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Cancer Immunotherapy Targets Based on Understanding the T Cell-Inflamed Versus Non-T Cell-Inflamed Tumor Microenvironment

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2.1 T Cell-Inflamed Versus Non-T Cell-Inflamed Tumor Microenvironment

Interrogation of the tumor microenvironment in metastatic melanoma was initially pursued to test the hypothesis that resistance mechanisms downstream from T cell priming in response to melanoma vaccines might dominate and enable tumor escape [1, 2]. Baseline biopsies of melanoma metastases were obtained and evaluated by gene expression profiling, to correlate with clinical outcome from vaccination. Two major subsets of tumor microenvironment could be identified that were largely characterized by the presence or absence of a transcriptional profile indicative of a pre-existing T cell infiltrate (Fig. 2.1). The T cell-inflamed subset of tumors was dominated by T cell markers and chemokines that likely mediate effector T cell recruitment [3–5]. Expression of the chemokines CCL2, −3, −4, −5 and CXCL9, −10 was observed to correlate with T cell presence, and each of these chemokines was sufficient to recruit $CD8^+$ effector T cells in vitro [3]. Recently, CXCR3-binding chemokines (such as CXCL9 and CXCL10) were found to be critical and necessary for trafficking of activated $CD8^+$ T cells into tumor sites [6]. Immunohistochemistry confirmed the presence of $CD8^+$ T cells, macrophages, as well as some B cells and plasma cells in the T cell-inflamed lesions [3]. This subset of tumors was remarkably distinct from the non-T cell inflamed subset, and the biology suggests that spontaneous T cell priming and recruitment into the tumor microenvironment had occurred in those patients, even prior to any therapy. Interestingly, reflecting back onto the original goal of this analysis, the clinical responders to vaccination were seen among patients with the T cell-inflamed phenotype [4]. Thus, it appears as though tumors capable of supporting recruitment of activated $CD8^+$ T cells are those that stand to benefit from interventions that increase the frequency of tumor antigen-specific T cells in the circulation, such as vaccination.

The T cell-inflamed subset of melanoma metastases is remarkably similar to the phenotype described in early stage colon cancer and other tumors in which activated T cells have been associated with favorable prognosis [7–9]. In several small studies of $HLA\text{-}A2^+$ patients, $CD8^+$ T cells specific for melanoma differentiation antigens such as Melan-A were identified from tumor sites using peptide-HLA-A2 tetramer analysis [10–12]. Therefore, at least a subset of T cells specific for tumor antigens is present among these infiltrates. The

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fact that the starting point for adoptive T cell approaches utilizing tumor-infiltrating lymphocytes (TIL), which have demonstrated approximately a 50% response rate in metastatic melanoma [13], is T cells harvested from the tumor, also supports the notion that tumor-specific T cells are present. However, the function of these T cells in situ is impaired. Various degrees of dysfunction of tumor antigen-specific T cells have been described upon analysis directly ex vivo [10–12]. Together, 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 T cell-inflamed melanomas. However, their function seems to be intact [10, 14], and these probably represent non-specifically recruited activated T cells migrating along chemokine gradients but not participating in tumor recognition. These observations suggest that a component of T cell dysfunction in the tumor microenvironment is antigen-specific and restricted to tumor-reactive T cells.

In contrast to the rich set of immune genes expressed in the T cell-inflamed tumor microenvironment phenotype, the non-T cell-inflamed tumors lacked this broad signature. In particular, there was a lack of T cell markers and of chemokines that can mediate T cell recruitment [3]. These tumors still contain macrophages and vascular endothelial cells, and work from others has indicated the presence of fibroblasts and extracellular matrix, and in some cases immature dendritic cells [15–19]. It is not yet certain whether tumors that lack spontaneous T cell infiltration are defective only at the level of initial T cell priming against tumor antigens or whether there are additional mechanisms that exclude activated T cells from migrating into the tumor microenvironment, but is seems plausible that both processes may be operational. The idea that both the priming and the effector phases of the anti-tumor immune response are defective in non-T cell-inflamed tumors is supported by recent data in genetically engineered mouse models. Tumors with poor T cell infiltration appear to have higher expression of several angiogenic factors [3, 19]. Vascular endothelial cells from T cell-infiltrated versus T cell-non-infiltrated tumors have been reported to have distinct gene expression profiles, and the endothelin B receptor has been identified as a vascular target in an ovarian cancer context [19]. Thus, effector T cell trafficking into the tumor microenvironment is complex, and depends on adhesion molecules and homing receptors expressed on vascular endothelial cells in concert with chemokines produced by tumor cells and/or stromal cells within the tumor microenvironment. This process is likely necessary for clinical response to immunotherapies in most instances.

