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Review

Cancer cell-intrinsic mechanisms driving acquired immune tolerance

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SUMMARY

Immune evasion is a hallmark of cancer, enabling tumors to survive contact with the host immune system and evade the cycle of immune recognition and destruction. Here, we review the current understanding of the cancer cell-intrinsic factors driving immune evasion. We focus on T cells as key effectors of anti-cancer immunity and argue that cancer cells evade immune destruction by gaining control over pathways that usually serve to maintain physiological tolerance to self. Using this framework, we place recent mechanistic advances in the understanding of cancer immune evasion into broad categories of control over T cell localization, antigen recognition, and acquisition of optimal effector function. We discuss the redundancy in the pathways involved and identify knowledge gaps that must be overcome to better target immune evasion, including the need for better, routinely available tools that incorporate the growing understanding of evasion mechanisms to stratify patients for therapy and trials.

INTRODUCTION

Cancer cells have an altered-self pattern of antigen expression and are subject to recognition and control by T cells as key immune effectors. The cancer immunity cycle describes an optimal scenario of immune recognition, immune activation, and anti-tumor response leading to cancer elimination.[1] However, the lethal growth of tumors in immune-competent patients demonstrates that cancer cells employ effective strategies to avoid immune destruction.

How is this achieved? One way to approach the problem is through consideration of physiological mechanisms that maintain immune tolerance to self.[2] To ensure recognition of a broad range of potentially pathogenic antigens by αβT cells, random diversification of T cell receptor (TCR) encoding genes takes place during thymocyte development.[3] This gives rise to both pathogen-reactive and self-reactive T cells. Central and peripheral tolerance mechanisms have evolved to limit the autoimmune consequences of self-reactive clones generated in this process. Peripheral tolerance mechanisms to regulate T cell activity are most clearly observed at immune-privileged sites[4,5] such as the brain, testis, eye, and pregnant uterus, where transplanted non-self-tissues are capable of avoiding immune rejection.

Tolerance can also be acquired at non-privileged sites, as demonstrated by the seminal experiments of Medawar and colleagues published 70 years ago.[6] Given their potential immunogenicity, cancer cells must similarly induce a state of tolerance to survive immune predation. The cancer immunity cycle describes a state within which tolerance has broken down or failed. A consideration of how cancer cells solve the challenge of surviving contact with the host immune system, through the perspective of peripheral tolerance and immune privilege, may help better understand and tackle immune evasion.

Immune surveillance is hypothesized to exert an evolutionary pressure on cancer development.[7] During an equilibrium phase of cancer-immune interaction, the survival of cancer cell clones capable of resisting rejection is favored, leading to natural selection of clones capable of escaping immune control. This evolutionary perspective implies the necessity of immune evasion for cancer development[8] and generation of tolerance as a key feature of the malignant phenotype.

Mechanisms to control T cell activity are crucial for the maintenance of tolerance. Therapeutic advances that act to enhance T cell anti-cancer function illustrate this point. Ground-breaking work into T cell regulatory mechanisms has revealed the role of inhibitory receptors (checkpoints) including PD-1,[9] CTLA-4,[10] and LAG-3[11] in limiting autoreactivity and T cell over-activation.[12–14] Antagonistic antibodies against these receptors—checkpoint immunotherapies—that reduce the negative control of T cell activity improve patient outcomes across multiple cancer types,[15–19] although at the cost of autoimmune side effects. The clinical success of checkpoint immunotherapies reveals the centrality of T cell regulatory mechanisms in determining tumor growth vs. rejection.

There are three interrelated, broad control mechanisms to impose immune tolerance; these are conceptually relevant to each stage of the cancer immunity cycle. Control over T cell localization is a mechanism employed at immune-privileged sites such

as the eye and brain to limit or exclude effector T cell entry to guard against the potentially catastrophic consequences of inflammation at these sensitive sites.[20,21] T cell target recognition requires TCR signaling upon peptide-major histocompatibility complex (MHC) binding. Decreased expression of MHC class I is employed by cells at immune-privileged sites including the brain and by placental trophoblasts to limit T cell reactivity.[22–24] T cell activation and differentiation to acquire full effector capability is closely controlled by antigen availability, interaction with antigen-presenting cells (APCs), and the extracellular inflammatory environment. These requirements collectively provide tolerogenic control mechanisms. For example, contributors to ocular immune privilege include maintenance of self-reactive T cells in an ignorant state with limited access to antigenic stimulation,[25] diversion of effector T cells to hypofunctional[26] or suppressive states,[27] and promotion of apoptotic T cell death[28] with similar mechanisms operating at other sites. Immune pressure favors selection of cancer cells that exploit these physiologically essential immune homeostatic processes to acquire a state of tolerance and thus gain a survival advantage.

How might cancer cells exploit tolerogenic strategies? In general, cancers accumulate a range of genetic and molecular alterations affecting their functional properties. Mutations in driver genes, chromosomal instability, and epigenetic alterations impact pathways related to cell signaling, metabolism, and apoptosis. Cancer genomic instability also results in the generation of subclonal variants and tumor heterogeneity.[29] These aberrations contribute to essential features of the malignant phenotype including properties necessary for cancer cells to achieve immune evasion[30] through exploitation of tolerance pathways. In contrast, cancers that arise from tissues that are physiologically more tolerogenic may achieve immune evasion without requiring additional genomic alterations. The immunotherapy resistance of germ cell tumors and glioblastomas could be related to the intrinsic properties of the cell of origin, although the profound immunotherapy sensitivity of cancers arising from placental trophoblast suggests deeper complexity.[31]

Here, we discuss how cancer cell-intrinsic mechanisms promote immune evasion. We frame this discussion around the three pillars of tolerance: control over T cell localization, antigen recognition, and acquisition of effector function, providing parallels to the setting of physiological (as opposed to pathological) tolerance. We focus primarily on T cells and their interactions with innate immune populations, examining how cancer cells interfere with immune recognition and activation pathways to achieve immune evasion. Considering cancer immune evasion as a state resulting from the exploitation of physiological tolerance pathways offers novel routes into resolving how evasion is achieved. In general, molecular understanding of the multiple pathways of immune evasion and their evolution strongly supports early therapeutic intervention. The redundant nature of the mechanisms involved in cancer immune evasion mirrors physiological tolerance and supports multi-targeted approaches including vaccination and strategies that are more robust to cancer cell evolution, for instance by targeting clonal antigens and mobilization of CD4 T cells in the setting of MHC class I loss. Taken together, the insights derived from this analysis may inform new therapeutic strategies and approaches to stratify patients for therapy.

Cancer cell control over T cell localization: Endothelial dysfunction

Surveillance and effector activity of T cells is proximity dependent and thus control over T cell localization is an essential mechanism of peripheral tolerance. Broadly, this is achieved through control over chemokine signaling and the maintenance of barriers to T cell trafficking. For instance, in the central nervous system, T cell tissue entry into brain parenchymal tissues is limited through microendothelial cell expression of the chemokine CXCL12 that delivers chemorepellent signals to T cells through CXCR4,[32] an immunomodulatory mechanism that is also employed by cancer cells. Reduced expression of endothelial adhesion molecules such as intercellular adhesion molecule (ICAM)-1 required for the migration of circulating T cells into brain tissue is a further control mechanism.[33] Physical barriers are also utilized physiologically. For instance, the blood-testis barrier generated by tight junctions between Sertoli cells serves to exclude T cells from contact with developing germ cells that express novel antigens following meiosis.[34]

Across cancers, $CD8^+$ and effector $CD4^+$ T cell infiltration is a predictor of patient outcomes[35,36] and response to checkpoint immunotherapy.[37–39] Tumor types with poor immune infiltration (immune cold) such as pancreatic cancer and microsatellite stable colorectal cancer respond poorly to checkpoint immunotherapy. Cancer cell evolution under immune pressure converges on multiple mechanisms to inhibit the trafficking of effector T cells into the tumor microenvironment (TME) and regulate T cell localization once within the tumor. Mirroring physiological processes, this is achieved by interfering with effector T cell localization through disrupting endothelial function, generation of stromal barriers, and modulating chemokine availability (Figure 1).

Lymphocyte adhesion to endothelial cells is an essential step in T cell trafficking into tumors. Work from the 1990s revealed that tumor-associated endothelial cells have reduced expression of adhesion molecules including ICAM-1, ICAM-2, E-selectin, and vascular cell adhesion molecule (VCAM-1).[40] This is mediated by the activity of pro-angiogenic agents produced by cancer cells such as vascular endothelial growth factor (VEGF),[41] which acts through various mechanisms including inhibition of endothelial nuclear factor (NF)-κB signaling.[42] Such "endothelial anergy" results in an impaired ability to mediate T cell extravasation and entry into the TME.[43] Cancer cell VEGF expression is increased by intratumoral hypoxia[44] and lies downstream of multiple signaling pathways that are dysregulated in cancer cells including PI3K-AKT,[45] which is negatively regulated by the tumor suppressor PTEN. Indeed, *PTEN* loss or *PI3K* activation are associated with limited T cell infiltration[46] and immunotherapy resistance.[47] Among patients with melanoma, *PTEN* loss is associated with reduced $CD8^+$ T cell infiltration and reduced response to anti-PD-1 therapy.[48] In a transplantable melanoma model, *Pten* silencing associated with increased expression of CCL2 and VEGF. Anti-VEGF therapy enhances T cell infiltration, in keeping with previous studies that found anti-angiogenic factors could enhance tumor immune infiltration.[49] VEGF expression can increase tumor endothelial cell Fas ligand (FasL) expression, contributing to T cell exclusion through elimination of T cells by apoptosis mediated by signaling through the death receptor Fas.[50]

