Cancer Immunology Research

T Cell–Inflamed versus Non-T Cell–Inflamed Tumors: A Conceptual Framework for Cancer Immunotherapy Drug Development and Combination Therapy Selection

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Abstract

Immunotherapies such as checkpoint-blocking antibodies and adoptive cell transfer are emerging as treatments for a growing number of cancers. Despite clinical activity of immunotherapies across a range of cancer types, the majority of patients fail to respond to these treatments and resistance mechanisms remain incompletely defined. Responses to immunotherapy preferentially occur in tumors with a preexisting antitumor T-cell response that can most robustly be measured via expression of dendritic cell and $CD8^+$ T cell–associated genes. The tumor subset with high expression of this signature has been described as the T cell–"inflamed" phenotype. Segregating tumors by expression of the inflamed signature may help predict immunotherapy responsiveness. Understanding mechanisms of resistance in both the T cell–inflamed and noninflamed subsets of tumors will be critical in overcoming treatment failure and expanding the proportion of patients responding to current immunotherapies. To maximize the impact of immunotherapy drug development, pretreatment stratification of targets associated with either the T cell–inflamed or noninflamed tumor microenvironment should be employed. Similarly, biomarkers predictive of responsiveness to specific immunomodulatory therapies should guide therapy selection in a growing landscape of treatment options. Combination strategies may ultimately require converting non-T cell–inflamed tumors into T cell–inflamed tumors as a means to sensitize tumors to therapies dependent on T-cell killing. *Cancer Immunol Res; 6(9); 990–1000. ©2018 AACR.*

Introduction

The immune system can detect and eradicate cancer cells. However, tumors acquire genetic mutations, induce immunosuppressive signaling pathways, and undergo epigenetic changes that lead to resistant phenotypes. This resistance manifests as a capacity to avoid immune recognition or disable antitumor components of immunity. At baseline, spontaneous antitumor T-cell response occurs in a fraction of patients with solid tumors. Although cancer in these patients continues to progress, the beneficial effect of antitumor immune engagement may persist during tumor progression. The biological processes associated with this spontaneous, although inadequate, induction of antitumor immunity correlate with improved clinical outcomes and may predict responsiveness to immunotherapy (1–3).

Across cancer, the majority of tumors lack a robust T-cell infiltrate prior to treatment. It is not clear why T cells infiltrate some tumors and not others. Fundamental to answering this question is a more thorough understanding of the essential events leading to a spontaneous antitumor T-cell response (Figure 1). Innate immune recognition of incipient neoplasms and activation of type I IFN signaling are among the most proximal events required to generate a *de novo* T-cell responses (4, 5). One major mediator of type I IFN generation is the cGAS/STING (stimulator of IFN genes) pathway, which is activated by cytosolic tumor-derived DNA. Activation of STING mediates innate immune sensing of cancer cells by tumor-infiltrating antigen-presenting cells (APCs; ref. 6). STING pathway activation in the tumor microenvironment leads to downstream type I IFN production, resulting in the recruitment and activation of dendritic cells, including the Batf3 (basic leucine zipper transcription factor ATF-like 3)-driven subset (4, 7). In turn, Batf3-lineage dendritic cells cross-present tumor-derived antigen to $CD8^+$ T cells and regulate T-cell recruitment to tumors (4, 8). To eradicate cancer cells, $CD8^+$ T cells must become appropriately activated, traffic to tumor tissue, overcome local mechanisms of immunosuppression, and maintain their effector function.

Negative regulatory pathways, such as programmed death 1 (PD-1)/ligand-1 (PD-L1) and cytotoxic T lymphocyte–associated protein 4 (CTLA-4), can be exploited by cancers to evade immune-mediated tumor clearance. Pharmacologic inhibitors of these checkpoints can reinvigorate tumor-reactive T cells and restore T cell–mediated tumor regression. Antibodies that target PD-1 or its ligand PD-L1 have demonstrated broad applicability across tumor types and have gained regulatory approval across several cancer types. Although some patients achieve dramatic and durable clinical responses with checkpoint inhibitors, most patients do not benefit from these treatments. Therefore, there remains a need to identify mediators of successful responses and resistance. The presence of a preexisting gene expression signature associated with dendritic cell and T-cell infiltrates within the tumor

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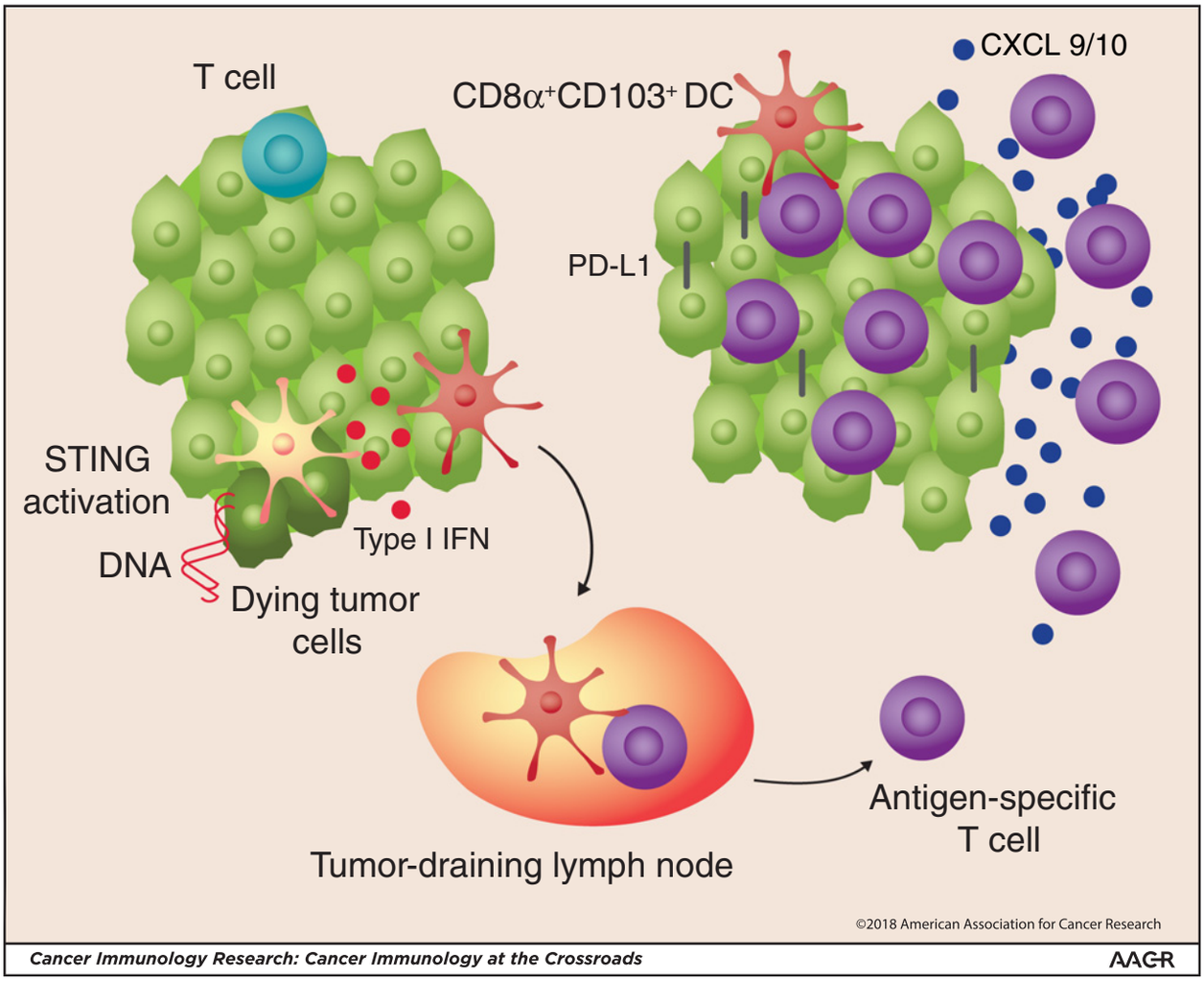