It has been argued that non-T cell-inflamed tumors might lack neoantigens for T cell recognition and therefore might not be immunogenic because they are not antigenic. A recent report has suggested that patients who failed to derive clinical benefit from the anti-CTLA-4 mAb ipilimumab might have fewer mutations and lack the antigens present in the tumors of responding patients [20]. However, we have recently analyzed exome sequencing versus germline data from the metastatic melanoma samples that are among The Cancer Genome Atlas data set. RNAseq data were used to categorize patients as having a T cell-inflamed versus a non-T cell-inflamed tumor microenvironment. The frequency of non-synonymous mutations, expression of cancer-testis antigen genes, and the expression of melanoma differentiation antigens were enumerated between these groups. No differences

were observed with any of these parameters between the two cohorts of patients [21]. These data indicate that lack of antigen expression is unlikely to explain the non-T cell-inflamed tumor microenvironment phenotype in melanoma. These data are encouraging, as they suggest that strategies to overcome the barrier of T cell migration into tumor sites might ultimately enable immunotherapy efficacy in non-T cell-inflamed tumors.

2.2 Negative Regulatory Pathways Impeding Immune Efficacy in T Cell-Inflamed Tumors

Because of the presence of dysfunctional T cells in the same microenvironment as antigen-expressing tumors cells, the T cell-inflamed subset of tumors was probed for candidate immune-inhibitory mechanisms that might contribute to T cell dysfunction in situ. Gene expression profiling data revealed the presence of transcripts encoding indoleamine-2,3-dioxygenase (IDO) in these tumors, a molecule that had previously been demonstrated to contribute to peripheral tolerance [22]. Interrogation for additional candidates revealed that these tumors also expressed PD-L1 and Foxp3 transcripts [23, 24]. Quantitative analysis of individual tumors revealed that the expression level of each of these three transcripts was significantly correlated, and that the degree of expression was also associated with T cell markers. Immunohistochemistry 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 (Fig. 2.2). However, non-T cell-inflamed melanomas generally lacked these factors. These observations suggested that these immune suppressive mechanisms might not be a property of the tumor cells themselves but rather immune-intrinsic negative feedback processes that follow the recruitment of activated $CD8^{+}$ T cells. Indeed, 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 [24]. Using laser capture microdissection, a correlation between IFN-γ production by TILs and local PD-L1 expression also was observed by Taube and colleagues in human tumors [25], supporting the notion that infiltrating T cells become activated by specific antigen and consequently produce IFN-γ and upregulate PD-L1 expression. The fact that these immune evasion mechanisms are part of the host response implies that targeting these pathways therapeutically should have an increased likelihood of efficacy because they are less dependent on tumor cell properties and the associated mutability that can frequently lead to therapeutic resistance.

