,106} Notably, LAG-3 expression was higher in specimens from patients who initially responded to PD-1 therapy but eventually progressed. Furthermore, LAG-3 has been observed to be co-expressed with PD-1 in exhausted or dysfunctional T cells in human

tumors like ovarian cancer and melanoma.^{100,107} Additionally, LAG-3 signaling can promote Treg populations through the release of immunosuppressive cytokines like transforming growth factor beta (TGF- β) and IL-10.¹⁰⁴ Collectively, the growing body of data suggests that LAG-3 upregulation is associated with primary and acquired resistance in different cancer conditions.^{104,108}

The immune checkpoint molecule TIM-3 is expressed in activated human T cells, NK cells, and monocytes.¹⁰⁰ High TIM-3 expression in several syngeneic models has been associated with acquired resistance toward PD-1 blockade.^{109–111} For example, Koyama et al. correlated acquired resistance after anti-PD-1 therapy with TIM-3 upregulation in a lung adenocarcinoma murine model.¹¹⁰ The same TIM-3 upregulation was observed in lung cancer patients who had PD following PD-1 blockade. Furthermore, TIM-3 expression was higher in the relapsed NSCLC patients when compared to those who were not treated with ICIs.¹¹⁰ Additionally, clinical data support that TIM-3 is universally co-expressed with PD-1 in TILs, enabling a more exhausted T cell phenotype.^{100,112} Indeed, a study associated the co-expression of PD-1 and TIM-3 with acquired resistance to anti-PD-1 therapy in NSCLC patients.¹⁰⁹

TIGIT expression is restricted to CTLs, Th, Tregs, and NK cells.^{113,114} TIGIT's inhibitory signaling occurs upon interaction with its main ligand CD155 (PVR), expressed on tumor-infiltrating myeloid cells and tumor cells.¹¹⁴ Since TIGIT binds to CD155 with a higher affinity than its competitive ligand CD226, TIGIT/CD155 interaction disrupts CD226 homodimerization, inhibiting CD226-mediated T cell activation.^{113,114} The TIGIT/CD155 complex can also reduce TCR-expression and TCR signaling.¹¹⁴ A study of melanoma correlated high CD155 and TIGIT expression with PD-1 and/or CTLA-4 primary and acquired resistance in patients with non-inflamed tumors and inflamed tumors with TMB-H.¹¹⁵ In addition, a study using a preclinical model of pancreatic adenocarcinoma (PDAC) supported the hypothesis that TIGIT blockade may overcome preexisting or acquired resistance to CD40a/PD-1 therapy.¹¹⁶

4.4 Immunosuppressive signaling

Tumor-cell extrinsic factors such as immunosuppressive cells, including Tregs and tumor-associated macrophages (TAMs), and inhibitory cytokines, such as TGF- β , also contribute to primary and/or acquired resistance in cancer (Figure 1d).^{1,3} In physiological conditions, the main function of Tregs is to maintain immune homeostasis; however, cancer exploits Treg cellular mechanisms to restrain the effector function of immunocompetent cells.¹¹⁷ Tregs can induce CTLA-4-mediated suppression of APCs, compete for IL-2 binding, produce anti-inflammatory cytokines, and express immune inhibitory molecules such as TIGIT, PD-1, TIM-3, and LAG-3.¹¹⁷ Indeed, a study in NSCLC, gastric cancer and melanoma have suggested the involvement of Tregs in resistance to anti-PD-1 immunotherapy.¹¹⁷ This study demonstrated that the balance

between PD-1⁺CD8⁺ T cells and PD-1⁺ Tregs in the TME can better predict the outcome of PD-1 therapy than PD-L1 tumor tissue expression or TMB.¹¹⁷

TGF- β is a pleiotropic cytokine that plays a key role in maintaining immune tolerance, yet is also involved in tumor evasion and immunotherapy resistance.^{118,119} A plethora of studies on solid tumors have correlated TGF- β signaling in the TME with different mechanisms underlying ICI resistance.^{118,119} For example, TGF- β can suppress TILs and at the same time induce high expression of PD-1 and PD-L1 in T cells and tumor cells, respectively.¹¹⁸ Additionally, a clinical study showed that high TGF- β gene signature can predict the failure of ICIs in gynecological cancer patients.¹¹⁹ The role of TGF- β in promoting primary resistance is yet to be understood; however, inhibition of TGF- β in a murine model refractory to anti-PD-1 improved antitumor response and survival benefits.^{118,120,121}