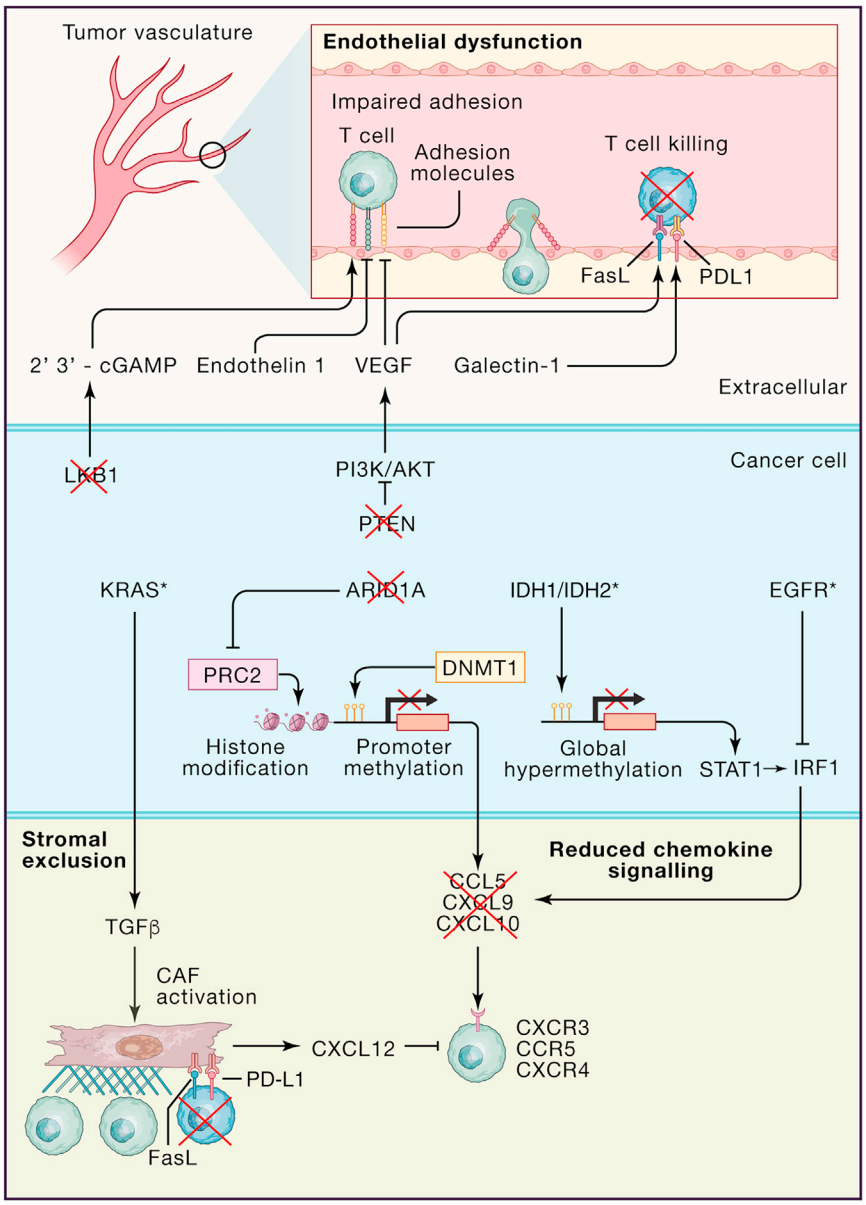

Figure 1. Mechanisms of T cell exclusion

Exclusion of T cells may be driven by endothelial dysfunction, cancer-associated fibroblast (CAF) activity, or altered chemokine signaling. PTEN loss results in enhanced PI3K/AKT signaling and increased cancer cell production of vascular endothelial growth factor (VEGF) that inhibits endothelial cell expression of adhesion molecules including intercellular adhesion molecule (ICAM)-1, ICAM-2, E-selectin, and vascular cell adhesion molecule (VCAM-1), limiting T cell extravasation. Cancer cell production of endothelin 1 and reduced 2′ 3′-cGAMP export due to loss of LKB1 also results in reduced endothelial cell adhesion molecule expression. VEGF additionally upregulates endothelial cell expression of FasL, promoting T cell death. Galectin-1 acts to increase endothelial cell expression of PD-L1 contributing to reduced T cell extravasation. Increased transforming growth factor β (TGF-β) signaling, which can be driven by cancer cell KRAS overactivity, activates CAFs to produce extracellular matrix components that limit T cell contact with cancer cell nests. CAFs can express FasL and PD-L2 contributing to T cell deletion. CAF CXCL12 contributes to T cell exclusion through chemorepulsion, acting on CXCR4. Cancer cell expression of CXCL9 and CXCL10 that support recruitment of T cells through CXCR3 signaling is limited by (1) PRC2 overactivity resulting in histone trimethylation at lysine 27 (H3K27me3), which may occur due to *ARID1A* mutation, (2) DNA methyltransferase activity resulting in promoter hypermethylation, (3) IDH1/IDH2 mutation resulting in global hypermethylation and reduced CXCL9 and CXCL10 expression through reduced STAT1 activity. *EGFR* mutation results in reduced IRF1 activity and suppressed CXCL10 and CCL5 that supports T cell recruitment through CCR5. CCL5 expression can additionally be reduced due to promoter methylation.

Loss-of-function mutations of *STK11* (encoding serine/threonine kinase 11; LKB1) mediate endothelial dysfunction that reduces T cell infiltration into the TME.[51] These mutations often co-occur with *KRAS* mutations in lung adenocarcinoma[52] and limit T cell infiltration through modulation of the cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) pathway. Activation of the cytosolic single-strand DNA sensor cGAS produces 2′3′-cGAMP that promotes STING activity, mediating downstream anti-pathogen effects such as interferon (IFN) production[53] through transcription factors including IRF3. LKB1 inactivation reduces STING expression, with emerging evidence that intracellular accumulation of cGAS and 2′3′-cGAMP is also limited.[53] Utilizing a microfluidic endothelial cell-cancer cell co-culture system, Campisi et al. found that LKB1 reconstitution restored 2′3′-cGAMP export and STING activation within endothelial cells, resulting in increased expression of adhesion molecules and T cell adhesion.[54] In addition to potential effects on tumor endothelial function as a mechanism of limiting T cell infiltration, loss of STING signaling in *KRAS/STK11*-mutated lung cancer reduces expression of CXCL10 and CCL5,[55] chemoattractants that enhance T cell tumor infiltration[56] and immunotherapy response.[57]

Cancer cell production of the vasoactive protein endothelin 1 is a further mediator of endothelial dysfunction. In ovarian cancer, increased expression of endothelin receptor B by endothelial cells associates with immune exclusion and worse survival.[58] Mechanistically, endothelin receptor B signaling suppresses ICAM-1 function required for T cell adhesion to endothelial cells.

Finally, T cell exclusion is attributed to the carbohydrate-binding protein galectin-1. Galectin-1 expression is increased in multiple cancer types in association with hypoxic signaling[59] and has immunomodulatory properties.[60] In a pan-cancer analysis of transcriptional data, Nambiar et al. found an inverse correlation between galectin-1 expression and predicted T cell infiltration, which was seen also in a mouse model of head and neck

cancer.[61] This was attributed to enhanced endothelial expression of PD-L1 mediated by STAT1 signaling, and antibody targeting of galectin-1 resulted in enhanced anti-PD-1 efficacy among tumor-bearing mice.

Collectively, these findings have formed the basis for clinical studies combining inhibitors of VEGF or VEGFR with checkpoint immunotherapies. For instance, combined therapy with the monoclonal antibodies atezolizumab and bevacizumab (targeting PD-L1 and VEGF, respectively) is approved for patients with hepatocellular cancer (the IMbrave150 study[62]) and non-squamous lung cancer in combination with chemotherapy (the IMpower150 study[63]). However, the contribution of bevacizumab is uncertain. Although in IMbrave150, there was no atezolizumab monotherapy arm, IMpower150 included a cohort treated with atezolizumab plus chemotherapy without bevacizumab. A direct comparison was not performed, but an updated analysis found that the addition of bevacizumab to chemoimmunotherapy did not appreciably enhance survival outcomes.[64] In a phase II study of atezolizumab with or without bevacizumab for renal cancer, patients with a low baseline T cell infiltrate (measured by gene expression) had a reduced response rate and poorer survival, not enhanced by the addition of bevacizumab.[65] These data indicate that anti-VEGF and anti-PD-L1 therapy may not be synergistic as expected, and any additional benefit of bevacizumab is not mediated by enhancing T cell recruitment in immune cold tumors. Thus, alternative approaches to act on endothelial dysfunction are required. Thus far, inhibitors of endothelin-A have been unsuccessful in trials,[66] and galectin-1-targeted agents are at an early phase of development.[67]

Stromal inhibition of T cell recruitment

T cell exclusion from the tumor or their limitation to stromal areas surrounding cancer cell nests serves as a second mechanism to achieve immune evasion through control over immune localization.

Transforming growth factor β (TGF-β) expression is increased in tumors and has an important role to play in this process through inhibition of T cell infiltration,[68,69] partly through induction of cancer-associated fibroblasts (CAFs).[70–72] CAFs are also induced by platelet-derived growth factor and Hedgehog ligands, Shh and Ihh, which are increased in *KRAS-G12D*-mutated murine pancreatic cancer models.[73]

TGF-β exists predominantly in a latent form associated with latency-associated peptide (LAP) and bound to the extracellular matrix (ECM) protein fibrillin or docked to the surface of cell types including regulatory T cells (Tregs),[74] platelets, and cancer cells through the transmembrane protein glycoprotein A repetitions predominant (GARP).[75] TGF-β signaling is increased in tumors through multiple processes, including the following: (1) LAP binding to the cell surface integrins αvβ6 and αvβ8, forcing TGF-β liberation upon cell contraction[76]; (2) TGF-β liberation through the activity of proteases such as matrix metalloproteases that are present within the TME[77]; and (3) increased TGF-β1 generation by cancer cells downstream of MEK-ERK-AP1 signaling in *KRAS-G12V*-mutated cancer models.[78] In addition to playing a role in mediating T cell exclusion, TGF-β also impacts T cell differentiation and dendritic cell (DC) function.