Figure 1.

Development of a spontaneous antitumor response and a T cell–inflamed tumor microenvironment. APCs take up tumor-derived DNA. Cytosolic DNA activates the STING pathway, resulting in the production of type I IFNs and recruitment and activation of Batf3-lineage DCs that express CD8α or CD103. In turn, an innate immune cascade is initiated leading to antigen presentation, cross-priming in the tumor-draining lymph nodes, and eventually recruitment of $CD8^+$ T cells to the tumor microenvironment. Tumor antigen-specific T cells are recruited by the chemokines CXCL9 and CXCL10. If the tumor is not eliminated, then T cells become dysfunctional and PD-L1 upregulation by tumor cells and immune-infiltrating cells is observed. The resulting adaptive immune response is damped by this counterregulation, which is characteristic of a T cell–inflamed tumor microenvironment.

microenvironment positively correlates with clinical response to several immunotherapies, including vaccines, ILs, and T-cell checkpoint blockade (9–11). Thus, understanding why tumors fail to develop this T cell–inflamed phenotype is paramount to combating treatment failure for many patients.

Tumors Develop along a Spectrum of T Cell–Inflamed to Non-T Cell–Inflamed Phenotypes

Gene expression profiling of baseline biopsies of melanoma metastases has revealed two major subsets of tumors characterized by high (T cell–inflamed) or low (non-T cell–inflamed) expression of genes indicative of a preexisting T-cell infiltrate (12, 13). T cell–inflamed tumors are characterized by type I IFN activation, immune potentiating chemokines that attract T cells, antigen presentation, cytotoxic effector molecules, and $CD8^+$ T cells (Fig. 1; ref. 13). These infiltrating $CD8^+$ T cells commonly have a dysfunctional phenotype, partially explaining the paradox of tumor progression despite the presence of cytotoxic T cells. Immune inhibitory pathways induced by IFNs, reflected by, for example, expression of PD-L1 and indoleamine-2, 3-dioxygenase (IDO) as well as by higher proportions of $FOXP3^+$ regulatory T cells (Tregs), are also present in the T cell–inflamed microenvironment (14). Mechanistic studies indicate that IFNγ produced from tumor-infiltrating T cells is required for the upregulation of these inhibitory factors. In contrast, the non-T cell–inflamed phenotype lacks expression of the type I IFN signature, $CD8^+$ T cells, and IFN-inducible inhibitory factors. Applying this model broadly, gene expression profiling, centered on IFNγ response, of

Table 1. Established gene signatures indicative of a T cell–inflamed tumor microenvironment

Gene signature	Genes
Gajewski T cell–inflamed signature (13)	IRF1, CD8A, CCL2, CCL3, CCL4, CXCL9, CXCL10, ICOS, GZMK, HLA-DMA, HLA-DMB, HLA-DOA, HLA-DOB
IFNγ-related gene signature (11)	CD8A, CCL5, CD27, CD274, PDCD1LG2, CD276, CMKLR1, CXCL9, CXCR6, HLA.DQA1, HLA.DRB1, HLA.E, IDO1, LAG3, NKG7, PSMB10, STAT1, TIGIT
T effector signature (81)	GZMA, GZMB, PRF1, EOMES, IFNG, TNF, CXCL9, CXCL10, CD8A, CD4, FOXP3, ICOS, CTLA4
Immune cytolytic activity (82)	GZMA, PRF

30 cancer types within The Cancer Genome Atlas (TCGA) database has revealed that all tumor types segregate into T cell–inflamed and non-T cell–inflamed subsets (15). This effect appears to be independent of nonsynonymous mutational load, indicating that failure to develop spontaneous T-cell infiltration is unlikely solely due to paucity of T-cell antigens. Rather, non-T cell–inflamed tumors lack evidence of antigen-presenting machinery, which is critical for $CD8^+$ T-cell priming (15).