Metabolic modulators such as indoleamine 2,3-dioxygenase 1 (IDO) and adenosine can also play a role in fostering immunosuppressive conditions in the TME that result in immunotherapy resistance (Figure 1d).^{104,122,123} IDO is an intracellular enzyme that catalyzes the reaction that converts tryptophan into kynurenine under normal physiological conditions. It is expressed only in select tissues (mucosal tissues, placenta, eye, and pancreas) and by a small population of immune cells (DCs and eosinophils).^{29,122} However, data confirmed IDO upregulation in CRC, breast cancer, prostate cancer, and esophageal cancer.²⁹ As such, expanding preclinical and clinical data correlated IDO upregulation with suppression of T cell function and ICI resistance.^{29,122,124}

Adenosine accumulates in the TME through the conversion of hydrolyzed forms of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) or adenosine monophosphate (AMP), and then into adenosine by ectonucleotidases (CD39 and CD73), which are overexpressed in different cancer types.¹²³ High levels of adenosine in the TME may also be due to mutations or hypoxic conditions that decrease the reduction of this metabolite (Figure 1d).¹²³ Another source of adenosine in the TME comes from nicotinamide adenine dinucleotide (NAD⁺) converted to AMP by CD38, a molecule that is upregulated by tumor cells and identified as another mechanism of resistance to PD-1 and PD-L1 checkpoint blockade (Figure 1d).¹²³

5. Strategies to overcome associated mechanisms contributing to immunotherapy resistance

As described above, response to ICI is contingent on the presence, abundance, and activity of tumor-reactive T cells. Treatments to induce effector T cell activation, infiltration, and function, therefore, are strategies that can be combined with ICI to enhance antitumor response or used after ICI therapy failure (Table 1).

5.1 Overcoming deficiencies in T cell priming

Lack of tumor antigen, defective antigen processing and presentation, and insufficient T cell–DC interaction all contribute to

Table 1. Clinical trials recruiting patients with primary refractory or acquired secondary resistance to prior immune checkpoint treatments.

Study ID	Malignancies	Phases	Combinatorial approach
NCT04577807	Melanoma	Phase II	Lerapolturev (formerly known as PVSRIPO) (live attenuated Sabin type 1 polio vaccine, targeting CD155) 125 anti-PD-1
NCT04239040	Neuroblastoma	Phase I	GVAX vaccine (irradiated GM-CSF secreting, autologous neuroblastoma cell vaccine) Nivolumab Ipilimumab
NCT03388632	Metastatic solid tumors	Phase I	IL-15 Nivolumab Ipilimumab
NCT05533697	Advanced solid tumors	Phase I/II	mRNA-4359 vaccine (encoding for concatemered PD-L1 and indoleamine 2,3-dioxygenase 1 (IDO1) antigens) 126 Pembrolizumab
NCT03474497	Metastatic NSCLC, melanoma, RCC, or HNSCC	Phase I/II	IL-2 Pembrolizumab Radiotherapy
NCT03739931	Solid tumors or lymphoma	Phase I	mRNA-2752 (a lipid nanoparticle encapsulating mRNAs encoding human OX40L, IL-23, and IL-36γ)
NCT05764395	Unresectable metastatic melanoma	Phase II	Rigosertib (ON01910) (in vitro inhibitor of PLK1) 127 Pembrolizumab
NCT05200143	Cutaneous melanoma	Phase II	Ipilimumab Nivolumab Cabozantinib
NCT05431270	Solid tumors	Phase I	PT199 (an anti-CD73 mAb) Anti-PD-1
NCT04493203	Advanced melanoma	Phase II	Nivolumab Axitinib
NCT05723055	Classical Hodgkin lymphoma	Phase II	Nivolumab Axatilimab
NCT03333616	Advanced Rare Genitourinary Tumors	Phase II	Nivolumab Ipilimumab
NCT04862455	Recurrent/metastatic HNSCC	Phase II	NBTXR3 (hafnium oxide-containing nanoparticles) Pembrolizumab
NCT03747484	Metastatic MCC	Phase I/II	FH-MCVA2TCR (gene-modified autologous MCPyV-specific HLA-A02-restricted TCR-transduced CD4+ and CD8+ T-cells) Pembrolizumab Interferon-1b
NCT03228667	NSCLC, SCLC, UC, HNSCC, MCC, melanoma, RCC, gastric cancer, cervical cancer, HC, MSI/MMR-D CRC	Phase II	N-803 Pembrolizumab Nivolumab Atezolizumab
NCT04879368	AGOC	Phase III	Regorafenib Nivolumab Docetaxel
NCT03161431	Melanoma	Phase I	Pembrolizumab SX-682 (CXCR1/CXCR2 inhibitor)