T cell restriction to stromal areas preventing their contact with cancer cells is seen in multiple tumor types[79,80] and associated with worse patient outcomes[35] and lack of response to immunotherapy.[81,82] CAFs are implicated in this process through deposition and organization of dense extracellular matrix components such as collagen I, collagen IV, and hyaluronan[83] to generate a barrier limiting T cell migration.[84,85]

CAFs also have an active role in mediating T cell exclusion. Noting phenotypic similarities between CAFs and lymphatic fibroblastic reticular cells (FRCs) that play a physiological role in deletional tolerance, Lakins et al. showed in a mouse lung cancer model that CAFs are capable of antigen cross-presentation and FasL-mediated $CD8^+$ T cell killing in a manner that could be blocked by anti-FasL treatment.[86] CAFs may also determine T cell exclusion through expression of CXCL12, which mediated a chemorepulsive effect on CXCR4-bearing T cells in a mouse pancreatic cancer model.[87] Finally, CAF expression of CXCL13 recruits tumor-promoting B cells in a mouse model of prostate cancer,[88] with evidence that interleukin (IL)-35 production by immunosuppressive B cells further contributes to T cell exclusion.[89]

Several areas of uncertainty are yet to be resolved. Single-cell RNA sequencing reveals high heterogeneity of CAFs; the relative contributions of distinct CAF subsets to immune evasion are unknown.[90–92] Related to this, although CAF depletion associates with enhanced immune responses in some settings,[93] depletion is associated with worse outcomes in others,[94] suggesting subset or context dependencies that are undefined. Finally, clinical trials targeting CAFs with various modalities failed to show benefits including with Hedgehog pathway inhibitors,[95] the TGF-β receptor kinase inhibitor galunisertib[96] and PEGylated-recombinant human hyaluronidase targeting the ECM.[97] A better understanding of CAF subset diversity may make this population more amenable as a therapeutic target.

Beyond the activity of CAFs, emerging evidence suggests that additional mechanisms may exist to limit tumor-infiltrating T cell contact with cancer cells. For instance, tumor-infiltrating macrophages recruited by chemokines produced by cancer cells may reduce T cell motility through long-lasting interactions, resulting in impaired migration to cancer cell nests.[98]

Reduced production of chemokines involved in T cell recruitment

Chemokine signaling plays an important role in T cell trafficking to inflammatory sites through receptors including CXCR3.[99] Differences in chemokine availability are well described between T cell inflamed vs. non-inflamed tumors, including for the CXCR3 ligands CXCL9, CXCL10, and CXCL11, which are produced downstream of IFN-γ signaling.[100–103] These chemokines may also be involved in establishing lymphocyte niches within tumors with potential relevance for immunotherapy response.[104–106] In a pan-cancer analysis of patients treated with checkpoint immunotherapy, *CXCL9* expression emerged as a predictor of patient response.[107] By decreasing expression of pro-inflammatory chemokines, cancer cells can limit T cell recruitment into the TME. Broader effects of altered patterns of chemokine expression additionally shape the myeloid compartment, altering the abundance and activity of populations including DCs, macrophages, and myeloid-derived suppressor cells (MDSCs) with roles in T cell recruitment, as discussed in later sections.

Epigenetic alterations are common in cancer cells, affecting gene transcription patterns relevant to multiple processes

related to cell growth and survival. Epigenetic alterations also modulate the immune environment through altered expression of T cell chemoattractants. One mechanism is through the activity of polycomb repressive complex 2 (PRC2)[108] that plays a role in inhibiting gene expression through histone H3 lysine 27 (H3K27) trimethylation. In primary colon[109] and ovarian cancer cells,[110] PRC2 activity reduced CXCL9 and CXCL10 expression through promoter H3K27 trimethylation. In ovarian cancer cells, reduced CXCL9 and CXCL10 expression associates with DNA methylation by the DNA methyltransferase (DNMT)1. In general, these effects are reversed by inhibitors including 5-AZA-2-deoxycytidine (5-AZA dC)[110] that suppresses DNMT1, and GSK126 that interferes with the PRC2 catalytic component enhancer of zeste homolog 2 (EZH2) methyltransferase activity. In both ovarian and colon cancer, patients with elevated tumor expression of the PRC2 catalytic component EZH2 and DNMT1 had reduced $CD8^+$ T cell infiltration and worse survival. Similarly, in human melanoma, EZH2 expression inversely correlates with T cell infiltration, and EZH2 silencing in a mouse melanoma model increased expression of CXCL9 and CXCL10.[111] Finally, the SWI/SNF chromatin remodeling complex component ARID1A represses EZH2 activity, and mutations in *ARID1A* associates with reduced immune infiltration and expression of *CXCL9* and *CXCL10* in human ovarian cancer specimens.[112]

Gain-of-function mutations in the isocitrate dehydrogenase genes *IDH1* and *IDH2* are implicated in reduced CXCR3 ligand production due to epigenetic reprogramming. These mutations are commonly seen in glioma and enhance conversion of α-ketoglutarate to 2-hydroxyglutarate,[113] resulting in genome-wide DNA hypermethylation.[114] *IDH*-mutated gliomas in human studies[115] and mouse models[116] have reduced cancer cell expression of *CXCL9* and *CXCL10* related to suppressed STAT1 signaling,[117] contributing to generation of an immune cold TME.

Loss of cancer cell expression of the chemokine ligand CCL5 associates with low immune infiltration across human cancer types. Analysis of pan-cancer microarray data revealed a correlation between expression of *CCL5* and *CXCL9* with *CD8A* as a marker of T cell infiltration.[118] Low *CCL5* expression associated with promoter methylation, and this could be reversed with 5-AZA dC in ovarian cancer cell lines. In a mouse ovarian cancer model, CCL5 produced by cancer cells promoted CXCL9 expression by tumor macrophages to enhance T cell recruitment. DNA hypomethylation in association with low T cell infiltration is also seen in human lung and head and neck squamous cancers bearing mutations of the methyltransferase encoding gene *NSD1*.[119] In mouse head and neck cancer cell lines, NSD1 inactivation altered chemokine profiles including reduced expression of CCL5. Increased β-catenin signaling is linked to suppressed CCL5 expression as well, as discussed later.

Non-epigenetic mechanisms also regulate cancer cell chemokine signaling. The ErbB family epidermal growth factor receptor (EGFR) is frequently mutated across cancer types including a subset of non-small cell lung cancers (NSCLCs) and predicts low response to anti-PD-1 therapy.[120,121] Although *EGFR* mutations associate with low tumor mutational burden and thus potentially reduce cancer immunogenicity, there is also an effect of reduced T cell recruitment. In a pan-cancer analysis of RNA sequencing data, *EGFR* mutations were enriched in T cell non-inflamed tumors.[122] Mechanistically, EGFR signaling reduced expression of *CCL5* and *CXCL10* in NSCLC cell lines via reduced activity of the IFN regulatory transcription factor IRF1.[123] Finally, low T cell infiltration associates with overactivity of the peroxisome proliferator-activated/retinoid X receptor (PPARγ/RXRα) transcription factor complex, secondary to chromosomal amplification or mutations commonly seen in urothelial bladder cancer.[124] In a mouse model of bladder cancer, PPARγ/RXRα overactivity reduced expression of *CCL5* and *CXCL10*, reduced T cell infiltration, and promoted partial resistance to anti-CTLA-4 or anti-PD-1 immunotherapy.

T cell recruiting chemokines are therapeutically attractive and may be modulated through a number of means. Early phase trials are exploring the use of a tumor-selective virotherapy platform modified to express CXCL9 and CXCL10.[125] A combination of the TLR3 agonist rintatolimod, IFN-α, and the cyclooxygenase (COX)-2 inhibitor celecoxib demonstrated activity in a phase I breast cancer study.[126] In mouse breast cancer models, inhibitors of cyclin-dependent kinases 4 and 6 (CDK4/6) such as palbociclib enhance cancer cell expression of CCL5, CXCL9, and CXCL10 and response to checkpoint immunotherapy, with a similar increase in chemokine expression by treated human breast cancer cells.[127] An early phase trial of palbociclib, the aromatase inhibitor letrozole, and pembrolizumab in breast cancer has shown activity,[128] and biomarker analysis revealed effects on circulating myeloid populations, suggesting a complex and currently insufficiently well-understood mode of action beyond stimulation of chemokine expression.[129]

Inhibition of target recognition: Suppression of MHC class I expression

T cells recognize cognate antigens through the TCR, following binding to peptide-MHC complexes upon APCs and target cells. Physiologically, several normal cell populations are of heightened immunogenicity by virtue of their genomic differences to self. These include germ cells that accumulate genetic differences through meiosis and placental trophoblasts that are derived from maternal and paternal genomic contributions and are hence semi-allogeneic. These populations are protected from immune destruction partly through suppression of MHC class I expression,[22,24] which is a tolerogenic mechanism additionally employed by brain neuronal populations[130] and ocular cells.[23]

Initial priming of T cell responses is mediated within lymphoid organs by APCs—mainly DCs that transport antigens to these sites and present peptides with additional signals that pattern subsequent T cell activation. T cell priming is dependent on antigen density, and sufficient quantities of antigen must reach draining lymphoid organs to elicit optimal T cell activation.[131,132] T cell "ignorance" results from lack of antigen availability.[133–135] Given these constraints, regulation of APC function,[136] tissue localization, and trafficking (for instance, at privileged sites[137]) are additional key physiological mechanisms to induce and maintain peripheral tolerance. For instance, DCs are limited from key ocular structures such as the retina, and lymphatic drainage is impaired.[138]