Preclinical studies targeting CTLA-4, PD-L1, and IDO have indicated that the therapeutic effect is associated with re-activation of $CD8^{+}$ T cells directly within the tumor microenvironment [26]. The major biologic correlate that is restored with blockade of these pathways is the ability of tumor-infiltrating $CD8^{+}$ T cells to produce IL-2 and to proliferate when analyzed ex vivo. In order to test whether the therapeutic effect required influx of new T cells at all, the drug FTY720 was utilized, which prevents new T cell egress from lymph nodes. In fact, both restoration of IL-2 production and proliferation of TIL as well as tumor regression were preserved despite FTY720 administration [26], arguing that the immediate

functional effects of checkpoint blockade can all be explained by re-activation of T cells already present within the tumor microenvironment. Consistent with these data, clinical response with anti-PD-1 mAb in metastatic melanoma was found to occur predominantly in patients with pre-existing T cell infiltrates in the region of PD-L1 upregulation [25, 27, 28]. Following anti-PD-1 administration, these $CD8^+$ T cells seemed to proliferate and expand, as indicated by Ki67 expression, and to penetrate deeply throughout the tumor [28]. Thus, the preponderance of clinical response with active immune-therapies also is likely mediated through restored function of pre-existing TIL.

In addition to the presence of PD-L1, IDO, and Tregs that likely mediate extrinsic suppression, a T cell-intrinsic mechanism is also likely contributing to tumor escape in the T cell-inflamed cancers. This phenomenon is similar to T cell anergy that has been characterized extensively using in vitro model systems, an observation which has provided a tool for identifying additional immune regulatory targets on dysfunctional T cells within the tumor microenvironment. In vitro experiments using $CD4^+$ Th1 clones as a model system have identified the transcription factor EGR2 as a critical mediator of T cell dysfunction [29]. EGR2 is induced following TCR ligation alone, and leads to upregulation of the lipid phosphatase diacylglycerol kinase, which in turn inhibits TCR-mediated Ras pathway activation [30]. Conditional EGR2-knockout T cells have shown improved anti-tumor activity in vivo [29], arguing for a functional relevance of this pathway in anti-tumor immunity. With this functional importance in mind, experiments were conducted to identify the full spectrum of EGR2 target genes in anergic T cells. Gene expression profiling of wild-type versus EGR2-deleted T cells was performed, to identify EGR2-dependent genes. In parallel, a genome-wide ChIPseq study was performed, to identify genes directly bound by EGR2. Merging these two datasets revealed 50 genes that characterized the EGR2 transcriptome [31]. Interestingly, several of these target genes encode surface receptors that allow phenotyping using flow cytometry, including LAG-3 and 4–1BB. LAG-3 is an inhibitory receptor with homology to CD4 that recognizes at least class II MHC as a ligand [32]. 4–1BB is a costimulatory receptor of the TNFR superfamily that engages the NF-κB pathway [33]. Returning to the tumor microenvironment, flow cytometric analysis revealed that a major population of $CD8^+$ TIL co-expressed LAG-3 and 4–1BB. All of these cells were also PD-1-positive. By cell sorting and stimulation in vivo, it was found that the $LAG\text{-}3^+4\text{–}1BB^+$ subset was the most dysfunctional as reflected by IL-2 production and proliferation. The majority of tumor-specific T cells were found to fall into this subset. Thus, these likely represent important markers for identifying the dysfunctional tumor antigen-specific T cell subset within the tumor microenvironment (Williams and Gajewski, manuscript in preparation). Administration of a blocking mAb against LAG-3 along with an agonistic Ab against 4–1BB showed profound anti-tumor activity in vivo. Anti-4–1BB also synergized therapeutically with either anti-CTLA-4 or anti-PD-L1 mAbs (Horton and Gajewski, unpublished data). Interestingly, all of these combination therapies also depend upon re-activation of T cells already present within the tumor microenvironment. These data suggest that thorough phenotypic analysis of dys-functional TIL should reveal the total array of immune regulatory receptors amenable to in vivo therapeutic targeting.

Because of the presence of multiple immune regulatory factors in the same T cell-inflamed tumor microenvironment, and based on preclinical evidence for synergistic efficacy, multiple

phase I/II trials are underway to evaluate key combinations. These include an IDO inhibitor combined with either anti-CTLA-4 or anti-PD-1 mAbs, anti-LAG-3 + anti-PD-1, and anti-4–1BB mAb in various combinations. The potential for combination immunotherapy to have superior efficacy is supported by recent data using anti-CTLA-4 + anti-PD-1, which revealed a higher response rate than either agent alone in metastatic melanoma, albeit with a higher rate of adverse events [34]. Over time, additional combinations that have comparable efficacy and perhaps decreased toxicity will hopefully be identified, both for melanoma and for other cancer types showing a fraction of patients characterized by the T cell-inflamed tumor microenvironment.

