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The next hurdle in cancer immunotherapy: Overcoming the non-T cell-inflamed tumor microenvironment

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Abstract

A growing body of evidence suggests that a major subset of patients with advanced solid tumors shows evidence for a T cell-inflamed tumor microenvironment. This phenotype has positive prognostic value for several types of early stage cancer, suggesting that the attempt by the host to generate an anti-tumor immune response reflects a biologic process associated with improved patient outcomes. In metastatic disease, the presence of this phenotype appears to be associated with clinical response to several immunotherapies, including cancer vaccines, checkpoint blockade, and adoptive T cell transfer. With the high rate of clinical response to several of these therapies, along with early data indicating that combination immunotherapies may be even more potent, it seems likely that effective immune-based therapies will become a reality for patients with a range of different cancers that physiologically support the T cell-inflamed tumor microenvironment in a subset of individuals. Therefore, one of the next significant hurdles will be to develop new therapeutic interventions that will enable these immunotherapies to be effective in patients with the non-T cell-inflamed phenotype. Rational development of such interventions will benefit from a detailed molecular understanding of the mechanisms that explain the presence or absence of the T cell-inflamed tumor microenvironment, which in turn will benefit from focused interrogation of patient samples. This iterative “reverse-translational” research strategy has already identified new candidate therapeutic targets and approaches. It is envisioned that the end result of these investigations will be an expanded array of interventions that will broaden the fraction of patients benefitting from immunotherapies in the clinic.

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1. Characteristics of the T cell-inflamed tumor microenvironment. The molecular identification of tumor antigens that can be recognized by host T cells in cancer patients (1, 2) prompted the development of vaccination strategies to increase the frequency of tumor antigen-specific T cells as a therapeutic approach. In addition, it provided tools that could be utilized to quantify tumor antigen-specific T cells in the blood and also at tumor sites (3). During the course of this work, two major observations were made. First, a number of patients showed spontaneously elevated frequencies of tumor antigen-specific T cells at

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baseline, prior to immunization. Second, only a minor subset of patients showed clinical activity, despite induction of increased specific T cell frequencies after vaccination (4, 5). Together, these observations generated a new conceptual advance—the hypothesis that resistance mechanisms at the level of the target tissue (the tumor microenvironment) might remain dominant despite the presence of abundant anti-tumor T cells circulating in the host.

To pursue this question in patients, we began with studying baseline biopsies of melanoma metastases for interrogation by transcriptional profiling. This analysis revealed two major subsets of tumors that were largely characterized by the presence or absence of a gene expression profile indicative of a pre-existing T cell-inflamed tumor microenvironment. The T cell-inflamed subset of tumors showed presence of T cell transcripts, chemokines that likely mediate effector T cell recruitment, macrophage activation markers, and a type I IFN transcriptional profile (6). Immunohistochemistry confirmed the presence of $CD8^+$ T cells, macrophages, and some B cells and plasma cells in these lesions (6). In several small series of HLA-$A2^+$ patients, $CD8^+$ T cells specific for melanoma differentiation antigens have been identified from tumor sites using peptide-HLA-A2 tetramer analysis. Therefore, at least a subset of T cells specific for tumor antigens is present among these infiltrates. In fact, this is arguably the starting point for adoptive T cell approaches utilizing tumor-infiltrating lymphocytes (TIL), which has consistently generated approximately a 50% response rate in patients with metastatic melanoma (7). However, functional analysis has indicated various degrees of dysfunction of these tumor antigen-specific T cells when analyzed directly ex vivo (8–10). These results suggest that the reason for tumor progression despite the presence of specific adaptive immunity in this subset of patients is likely secondary to immune suppressive mechanisms acting at the level of the tumor microenvironment. Interestingly, in some cases the presence of memory virus-specific $CD8^+$ T cells also has been observed in these T cell-inflamed tumors. However, their function seems to be intact (8, 11), and these probably represent non-specifically recruited activated T cells migrating along chemokine gradients but not participating in tumor recognition. Thus, a component of antigen-specificity to the T cell dysfunction in the tumor microenvironment appears to be operational.