NSCLC: non-small cell lung carcinoma, RCC: renal cell carcinoma, HNSCC: head and neck squamous cell carcinoma, SCLC: small cell lung carcinoma, MCC: Merkel cell carcinoma, PLK1: polo-like kinase 1, UC: urothelial carcinoma, HC: hepatocellular carcinoma, MSI: microsatellite instability, MMR-D: mismatch repair deficiency, AGOC: advanced gastro-esophageal carcinoma.

deficient T cell priming and activation that undermines the effect of immunotherapy. Anticancer treatments that induce immunogenic cell death (ICD), such as oncolytic viruses, radiotherapy, and certain types of chemotherapy and small-molecule inhibitors, can bridge this gap. Tumor cells undergoing ICD release antigens and damage-associated molecular patterns (DAMPs), allowing for the recruitment and maturation of APCs, the presentation of targetable antigens to effector T cells, and subsequent induction of antitumor immune responses and immune memory (Figure 2a).¹²⁸ Furthermore, chemotherapy and radiotherapy can upregulate the antigen processing machinery, promote the expression of MHC class I, and elicit the expression of death receptors, rendering the tumor cells that survived the treatment more susceptible to immune attack (Figure 2a).^{129,130} Several preclinical studies, including those on ICI-resistant tumor models, demonstrate that induction of ICD can potentiate the efficacy of ICI therapy.^{131–133} The exact role of ICD is yet to be elucidated in a clinical setting, but growing evidence suggests that ICD inducers may play an important role in the antitumor effect of ICIs.^{134–136} Most inducers of ICD, such as CAR T cells, are not “targeted,” at

least not to the same degree as checkpoint blockade; therefore, future studies on the immunotherapy field should focus on developing a consensus on the importance of defining a more generalized approach. Ongoing clinical trials are investigating the safety and the effect of combining ICD-inducers with ICI therapy, including in cancers that are refractory to checkpoint blockade (NCT03474497; Table 1).

5.2 Increasing the number of tumor-reactive T cells

Achieving high numbers of infiltrating tumor-reactive T cells is crucial for ICI response. The most direct way to increase the number of effector cells is via adoptive cell transfer (ACT) using TILs, TCR-engineered T cells, or chimeric antigen receptor (CAR)-engineered T cells. ACT with TILs involves isolating lymphocytes from resected tumors, expanding the cells *ex vivo*, and selecting for tumor-reactivity before re-infusing the TILs into patients.^{137,138} In a clinical study, TIL therapy was shown to induce durable responses in patients with metastatic melanoma. Notably, 11 patients enrolled in