2.3 Innate Immune Mechanisms Bridging to Spontaneous Anti-Tumor T Cell Responses

Expanding the efficacy of immunotherapies will require a better understanding of the mechanisms mediating the non-T cell-inflamed tumor microenvironment. As a first approach, possible innate immune pathways involved in generating the T cell-inflamed tumor microenvironment when it does occur have been pursued. 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 for productive adaptive immunity. In the context of infectious agents, this is typically through stimulation of Toll-like receptors (TLRs) by pathogen-associated molecular patterns (PAMPs), such as endotoxin that is recognized by TLR4 [35]. 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 implicated. Melanoma gene expression profile data were interrogated for evidence of innate immune pathway activation. A major clue came from the observation that tumors that contained a T cell infiltrate also showed evidence for a transcriptional signature known to be induced by type I IFNs [3, 36]. Armed with that information, mouse mechanistic experiments were carried out to determine whether type I IFN signaling on host cells was necessary for spontaneous priming of $CD8^+$ T cells against tumor-associated antigens. In fact, type I $IFNR^{-/-}$ mice, or mice deficient in the downstream transcription factor Stat1, showed markedly reduced T cell responses against tumor-associated antigens in multiple transplantable tumor models [36]. 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 CD8α or CD103 [36–38]. In addition, IFN-β 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 results in IFN-β production, which in turn was necessary for complete DC activation and $CD8^+$ T cell priming to give rise to the T cell-infiltrated tumor microenvironment phenotype [39].

These observations led to the next level question of identifying the receptor system and putative ligands that induce IFN-β 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, most of the innate immune pathways that have been implicated in various infectious disease models to promote type I IFN production were ruled out as being essential. By process of elimination, this pointed to the STING pathway as an important candidate. STING is an adapter that is activated by cyclic dinucleotides generated by cGAS, which in turn is directly activated by cytosolic DNA [40–42]. This pathway has been implicated in the sensing of DNA viruses, but also in selected autoimmune models [43, 44]. Moreover, activating mutations of STING have been identified in human patients with a vasculitis/pulmonary inflammation syndrome characterized by increased type I IFN production [45]. Indeed, the use of $STING^{-/-}$ mice revealed that spontaneous T cell priming against tumor antigens was markedly reduced in vivo, and rejection of immunogenic tumors was ablated [46]. Moreover, tumor-derived DNA was detected within the cytosol of a major population of tumor-infiltrating DCs, which was associated with STING pathway activation and IFN-β production. Therefore, the host STING pathway appears to be a major innate immune sensing pathway that is activated in the tumor context to drive DC activation and subsequent T cell priming against tumor-associated antigens in vivo. Several additional tumor model systems have confirmed a role for the STING pathway in antitumor immunity in vivo [47–49].

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 the cGAS/STING axis for promoting immune-mediated tumor control, particularly in the non-inflamed tumor subset. Recent studies have pursued intratumoral injection of STING agonists. 5,6-Dimethylxanthenone-4-acetic acid (DMXAA) is a flavonoid compound that was previously shown to have anti-tumor activity in mouse models [50]. This drug ultimately failed in humans when combined with chemotherapy in a Phase 3 trial in non-small cell lung cancer [51]. Structure-function studies demonstrated that DMXAA is a direct ligand for mouse STING [52, 53]. 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. Recent evidence has confirmed that DMXAA is a strong agonist of the mouse STING pathway in vitro and in vivo. Intratumoral injection of DMXAA markedly augmented endogenous priming of tumor antigen-specific $CD8^+$ T cells and caused complete tumor elimination. Rejection was completely dependent on host 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 that also bind mouse STING and showed similarly potent efficacy in preclinical tumor models [54]. These agents will be attractive for clinical translation as a potential strategy to initiate de novo inflammation, DC activation, and T cell priming especially in non-T cell-inflamed tumors.