More detailed analysis of the T cell-inflamed subset of tumors revealed the presence of transcripts encoding indoleamine-2,3-dioxygenase (IDO), PD-L1, and FoxP3 (12, 13). Quantitative analysis of individual tumors revealed that the expression level of these three transcripts was positively correlated. IHC confirmed that PD-L1 and IDO protein expression, and also nuclear $FoxP3^+CD4^+$ cells, were found within T cell-inflamed tumors in the same region as $CD8^+$ T cells. Mouse mechanistic studies confirmed that $CD8^+$ T cells were required for the upregulation of all of these three factors within the tumor microenvironment. For PD-L1 and IDO induction, the requisite factor produced by the $CD8^+$ T cells was IFN-γ. For $FoxP3^+$ Tregs, production of the chemokine CCL22 was identified, which mediated Treg recruitment into tumor sites (13). Together, these data suggest that the involvement of these three immune-inhibitory mechanisms in T cell-inflamed tumors is not pre-existing and driven by the tumor cells, but rather is driven by the activated $CD8^+$ T cells themselves.

The original hypothesis in the context of our melanoma vaccine studies was that the responding patients might have low expression of immune inhibitory mechanisms in the tumor microenvironment and resistant patients the highest expression. On the contrary, the opposite was the case. A baseline T cell-inflamed tumor microenvironment (that includes presence of PD-L1, IDO, and Tregs) was positively associated with clinical benefit from these vaccines (14–16). Thus, the ability of a tumor to produce chemokines and recruit activated T cells into the tumor microenvironment appears to be instrumental for clinical benefit. Similar results have been observed in patients treated with high-dose IL-2 (17) and also with the anti-CTLA-4 mAb ipilimumab (18). Most strikingly, clinical response with anti-PD-1 mAb, which is blocking PD-L1/PD-1 interactions directly within the tumor microenvironment, was found to occur almost exclusively in patients with pre-existing T cell infiltrates in the region of PD-L1 upregulation (19–21). Following anti-PD-1 administration, these $CD8^+$ T cells seemed to proliferate and expand to penetrate throughout the tumor, which was correlated with tumor regression (21). These observations are consistent with our own mouse model data which demonstrated that tumor regression upon checkpoint blockade was almost completely mediated by re-activation of $CD8^+$ T cells directly within the tumor site to be able to proliferate and produce IL-2 (22).

Combination immunotherapies are being developed and evaluated as an attempt to improve clinical benefit further (22–24). This is logical, as multiple immune inhibitory mechanisms appear to be present concurrently within the T cell-inflamed tumors, so blockade of two or more pathways could be synergistic. Preclinically, we have shown that concurrent doublets of anti-CTLA-4 +/− anti-PD-L1 +/− an IDO inhibitor each was synergistic in the B16 melanoma model in vivo (22). Interestingly, each of these combination therapies involved re-acquisition of IL-2 production and proliferation by $CD8^+$ T cells directly within the tumor microenvironment. In fact, therapeutic effects were preserved even in the presence of FTY720 blockade, which prevents exit of new T cells from lymph nodes. Therefore, as clinical development of each of these doublets is proceeding in advanced cancer patients (25, 26), our working model suggests that they may indeed have increased clinical activity but that this clinical benefit may be restricted to the context of the T cell-inflamed tumor microenvironment phenotype. Therefore, new therapeutic approaches will likely be needed to support immunotherapy efficacy in patients with the non-T cell-inflamed tumor microenvironment. Rational development of such approaches will benefit from more detailed knowledge regarding the underlying mechanisms that explain the presence or absence of a spontaneous T cell infiltrate.

2. Mechanisms of innate immune sensing that translate into spontaneous T cell priming and T cell infiltration into tumors. As a first approach toward understanding the underlying mechanisms that control the T cell-inflamed tumor phenotype, we interrogated our melanoma gene expression profile data for evidence of innate immune activation pathways. In general, in order for adaptive T cell responses to be induced against an antigen, dendritic cells (DCs) or other involved antigen-presenting cells (APCs) need to be activated themselves by additional molecular entities. In the setting of infectious agents, this is often through stimulation of Toll-like receptors (TLRs) by pathogen-associated molecular patterns (PAMPs), such as endotoxin that is recognized by TLR4 (27). However, it had not been clear what factors might provide innate immune signaling in the context of sterile tumors in

which infectious agents are not involved. Our melanoma data indicated that tumors that contained a T cell infiltrate also showed evidence for a transcriptional signature known to be induced by type I IFNs (6, 28). We therefore carried out mouse mechanistic experiments to determine whether type I IFN signaling on host cells was necessary for spontaneous priming of $CD8^+$ T cells against tumor-associated antigens. This indeed was the case. Type I $IFNR^{-/-}$ mice, or mice deficient in the downstream transcription factor Stat1, showed markedly reduced T cell responses in multiple transplantable tumor models (28). The requirement for type I IFN signaling was mapped to the level of APCs, and indeed specific deletion of the type I IFNR in DCs was sufficient to reproduce this defect. Mixed bone marrow chimera experiments demonstrated that the specific subset of DCs involved in this effect was the Batf3-driven lineage that expresses $CD8^+$ or CD103 (28–30). In addition, IFN-γ production was found to be induced in DCs upon implantation of a tumor in vivo. These data suggest that early innate immune recognition of cancer cells in vivo involved activation of a pathway that resulted in IFN-γ production, which in turn was necessary for complete DC activation and $CD8^+$ T cell priming against tumor antigens to give rise to the T cell-infiltrated tumor microenvironment phenotype (31).