A baseline T cell–inflamed tumor microenvironment correlates with responsiveness to checkpoint blockade and adoptive cell therapy (11, 16). In contrast, the non-T cell–inflamed phenotype correlates with treatment resistance. Gene expression profiling of melanoma biopsies collected from patients before ipilimumab treatment indicates that tumors with preexisting immune activation are more likely to respond to this drug (16). Similarly, multiple T cell–inflamed gene signatures (Table 1) correlate with clinical response (or inversely with lack of response) to PD-1/L1 blockade across a variety of tumor types (11). These T cell–inflamed gene signatures could provide a clinical-grade predictive tool to evaluate patient samples in ongoing clinical trials and inform use of antibodies to PD-1/L1 (11). However, it is also clear that although the T cell–inflamed signature is associated with responses, it is not always sufficient to achieve clinical benefit from anti–PD-1/L1. Nonresponding patients appear to fall into at least two categories: those lacking a preexisting inflamed phenotype and those possessing an inadequate antitumor T-cell response that failed to control tumors upon anti–PD-1 therapy. These findings raise the possibility that distinct mechanisms of resistance mediate treatment failure in the inflamed compared with non-T cell–inflamed tumors.

Elucidating the mechanisms that determine why a given tumor possesses a T cell–inflamed or non-T cell–inflamed microenvironment should enable the development of therapeutic solutions to combat resistance to immunotherapy. Conceptually, resistance encompasses both tumor cell-intrinsic and tumor-extrinsic factors. Tumor cell-intrinsic factors could include genomic alterations in cancer cells, including somatic mutations in immune genes, antigen repertoire, and distinct oncogenic pathways that mediate immune evasion. Tumor cell-extrinsic factors of resistance may encompass nonmalignant cells, stromal tissue, and the molecular and cellular compartments of the immune system, including inhibitory immune checkpoints. Germline genetic differences, including polymorphisms in immune regulatory genes, may also impact tumor control (17, 18), whereas environmental factors, such as commensal microbiota, further modulate host immune function, potentially determining responsiveness to immune checkpoint therapy (19–21). Efforts are currently underway to enable T-cell trafficking into noninflamed tumors and restore immune function by targeting the various factors that regulate antitumor immunity.

Loss of Antigen Presentation and Defective IFNγ Signaling Mediate Tumor Resistance

A significant proportion of patients who initially respond to anti–CTLA-4 or anti–PD-1 antibodies will relapse over time, despite receiving continued therapy. Cancer cells may escape T-cell recognition due to defective tumor antigen presentation via genetic or epigenetic changes affecting the antigens themselves or the antigen presentation machinery. Early studies found evidence that patients with melanoma who initially responded to immunotherapies with cytokines and adoptive T cell–based therapies might have developed acquired resistance through loss of β_2-microglobulin (B2M), the required subunit necessary for surface expression of MHCI (22). Loss or disabling mutations of both copies of B2M is likely an efficient mechanism for tumor escape from T-cell recognition, because stable presentation of peptide antigen by MHC class I molecules does not occur in the absence of B2M. Analysis of longitudinal tumor biopsies from patients with metastatic melanoma treated with either anti–CTLA-4 or anti–PD-1 therapy identified a subset of patients who initially responded and then progressed with resistant tumors no longer expressing B2M (23). In this group of patients, B2M defects were enriched in pretreatment biopsies from nonresponders relative to responders. One case of metastatic melanoma with acquired resistance to PD-1 blockade was associated with loss of B2M (24). In addition, acquired B2M loss has been identified in one patient with lung cancer who developed resistance to combination therapy with anti–CTLA-4 and anti–PD-1, and in resistant brain metastases in two patients with mismatch repair–deficient colorectal cancer that acquired resistance to PD-1 inhibitors (25, 26). Thus, B2M loss contributes to a T cell–resistant phenotype and may mediate resistance to immune checkpoint inhibitors.

Loss of function mutations in B2M and HLA genes has been observed in a range of cancers, and might be more likely to arise under selective pressure imposed by the immune system (27–30). A case study described a patient with metastatic colorectal cancer who developed secondary resistance to adoptive T-cell therapy targeting mutated KRAS, presumably through loss-of-heterozygosity of HLA-C*08:02, the restriction element required for recognition by KRAS-specific T cells (31). Having greater diversity (heterozygosity) in the repertoire of HLA-genes (HLA-A, -B, -C) compared with homozygosity for one or more HLA genes was associated with improved overall survival after anti–CTLA-4 and/or anti–PD-1 blockade (32). These findings suggest that the success of immune checkpoint inhibitors appears to be in part dependent on expressing and maintaining the maximum number of different HLA alleles, conferring a greater ability to present diverse tumor antigens to T cells. In most cases, the initial mutations at HLA loci are not likely to be sufficient to result in resistance to immune checkpoint inhibitors; however, HLA mutations indicate that the tumors are on a resistance continuum and are evolving a means of immune escape even prior to therapy.

Defective IFNγ signaling in cancer cells has been reported in patients unresponsive to immune checkpoint inhibitors. Genomic defects in IFNγ pathway genes occurred at higher frequencies in melanoma tumors from anti–CTLA-4 nonresponders relative to responders (33). Defects in IFNγ signaling, such as through

inactivating mutations in Janus kinases (JAK1 or JAK2), has likewise been proposed to contribute to resistance to anti–PD-1 therapy (24, 34, 35). Two patients with melanoma who initially responded to anti–PD-1 but later relapsed were discovered to have mutations in JAK1 or JAK2 (24). Genome-scale CRISPR-Cas9 mutagenesis screens of cancer cells have confirmed that defects in antigen processing and presentation machinery and IFNγ signaling promote resistance to T cell–based immunotherapies (36, 37). The deleterious consequences of defective IFNγ signaling are multifactorial, and likely involve resistance to the antiproliferative effects of IFNγ, impaired antigen presentation, the inability to reactively express PD-L1, and failed recruitment of T cells into the tumor.

Tumor-Intrinsic Oncogene Pathways Mediate T-cell Exclusion

Genetic and epigenetic alterations in cancer cells function beyond controlling intrinsic properties of cellular biology, such as cell growth and survival. They additionally may modulate immune cell function to evade destruction (38). Comparing the mutational landscape of tumors to the presence of an activated immune response has led to the identification of tumor-intrinsic oncogenic pathways that appear to mediate T-cell exclusion from the tumor microenvironment. It appears that oncogenic pathways may be major determinants of primary and secondary resistance to immunotherapy. Therefore, it might be useful to target these oncogenic pathways pharmacologically, both for direct cytotoxic effects and to promote antitumor immune responses.