the study had failed CTLA-4 treatment. Of the 11, five patients experienced complete regression while two experienced partial regression after TIL ACT.^{137,138} TCR T cells are peripheral T cells that are engineered to express tumor antigen-specific TCRs that are modified for improved expression and function.¹³⁹ As such, TCR T cells rely on antigen presentation via MHC molecules but can be generated to target antigenic peptides of intracellular and extracellular origins. Clinical studies using TCR T cells directed against the cancer-testis antigen NY-ESO-1 resulted in promising responses in different tumor types.^{140–142} Preclinical studies combining NY-ESO-1 TCR T cells with ICI demonstrated improved antitumor activity related to increased T cell efficacy (Figure 2b).^{143,144} However, in a pilot trial, the combination of NY-ESO-1 TCR T cell and NY-ESO-1 DC vaccine resulted in transient antitumor response and the addition of ipilimumab to the combination did not improve clinical benefit,¹⁴⁵ indicating that optimization may be warranted. Clinical trials evaluating the efficacy of TCR T cells with anti-PD-1 or anti-PD-L1 are currently underway.¹⁴⁶ CAR T cells express receptors composed of an antibody-derived single-chain variable fragment for antigen recognition fused to an intracellular signaling domain derived from T cell signaling proteins.¹⁴⁷ Contrary to TCR T cells, CAR T cells are MHC-independent and can only target cell surface antigens (Figure 2b). Advancements in this ACT platform have led to six approved CAR T cell therapies for hematological cancers. In solid tumors, however, the efficacy of CAR T cells is severely limited partly due to the immunosuppressive environment that the adoptively transferred cells encounter.¹⁴⁸ Hence, a great deal of effort is being exerted on studying the application of CAR T cells with ICI. Furthermore, the role of checkpoint blockade in ACT efficacy is paramount; thus, autologous T cells and CAR T cells with knocked out or disrupted PD-1 are being developed and evaluated.^{149,150}

Cancer vaccines can amplify a preexisting response by expanding tumor-specific T cell populations, broadening the T cell repertoire, and promoting the transport of T cells to tumor lesions (Figure 2c).^{151,152} Cancer vaccines are typically composed of soluble tumor antigens (e.g., oncoviral, oncofetal, cancer-testis, or neoantigens), formulation (e.g., peptide, nucleic acid, or whole tumor), delivery vehicle (e.g., liposome, cell-based, or viral-based), and an immune adjuvant (e.g., CD40 agonist, TLR agonist, or GM-CSF). Although monotherapy activity of cancer vaccines has so far been limited, its ability to sensitize host immunity to the tumor can complement ICI therapy, and this prospective synergistic activity is the focus of several clinical trials¹⁵³ (Table 1). The phase 1 Lipo-MERIT Trial (NCT02410733) aimed to evaluate the efficacy and safety of a tetravalent RNA vaccine (targeting the tumor antigens NY-ESO-1, MAGE-A3, tyrosinase, and TPTE) with or without PD-1 blockade in metastatic melanoma.¹⁵⁴ Partial responses were observed in both vaccine monotherapy (12%) and vaccine plus anti-PD-1 treatment (35.3%). Notably, patients with disease that progressed under ICI treatment achieved partial responses with the monotherapy or the combination. Furthermore, the response was associated with the

induction of T cells specific to at least one vaccine-targeted antigen and the formation of memory T cells. The data suggest that cancer vaccines may be a strategy to overcome ICI resistance by synergizing with ICI in ICI-experienced tumors.

Immunostimulatory cytokines may also be key combination partners for ICI. One example is IL-12, which is a pro-inflammatory cytokine produced by APCs that promotes Th1 polarization, enhances effector cell recruitment, and boosts CD8⁺ T cell and NK cell cytolytic functions (Figure 2d).^{155–157} Intratumoral administration of IL-12 in conjunction with CTLA-4 blockade has been shown to act synergistically to eradicate murine glioblastoma.¹⁵⁸ Moreover, in murine tumor models resistant to anti-PD-1/anti-PD-L1 therapy, tumor-targeted NHS-IL12 (IL-12 fused to an antibody that binds exposed DNA commonly found in necrotic tumors) when administered with a histone deacetylase inhibitor potentiated CD8⁺ T cell-dependent antitumor activity and provided survival benefit.³³ However, a phase 1b study evaluating the combination of NHS-IL12 and avelumab was discontinued due to lack of efficacy (NCT02994953). Several active studies that involve NHS-IL12 with anti-PD-L1/anti-TGF- β fusion protein are ongoing but have yet to post results (NCT04287868, NCT04303117).