Cancer cells bear distinct patterns of antigen expression recognized as non-self by T cells. Cancer antigenicity is determined by the expression of neoantigens that may result from DNA mutations,[139–143] aberrant splicing or translation,[144,145] or expression of viral proteins. Additionally, tumor-associated

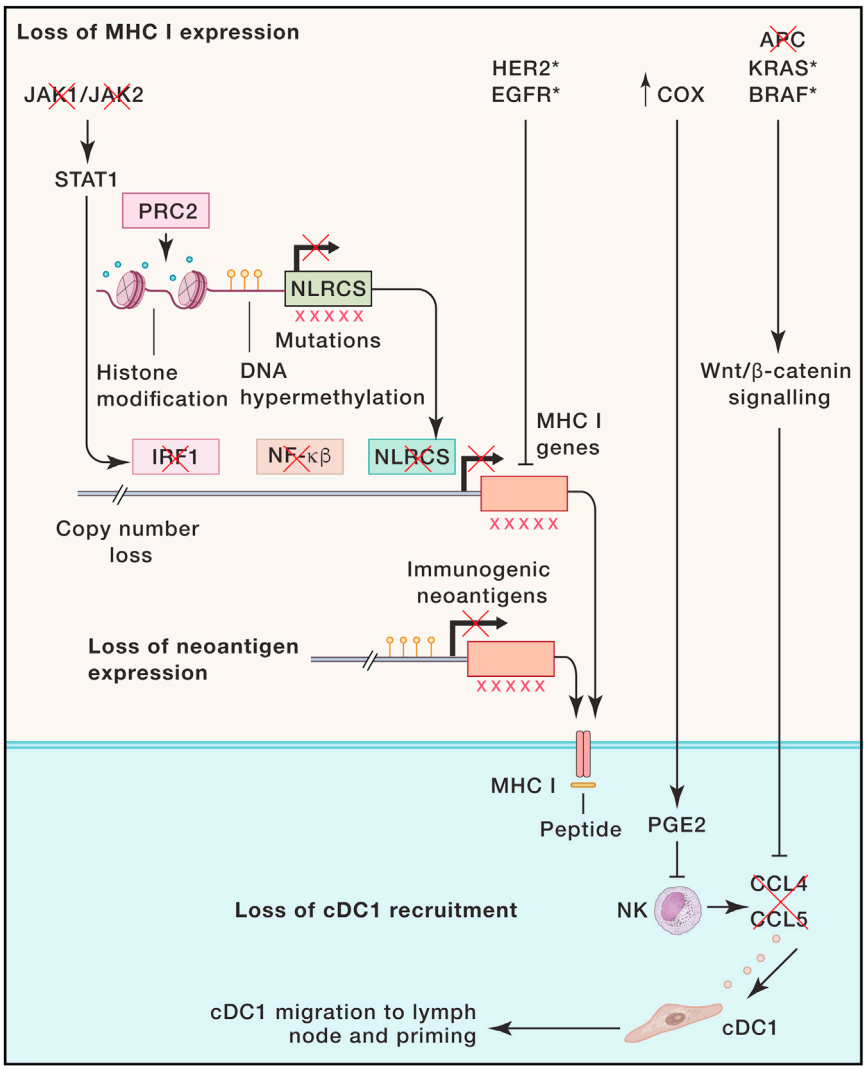

Figure 2. Mechanisms of reduced T cell antigen engagement

Reduced antigen engagement can be due to loss of cancer cell MHC class I expression, loss of neoantigen expression, and loss of conventional type 1 dendritic cell (cDC1) recruitment required for priming of T cells. MHC class I loss can occur due to reduced *NLRC5* activity caused by mutations, promoter hypermethylation, and PRC2 mediated generation of repressive H3K27me3 histone marks. NLRC5 loss results in reduced expression of MHC class I-related genes, which can also be inhibited by JAK1/JAK2 mutations resulting in reduced STAT1 mediated IRF1 activity, *HER2/EGFR* mutations, reduced NF-κB signaling, copy-number losses, and mutations within the MHC class I genes themselves. Neoantigen downregulation can occur due to mutations and promoter hypermethylation. Reduced recruitment of cDC1s can occur due to mutational loss of *APC* or KRAS/BRAF mutations resulting in increased Wnt/β-catenin signaling and suppression of the chemoattractants CCL4 and CCL5. Enhanced cyclooxygenase (COX) activity results in upregulated PGE2 production that impairs natural killer (NK) cell release of CCL5.

antigens may arise from increased expression of proteins that are usually tissue restricted, expressed during embryonic development,[146] or limited to reproductive tissues.[147,148] Neoantigen load and predictors of immunogenicity such as self-dissimilarity are predictors of patient outcome and immunotherapy response.[142,149–155]

In general, cancer genomic and clonal diversification resulting in tumor heterogeneity[156] occurs through mechanisms[157] including defective DNA damage repair,[158] replication stress,[159] APOBEC3 enzyme expression,[160,161] and epigenetic dysregulation.[162] Under immune pressure, these processes can alter the cancer cell antigenic landscape resulting in the loss of immunogenic targets for immune control and conversely result in diversification and T cell activation-induced hypofunction or death. The potential clinical relevance of genomic instability is, for instance, illustrated by the finding that the abundance of clonal neoantigens shared by all cancer cells is an important predictor of immunotherapy response.[107,143] In contrast, excess stimulation through TCR signaling can also have detrimental effects on T cell function.

Cancer cell evolution converges on mechanisms to limit T cell antigen engagement including suppression of MHC class I expression, neoantigen loss, and control over DC recruitment (Figure 2). Loss or reduced expression of MHC class I is seen in various cancer types[163–165] and correlates with worse patient outcomes and acquired resistance to various immunotherapeutic modalities including check point blockade, vaccination,[166,167] and adoptive cell therapy.[168,169] Decreased MHC class I expression in cancer is associated with loss of key components of the antigen processing and presentation machinery due to mutations and epigenetic alterations affecting genes encoding MHC class I heavy chains,[170,171] β_2-microglobulin,[172] tapasin,[173] and transporter associated with antigen processing (TAP),[174] which plays a role in peptide loading. The MHC class I transactivator NLRC5, a transcription factor that activates expression of multiple MHC class I-related genes, is also subject to genetic and epigenetic loss in cancer cells,[175] such as via EZH2-generated repressive H3K27me3 histone marks at the *NLRC5* promoter.[176,177]

Transcriptional inhibition of MHC class I expression may occur due to altered IFN signaling. Both type I and type II IFN signaling pathways promote expression of MHC class I through receptor activation of Janus kinases (JAKs) and downstream STAT1- and IRF1-mediated transcription of genes involved in antigen presentation.[178] Resistance to anti-PD-1 therapy in melanoma associates with *JAK1* and *JAK2* loss through copy-number alterations and mutations, resulting in insensitivity to IFN-γ mediated increase in MHC class I expression.[179,180] Cancer cells can harbor defects in IFN-γ signaling pathway components,[181,182] particularly associated with immunotherapy resistance[183,184]—

although in some settings, loss of IFN-γ signaling can sensitize cancer cells to immune control.[185,186] Increased expression of the ErbB family receptor tyrosine kinases human epidermal growth factor receptor 2 (HER2)[187–189] and EGFR[190] along with decreased NF-κB activity[191] can also decrease MHC class I expression in cancer cells.

A common mechanism underlying decreased MHC expression is loss of heterozygosity at the HLA locus, secondary to focal or arm-level losses at chromosome 6p21, which occurs in 17% of cases across cancer types and 40% of patients with NSCLC.[164,192] Patients with lung cancer exhibit frequent loss of HLA heterozygosity, more frequently as a subclonal event shared by a fraction of cancer cells. HLA loss of heterozygosity associates with neoantigen burden and may be enriched in metastases. Loss of MHC class I expression over the course of cancer evolution is also seen in melanoma.[193]

Of note, melanoma,[194] lung,[195] colorectal,[196] and gestational cancers[31] with MHC class I suppression can remain sensitive to anti-PD-1 therapy. Along with data suggesting that HLA loss of heterozygosity is a late event in cancer evolution, these findings point to the importance of other immune mechanisms in controlling cancers with low MHC expression. These mechanisms are not well understood but may include the activity of effector populations including $CD4^+$ T cells,[197] natural killer (NK) cells,[198] and γδT cells[199] that act independently of MHC class I.

Loss of MHC class I expression poses an important challenge for current $CD8^+$ T cell therapy and vaccination approaches. Although adoptive transfer of TCR-modified T cells (TCR-T cells) is an area of active research,[200] a major limitation of this approach is that the transferred cells are HLA restricted. Even in the context of non-TCR-modified T cell therapy, immune escape may result from decreased HLA expression.[169] Because immune pressure is high during TCR-T cell therapy, loss of either the target antigen or target HLA could result in immune escape. Although HLA loss is usually a subclonal event, immune editing of the remaining population could drive the emergence of dominant subclones that escape immune control. Targeting a broad range of antigens recognized by multiple MHC class I molecules and focusing on clonally expressed variants is consequently an attractive approach to mitigate this.