An alternative approach 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, augment specific T cell priming, and support T cell-mediated tumor control in vivo [55]. Based on the observation 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, recent data have revealed that the therapeutic efficacy of radiation was

largely ablated in STING$^{-/-}$ hosts. This defect was associated with blunted type I IFN induction and markedly reduced T cell priming. In contrast, no defect in the therapeutic effect of radiation was observed using mice lacking specific TLR signaling pathways [56]. 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.

2.4 Reverse-Translational Research to Identify New Therapeutic Angles for Non-T Cell-Inflamed Tumors

An additional major strategy for identifying molecular mechanisms that control the presence or absence of a T cell-inflamed tumor microenvironment is to interrogate categories of genomic heterogeneity directly from patients. By clustering patients has having a T cell-inflamed versus non-T cell-inflamed tumor microenvironment using gene expression profiling as a defined phenotype, reproducible genetic or genomic patterns can be identified as a correlation. 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 tumor in a given subject might contain or lack spontaneous T cell infiltration. These categories are differences in accessory oncogene pathways within the tumor cells based on somatic mutational events, germline polymorphisms in immunoregulatory genes that could set thresholds for immune cell activation, or environmental differences that could have global effects on immune functionality. Regarding the latter category, the major phenomenon that has recently garnered interest is the impact of the intestinal microbiome on systemic immune responses in the host. Importantly, each of these parameters is measurable in individual patients. Somatic heterogeneity in tumors can be assessed through exome sequencing and pathway analysis, germline heterogeneity in the host can be evaluated using SNP arrays on peripheral blood cells, 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. These analyses require prospective tissue collection from patients embarking on immunotherapy treatments—fresh tumor biopsies, peripheral blood specimens, and stool samples. Broad-based tissue banking from cancer patients participating in immunotherapy clinical trials should be supported and represents a rich discovery opportunity to identify mechanisms of immunotherapy success versus resistance.

Such an analysis has been initiated in metastatic melanoma patients, focusing first on somatic differences at the level of the tumor itself. Using a series of 266 melanoma metastases, tumors were categorized based on the presence or absence of the gene signature indicative of the T cell-inflamed tumor microenvironment [3]. 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 Wnt/β-catenin pathway. Some tumors had activating mutations in β-catenin itself, some had inactivating mutations in negative regulators of β-catenin, and some showed over-expression of Wnt7B or Frizzled 3. 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-intrinsic β-catenin activation was sufficient to exclude T cell infiltrates in vivo. The molecular mechanism was narrowed down to a loss of chemokines that mediate recruitment of Batf3-lineage DCs into the tumor microenvironment, leading to defective T cell priming. The therapeutic activity of anti-CTLA-4 + anti-PD-L1 mAb normally seen was lost when tumors additionally expressed active β-catenin [57]. Thus, the Wnt/β-catenin pathway is the first identified tumor-intrinsic oncogene pathway to result in immune exclusion and resistance to immunotherapy. 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.

Ongoing work also investigates germline polymorphisms as they relate to the presence or absence of the T cell-inflamed tumor microenvironment. There is precedent for germline genetic differences influencing response to immunotherapy. A SNP in the gene encoding the chemokine receptor CCR5 was identified that was associated with clinical response to high-dose IL-2 [58]. 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 [59]. Numerous polymorphisms have been identified in immune regulatory genes that are associated with various types of autoimmunity, including lupus [60], and many patients who are treated with immune-potentiating drugs do develop autoimmune-like adverse events. Thus, it is attractive to consider that specific germline SNPs might be associated either with clinical response or with side effects upon treatment with anti-CTLA-4 or anti-PD-1 mAbs.