The first tumor-intrinsic oncogene pathway mediating immune exclusion identified was the WNT/β-catenin pathway in metastatic melanoma. This observation was made after analysis of a metastatic melanoma dataset from TCGA wherein each sample was categorized as either T cell–inflamed or non-T cell–inflamed (13). Comparing these samples on the basis of RNA expression, DNA exome sequencing, and pathway analysis revealed that nearly one half of the non-T cell–inflamed tumor subset showed increased activation of the WNT/β-catenin signaling pathway. Mechanistic studies using genetically engineered mouse models confirmed that melanomas with increased WNT/β-catenin activation lacked tumor-infiltrating T cells, mimicking the noninflamed phenotype observed in patients with melanoma (8, 13). This effect was due to failed recruitment of Batf3-lineage dendritic cells into tumors, leading to impaired recruitment of T cells to the tumor microenvironment and resistance to checkpoint blockade and adoptive T-cell transfer (8, 13). A similar analysis of urothelial bladder cancers in TCGA database revealed that non-T cell–inflamed bladder tumors associated with increased WNT/β-catenin pathway activation (39). Thus, pharmacologic inhibition of WNT/β-catenin signaling may be a therapeutic strategy to restore T-cell entry into at least some non-T cell–inflamed tumors and facilitate response to checkpoint inhibitors. The prevalence of aberrant WNT/β-catenin pathway activation across cancer types is not yet definitively defined, but is likely relevant for other tumor types (40).

Multiple oncogene pathways, beyond WNT/β-catenin, likely give rise to immune resistance (Table 2). Also, loss of the tumor suppressor PTEN in metastatic melanoma correlates with decreased intratumoral T-cell infiltration and reduced responsiveness to PD-1 inhibitor therapy (41). The non-T cell–inflamed phenotype is significantly associated with PTEN gene deletions and loss-of-function mutations, which may correspond with increased activation of the PI3K/AKT pathway. In preclinical murine studies where spontaneously induced melanomas lack PTEN expression, treatment with a PI3Kβ inhibitor improved the efficacy of checkpoint blockade (anti–PD-1 and anti–CTLA-4; ref. 41). These findings provide a rationale for developing a therapeutic approach using specific PI3K inhibitors in combination with checkpoint inhibitors for melanomas with PTEN loss. Genetic alterations in PTEN and the WNT/β-catenin pathway appear to be mostly nonoverlapping (only 2% of noninflamed melanomas possessed alterations in both pathways), indicating that these may represent distinct mechanisms of immune evasion (41).

Table 2. Oncogenic pathways associated with immune exclusion and immunosuppression within the tumor microenvironment and their relevant cancer types

Oncogenic pathways	Cancer type
WNT/β-catenin activation	Melanoma (75) Bladder cancer (39)
PTEN loss/PI3K-AKT activation	Melanoma (41) Glioma (83) Sarcoma (84)
PPARγ/RXRα activation	Bladder cancer (39, 42)
Isocitrate dehydrogenase gain-of-function mutations (IDH1 and IDH2)	Lower grade gliomas (51)
FGFR3 activation	Bladder cancer (39)
MYC activation	Acute lymphoblastic leukemia, hepatocellular carcinoma, melanoma, NSCLC (43)
STAT3 oncogenic signaling	NSCLC (85)
AXL receptor tyrosine kinase expression	Breast cancer (86) Melanoma (87)
LKB1 (also known as STK11) loss of function	Endometrial cancer (49) NSCLC (50)
TP53 loss of function	Breast cancer (estrogen receptor-negative; ref. 45)

Beyond metastatic melanoma, tumor-intrinsic oncogenic signaling pathways associated with non-T cell–inflamed tumors have also been identified in other malignancies. Studies of T-cell exclusion in muscle-invasive bladder cancer have identified the peroxisome proliferator-activated receptor γ (PPARγ) and FGFR3 pathways, in addition to WNT/β-catenin signaling, as possible drivers of the non-T cell–inflamed phenotype (39). Mechanistic studies using an *in vivo* tumor model suggest that increased PPARγ/retinoid x receptor alpha (RXRα) activity within tumor tissue results in reduced $CD8^+$ T-cell infiltration and contributes to immunotherapy resistance (42). Activation of the PPARγ pathway *in vitro* leads to a reduction in IL6, IL8, CCL2, CCL5, TNF, and CXCL10. Conversely, pharmacologic inhibition of PPARγ increases expression of CCL2 and IL8 (42). Additional preclinical studies are needed to assess whether pharmacologic inhibition of PPARγ restores T-cell infiltration and response to immunotherapy against tumors with increased PPARγ/RXRα activity.

The MYC oncogene regulates tumor cell expression of two immune checkpoints involved in cancer cell immune evasion, PD-L1 and CD47 (43), the latter of which is an antiphagocytic protein that inhibits the ingestion of tumor cells by macrophages and dendritic cells, ultimately impairing the ability of APCs to prime effector T cells. In an animal model of MYC-induced T-cell acute lymphoblastic leukemia (T-ALL), MYC overexpression

resulted in upregulation of PD-L1 and CD47 by tumor cells, whereas MYC inactivation in tumors decreased expression of these molecules and enhanced the antitumor immune response (43). MYC also regulates the expression of PD-L1 and CD47 in human cancer types, including T-ALL, hepatocellular carcinoma, melanoma, and non–small cell lung cancer (NSCLC; ref. 43). It remains to be determined whether MYC-driven cancers are more susceptible to PD-1/L1 blockade or whether therapeutic CD47-blocking antibodies (44) have activity against such tumors.

Inactivating mutations in specific tumor suppressor genes have been associated with reduced immune infiltration. For example, in a cohort of patients with breast cancer, loss of heterozygosity or mutations in TP53 in a subset of estrogen receptor (ER)–negative breast cancer and basal-like breast tumors is associated with decreased expression of cytotoxic T-cell signature genes, corresponding to low T-cell infiltration (45). Confirming a mechanistic link between TP53 status and immune infiltration in ER-negative breast cancer will require additional functional studies; however, mechanistic data from a different tumor context have linked p53 status and innate immune activation (46). In a p53-deficient murine model of hepatocarcinoma, restoration of p53 expression resulted in upregulation of inflammatory cytokines, an intratumoral innate immune response and tumor regression.