Immune escape via suppression of neoantigen expression

In addition to the loss of MHC class I expression, early work in mouse models suggested that cancer cells can reduce expression of T cell epitopes under selection pressure as a mechanism of immune escape.[201] Identification of T cell-recognized antigens expressed by human cancers[202,203] allowed this concept to be evaluated in this setting. For instance, expression of the immunogenic cancer differentiation antigen Melan-A in a melanoma sample was subsequently lost at the RNA and protein level from recurrent disease resected years later.[204] Decreased expression of tumor-associated antigens associated with gene methylation[205–207] related to DNMT activity,[208] in addition to mutations as shown for MAGE family and other cancer-testis antigens.[209] Antigen loss due to mutations is commonly seen following chimeric antigen receptor (CAR) T cell therapy of hematological malignancies—for instance, CD19-directed CAR-T therapy of B cell acute lymphoblastic leukemia.[210]

Neoantigens can also be eliminated during the course of cancer evolution. Patients with NSCLC who fail checkpoint immunotherapy with anti-PD-1 or combined anti-PD-1 with anti-CTLA-4 therapy exhibit deletion of predicted neoantigens, mostly through single-base substitutions.[211] Based on predicted MHC class I binding affinity, eliminated neoantigens appear more immunogenic than those that are gained or retained. Single-base substitutions in this setting focus on MHC-binding anchor residues critical for peptide-MHC class I interaction. Eliminated neoantigens elicited a T cell response among autologous circulating T cells *in vitro*, supporting their immunogenicity and loss secondary to immune pressure rather than as random events. Additionally, inactivating mutations of *STK11* associate with impaired cancer cell antigen processing and presentation.[212]

In the TRACERx study,[213] patients with early-stage, surgically resected NSCLC are followed longitudinally to shed light on mechanisms of tumor evolution. Work from this study further highlights how loss of neoantigen expression may occur through multiple mechanisms. Neoantigen editing can follow copy-number loss and reduced transcription partly due to promoter hypermethylation, both occurring as subclonal events.[214] Patients with genomic evidence of immune evasion mechanisms have worse survival outcomes. Finally, RNA editing and epigenomic dysfunction resulting in copy-number-independent allele-specific gene expression may additionally result in reduced neoantigen expression, although further work is required to characterize this.[215]

In general, cancers with high intratumoral heterogeneity marked by a predominance of subclonal mutations have worse outcomes.[143,216] High intratumoral heterogeneity generated by mixtures of clones in a mouse melanoma model associates with immune evasion.[216] Because by definition subclonal neoantigens are expressed by a subset of cancer cells, one mechanism relating increased intratumoral heterogeneity and immune evasion is through low availability of individual neoantigens resulting in immune ignorance. This concept is supported by pre-clinical models showing that immunogenic antigens must be expressed by a sufficiently high fraction of cancer cells to elicit subclone rejection,[217] emphasizing the relationship between genomic tumor heterogeneity and the anti-cancer immune response.

A second mechanism may be through expansion of dominant T cell clones that subsequently lose their target antigen. This concept is supported by the observation across studies that the fraction of infiltrating lymphocytes identified as cancer specific is low, even among T cells that have phenotypic characteristics of persistent antigen exposure, including high expression of PD-1,[218] the transcription factor TOX,[219–221] and CD39.[222–224] Following antigen loss, expanded clones with a tissue-resident phenotype may remain locked within limited tumor niches[225] and pose a barrier to entry of new, potentially antigen-reactive populations. Indeed, the replacement of pre-existing tumor-infiltrating clones is implicated in successful anti-PD-1 therapy.[226] Pre-existing clones may inhibit entry or activity of new clones, for instance, by acting as a sink for cytokines critical for cell proliferation and survival.[227]

The concept that pre-existing immunity may inhibit the development of new responses is described in the context of viral infection. Aging in association with cytomegalovirus (CMV) infection is associated with systemic immune dysfunction termed "immunosenescence," which includes reduced responsiveness to vaccination,

increased infection risk, and infection severity.[228] This is partly attributed to massive inflation of CMV-specific memory T clones, resulting in repertoire shrinkage as the T cell compartment is skewed toward CMV reactivity.[229,230] Immune imprinting or "original antigenic sin" is a related mechanism described in viral infections, whereby optimal T cell responses to a viral escape variant or different serotype are limited by pre-existing responses to the original variant.[231,232] This effect is related to preferential activation of pre-existing, lower affinity T cell responses over priming of new clones, but whether this is relevant in the context of neoantigen loss during cancer evolution is unknown. Recent data suggesting that clones reactive to subdominant neoantigens are maintained in a hypofunctional, early differentiated state, additionally suggesting the potential for competition between clones that shape their functional capability.[233]

Overall, cancer genomic instability may thus result in generation of an immune microenvironment with limited capability to support ongoing surveillance. Several therapeutic modalities are relevant in this context. Progress toward the development of effective, personalized neoantigen vaccines[234–236] has been driven by advances in high-throughput genomics, MHC class I ligand identification, predictive modeling of peptide-MHC interactions, and vaccine technology, particularly around delivery strategies and adjuvants, to elicit optimal immune responses. This approach could be particularly valuable for tumors characterized by a low-antigen dose due to suppressed expression or diversification. However, tumor heterogeneity could again present a barrier to success, because the targeting of subclones may be ineffective. Approaches targeted to clonally expressed neoantigens should therefore be prioritized, because these are less likely to be eliminated.[237]

Impaired target recognition through suppression of DC recruitment

Across studies, very few neoantigens are recognized by infiltrating lymphocytes, even in cancers with high predicted neoantigen load.[238,239] This may partly be related to technological limitations in neoantigen prediction and identification of reactivity. However, studies of vaccination with neoantigen-loaded DCs in patients with melanoma show that this modality can elicit T cell responses that are otherwise undetectable,[240] in support of immune ignorance[241] as a contributor to the low fraction of targeted neoantigens, suggesting that T cell ignorance is reversible. Cross-presenting conventional type 1 DCs (cDC1s) are central to priming T cell responses and have emerged as a key positive regulator of anti-cancer immunity in this regard.[242]

One mechanism by which cancer cells can exert control over DC activity is through inappropriate activation of Wnt/β-catenin signaling. This pathway is involved in multiple oncogenic processes including proliferation,[243] migration,[244] and apoptosis[245] and is commonly dysregulated in melanoma and other cancer types through inactivating mutations of *APC* or gain-of-function mutations of drivers including *KRAS* and *BRAF*.[246] Studying transcriptional data from T cell inflamed vs. non-inflamed melanoma samples, Spranger et al. found Wnt/β-catenin signaling to be upregulated in the latter,[247] a finding subsequently confirmed across cancer types.[248] Mechanistically, this is supported by work in a genetically engineered mouse model of melanoma with locally inducible expression of stabilized β-catenin, the downstream mediator of canonical Wnt signaling. Elevated β-catenin activity is associated with the near absence of T cell infiltration, which is related to reduced CCL4 expression and lower cDC1 recruitment.[249]

Subsequent work in a mouse hepatocellular cancer model confirmed a link between β-catenin upregulation and immune escape through defective DC recruitment,[250] mediated by reduced CCL5 expression. In keeping with this, pharmacological Wnt inhibition is capable of enhancing T cell infiltration in mouse tumor models.[251,252]

The link between CCL5 expression and cDC1 recruitment has independently been shown, revealing a further mechanism of cancer control over the immune TME.[253] Multiple cancer types upregulate COX enzymes, resulting in upregulation of the prostanoid prostaglandin E2, which is associated with immune evasion.[254] In a mouse model of melanoma, cDC1s accumulate in COX-deficient tumors, related to loss of prostaglandin E2-mediated inhibition of NK cell produced CCL5 and the chemoattractant XCL1, that mediate cDC1 recruitment to the TME. In addition to reduced priming and immunological ignorance, loss of cDC1 production of chemokines including CXCL9 and CXCL10[249] may impair immune responses by reducing T cell recruitment. The relative contributions of these mechanisms are not well understood. Finally, although control over DC trafficking may play a role in maintenance of CNS tolerance,[255] whether tumors act to limit DC migration to lymph nodes is not well characterized.

Limiting the attainment of optimal T cell effector function

T cell fate and effector function is patterned by the integration of multiple positive and negative environmental signals. In contrast to the acquisition of optimal effector function, for instance in the setting of acute viral infection, signaling conditions may alternatively promote T cell differentiation into hypofunctional or suppressive states.

In addition to antigen dose, the kinetics of TCR stimulation are an important regulator of T cell functional capability, with chronic stimulation resulting in a loss of effector function termed "exhaustion."[256] Physiologically, exhaustion may be considered a mechanism to sustain tolerance by limiting T cell overactivity under certain conditions. For instance, persistent exposure to immunogenic fetal antigens during pregnancy results in maternal T cell exhaustion, characterized by upregulation of co-inhibitory receptors such as PD-1.[257] Blockade of PD-L1 signaling results in fetal resorption in allogeneic mouse pregnancies,[258] indicating the importance of T cell exhaustion in maintaining gestational tolerance in addition to cancer immune evasion.

Along with TCR signaling, T cell functional capability is shaped by factors including signaling through co-inhibitory and co-stimulatory receptors, cytokine availability, and metabolic conditions. Myeloid populations including DCs and macrophages in addition to CD4 Tregs are key determinants of the balance between these cues. The particular importance of Tregs to peripheral tolerance is highlighted by the occurrence of severe autoimmunity among patients with mutations in the Treg transcription factor FOXP3, as part of the immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome.[259] Treg recruitment and activation are likewise key mediators of cancer immune evasion, discussed below. Mechanistically, Tregs inhibit effector T cell activity by altering local

cytokine conditions through production of suppressive mediators such as IL-10, TGF-β, and IL-35, which have roles in both physiological tolerance and cancer immune evasion.[260,261] Tregs can additionally outcompete effectors for access to IL-2.[262] Tregs can furthermore alter local metabolic conditions through production of the immunosuppressive metabolite adenosine via dephosphorylation of ATP by the ectonucleases CD38,[263] CD39, and CD73,[264] with similar mechanisms described in cancer.[265] Treg-mediated immune suppression can also be achieved through CTLA-4-mediated sequestration of DC expressed co-stimulatory ligands CD80/CD86,[266] although the significance of this specific mechanism is less well known in the context of cancer.