The third category of biomarkers is the composition of the intestinal microbiota. The group of Trinchieri and colleagues has 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 [61]. In addition, Zitvogel and colleagues reported that the immune-potentiating effect of cyclophosphamide is associated with translocation of commensal bacteria [62]. These early data have prompted a comprehensive analysis of the intestinal microbiota using 16S rRNA sequencing from patients undergoing treatment with immunotherapeutics. Restoring the presence of specific commensal that maximize anti-tumor immunity, such as through the use of a probiotic, represents an additional future immunotherapeutic strategy.

2.5 Conclusions and Future Directions

The paradigm of the T cell-inflamed and non-T cell inflamed tumor microenvironment has provided a useful working model for identifying therapeutic targets for immunotherapy, understanding mechanisms of response versus resistance, and pursuing new strategies for overcoming resistance to expand the range of immunotherapy efficacy. Inasmuch as many of

these concepts have been explored predominantly in melanoma, there is a rich opportunity to investigate these principles similarly in other tumor types. A summary of candidate interventions to improve immunotherapy efficacy to include the non-T cell-inflamed tumor microenvironment based on these principles is illustrated in Fig. 2.3. One could envision intratumoral administration of innate immune activators such as STING agonists, to trigger de novo DC activation and T cell priming and recruitment. Tumor-focused radiation also may have these effects. If specific oncogene pathways are activated such as β-catenin, then targeted inhibitors could be administered to block such pathways and restore immune cell entry. If unfavorable germline genetics are identified, specific gene products might be amenable to pharmacologic manipulation as well. Finally, if commensal bacteria are identified that might amplify host anti-tumor immunity, then probiotics could be developed to improve T cell infiltration and clinical efficacy of immunotherapies such as anti-PD-1. Ultimately, combination therapies will likely provide the broadest and deepest clinical benefit.

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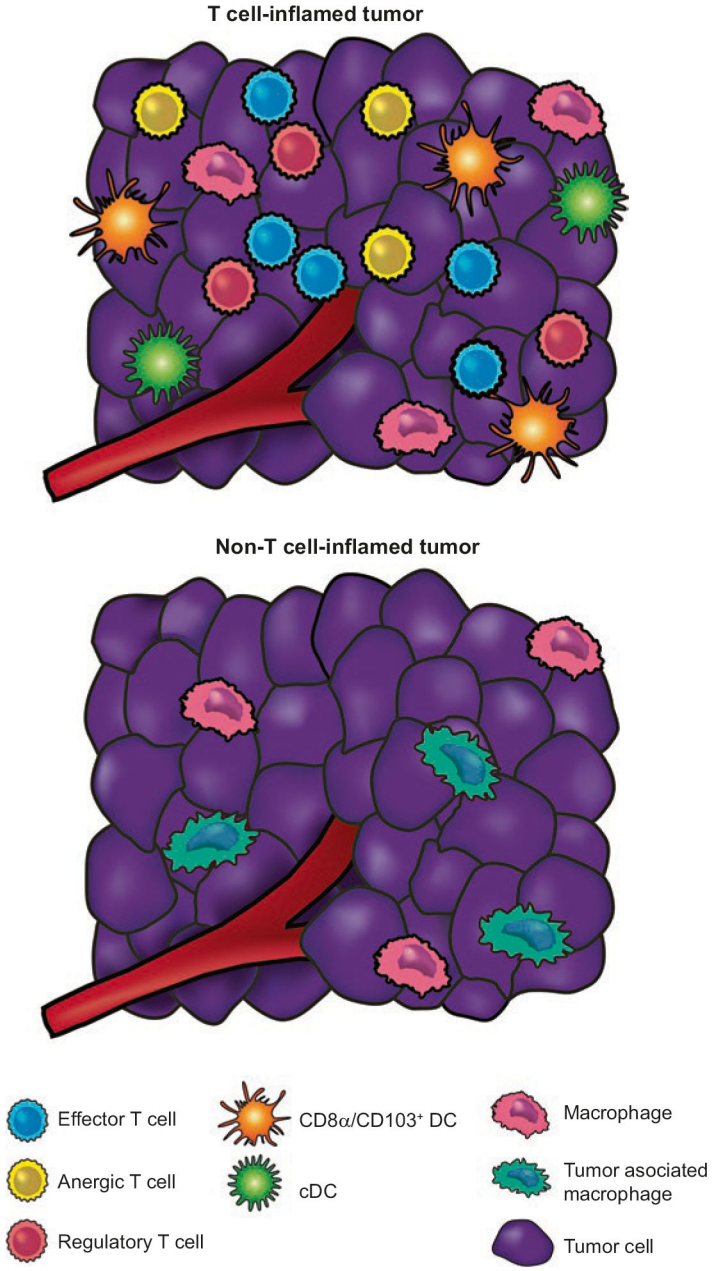