Another cytokine that has the potential to elevate ICI activity is IL-15, which plays an important role in the activation, proliferation, survival, and function of NK and cytotoxic CD8⁺ T cells.^{159–161} In colon and prostate murine tumor models, the dual blockade of CTLA-4 and PD-L1 in combination with recombinant IL-15 (rIL-15) treatment resulted in tumor growth suppression associated with cytotoxic T cell activity, IFN γ secretion, and inhibition of Treg functions.^{162,163} Based on these findings, a phase 1 trial has been designed to test the safety of combining rIL-15 with nivolumab and ipilimumab in patients with refractory cancers (NCT03388632).¹⁶⁰ Due to the short lifespan of rIL-15 *in vivo*, N803, an IL-15 superagonist composed of IL-15 mutant (IL-15N72D) complexed to a dimeric sushi domain of IL15Ra (IL-15 RaSu) and fused to an IgG-Fc fragment, was developed.^{161,164} The combination of N803 and ICI has been the focus of several preclinical and clinical studies. In syngeneic breast and colon murine tumor models, the combination of N803 and PD-L1 blockade promoted the activation, proliferation, and cytotoxicity of CD8⁺ T cells and NK cells, resulting in decreased tumor burden (Figure 2d).¹⁶⁵ Meanwhile, in an oral squamous cell carcinoma model, N803 plus PD-1 blockade had moderate therapeutic efficacy that was further enhanced with the adoptive transfer of a PD-L1-targeted CAR-NK.¹⁶⁶ In the clinic, a completed phase 1/phase 2 study in NSCLC patients demonstrated that N803 and nivolumab were well-tolerated.¹⁶⁷ Furthermore, six (29%) of the 21 patients enrolled in the study achieved an objective response. Eleven of the 21 patients had relapsed after PD-1 blockade therapy and, of the 11, three (27%) had partial responses and seven (64%) had stable disease. Similarly, an ongoing phase 2b aims to evaluate the safety and efficacy of N803 plus ICI in patients who have progressed after PD-1/PD-L1 treatment (NCT03228667; Table 1). In this trial, patients

with disease progression with N803 + ICI roll over to a new cohort and receive N803, PD-1/PD-L1 checkpoint inhibitor, and PD-L1-targeted CAR-NK.

The pathophysiological properties of tumors result in physical barriers that prevent the infiltration of effector cells. One impediment is the tumor vasculature, which is characterized by abnormal endothelial cells, chaotic vessel growth, leakiness, and erratic blood flow.¹⁶⁸ Tumor vascular endothelial cells also express the death ligand FasL, killing extravasating CD8⁺ T cells while sparing Tregs that are not as sensitive to Fas-mediated apoptosis.¹⁶⁹ Furthermore, VEGF, the critical driver of angiogenesis, promotes an immunosuppressive ecosystem in the TME.¹⁷⁰ The first anti-angiogenic drug, bevacizumab, an anti-VEGFmAb, was initially approved in 2004 as a treatment for colorectal cancer in combination with chemotherapy.^{171,172} Since then, research has been conducted to improve patient response to bevacizumab and other antiangiogenic drugs, mainly monoclonal antibodies and small-molecule tyrosine kinase inhibitors that target VEGF/VEGFR and other angiogenic pathways, in combination with other anti-cancer agents.¹⁷³ Several murine tumor models posit that the combination of ICI with anti-angiogenic agents results in better antitumor effects, associated with normalized vasculature, increased T cell infiltration, improved antigen presentation, and decreased immunosuppressive cell populations (Figure 2e).^{174–176} The combination of ICI with antiangiogenic agents has also achieved success in the clinic. For instance, the open-label, randomized phase 3 IMbrave150 (NCT03434379) study in patients with unresectable hepatocellular carcinoma showed that atezolizumab, an anti-PD-L1 antibody, with bevacizumab resulted in superior overall and progression-free survival outcomes than standard-of-care sorafenib, a multikinase inhibitor.^{177,178} This trial informed the FDA approval of this combination in hepatocellular carcinoma in 2020.¹⁷⁹ In addition, a phase 1b clinical trial (NCT02715531) demonstrated that in patients with unresectable hepatocellular carcinoma, atezolizumab plus bevacizumab significantly prolonged progression-free survival compared with atezolizumab monotherapy.¹⁸⁰ Collectively, these studies show that the combination of ICI with an antiangiogenic agent results in improved clinical benefit when compared to monotherapy with either treatment.