The serine/threonine liver kinase B1 (LKB1; also known as STK11) is a tumor suppressor gene mutated in a diverse range of cancer types, and LKB1 loss has been associated with worse prognosis in several cancer types (47, 48). Loss of LKB1 may mediate immune evasion through the recruitment of inhibitory cell populations to the tumor microenvironment. Analysis of LKB1-deficient human endometrial tumors revealed increased expression of the chemokine CCL2 by tumor cells and increased macrophage density in the tumor microenvironment (49). In a model of murine endometrial cancer, homozygous deletion of LKB1 in endometrial epithelium resulted in aberrant CCL2 expression by tumors, facilitating recruitment of protumorigenic macrophages. In a KRAS-driven NSCLC model, loss of LKB1 resulted in elevated IL6 production, increased intratumoral accumulation of immunosuppressive neutrophils, and reduced tumor-infiltrating lymphocytes (50). Phenotypic analysis of the $CD4^+$ and $CD8^+$ T cells within these $LKB1^{-/-}$ tumors revealed higher expression of PD-1, CTLA-4, TIM-3, and LAG-3, indicative of a dysfunctional phenotypic state. However, treatment of these tumors with blocking antibodies against PD-1, CTLA-4, and TIM-3 did not show efficacy, indicating that these checkpoint pathways are not major determinants of the immune evasion mediated by tumor-intrinsic LKB1 loss, at least in this model. Treatment with a neutrophil-depleting antibody or an IL6-blocking antibody resulted in increased T-cell infiltration into LKB1-null tumors and improved tumor control. Collectively, these findings suggest that targeting aberrant cytokine/chemokine signaling or immunosuppressive cell populations might be a promising therapeutic strategy against LKB1-deficient tumors.

Mutations in the isocitrate dehydrogenase *IDH1* and *IDH2* genes may facilitate escape from immunosurveillance in a subset of malignant gliomas (51). Mutations in IDH are frequent in low-grade gliomas, occurring in up to 70% to 80% of these tumors (52). Mutations in IDH confer gain-of-function activity by converting α-ketoglutarate to the oncometabolite R-2-hydroxyglutarate, which coordinates epigenetic changes that promote malignant transformation (53). Gene expression profiling of gliomas revealed reduced expression of T cell–associated genes and IFNγ-inducible chemokines in the IDH-mutated gliomas compared with gliomas expressing wild-type IDH (51). Mice possessing gliomas with mutated IDH recapitulated the immunophenotype of human IDH-mutated gliomas and demonstrated poor $CD8^+$ T-cell infiltration. R-2-hydroxyglutarate limited intratumoral production of chemokines CXCL9 and CXCL10, resulting in decreased T-cell recruitment into murine IDH-mutated gliomas. A selective inhibitor that blocks the ability of mutated IDH-1 to produce the oncometabolite R-2-hydroxyglutarate restored chemokine expression, promoted T-cell infiltration, and increased the efficacy of therapeutic peptide vaccination against IDH-mutated gliomas instilled in the brains of mice (51). Thus, inhibitors of mutant IDH are potential therapeutic candidates to reverse immune evasion in gliomas and might be integrated into immunotherapies for patients with IDH-mutated tumors.

Activation of oncogenes or loss of tumor suppressor genes not only exerts an intrinsic influence on the biology and behavior of cancer cells, but appears to also interfere with the induction and effector function of antitumor immune responses. Whether acquiring immune evasive oncogenic pathways following initial response to immunotherapy drives tumor recurrence is not known. Increasing evidence indicates that it might be possible to therapeutically target a particular oncogenic signaling pathway operating within a non-T cell–inflamed tumor to reengage antitumor immune defenses. Future studies are needed to gain a more comprehensive understanding of the various oncogenic-driven mechanisms of immune escape across cancer types.

Genomic Determinants of Response

Genomic determinants of T-cell reactivity against the tumor include tumor mutational load (total number of mutations per coding region of tumor genome) and neoantigen density. Immune checkpoint inhibitors have shown efficacy in melanoma and NSCLC, cancers that harbor higher numbers of somatic mutations relative to other tumor types. In addition, the number of somatic nonsynonymous mutations tends to be higher in patients who derive durable clinical benefit from CTLA-4 inhibitors in melanoma (54) and PD-1 inhibitors in NSCLC (55). On the other hand, high mutation burden is also not sufficient to drive a response to immune checkpoint blockade: A subset of nonresponders had a high tumor mutation load and some responders expressed few mutations. The absolute or threshold number of nonsynonymous mutations needed by a specific tumor type to predict responsiveness remains unclear, limiting the use of tumor mutation burden as a predictor of response to immune checkpoint inhibitors.

DNA repair deficiency has emerged as a predictive biomarker of response to anti–PD-1 therapy (56). Tumors with DNA mismatch repair (MMR) defects, such as microsatellite instability (MSI)-high/MMR-deficient cancers, have an exceptionally high number of somatic mutations and are highly responsive to anti–PD-1 blockade (26, 57). Accordingly, pembrolizumab has gained approval for use in MSI-high/MMR-deficient solid tumors independent of the cancer tissue of origin. Factors beyond tumor mutation load affect endogenous infiltration in a range of tumor types as suggested by prior data showing no correlation between the presence of the T cell–inflamed phenotype and the number of nonsynonymous mutations observed (15). In a genomic analysis of colorectal cancers, a proportion of untreated MSI-high colorectal cancers had mutations associated with WNT/β-catenin

activation and a corresponding decrease in tumor-infiltrating lymphocytes (30). A high frequency of mutations was also identified in antigen presentation machinery in MSI-high tumors. Additional studies are needed to confirm whether these mutations in MSI-high tumors correlate with resistance to anti–PD-1 therapy. Immune checkpoint inhibitors might have activity in other DNA repair–deficient settings, beyond MSI, including tumors with mutations in BRCA1/2 and DNA damage sensor kinases. Response rates to anti–PD-1 therapy in urothelial cancers with deleterious alterations in DNA damage response and repair genes were higher than in tumors lacking these mutations (58).