Fig. 2.1.

Immunologic composition of the T cell-inflamed versus non-T cell-inflamed tumor microenvironments. The T cell-inflamed tumors contain variable numbers of $CD8^+$ T cells and CD8α/CD103-lineage DCs, but also possess the highest density of $FoxP3^+$ Tregs. In addition, many of the conventional T cells have a dysfunctional anergic phenotype. In contrast, the non-T cell-inflamed tumors lack these elements but still contain blood vessels, fibroblasts, and macrophages that help support tumor growth. Recruitment of $CD8^+$ effector cells is largely dependent on the chemokines CXCL9 and CXCL10, which engage the receptor CXCR3. Treg recruitment is primarily driven by CCL22, which is in part produced by activated $CD8^+$ T cells

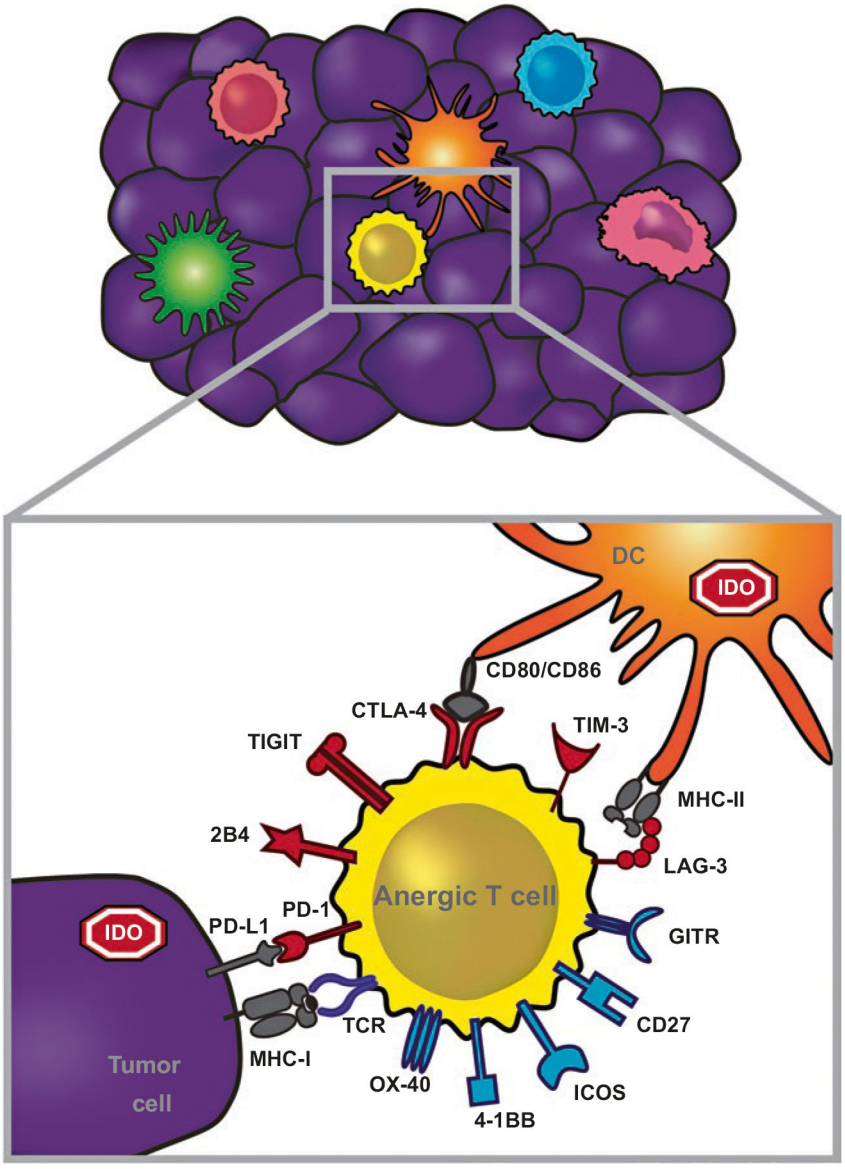

Fig. 2.2.

Immunotherapeutic targets that are preferentially relevant for the T cell-inflamed tumor microenvironment subset. T cell-inflamed tumors contain activated $CD8^+$ T cells but also express IDO and PD-L1, which inhibit T cell function. The dysfunctional/anergic