Suppressive myeloid populations may similarly exert modulatory effects through control over the cytokine milieu and adenosine production,[267] in addition to tryptophan depletion and generation of nitric oxide through iNOS expression.[268] Notably, the physiological immunomodulatory role of the enzyme indoleamine 2,3-dioxygenase (IDO) in tryptophan deletion was first described in the context of placental trophoblast-mediated gestational tolerance[269] and later described as a suppressive factor in cancer.[270] Effector suppression can additionally be achieved by inducing T cell death, for example, through Fas signaling, which plays a role in maintenance of immune privilege in the testis[271] and eye[28] and is also employed by suppressive myeloid populations as a mechanism to deplete T cells in the tumor microenvironment.[272]

Downregulation of co-stimulatory ligands by immature or tolerogenic DCs can result in incomplete T cell activation and an "anergic" hypofunctional state[273] or deletion as a further contributor to maintenance of self-tolerance at diverse sites. At the extreme, effectors may be diverted toward a Treg fate. This is an antigen-specific mechanism of ocular tolerance[27] and may additionally be mediated by tolerogenic plasmacytoid DCs (pDCs) physiologically[274,275] and in cancer.

Finally, target cells may escape immune destruction through expression of inhibitory factors including PD-L1, FasL, IDO, and non-classical MHC class I molecules such as HLA-G that have immunomodulatory properties. Physiologically, these mechanisms are employed by placental trophoblasts to maintain gestational tolerance.[269,276–278]

Cancer mechanisms to suppress T cell effector function are shown in Figure 3.

Antigen diversity as a driver of T cell dysfunction

Multiple studies now suggest that early differentiated T cells that retain expression of the transcription factor TCF7 and have intermediate/low expression of inhibitory receptors such as PD-1 retain fitness to sustain immune responses in chronic viral infection, autoimmunity, and cancer[279–283] and mediate immunotherapy response in cancer.[284,285] In the context of persistent antigen encounter across disease states, these cells become functionally exhausted with characteristic epigenetic, transcriptional, and metabolic features.

In addition to aiding in the development of hallmark features of the malignant phenotype, cancer genomic instability leads to increased diversity of antigen expression. Although simultaneously providing targets for T cell anti-cancer function, if cancer cells are not eliminated, antigen diversification can ultimately result in immune system failure as functional, early differentiated T cells are depleted and transition toward an exhausted state. Similarly, an antigen-driven process of skewing from early to later differentiated, dysfunctional states occurs in chronic viral infection, including HIV, and is attributed to systemic decline in immune function.[286,287]

The notion that the magnitude of antigen stimulation may correlate with immune failure was recently explored in NSCLC within the TRACERx study. An inverse correlation was demonstrated between the abundance of early vs. dysfunctionally differentiated T cell populations and the degree of skewing in favor of dysfunctional populations is associated with worse survival outcomes.[288] Notably, skewing occurs in association with tumor mutational burden as a proxy for neoantigen load but only when considering the abundance of clonal but not subclonal mutations. This suggests that high-dose, persistent antigen encounter may not only provide fuel for both immune activation but also eventual exhaustion and immune failure. As with antigen loss discussed above, accumulation of dysfunctional cells adapted to tissue residency may inhibit entry of new clones required to sustain ongoing immune responses. Although it is clear that neoantigen-derived epitopes can contribute to tumor control, the dual role of cancer antigens as both targets for a response and drivers of dysfunction is an area that warrants further exploration.

Effector diversion and recruitment of suppressive populations

Tumor-infiltrating suppressive populations are important contributors to immune evasion. Tregs play multiple roles in immune suppression, and their abundance correlates with worse cancer outcomes.[289] Depletion of this population with anti-CTLA-4 or anti-CD25 directed antibodies is of therapeutic benefit in mouse models.[290–292] Additionally, tumor-associated inflammation promotes the activation and recruitment of suppressive myeloid populations including subsets of tumor-associated macrophages and heterogeneous immature myeloid cells with immune inhibitory properties termed MDSCs.[293]

Cancer cells can enhance the abundance of Tregs and suppressive myeloid populations through their conversion from other cell types, in addition to cytokine-mediated expansion and chemokine-mediated recruitment that exploits their characteristic chemokine receptor profiles.

KRAS overactivity in mouse and human cancer has been implicated in enhanced TGF-β/IL-10 production through MEK-ERK-AP1 signaling, resulting in conversion of $CD4^+$ T cell effectors to Tregs.[78,294] In general, this is subject to the activity of other cytokines such as IL-21.[295] Human cancer studies have reported TCR sharing between effector and Treg populations, although the degree of overlap is low,[296] and the contribution of Treg conversion to immune evasion is unknown.

Kras mutation may additionally enhance recruitment and expansion of Tregs and suppressive myeloid populations. For example, orthotopic implantation of *Kras-G12D* mutant pancreatic ductal epithelial cells resulted in expansion of immunosuppressive MDSCs, mediated by cancer cell production of granulocyte-macrophage colony-stimulating factor (GM-CSF),[297] suggesting a mechanism of immune evasion early in cancer development. Other labs have similarly reported MDSC recruitment in KRAS-driven mouse models of pancreatic cancer.[298] In a lung cancer model, *Kras-G12D* mutation was associated with recruitment of Tregs, MDSCs, and suppressive tumor-associated macrophages.[299] This was attributed to STAT3-mediated cancer cell IL-6 expression, with antibody

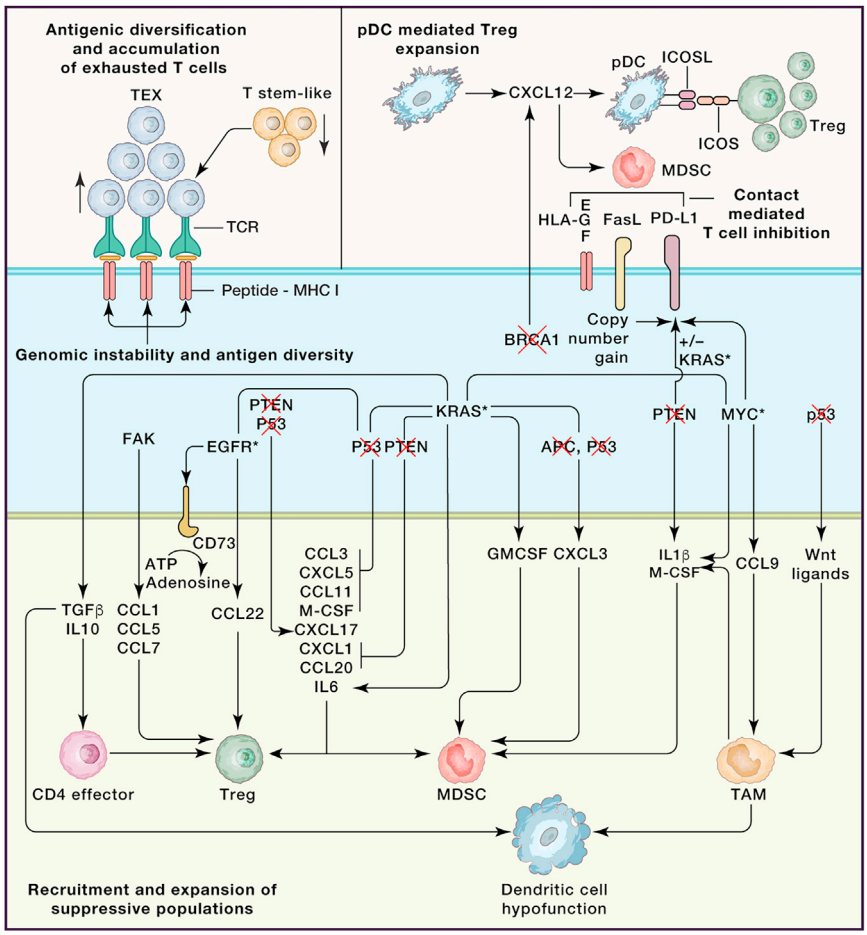

Figure 3. Suppressed attainment of T cell effector function

T cell inhibition can be caused by chronic antigen exposure and exhaustion, plasmacytoid-mediated regulatory T cell (Treg) expansion, recruitment and expansion of suppressive populations by cancer cell cytokine and chemokine production, contact-mediated T cell inhibition, and dendritic cell hypofunction. Genomic instability drives antigenic diversification, high antigen load, and T cell exhaustion. Exhausted T cells accumulate in the TME and may limit entry of stem-like cells required to sustain immune responses. Plasmacytoid DCs are recruited by CXCL12 expressed by CAFs and cancer cells, enhanced by *BRCA1* mutation. Treg expansion is driven by pDCs through ICOSL:ICOS interaction. Cancer cell chemokine and cytokine signaling recruits suppressive populations through FAK overactivity resulting in CCL1, CCL5, and CCL7 expression resulting in Treg recruitment; *EGFR* mutation resulting in CCL22 expression and Treg recruitment; recruitment of both myeloid-derived suppressor cells (MDSCs) and Tregs through *PTEN/TP53* mutation resulting in CXCL17 overexpression; *EGFR/TP53* or *KRAS/TP53* mutation resulting in overexpression of CCL3, CXCL5, CCL11, and M-CSF; KRAS/PTEN mutation resulting in CCL1 and CCL20 overexpression; and *KRAS* mutation driving IL-6. MDSC recruitment and expansion can be driven by mutations of *KRAS* resulting in increased GM-CSF production, mutation of *KRAS/APC/P53* resulting in CXCL3 overproduction, *PTEN* mutation, and IL-1β/M-CSF production and *KRAS/MYC* mutation resulting in IL-1β production. *KRAS/MYC* mutation can upregulate CCL9 that promotes tumor-associated macrophage (TAM) production of IL-1β, and TAM recruitment can also be increased by Wnt ligand production in *TP53*-mutated cancers. Treg expansion can also occur due to conversion from effectors through TGF-β and IL-10 signaling downstream of *KRAS* mutation. *EGFR* mutation additionally promotes CD73 expression and adenosine generation that can recruit Tregs among other immune suppressive actions. Contact mediated T cell inhibition can be through PD-L1 expression upregulated by *MYC* and *PTEN* mutation with or without *KRAS* mutation and copy-number gains. Expression of FasL can also promote contact inhibition. Dendritic cell hypofunction can be mediated by TAMs and TGF-β/IL-10.