These observations led to interrogation of the next level of the problem, which is identifying the receptor system and putative ligands that induce IFN-γ production by host DCs in the context of a growing tumor in vivo. By using a series of knockout mice specifically lacking TLRs, the adaptors MyD88 or Trif, the intracellular RNA sensing pathway involving MAVS, or the extracellular ATP sensing receptor P2X7R, we were able to rule out most of the receptor systems that have been implicated in various infectious disease models. By process of elimination, this left us with the STING pathway as an important candidate. STING is an adapter that is activated by cyclic dinucelotides generated by cGAS, which in turn is directly activated by cytosolic DNA (32–34). This pathway has been implicated in the sensing of DNA viruses, but also in selected autoimmune models (35, 36). Moreover, activating mutations of STING have recently been identified in human patients with a vasculitis/pulmonary inflammation syndrome that is characterized by increased type I IFN production (37). Indeed, through the use of $STING^{-/-}$ mice we demonstrated that spontaneous T cell priming against tumor antigens was markedly reduced in vivo, and rejection of immunogenic tumors was ablated (38). We found evidence for tumor-derived DNA within the cytosol of a major population of tumor-infiltrating DCs, which was associated with STING pathway activation and IFN-γ production. Therefore, the host STING pathway appears to be a major innate immune sensing pathway that detects the presence of a tumor to drive DC activation and subsequent T cell priming against tumor-associated antigens in vivo. Recently, several additional tumor model systems have confirmed a role for the STING pathway in anti-tumor immunity in vivo (39–41). The realization of the importance of this particular innate immune pathway in the cancer context is generating new therapeutic strategies that might be utilized to activate or mimic this pathway for promoting immune-mediated tumor control, particularly in the non-inflamed tumor subset.

3. Candidate molecular mechanisms that could explain T cell exclusion through genomic analysis of inter-patient heterogeneity. In parallel to investigations into differences in innate immune sensing, additional potential mechanisms for the presence or absence of a T cell-

inflamed tumor microenvironment are being probed through genomic analysis of patients with melanoma. In principle, the variable presence of a spontaneous T cell infiltrate in tumors can be viewed as a phenotype, and therefore correlations with genomic heterogeneity can be sought. Because the T cell-inflamed tumor microenvironment is also a good predictive biomarker for response to immunotherapies such as anti-PD-1, this question can also be viewed as a pharmacogenomic analysis for mechanisms of primary resistance to these agents. Based on these notions, three potential sources of inter-patient heterogeneity could be envisioned that have the potential to influence whether a given tumor in a given subject might contain or lack spontaneous T cell infiltration. These categories are germline polymorphisms in immune-regulatory genes, differences in accessory oncogene pathways within the tumor cells based on somatic mutational events, or environmental differences that could have global effects on immune functionality. Regarding the latter category, the major process that has garnered interest is the impact of the intestinal microbiome on overall immune responses in the host. Importantly, each of these parameters is measurable in individual patients. Germline heterogeneity can be evaluated using SNP arrays on peripheral blood cells, somatic heterogeneity in the tumors can be assessed through exome sequencing, and patterns of differences in the intestinal microbiome can be identified through 16S ribosomal RNA sequencing on stool samples. Associations between individual sequences and either the T cell-inflamed tumor microenvironment or clinical outcome to immunotherapy can then be investigated. Implicit in this discover process is that the analyses can only be performed if the proper tissues are collected and banked from patients at baseline—peripheral blood, fresh tumor biopsies, and stool samples. Therefore, broad-based tissue banking from cancer patients participating in immunotherapy clinical trials is required for approaching these questions.