A focus has been placed on interrogating baseline tumor biopsies for genomic and immune predictors of response and resistance to immunotherapies. When feasible, longitudinal analysis of tumor prior to initiating treatment, early on treatment, and at disease progression may help to identify better predictive biomarkers and to uncover mechanisms of response and resistance. In a cohort of patients with melanoma treated with sequential anti–CTLA-4 followed by PD-1 blockade, analyses of early on-treatment biopsies (i.e., within the first 2–3 cycles of treatment) revealed that response to therapy correlated with an influx of $CD8^+$ T cells, upregulation of regulatory molecules (PD-1, PD-L1, LAG3), expression of antigen presentation molecules, activation, and higher clonality (narrow TCR repertoire, more clonal population; refs. 59–61). In contrast, immune profiling of pretreatment biopsies did not predict clinical response to sequential monotherapy in this cohort of patients. The peripheral blood can also readily be sampled to identify immune profiles associated with therapeutic efficacy. A report using high-dimensional single-cell mass cytometry on peripheral blood samples of patients with melanoma before and while on therapy found that response to anti–PDL-1 correlated with increased frequency of monocytes ($CD14^+CD16^-HLA\text{-}DR^{hi}$) prior to treatment (62). The lymphocyte compartment was not vastly different prior to therapy although in responding patients, T cells exhibited an activated phenotype during treatment. On-treatment tumor biopsies and blood-based assays might identify those who will respond, supporting continuation of therapy, whereas alternative or combination therapies can be initiated earlier in those not likely to respond. Resistant tumors can also be sampled at the time of progression while on therapy to possibly identify drivers of immune resistance.

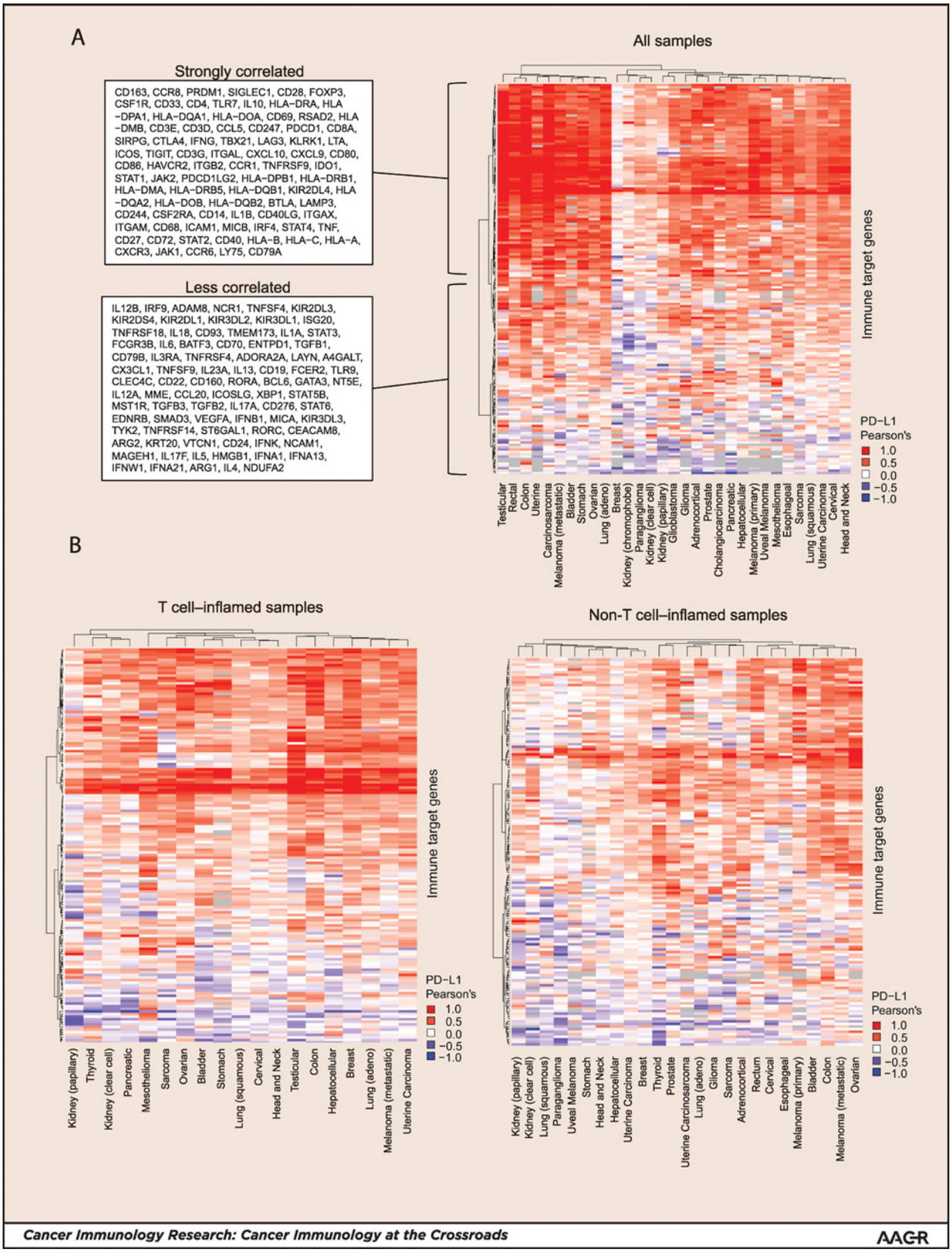
Baseline tumor mutational load and immune profiles between responders and nonresponders can overlap, making these assays imperfect predictors of response. In such cases, a combined approach that analyzes tumor genomics, immune profile, germline genetics, and perhaps even commensal microbial composition (19, 21, 63, 64) will maximize our ability to predict which patients will respond to immunotherapies.