blockade of this cytokine resulting in a reduced abundance of Tregs and suppressive myeloid populations. IL-6 may enhance proliferation of Tregs and MDSC recruitment in this context.[300] Combined *Kras* mutation and *Pten* loss is additionally associated with Treg and MDSC recruitment through NF-κB-mediated production of chemokines including CXCL1 and CCL20.[301] *Kras* mutation in a mouse colon cancer model of *Apc* and *Trp53* loss also resulted in MDSC recruitment through cancer cell loss of IRF2 and consequent upregulation of CXCL3 that binds MDSC expressed CXCR2.[302] Finally, the kinase FAK is upregulated in multiple cancer types and additionally enhanced Treg recruitment in a squamous cell carcinoma model associated with upregulation of chemokine-encoding genes such as *Ccl1*, *Ccl5*, and *Ccl7*.[303]

Other cancer-driver gene mutations are implicated in expansion and recruitment of suppressive populations. In addition to an association with reduced tumor mutational burden and limited effector T cell infiltration secondary to reduced production of chemoattractants, *EGFR* mutation in human lung adenocarcinoma is associated with Treg enrichment via upregulation of CCL22,[123] which acts through the Treg receptor CCR4.[304] *EGFR* mutation is also associated with a dampened immune environment in NSCLC and cancer cell expression of *NT5E* (encoding CD73),[305] driving adenosine production that enhances Treg function among other suppressive effects. In mouse prostate cancer models, enhanced recruitment of Tregs, MDSCs, and suppressive macrophages is described in the context of *Pten/Tp53* deficiency,[306] in keeping with a previous report that *Pten* deficiency enhances MDSC intratumoral expansion mediated by IL-1β and macrophage colony-stimulating factor (M-CSF) production.[307] Immune suppression due to enhanced IL-1β expression by intratumor macrophages is also described secondary to *Trp53* loss in a breast cancer model, associated with upregulated Wnt ligand secretion.[308] Deficiency of *Trp53* in the context of *Kras-G12D*-driven pancreatic or *EGFR*-mutated lung cancer models additionally is shown to promote recruitment of suppressive myeloid cells associated with production of CXCL1, CCL3, CXCL5, CCL11, and M-CSF.[309]

A variety of immune regulatory functions have been attributed to the *Myc* oncogene,[310] including recruitment of suppressive cell populations. In a mouse model of *Kras-G12D*-mutated lung cancer with inducible *Myc* expression, CCL9 upregulation resulted in recruitment of PD-L1 expressing, suppressive macrophages linked to T cell exclusion.[311] Upregulation of IL-1β expression

with potential effects on MDSC recruitment was additionally observed in a *Myc*-mutated model of mouse pancreatic cancer.[312]

In addition to potential effects on endothelial function and antigen presentation, *STK11* mutations are associated with recruitment of immunosuppressive neutrophils in mouse and human studies.[313,314]

Another mechanism by which cancer cells may promote Treg expansion and activation is through effects on pDCs. These are a heterogeneous population associated with production of type I IFNs (IFN-α and IFN-β) with roles in both anti-tumor and anti-viral immunity. However, pDCs also have a physiological role in maintaining peripheral tolerance associated with Treg induction.[315] In tumors, pDC accumulation is associated with cancer cell CXCL12 production,[316] described to be upregulated in *Brca* deficiency in a mouse breast cancer model and additionally associated with MDSC recruitment.[317] Abundance of pDCs is linked with worse outcomes, [318,319] which may partly be related to their role in driving Treg expansion, for instance through enhanced ICOSL signaling.[320–322] TGF-β- and PGE2-mediated downregulation of pDC type I IFN production is further associated with Treg expansion in mouse[323] and human studies.[324]

Downregulation of co-stimulatory ligands by immature or tolerogenic DCs can result in incomplete T cell activation and an anergic hypofunctional state[273] or deletion. In addition to effects on pDCs resulting in Treg expansion, cancer cells can directly and indirectly impair DC immunostimulatory function. In a mouse ovarian cancer model bearing *Kras-G12D/Trp53* mutations, the stimulatory potential of infiltrating DCs declined over time, associated with their increased arginase activity. Loss of DC stimulatory function was related to cancer cell-derived TGF-β and PGE2,[325] with similar findings in a lung cancer model.[326] In a study of the role of macrophages in a chemotherapy-resistant mouse breast cancer model, this population was a major source of IL-10, found to inhibit DC function through reduced IL-12 production required for optimal CD8 T cell activation.[327] Tumor produced factors other than cytokines also interfere with DC function. For instance, in murine tumor models, cancer cell production of the extracellular actin-binding protein gelsolin was found to inhibit cDC1 cross-presentation by blocking the DNGR-1 receptor, although the mechanisms regulating gelsolin production are not well known.[328]

Direct induction of T cell death and co-inhibitory signaling

In addition to production of soluble factors such as TGF-β, IL-10, and adenosine that have broad immune suppressive properties, cancer cells can directly block T cell acquisition of effector function through expression of co-inhibitory ligands and induction of cell death. PD-L1 is expressed downstream of IFN signaling[329] and is upregulated in association with tumor T cell infiltration,[330] as a mechanism of acquired immune resistance. Cancer cells can additionally upregulate PD-L1 signaling as a result of genetic defects. Upregulation of PD-L1 is attributed to *MYC* overactivity in various human cancers,[331] HER2 signaling through MEK in breast cancer,[332] and *KRAS* activation among patients with NSCLC[333] attributed to PD-L1 mRNA stabilization.[334] PD-L1 upregulation is observed in multiple human cancers lacking PTEN[335,336] and may additionally be upregulated due to copy-number gains at 9p24.1[337] and promoter demethylation.[338]

Cancer cells additionally upregulate FasL,[339–341] which can bind to the death receptor Fas expressed by activated T cells resulting in apoptosis through caspase 8 activation.[342] The molecular mechanisms regulating FasL overexpression are understudied. In general, although T cell death is important in multiple physiological mechanisms of tolerance, the contribution of T cell death receptor signaling to cancer immune evasion is unclear. Although studies have focused on agonizing death receptor signaling to drive cancer cell death, antagonistic antibodies to block T cell death have not been investigated in clinical trials. Other than Fas, T cell death may be mediated by other tumor necrosis factor (TNF) family receptors such as TNFR2, although this receptor can mediate both activation and death signaling.[343] Early phase clinical trials of both antagonistic (NCT05569057) and agonistic (NCT05238883) anti-TNFR2 antibodies are underway.

CONCLUDING REMARKS

Progress in single-cell techniques, genomics, and spatial approaches such as transcriptomics and imaging along with computational tools to analyze and integrate these high-dimensional datasets has enabled a rapid advance in our understanding of the tumor microenvironment. Analysis of human samples particularly in longitudinal and treatment studies has shed new light on the problem of cancer immune evasion. Ongoing autopsy studies are in addition gaining prominence.[344] The picture that emerges is of heterogeneity at multiple levels of organization (cell phenotypic, genomic, and spatial) and the complexity of cellular interactions through which cancers redirect physiological mechanisms to impose a state of immune tolerance necessary for their survival. These mechanisms broadly center around impairments of T cell tissue localization, antigen encounter, and acquisition of effector function (Figure 4).

By analogy with peripheral tolerance, three themes emerge. First, immune evasion results from layered, overlapping mechanisms that act in concert within an individual tumor, rather than single critical points of control, and these show tissue specificity. Second, the final common pathways of tolerance can be acquired through diverse mechanisms that are hierarchically organized and can often be traced back to key cancer cell-intrinsic properties. Third, cancer cell evolution convergences on immune evasion strategies that either replicate or mimic pathways of peripheral tolerance. This suggests the study of physiological tolerance mechanisms, and their breakdown in autoimmunity may provide further insights into cancer immune evasion pathways.