There is precedent for considering these sources of variability among cancer patients with respect to immunotherapy responsiveness. The first germline polymorphism investigation described was analysis of a SNP in the gene encoding the chemokine receptor CCR5. Indeed, a CCR5 polymorphism was identified that was associated with clinical response to high-dose IL-2 (42). More recently, a polymorphism in the IRF5 gene was identified that was associated with clinical benefit in a cohort of patients treated with T cell-adoptive transfer (43). Regarding somatic differences in the tumor, activated Stat3 has been shown to block chemokine expression in tumor cell lines (44, 45), and if active in human cancers could contribute to lack of T cell recruitment. A potential functional role for the intestinal microbiota also has been implicated. Trinchieri and colleagues found in a mouse model that treatment with anti-bacterial antibiotics, which altered intestinal microbial composition, reduced the therapeutic efficacy of immunotherapy with the TLR9 agonist CpG combined with anti-IL-10R antibody in a transplantable tumor model (46). These early data support the notion that a comprehensive analysis of these sources of heterogeneity in cancer patients will likely be informative.

In metastatic melanoma patients, we have begun this line of inquiry by focusing on somatic differences at the level of tumor cells associated with absence of a T cell-inflamed tumor microenvironment phenotype. The rationale is that identification of accessory oncogene pathways that mediate immune exclusion will be attractive pathways to target pharmacologically, both for a direct anti-tumor effect and also for promoting host immune

recognition. Using a series of 266 melanoma metastases, segregation was performed based on the presence or absence of the gene signature indicative of the T cell-inflamed tumor microenvironment, as we have described previously (6). These same tumors were also subjected to exome sequencing, as well as pathway analysis using the Ingenuity platform based on gene expression patterns in the non-T cell-inflamed subset. Strikingly, these data indicated that nearly one-half of the non-T cell-inflamed tumors showed evidence of activation of the β-catenin pathway. Some of these were associated with activating mutations in β-catenin itself, some with inactivating mutations in negative regulators of β-catenin, and some through over-expression of a Wnt ligand or Frizzled receptor. Using a genetically engineered mouse model in which melanomas were induced that either did or did not include conditionally expressed active β-catenin, mechanistic experiments confirmed that tumor cell-β-catenin activation was sufficient to exclude T cell infiltrates in vivo (Spranger and Gajewski, manuscript submitted). These data suggest that pharmacologic strategies to block β-catenin activity might not only be directly therapeutic against tumor cells, but additionally might support a positive interaction with host immunity.

4. Potential therapeutic strategies to initiate productive innate immune activation and overcome T cell exclusion. Based on preliminary insights gained from the above mentioned studies, several strategies to induce or restore de novo endogenous immune responses against tumors are being considered (summarized in Table 1). Inasmuch as non-T cell-inflamed tumors appear to lack evidence of a type I IFN transcriptional signature, strategies to promote robust innate signaling via APCs in the tumor microenvironment might facilitate improved cross-priming of tumor antigen-specific $CD8^+$ T cells and also augment chemokine production for subsequent effector T cell trafficking. Because of the recently discovered role for the host STING pathway in mediating endogenous innate immune activation against tumors, recent studies have pursued intratumoral injection of STING agonists. 5,6-Dimethylxanthenone-4-acetic acid (DMXAA) is a flavonoid compound that was shown to have anti-tumor activity in mouse models (47). This drug ultimately failed to show benefit when combined with chemotherapy in a Phase 3 trial in patients with non-small cell lung cancer (48). Structure-function studies of mouse and human STING demonstrated that DMXAA is a direct ligand for mouse STING (49, 50). However, sequence differences in human STING rendered it unable to bind DMXAA, therefore abrogating its activity in human cells and explaining the lack of clinical activity of this compound. We found that DMXAA is a strong agonist of the mouse STING pathway in vitro and in vivo. Intratumoral injection of DMXAA augmented endogenous priming of tumor antigen-specific $CD8^+$ T cells around 20-fold and caused complete tumor elimination. Rejection was completely dependent on STING, and most of the effect depended on T cells and type I IFNs. New STING agonists that stimulate all known human STING polymorphic variants have been developed and will be attractive for clinical translation (Corrales and Gajewski et al, manuscript submitted).

A second approach for promoting innate immune activation directly within the tumor microenvironment has been to provide an increased local concentration of type I IFNs. A major challenge is developing strategies for systemic delivery of type I IFNs that give rise to local accumulation in the tumor microenvironment. One strategy approach has been through the use of tumor-targeting monoclonal antibodies (mAbs) coupled to IFN-γ as a payload.