Tackling Resistance in T Cell–Inflamed Tumors

Anti–PD-1 or anti–CTLA-4 immunotherapy treatment failures do occur even when baseline infiltrates are present. Multiple costimulatory and inhibitory pathways such as TIM-3, Lag-3, TIGIT, and many others regulate the net amplitude and duration of responses, offering many permutations for combination therapy in T cell–inflamed tumors (65). The upregulation of TIM-3 has been identified at the time of secondary resistance to anti–PD-1 therapy in a series of patients with lung cancer (66). In a murine lung cancer model, the addition of a TIM-3–blocking antibody to anti–PD-1 restored tumor control in mice with acquired resistance to anti–PD-1 treatment (66). Acquiring tumor biopsies and immune profiling at the time of relapse might help to identify newly upregulated immune checkpoints that can be targeted to restore tumor immune control. Beyond checkpoints, potentially immunosuppressive cells types, including $FoxP3^+$ Tregs, myeloid-derived suppressor cells, and tumor-associated macrophages, are also associated with the T cell–inflamed tumor microenvironment and may limit killing of cancer cells.

We have observed that many if not most immunotherapy drug targets currently being evaluated in clinical trials reflect the T cell–inflamed phenotype across tumor types within TCGA. To investigate this, we initially developed a nonexclusive list of 166 immunotherapy relevant genes and compared their expression with *PD-L1* across all solid tumors (see methods described in Fig. 2 legend). We observed strong correlations, as measured by Pearson *R* coefficients, for most of these immune target genes, with *PD-L1* in all tumors except for in kidney cancers (Fig. 2A). We then grouped the gene list into those more and those less correlated with *PD-L1*. As expected, IFNγ-inducible genes such as *IDO1*, JAKs/STATs, and HLAs as well as checkpoint genes such as *LAG3*, *CTLA-4*, and *ICOS* were strongly correlated. In contrast, genes associated with immunosuppression such as *TGFB1* and *VEGFA* were less correlated. The family of KIR genes does not appear to strongly correlate with *PD-L1*. We then divided the RNA sequencing data for all samples from solid tumors of TCGA by the T cell–inflamed versus non-T cell–inflamed gene signature and compared the expression of the 166 immunotherapy-relevant genes with *PD-L1* in each group (Fig. 2B). Through this approach, we observed stronger correlations between immunotherapy-relevant genes and *PD-L1* in T cell–inflamed tumors than in the non-T cell–inflamed group. These data suggest that most combination immunotherapies focused on increasing T-cell antitumor activity should be directed toward tumor types and patients with a baseline IFNγ-associated T cell–inflamed tumor microenvironment. Testing these combinations has seemingly less utility in non-T cell–inflamed tumors. Employing such a gene expression profiling screen prior to patient entry would have the potential to enrich clinical trials for patients likely to respond and perhaps accelerate the development of combination therapies.

In efforts to target T cell–inflamed tumors, various preclinical and clinical studies are testing whether depleting inhibitory cell subsets or interfering with their function improves tumor rejection (67). The IDO protein is a tryptophan-catabolizing enzyme involved in acquired immune tolerance to tumors, and inhibitors of the IDO pathway may improve antitumor activity when combined with checkpoint inhibitors (68). Despite initial optimism, the first phase III trial of an IDO1 inhibitor with anti–PD-1 failed to show any improvement over anti–PD-1 therapy alone in unselected patients with unresectable or metastatic melanoma suggesting that patient selection may be important with combination immunotherapy. IDO inhibition had a strong preclinical rationale and showed promise in smaller studies prior to the negative phase III results. Nonetheless, the IDO inhibitor BMS-986205 is still being investigated in bladder cancer, despite the negative data with epacadostat in melanoma. The target may be important in the right patient population; however, the negative phase III data highlight the need for developing biomarkers as well as immuno-oncology drugs. Antibodies targeting LAG3 have also entered phase III trials. In contrast with IDO inhibitor studies,



phase II studies selected patients based on the presence of LAG3 protein by IHC, making the presence of the T cell–inflamed tumor microenvironment much more likely. Multiple clinical trials are in the pipeline to examine the therapeutic potential of various combination therapies with anti–PD-1/L1 and anti–CTLA-4 drugs (67). The key to this effort will be to use tumor samples and blood-based assays to determine why some patients respond and others do not.

Conventional chemotherapy drugs and targeted anticancer agents are being probed for their ability to promote the antigenicity, immunogenicity, and susceptibility of malignant cells to enhance immune killing (69). For example, the combination of oxaliplatin and cyclophosphamide therapy elicits an immunogenic phenotype in cancer cells that may be advantageous for combination with immunotherapy (70). In a genetically engineered murine model of lung adenocarcinoma, this chemotherapy regimen increased the therapeutic activity of anti–CTLA-4 and anti–PD-1 treatment against tumors lacking baseline T cells (70). Chemotherapy-induced tumor control was dependent on $CD8^+$ T cells and innate immune signaling through Toll-like receptor-4 (TLR-4). The concept of chemotherapy-induced immunogenic cell death may have been reflected in the results from the Keynote-189 trial, in which chemotherapy combined with pembrolizumab was compared with chemotherapy plus placebo in first-line NSCLC. In this study, the combination of chemotherapy and pembrolizumab led to 69% 12-month survival compared with 49% for chemotherapy with placebo (HR for death = 0.49; 95% CI, 0.38–0.64; $P < 0.001$). The magnitude of benefit with the chemo-immunotherapy combo suggests synergy in the two modalities, and improvement was seen across all PD-L1 subgroups (71). Beyond chemotherapy, an additional modality for promoting innate immune activation in the tumor microenvironment may be directed fractionated radiation. The tissue-damaging effects of radiation lead to the release of danger-associated molecular patterns that activate the innate immune system and recruitment of inflammatory cells into irradiated tumor tissue (72). Targeted radiation induces type I IFN production and enhances priming with the potential to improve immune-mediated tumor control (72, 73). The efficacy of radiation is also in part dependent on DNA-sensing and type I IFN induction mediated by the STING pathway (74). Numerous clinical trials aim to harness the immune-potentiating effects of localized radiation to extend the therapeutic reach of immunotherapy (72). It is not yet clear whether radiotherapy combined with checkpoint blockade will yield the best synergy in T cell–inflamed or non-T cell–inflamed tumors.