The extracellular matrix is another physical barrier that effector immune cells must overcome to effectively attack tumor cells. In addition to regulating the migration of T cells, collagen produced by cancer-associated fibroblasts, macrophages, and tumor cells can impair immune activity through interactions with leukocyte-associated immunoglobulin-like receptor-1 (LAIR-1).¹⁸¹ LAIR-1 activation and signaling on T cells, NK cells, monocytes, and DCs result in the inhibition of these immune cells.^{52,182–184} An experimental antibody, NC410, that competes with LAIR-1 for collagen-binding is currently being evaluated in a phase 1 study in patients with advanced and metastatic solid tumors, such as ovarian, gastric, and colorectal cancer (NCT04408599). In murine EMT6 breast and MC38 colon cancer models, the combination of NC410 and an anti-PD-L1/anti-TGF- β

fusion protein antibody (bintrafusp alfa, formerly M7824) improved antitumor activity by remodeling the collagen matrix, enhancing T cell tumor infiltration, and skewing the tumor-associated macrophages from the immunosuppressive M2 phenotype to immune-favorable M1 (Figure 2f).³² This preclinical study underscores the valuable contribution of targeting the extracellular matrix in facilitating ICI therapy.

5.3 Repolarization of the immunosuppressive microenvironment

The immunosuppressive tumor microenvironment is comprised of cellular components and soluble factors that promote tumor progression and contribute to immune resistance. The abundance of myeloid-derived suppressor cells (MDSCs), Tregs, TAMs, and other immunosuppressive cells in the peripheral blood or in the tumor lesion has been associated with poor prognosis in cancer patients.¹⁸⁵ Immunosuppressive cells, together with tumor cells and stromal cells, can produce inhibitory cytokines (e.g., IL-10 and TGF- β) and factors (e.g., arginase, IDO, and collagen) and express checkpoint ligands to dampen the immune response.^{185,186}

Several immuno-oncology agents that deplete or reprogram immunosuppressive populations by targeting markers overexpressed on those cells are under development and have the potential to synergize with ICIs. Antibodies that target CD33 (gemtuzumab ozogamicin, BI 8,366,858) and TRAIL-R2 (DS-8273a) were demonstrated to eliminate MDSCs (Figure 2g).^{187–189} A phase 1 trial studying the combination of DS-8273a and nivolumab has recently been completed, but results are yet to be published (NCT02983006). Likewise, targeting colony stimulating factor-1 receptor (CSF-1 R), which regulates monocyte migration, proliferation, and differentiation into TAMs, has been shown to reduce TAM populations (Figure 2g).¹⁹⁰ The safety and efficacy of axatilimab, an anti-CSF1-R antibody, in combination with nivolumab in patients with refractory/relapsed classical Hodgkin lymphoma is the subject of an ongoing phase 2 trial (NCT05723055, Table 1). Targeting MARCO, which is expressed on immunosuppressive M2-like TAMs, reprogrammed the macrophages into a pro-inflammatory phenotype and enhanced the antitumor efficacy of anti-CTLA-4 in melanoma and colon carcinoma models.¹⁹¹ Studies on mouse models also suggest that depletion of Tregs using anti-CD4, anti-CD25, or anti-GITR (glucocorticoid-induced tumor-necrosis-factor receptor (TNFR)-related protein) antibodies in combination with ICI improves CD8⁺ T cell activity, resulting in control of tumor growth (Figure 2g).^{192–194} However, several phase 1 trials demonstrated that anti-GITR (MK-4166, MK-1248, or TRX518) in combination with anti-PD-1 (pembrolizumab or nivolumab) only resulted in limited clinical responses.^{195–197} A study investigating the safety and efficacy of another anti-GITR mAb, REGN6569, plus PD-1 blockade with cemiplimab is underway with results pending (NCT04465487). However, based on what is currently known, additional work will be required to translate the benefits of anti-GITR and ICI combination into an effective therapy.¹⁹⁸