Indeed, either anti-Her2 or anti-EGFR mAbs coupled to IFN-γ led to tumor regression in the appropriate models, through a mechanism that was completely T cell-dependent. Conditional type I IFNR$^{-/-}$ mice lacking type I IFN signaling specifically on CD11c^{+} cells lost the therapeutic effect with this strategy, arguing that the activity was driven through host immune priming (51). Thus, transient expression of low doses of IFN-γ within the tumor microenvironment appears to facilitate adaptive immunity to tumors. Interestingly, the mechanism of anti-tumor activity of type I IFNs appears to vary depending on the delivery strategy utilized, which likely is related to the dose and duration of type I IFN presence within the tumor microenvironment. Transfection of tumor cells to express high levels of IFN-γ led to tumor regression that was largely independent of host adaptive immunity. Rather, this approach led to an elimination of the tumor vasculature, consistent with an anti-angiogenic effect. Most of the therapeutic effect was preserved in RAG$^{-/-}$ and NK cell-depleted mice, and bone marrow chimera experiments revealed a major role for type I IFN signaling on non-hematopoietic cells. Moreover, conditional type I IFNR$^{-/-}$ mice lacking type I IFN signaling exclusively on Tie2^{+} cells lost the therapeutic effect of high-dose IFN-γ, arguing for a direct effect on the tumor vasculature (52). Optimal combinations with type I IFNs and T cell-directed immunotherapies in the future may depend on a careful consideration of the dose and schedule of IFN-γ or IFN-γ being used.

An additional option for promoting appropriate innate immune activation in the tumor microenvironment is through targeted radiation. Directed radiation to the tumor site also appears to induce type I IFN production, to augment specific T cell priming, and to support T cell-mediated tumor control (53). Based on our finding that the STING pathway was critical for spontaneous innate immune sensing of tumors in vivo, it was of interest to determine whether the STING pathway was also important for the therapeutic effect of radiation. Indeed, the efficacy of radiation was largely ablated in STING$^{-/-}$ hosts, which was associated with defective immune priming. In contrast, no defect was observed using mice lacking specific TLR signaling pathways (54). Thus, radiation may facilitate the proper acquisition of tumor-derived DNA by host DCs in the tumor microenvironment, thereby leading to improved T cell priming as well as coordination of the effector phase of the anti-tumor immune response.

Aside from strategies to directly engage or mimic the STING pathway, interventions aimed at initiating a tertiary lymphoid structure within the tumor microenvironment also are being explored. The TNF superfamily member LIGHT can engage the LTβR on stromal cells in essentially in any tissue when expressed as a transgene in vivo (55). Expression of LIGHT in tumor cells has been shown to induce chemokine production, T cell priming, improved T cell trafficking, and tumor rejection in vivo (56–58). These data are consistent with the observation that some cancer patients show evidence for spontaneous tertiary lymphoid structures that may be associated with improved clinical outcome (59).

An additional consideration for the T cell-poor subset of tumors is whether lack of T cell-based inflammation might be explained by the presence of dense stroma. The non-T cell-inflamed subset of tumors may express higher levels of pro-angiogenic factors and have altered vasculature that is not permissive for effector cell trafficking. Coukos and colleagues performed gene expression profiling of vascular endothelial cells in the ovarian cancer

tumor microenvironment and found distinct phenotypes associated with the presence or absence of a T cell infiltrate (60, 61). One of these endothelial cell gene products, the endothelin B receptor, could be manipulated towards improved T cell trafficking. A high density of fibroblasts, tumor-supporting macrophages, and extracellular matrix also may restrict T cell access. Tumor cells established in stroma are much more difficult to reject immunologically compared to a poorly established tumor cell suspension in mouse models (62, 63). However, it may be feasible to manipulate the stromal composition in solid tumors. In a pancreatic cancer context, agonistic anti-CD40 antibody has been shown to activate macrophages that enter the tumor microenvironment, remodel the stroma, and render the tumors more responsive to chemotherapy (64).

Emerging data from genomic studies of human cancer patients bearing a T cell-inflamed versus non-T cell-inflamed tumor microenvironment may reveal the ultimate information about candidate therapeutic targets for creating T cell-based inflammation in cases in which it fails to occur spontaneously. Gene products expressed in host immune cells with altered function because of specific polymorphisms might be subjectable to pharmacologic manipulation to facilitate initiation of anti-tumor immunity. Signaling pathways active within tumor cells that result in immune exclusion (such as β-catenin) might be drugable, in order to reverse mechanisms of T cell exclusion and catalyze T cell priming and trafficking into the tumor microenvironment. Finally, selected species of commensal bacteria that might support improved systemic immune responses against tumor-associated antigens could conceivably be administered as a probiotic towards improved immune-mediated tumor control. Several of these strategies are expected to enter clinical testing in the near future.