Overcoming the Non-T Cell–Inflamed Phenotype

Non-T cell–inflamed tumors are more likely to be refractory to immune checkpoint inhibitors and adoptive cell therapy due an immune-excluding tumor microenvironment (8, 11, 41, 75). In addition to targeting tumor-intrinsic oncogenes, such as WNT/β-catenin, several strategies to induce or directly activate the innate immune system within the tumor microenvironment are under investigation. Particularly, pharmacologic agonists of TLRs and (76) the STING pathway have already entered clinical development (76). Intratumoral administration of innate immune agonists provides a potential therapeutic strategy to initiate type I IFN production, endogenous priming, and immune cell trafficking into tumors, converting noninflamed tumors into inflamed tumors. Evidence from an *in vivo* model of non-T cell–inflamed melanoma suggests that innate immune activation can reverse oncogene-driven exclusion through direct intratumoral injection of dendritic cells activated by the TLR-3 agonist polyinosinic-polycytidylic acid (PolyI:C; ref. 8). Preclinical data indicate that intratumoral injection of dendritic cells can restore responses to combined anti–CTLA-4 and anti–PD-1 therapy in otherwise refractory non-T cell–inflamed tumors (13). As central regulators of antitumor immune responses, intratumoral dendritic cells may be predictive biomarkers of response to immunotherapy and therapeutic targets to induce responses. Tertiary lymphoid structures (TLS), or lymphoid aggregates, have been identified in or surrounding tumor tissue of various cancer types and have correlated with better overall survival (77). TLSs appear to be sites of immune activation and regulation, but not foci of tumor cytolysis. In pancreatic adenocarcinoma clinical trials, irradiated, granulocyte-macrophage colony-stimulating factor (GM-CSF) secreting, allogeneic pancreatic tumor vaccine converted a nonimmunogenic tumor into an immunogenic tumor by inducing TLSs in the tumor microenvironment (78). In response to a vaccine, IFNγ and PD-L1/PD-1 were upregulated, providing a rationale to combine the vaccination protocol with anti–PD-1 blockade in patients whose tumors do not otherwise have T cells.

Upstream from TLRs and STING activation, administration of oncolytic viruses holds promise as combination partners for checkpoint blockade in non-T cell–inflamed tumors. Oncolytic viruses directly kill target cancer cells and induce systemic antitumor immunity, at least in part through innate immune activation. Talimogene laherparepvec (T-VEC) is a recombinant attenuated herpes simplex virus-1 engineered to express GM-CSF

Figure 2.

Expression of PD-L1 is positively correlated with expression of immunotherapy-relevant target genes across solid tumors from TCGA. **A,** Heatmap of Pearson *R* coefficients between PD-L1 expression and immune target genes by tumor type. Immune target genes were separated into those strongly correlated with PD-L1 and those less strongly correlated. **B,** Heatmaps of Pearson *R* coefficients between PD-L1 expression and immune target genes in non-T cell–inflamed tumors and T cell–inflamed tumors. Methods: Gene expression correlation analysis. Gene expression data (release date February 4, 2015) were downloaded for 30 solid tumor types from TCGA (acute myeloid leukemia, diffuse large B-cell lymphoma, and thymoma were excluded because of high tumor intrinsic immune cell transcripts). Skin cutaneous melanoma had both primary and metastatic samples available, whereas the other 29 cancers had only primary tumors available. A total of 9,555 tumor samples were included in the analysis and processed as described previously (15). Data were normalized across all samples and the patients were categorized into non-T cell–inflamed (cold), intermediate (med), and T cell–inflamed (hot) tumor groups using a previously defined 160-gene T cell–inflamed signature. A list of 166 immune molecules representative of the interactions between tumor cells and immune cells in the tumor microenvironment were selected and correlated with *PD-L1* (also known as CD274). For each tumor type, Pearson product–moment correlation coefficient *r* was computed between the gene expression of each immune molecule and *PD-L1* and used for clustering the genes by hierarchical unsupervised clustering with Euclidean distance. The genes were clustered into two distinct groups consisting of (i) strongly correlated genes such as *IFNG* and *FOXP3*, and (ii) less correlated genes such as *TGFB1* and *VEGFA*.

locally within target malignant tissue to recruit T cell–priming APCs (79). T-VEC is adapted for selective cancer cell replication and is approved for the treatment of unresectable melanoma (79). Combination clinical trials with anti–CTLA-4 and with anti–PD-1 suggest potential for additive benefit. The combination of T-VEC and pembrolizumab may increase the response rate in patients with metastatic melanoma to as high as 62%, including 33% with complete responses (80). Clinical responses occurred in a fraction of patients whose pretreatment tumor tissue lacked expression of the T cell–inflamed signature. Thus, oncolytic viruses may provide a means to induce a robust immune response locally within the tumors, even in tissue lacking a baseline infiltrate. One limitation of these therapies is the requirement for direct injection into tumors.

Dividing tumors into those that are, or are not, T cell–inflamed provides a framework for rational combination immunotherapy development. Development of combination immunotherapies for T cell–inflamed tumors seems the most straightforward. Targeting of secondary immune checkpoints, or other aspects of T-cell function within the T cell–inflamed tumor microenvironment, are clearly approaches worth prioritizing and might be more easily selected with biomarkers. The non-T cell–inflamed tumor microenvironment, however, may be the most common primary resistance phenotype observed across various cancer types. Therefore, understanding the mechanism of immune evasion in such cases will be essential to overcome the limitations of current immunotherapies. Even the most potent inducers of immune priming may need to be coadministered in novel combinations, such as those that target immune-exclusionary oncogene pathways, for optimal and sustained therapeutic effect. Given the various discrete mechanisms of immune resistance that can manifest during tumor progression, understanding immune resistance and tailoring treatments requires analysis of tumors at baseline and during progression.

Disclosure of Potential Conflicts of Interest

R.F. Sweis is a consultant/advisory board member for Bristol-Myers Squibb, Eisai, and Exelixis. J.J. Luke is on the scientific/advisory board at TTC Oncology; reports receiving commercial research grants from Array, Palleon, and CheckMate; and is a consultant/advisory board member for 7 Hills, Aduro, EMD Serono, IDEAYA, Janssen, Merck, NewLink, Novartis, RefleXion, Spring Bank, Syndax, WntRx, Acytm, Alphamab Oncology, Array, AstraZeneca, Bristol-Myers Squibb, Castle, CheckMate, and Compugen. No potential conflicts of interest were disclosed by the other authors.

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