Commentary

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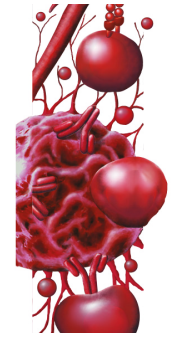
Immunotherapy



The T-cell-inflamed tumor microenvironment as a paradigm for immunotherapy drug development

Daniel J Olson*,[1] & Jason J Luke[1]

[1]Department of Medicine, Section of Hematology/Oncology, The University of Chicago, 5841 South Maryland Ave, MC 2115, Chicago, IL 60637, USA

*Author for correspondence: Tel.: +1 773 834 4183; Fax: +1 773 702 3163; dolson2@medicine.bsd.uchicago.edu

"The concept of T-cell-inflamed and noninflamed tumor offers a framework to rationally design novel therapies to overcome resistance to immunotherapy, and to segregate patients to the rational combinatorial treatments."

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The development of immunotherapy in solid tumors has reframed treatment landscapes across multiple tumor types. Despite improvements in median clinical outcomes, most patients do not respond, highlighting the need for improved patient selection as well as strategies to overcome mechanisms of resistance. In patients whose tumors respond to immunotherapy, an active immune response can commonly be observed prior to treatment. This is characterized by infiltrating antigen-specific T cells and elements of a feed-forward type I and type II interferon-associated gene expression loop. This phenotype has been described as the T-cell-inflamed tumor microenvironment (TME) and appears to robustly, though incompletely, differentiate responding and nonresponding tumors. This model of T-cell-inflamed versus non-T-cell-inflamed tumors provides a potential framework from which to design rational treatment strategies to address resistance to immunotherapy.

T-cell-inflammation in the TME

The T-cell-inflamed TME is characterized by elevated expression of gene transcripts associated with type 1 interferon, as well as promigratory chemokines that result in recruitment of activated $CD8^+$ effector T cells into the TME [1]. While multiple biomarkers have been described for effective cancer immunotherapy, gene expression profiling centered on interferon and T-cell-associated genes may be the most robust predictor of immunotherapy efficacy [2,3]. Paradoxically, a baseline T-cell-inflamed phenotype does not necessarily convey an antitumor effect in and of itself. The spontaneous infiltration of $CD8^+$ cells and IFNγ secretion can lead to upregulation of local negative immunoregulatory mechanisms that suppress T-cell effector function. Some of these mechanisms may include PD-L1 expression, modification of TME metabolism by indoleamine-2,3-dioxygenase and other enzymes, as well as recruitment of $FOXP3^+$ Treg and other immunosuppressive cells types that exert negative feedback on the initial T-cell response [4]. This local immunosuppression may then facilitate tumor escape and proliferation despite the initial inflamed TME.

Across multiple tumor types and immunotherapeutic modalities, the description of the T-cell-inflamed TME by gene expression profiling correlates with efficacy and conversely non-T-cell-inflamed gene signatures correlate with a lack of response [2,3]. In both a binary and continuous fashion, a T-cell-inflamed gene signature can serve as a paradigm from which to characterize mechanisms of tumor-immune evasion and to help guide the development of novel therapeutic strategies to overcome resistance to immunotherapy. In one fashion, this includes a focus on overcoming resistance mechanisms within moderately to highly T-cell-inflamed-tumors while alternatively a focus may be on immune priming to stimulate the initial steps necessary to convert a highly non-T-cell-inflamed to a T-cell-inflamed tumor.

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Enhancing response in a T-cell-inflamed TME

While the T-cell-inflamed TME is enriched in tumors that respond, not all patients with a T-cell-inflamed tumors respond to immunotherapy, and some responders may later develop resistance [5]. Selecting patients prior to, or early on, treatment by T-cell-inflamed gene expression offers an opportunity to target the mechanisms of resistance within an inflamed tumor. Much as CTLA4 and PD-L1 are physiologic immune checkpoints against autoimmunity, additional checkpoints and regulatory mechanisms serve to negate the potential for self-antigen recognition and may limit antitumor-immune responses. Some of these checkpoints have become attractive targets to overcome resistance within T-cell-inflamed tumors [6] with examples including LAG3 and TIM3.

Lymphocyte activation gene 3

Lymphocyte activation gene 3 (*LAG3*) is a cell surface molecule expressed on lymphocytes associated with decreased cytolytic function. LAG3 was originally described in a role potentiating $CD4^+$ Treg function, but it has also been demonstrated to have a direct regulatory effect on $CD8^+$ effector cells – specifically via mediation of tolerance to tumor antigens [7]. *LAG3* expression rises as part of a feedback loop in response to IFNγ and is expressed simultaneously or in parallel with other immune checkpoints such as PD-L1. In murine models, inhibition of *LAG3* in combination with PD1 demonstrates a synergistic benefit, differentiating from single treatment with anti-PD1 or anti-CTLA4 [6]. These observations were the rationale for early-phase clinical trials of anti-LAG3 with anti-PD1 therapies that have shown promising objective responses in patients who progressed on or after prior anti-PD1/L1 and whose tumors expressed *LAG3* by immunohistochemistry. Importantly, the safety profile of the combination treatment has been similar to anti-PD1 monotherapy [8].