T cells in the tumor microenvironment also can express an array of additional inhibitory receptors, including LAG-3, Tigit, Tim3, and 2B4. But in addition, these T cells also para-doxically express costimulatory receptors, including 4–1BB, Ox40, ICOS, GITR, and CD27. Both blockade of inhibitory receptors and ligation of costimulatory receptors are being developed as cancer therapeutics. Additional candidate immune suppressive factors not shown here that have yet to be effectively targeted clinically include TGF-β, IL-10, iNOS, and PGE_2

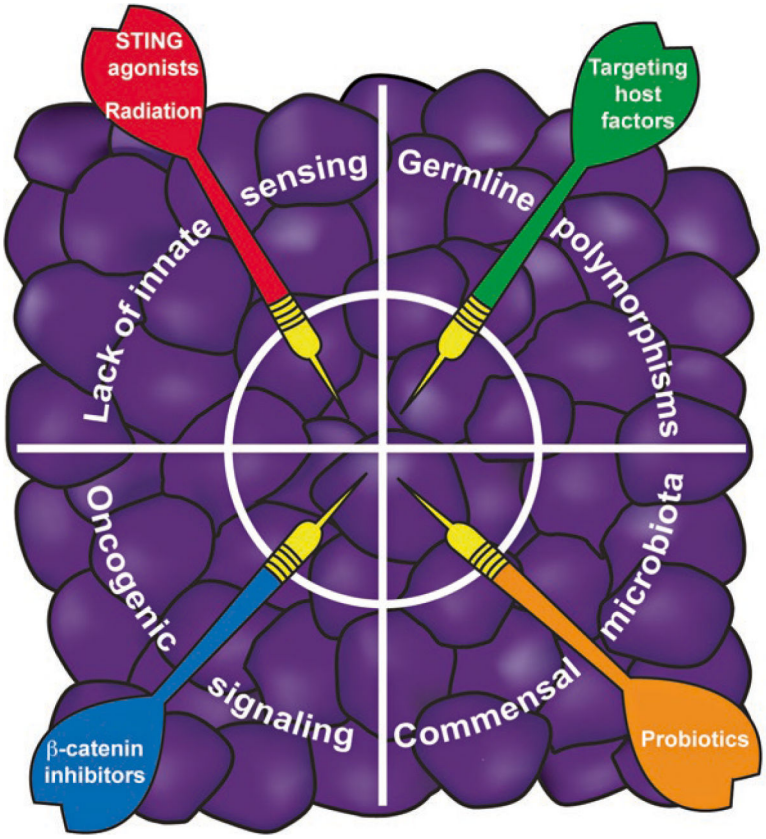

Fig. 2.3.

Summary of four types of strategies that could be considered to overcome the barrier of the non-T cell-inflamed tumor microenvironment. It is envisioned that intratumoral administration of innate immune activators or local tumor radiation, modulators of host polymorphic gene products, blockade of immune-exclusionary oncogene pathways, and delivery of probiotics that amplify anti-tumor immunity all could be considered for ultimate clinical translation