5. Future prospects for combination immunotherapies to include benefit for patients with the non-T cell-inflamed tumor microenvironment. The ongoing development of novel strategies to initiate or restore anti-tumor T cell responses in the setting of the non-T cell-inflamed tumor microenvironment ultimately may run into the same type of functional limitation as endogenous T cell responses—the dominant inhibitory effect of immune suppressive mechanisms.

Recruitment of new activated T cells into tumor sites is expected to result in upregulation of PD-L1 and IDO expression through local IFN-γ production, and also Treg recruitment via chemokines such as CCL22. Type I IFNs as part of innate immune activation also can contribute to immunosuppressive negative feedback regulation. Thus, even the most potent inducers of local immune priming may need to be combined with inhibition of negative regulatory pathways for optimal therapeutic effects in the clinic. Preclinical data with local radiation already has supported this notion. Radiation was found to upregulate local PD-L1 expression, and combined blockade with anti-PD-L1 mAb was therapeutically synergistic in vivo (65). As such, it is envisioned that, as new approaches for initiating the T cell-inflamed tumor microenvironment are pursued, combination therapies will likely be required. Optimistically, one could imagine substantially expanding the subset of patients with melanoma and multiple other tumor types that currently show fractional responses to anti-PD-1/PD-L1 mAbs in the clinic.

Several additional considerations must be kept in mind as such strategies to facilitate *de novo* immune responses are developed. First, much has been discussed recently regarding the abscopal effect that can be seen with radiation or other local therapies (66). With this phenomenon, local treatment of one tumor can lead to rejection of non-treated tumors through an immune-mediated mechanism. However, our model would suggest that an abscopal effect should only be possible if the non-treated tumors have the T cell-inflamed tumor microenvironment phenotype, as only then will the proper chemokine production and endothelial cell activation be supportive of effector T cell entry. Thus, interventions aimed at initiating a T cell-inflamed tumor microenvironment *de novo* may have to be applied to all major metastatic sites. Second, an underlying presumption is that the non-T cell inflamed tumor subset still expresses antigens that can be recognized by T cells. A recent report has suggested differential expression of mutated antigens in melanoma patients who have favorable or unfavorable clinical outcome to the anti-CTLA-4 mAb ipilimumab (67). However, we have utilized TCGA melanoma data to analyze the number of non-synonymous mutations in T cell-inflamed and non-T cell-inflamed phenotype tumors. These preliminary data have indicated that both phenotypes express an identical range of mutations, suggesting that neoantigens are likely to be comparably expressed. In addition, a similar proportion of tumors expressing MAGE genes was observed. These data support the notion that non-T cell inflamed tumors do indeed express candidate antigens and so antigen expression should not be rate-limiting.

Summary

A major subset of melanomas and other tumors shows evidence of spontaneous T cell priming and a T cell-inflamed tumor microenvironment. Immune-mediated destruction of these tumors appears to be held in check by negative regulatory mechanisms that include PD-L1/PD-1 interactions, IDO, and Tregs. New therapeutic interventions aimed at blocking these inhibitory pathways are beginning to have clinical success, in particular antibodies blocking PD-1 or PD-L1. Activity appears to be predominantly restricted to patients having a T cell-inflamed tumor microenvironment, through reacquisition of proliferative capacity by tumor-infiltrating $CD8^+$ T cells. The non-T cell-inflamed tumor microenvironment may require new interventions to make them amenable to currently active immunotherapies. Innate immune activators such as STING agonists, focal radiation, modulators of tumor stroma, targeting immunosuppressive oncogene pathways, and modulation of the intestinal microbiota are all being considered as strategies for improving endogenous immune activation against tumors in vivo. Combination therapies may ultimately be required to drive *de novo* immune responses concurrently with blocking inhibitory pathways, to maximize the fraction of patients responding to immunotherapies in the clinic.

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Table 1

Strategies being considered to provoke T cell-based inflammation in the tumor microenvironment.

Goal	Candidate intervention
1. Promote innate immunity/type I IFNs	Intratumoral STING agonists TLR agonists Local radiation Targeted IFN-α/IFN-β
2. Induce tertiary lymphoid structures	Intratumoral LIGHT
3. Modulate stroma	Anti-CD40 EtB receptor inhibitors
4. Inhibit immunosuppressive oncogene pathways	β-catenin inhibitors Stat3 inhibitors
5. Modulate microbiota	Probiotics