T-cell immunoglobulin mucin

T-cell immunoglobulin mucin (TIM3) is transmembrane protein that is expressed preferentially on IFNγ-secreting Th1 cells where it plays a key role in attenuating immune responses. Binding of TIM3 to its canonically described ligand, galectin 9, leads to apoptosis of Th1 cells [9]. TIM3 is also expressed on $CD8^+$ T-effector cells in the context of T-cell anergy. For example, TIM3+ tumor-infiltrating lymphocytes have been found to be simultaneously expressed with PD-L1, especially among those tumor-infiltrating lymphocyte subsets with a dysfunctional phenotype characterized by lack of proliferation and cytotoxic function. This observation generated the hypothesis that combination checkpoint blockade with anti-TIM3 with anti-PD1 may restore $CD8^+$ cell function. Multiple ongoing studies of anti-TIM3 antibodies are being evaluated in early-phase clinical trials, both as monotherapy and combined with anti-PD1 therapies.

Direct activation in the non-T-cell-inflamed TME

In contrast with T-cell-inflamed tumors, non-T-cell-inflamed tumors demonstrate an absence of adaptive immunity and targeting negative regulatory mechanisms is unlikely to be effective. In these non-T-cell inflamed tumors, novel approaches are more likely to succeed by first addressing this lack of baseline adaptive immune response. Particularly, treatments that may directly stimulate innate immune responses and type-1 IFN may drive downstream IFNγ leading to the generation of a T-cell-inflamed phenotype. Key examples highlighting such strategies are outlined in the following sections.

Talimogene laherparepvec

Talimogene laherparepvec (T-VEC) is a modified oncolytic virus built from the genetic framework of the HSV-1. The HSV-1 itself is genetically altered to attenuate its own virulence and to secrete human granulocyte macrophage colony-stimulating factor (GM-CSF). T-VEC is administered via direct intratumoral injection and upon lysis of tumor cells, secretion of GM-CSF is proposed to lead to trafficking and activation of antigen-presenting cells. These augmentations of tumor-sensing and antigen-exposure facilitate the creation of an adaptive, T-cell response within the TME and possibly in distant antigenically similar tumors (noninjected sites). The Phase III OPTiM study of T-VEC compared with GM-CSF demonstrated a survival benefit in those patients with limited unresectable or metastatic disease and T-VEC was the US FDA approved for this indication [10]. Given the efficacy of T-VEC monotherapy and its role in driving T-cell priming, T-VEC has been introduced in combination studies with anti-CTLA4 and anti-PD1. An initial Phase Ib combination of anti-PD1 with T-VEC resulted in a greater than 50% clinical response rate and an ongoing Phase III trial of pembrolizumab + T-VEC versus pembrolizumab alone will seek to further evaluate the broader efficacy of this combination [11]. Of note, patients who responded

to the combination of T-VEC and anti-PD1 showed increased IFNγ signatures regardless of their baseline IFNγ signatures supporting the hypothesis that the combination can augment the development of the T-cell-inflamed TME [12].

Toll-like receptor agonists

These receptors, expressed on innate immune cells, recognize pathogen-associated molecular patterns and mediate the production of proinflammatory cytokines, such as type-1 IFN, upon ligand binding [13]. It has been hypothesized that Toll-like receptor (TLR) agonists used in combination with checkpoint inhibition may generate responses in non-T-cell-inflamed tumors. In a Phase Ib study of a SD-101, a TLR9 agonist, in combination with anti-PD1, this combination was shown to be well tolerated with a responses rates of 60% in the frontline setting in advanced melanoma [14]. Similarly, the combination of ipilimumab with the TLR9 agonist, IMO-2125 in anti-PD1 refractory patients has been demonstrated to be safe, with a response rate of 47% and suggestion that the combination may be able to overcome immune exclusion in some patients [15]. The TLR8 agonist, resiquimod, has also been studied for treatment of in-transit melanoma lesion where it both generated clinical responses, and was associated with activation of dendritic cells and IFN signaling [16]. These mechanistic studies and initially promising response rates seen in the Phase Ib/II studies support further investigations of these combinations.