Blocking the recruitment and migration of immunosuppressive cells can also effectively repolarize the TME and sensitize tumors to ICI. For example, targeting CXCR1 and CXCR2, which are receptors to chemokines essential for MDSC and tumor-associated neutrophil (TAN) recruitment, can prevent MDSC and TAN accumulation, enhance effector cell function, and augment the antitumor activity of PD-1/PD-L1 blockade in preclinical models (Figure 2g).^{199–202} SX-682, a small molecule dual inhibitor of CXCR1 and CXCR2, is currently being investigated in combination with anti-PD-1 (nivolumab, pembrolizumab, or tislelizumab) in patients with colorectal cancer (NCT04599140), melanoma (NCT03161431; Table 1), pancreatic cancer (NCT05604560), and non-small cell lung cancer (NCT05570825).

Lastly, another potential strategy to re-invigorate effector cells in tandem with ICIs is to target immunosuppressive factors in the TME. IDO is an enzyme involved in tryptophan catabolism, converting tryptophan to kynurenine. In cancer settings, the depletion of tryptophan and accumulation of kynurenine promotes Treg activation and inhibits NK and CD8⁺ T cell activation, thereby resulting in immunosuppression (Figure 2g).^{203,204} The IDO inhibitor, epacadostat, showed promising anti-tumor activity when combined with pembrolizumab and nivolumab in phase 1/2 trials.²⁰⁵ However, a phase 3 study of epacadostat plus pembrolizumab did not improve progression-free or overall survival in patients with unresectable or metastatic melanoma.^{205,206} Another metabolite that accumulates in the TME and plays an important role in immunosuppression is extracellular adenosine (eADO). The adenosine pathway ultimately results in the conversion of extracellular ATP to eADO via ectonucleotidases CD39 and CD73, which are overexpressed in tumors.²⁰⁷ Ligation of eADO to adenosine receptors (A_{2A} and/or A_{2B}) on effector cells results in the reduction in CD8⁺ T cell function, differentiation of naïve CD4⁺ T cells to Treg cells, and decreased NK proliferation, survival, and function.¹²³ Immuno-oncology agents targeting CD39, CD73, and adenosine and their potential combinations have been reviewed recently by Zahavi and Hodge (Figure 2h).¹²³ Cytokines that support the immunosuppressive milieu can also be targeted to enhance ICI activity. TGF- β is expressed by tumor cells, stromal cells, and immunosuppressive cells that can attenuate CD8⁺ T cell function,^{208,209} inhibit CD8⁺ T cell infiltration,²¹⁰ expand Tregs,²¹¹ and polarize neutrophils into pro-tumor populations.²¹² The value of simultaneous blockade of PD-1/PD-L1 axis and TGF- β is underscored by the development of bintrafusp alfa, which is a fusion antibody composed of an anti-PD-L1 antibody fused to the extracellular domain of human TGF- β receptor II (Figure 2g). In preclinical studies, monotherapy with bintrafusp alfa was shown to effectively trap TGF- β and bind PD-L1, resulting in decreased tumor burden and prolonged survival.^{213,214} Furthermore, treatment with bintrafusp alfa had superior anti-tumor activity than treatment with either anti-PD-L1 or TGF- β , highlighting the importance of co-targeting these two inhibitory molecules. A recent publication by Gameiro et al. reviews past and current clinical trials involving bintrafusp alfa.²¹⁵

Conclusion

To date, the benefits of cancer immunotherapy are limited to certain cancer types, mainly because of the heterogeneity intrinsic to different tumor types and to the mechanisms promoting primary and acquired resistance. Efforts made in the field to overcome CBR-related unresponsiveness to ICI therapy have been focussed on overcoming T cell-related deficiencies and/or suppressing immunosuppressive populations in the TME. However, additional effort is needed to close the gap in knowledge in identifying additional checkpoints that may be abrogating the effectiveness of ICI therapy as well as unknown mechanisms underlying CBR. More detailed guidelines are needed as well to follow mixed responses after ICI therapy and to define primary and acquired resistance more accurately, not only in the setting of a clinical trial but also in the real-world after agent approval.

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