In cancer, the notion of redundancy and tissue specificity in immune evasion mechanisms is supported by the observation that although targeting individual nodes (e.g., the inhibitory PD-1/PD-L1 interaction) can be effective, only a subset of patients achieve a durable remission. This suggests an underlying utilization of multiple immune evasion mechanisms. Defining evasion signatures will enable better patient classification into biologically relevant subgroups to aid rational therapy selection. This notion is strikingly illustrated among patients with colorectal cancer treated with anti-PD-1 immunotherapy. Although responses are rare among patients with DNA mismatch repair (MMR)-proficient tumors,[19] the relatively small subgroup of patients with defective MMR, associated with high mutational burden and neoantigen load, obtain a high rate of response and a significant survival benefit.[345]

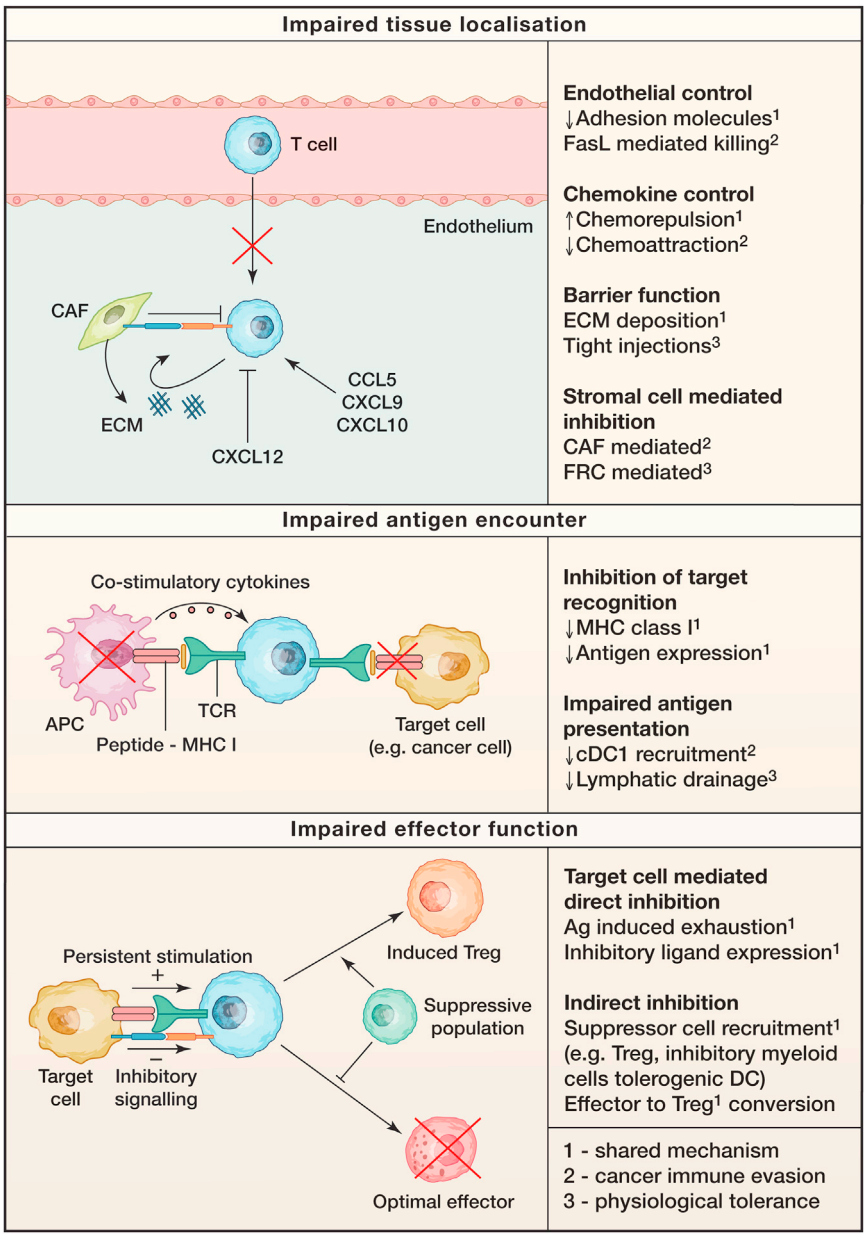

Figure 4. Pillars of physiological tolerance and cancer immune evasion

Selected mechanisms are represented in each category, denoting whether they relate to physiological immune tolerance, cancer immune evasion, or both. FasL, Fas ligand; CAF, cancer-associated fibroblast; ECM, extracellular matrix; FRC, fibroblastic reticular cell; cDC1, conventional dendritic cell 1; Treg, regulatory T cell.

Even in clinical trials considered to be negative, the minority of patients who benefit may belong to subgroups that are currently unidentified. This suggests a need for more sophisticated means to stratify patients into therapeutically relevant groups characterized by immune evasion signatures.

Several studies have suggested the outlines of how evasion signatures may be defined. For instance, by categorizing tumors according to the pattern of T cell infiltration,[35] a combination of PD-L1 expression and T cell infiltration,[346] tumor mutational burden and T cell infiltration,[347] or the evaluation of parameters including immunogenicity, metabolic state, and inhibitory features among others.[348] A framework considering T cell localization, antigen recognition, and acquisition of effector function along with subprocesses within these categories may be helpful.

A major barrier to translation is that in contrast to the capabilities of modern high-dimensional and single-cell approaches to interrogating sample biology, routine diagnostics are limited by tissue sample availability and the relatively low-resolution analysis techniques currently employed. For example, immune evasion mechanisms evolve over time as demonstrated by the acquisition of treatment resistance, but same site repeat biopsies are challenging in the routine setting. Furthermore, analysis of fresh tissue at a single-cell level is impractically time-consuming and resource intensive. Finally, routine analysis of more readily available histopathology and radiology data generally involves visual assessment with limited quantification.

These factors limit the resolution with which immune escape patterns and their underlying mechanisms can be identified and tracked over time. Advances in artificial intelligence applied to routinely collected data, for instance in the fields of digital pathology[349] and radiomics, along with the development of infrastructure to support genomic medicine, will offer new tools to enhance the capability of routine diagnostics. A key area of future research is how to leverage and integrate these advances to enable higher-resolution characterization of cancer immune evasion mechanisms to enhance routine care.

This will require clinical trials to deeply characterize known mechanisms using existing high-dimensional approaches, along with integrated analysis of paired routinely gathered data to generate multi-modal signatures of actionable immune evasion phenotypes. The importance of intratumoral heterogeneity is increasingly well recognized and should be addressed as part of these efforts. Patient subclassification in this manner will guide rational therapy selection and provide an expandable framework for future clinical trials, for instance as part of multi-arm multi-stage designs capable of testing a number of interventions in different subgroups in an efficient manner.[350]

Despite huge progress in our understanding of immune evasion mechanisms within the complex ecosystem of the

TME, it is striking that further translational progress remains relatively limited in the era of checkpoint immunotherapy. Although a deeper mechanistic understanding has led to the development of agents with a high degree of target specificity, arguably the greatest therapeutic advance has come from combining broadly acting cytotoxic chemotherapy and checkpoint immunotherapy for patients with lung,[351] breast,[18] and upper gastrointestinal tract malignancies.[352] The mechanisms underlying why this combination is effective are incompletely understood, and chemotherapy has multiple potential impacts on cancer-immune interactions. One possible explanation is that chemotherapy has a lymphodepleting effect on intratumor immune cell populations, allowing entry and expansion of new clones[226] driven by checkpoint immunotherapy. Alternatively, chemotherapy may encourage immunogenic cell death,[353] resulting in enhanced priming or increase or alter neoantigen expression on MHC class I.[354] Finally, chemotherapy may have immunomodulatory effects by acting on Tregs,[355] MDSCs,[356] and DCs.[357] Chemotherapy-induced perturbation of multiple pathways may be relevant to the enhanced efficacy of chemoimmunotherapy combinations, in the context of overlapping and redundant immune evasion mechanisms.

Although current treatment protocols have combined pre-existing chemotherapy regimens with checkpoint immunotherapy, future work should focus on determining the optimal cytotoxic agents for combination based on their modulatory effects, along with work to optimize their dosage,[357] frequency, and duration of therapy to enhance potential synergistic effects and minimize toxicities of combination therapy. Recent advances in clinical trial design to determine optimal treatment parameters should aid these efforts.[358,359]

In general, immune evasion mechanisms are hierarchically organized: for example, oncogene activation can drive expression of chemokines that recruit suppressive cell populations resulting in inhibition of T cell activity through the activity of key effector molecules. Which level is most sensitive to therapeutic intervention with targeted agents and how many nodes need to be targeted? Driver mutations are appealing targets in this regard, as top-level central nodes are implicated in multiple immune evasion mechanisms. However, progress toward improving therapeutic outcomes by combining small molecule inhibitors with checkpoint immunotherapy has been limited. For instance, multiple early phase studies of EGFR inhibitors plus checkpoint immunotherapy agents including anti-PD-L1 and anti-PD-1-directed agents have shown response rates and progression-free survival broadly similar to what is observed with EGFR inhibitors alone,[360] suggesting redundant evasion mechanisms as one explanation. Alternatively, once the TME is programmed, the suppressive state is likely maintained by other factors, and targeting oncogenic signaling may no longer be effective to reverse this. Trials exploring combinations of PI3Kβ inhibitors with anti-PD-1 agents for *PTEN*-mutated cancers[361,362] and *KRAS-G12C* inhibitors such as adagrasib or sotorasib plus anti-PD-1 or anti-PD-L1[363] are ongoing and will hopefully shed light on this.

Future progress may come from combinations that target key adverse features of the suppressive TME, for instance, the abundance and activity of immune inhibitory cell populations. Tregs are one particularly attractive target in this regard. This population may be targeted with agents that interfere with their chemokine receptor-mediated recruitment,[364] the conversion from effectors for instance through TGF-β signaling,[365] and functional activity for instance by blocking adenosine production or signaling.[265] The effects of anti-CTLA-4 may partly be mediated through intratumor Treg depletion,[291] and a depleting anti-CD25 antibody that exploits the potential for this approach[290,292] is currently in clinical trials. The strategy of Treg depletion is particularly attractive in combination with modalities that enhance T cell infiltration because these approaches may equally recruit this population. However, it is likely that combinations of several agents may eventually be required.

Overall, translation of key findings to clinical practice will likely require an approach that accounts for the particular pattern of evasion mechanisms employed by each tumor, considering microenvironmental heterogeneity and its evolution over time and with therapy. Although targeting individual nodes may be effective in selected subgroups, negative immune regulatory networks are likely sustained with a high degree of redundancy, and targeting more broadly acting processes or use of multiple drug combinations may be required. Such approaches will require careful consideration of toxicities. Better patient stratification tools are required, leveraging routinely collected data in the context of modern trial designs that can efficiently test multiple hypotheses in platform studies.

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