Stimulator of interferon gene pathway

The observation that a subset of tumors harbor a baseline T-cell infiltrate spurred interest in the mechanisms that generated the trafficking of T cells into the TME. Specifically, the question centered on what innate pathways led to tumor detection, and then signaled the adaptive immune response to generate a T-cell-inflamed TME. Interrogation of multiple immune-sensing pathways that may induce type-1 IFN production in response to cancer revealed the importance of the cGAS/stimulator of interferon gene (STING) pathway. STING activates in response to the detection of cytosolic tumor-derived DNA leading to increased IFN expression and secretion [17]. The intratumoral delivery of cyclic dinucleotide direct STING agonists in murine models has suggested powerful antitumor responses mediated by robust $CD8^+$ T-cell infiltrates and improved animal survival [17]. Human STING agonists are now in early-phase clinical trials as monotherapies as well as in combination with anti-PD1. In a non T-cell-inflamed tumor, such combinations could potentially generate a T-cell-inflamed phenotype, and then overcome the local immune suppressive mechanisms – ultimately with the hope to generate a durable clinical benefit for patients who would not otherwise respond checkpoint therapy.

Radioimmunotherapy

Radiotherapy, beyond its direct cytotoxic effect, also directly affects the TME, and may serve as a therapy capable of overcoming resistance in non-T-cell-inflamed tumors. Induction of cell death and its resulting immunogenic inflammation may offer an opportunity to augment the generation of antitumor immunity [18]. Ablative radiation can independently achieve an antitumor effect via direct, lethal DNA damage; however, ablative radiation can additionally induce tumor regression via T-cell-mediated antitumor effect via the stimulation of type-1 IFN signaling [19]. Radiation thus may be an attractive therapeutic strategy as a combinatorial partner with checkpoint immunotherapy to overcome resistance in noninflamed tumors. Questions do remain about the optimal radiation fractionation strategy, as well as the potential for radiation to also induce migration of immunosuppressive cells into the TME. This is an active area of study with the first prospective study of pembrolizumab and multisite stereotactic body radiotherapy in advanced solid tumor patients showed that the combination was well tolerated [20].

The successes of immunotherapy have been driven in part by an improved understanding of the tumor–immune interface and the mechanisms that mediate immune recognition of cancer. While these developments have been important, there remain a majority of patients who do not derive durable responses from immunotherapy. The concept of T-cell-inflamed and noninflamed tumor offers a framework to rationally design novel therapies to overcome resistance to immunotherapy, and to segregate patients to the rational combinatorial treatments. As the complexities of the tumor-immune relationship are further elucidated, more precise immune-modulatory therapeutics have the potential to expand the population of patients who can benefit from immunotherapy.

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References

1. Harlin H, Meng Y, Peterson AC *et al.* Chemokine expression in melanoma metastases associated with CD8+ T-cell recruitment. *Cancer Res.* 69(7), 3077–3085 (2009).
2. Ayers M, Lunceford J, Nebozhyn M *et al.* IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade. *J. Clin. Invest.* 127(8), 2930–2940 (2017).
3. Ulloa-Montoya F, Louahed J, Dizier B *et al.* Predictive gene signature in MAGE-A3 antigen-specific cancer immunotherapy. *J. Clin. Oncol.* 31(19), 2388–2395 (2013).
4. Spranger S, Spaapen RM, Zha Y *et al.* Up-regulation of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. *Sci. Transl. Med.* 5(200), 200ra116–200ra116 (2013).
5. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell* 168(4), 707–723 (2017).
6. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* 12(4), 252–264 (2012).
7. Grosso JF, Kelleher CC, Harris TJ *et al.* LAG-3 regulates CD8+ T cell accumulation and effector function in murine self- and tumor-tolerance systems. *J. Clin. Invest.* 117(11), 3383–3392 (2007).
8. Ascierto PA, Melero I, Bhatia S. Initial efficacy of anti-lymphocyte activation gene-3 (anti–LAG-3; BMS-986016) in combination with nivolumab in patients with melanoma previously treated with anti-PD-1/PD-L1 therapy. (2017).
9. Freeman GJ, Casasnovas JM, Umetsu DT, DeKruyff RH. TIMgenes: a family of cell surface phosphatidylserine receptors that regulate innate and adaptive immunity. *Immunol. Rev.* 235(1), 172–189 (2010).
10. Andtbacka RHI, Kaufman HL, Collichio F *et al.* Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. *J. Clin. Oncol.* 33(25), 2780–2788 (2015).
11. Long GV, Dummer R, Ribas A *et al.* Efficacy analysis of MASTERKEY-265 Phase 1b study of talimogene laherparepvec (T-VEC) and pembrolizumab (pembro) for unresectable stage IIIB-IV melanoma. *J. Clin. Oncol.* 34(15), 9568 (2017).
12. Ribas A, Dummer R, Puzanov I *et al.* Oncolytic virotherapy promotes intratumoral t cell infiltration and improves anti-PD-1 immunotherapy. *Cell* 170(6), 1109–1119.e10 (2017).
13. Amos SM, Pegram HJ, Westwood JA *et al.* Adoptive immunotherapy combined with intratumoral TLR agonist delivery eradicates established melanoma in mice. *Cancer Immunol. Immunother.* 60(5), 671–683 (2011).
14. Ribas A, Medina T, Kummar S *et al.* Abstract CT139: durability of responses to the combination of SD-101 and pembrolizumab in advanced metastatic melanoma: results of a Phase Ib, multicenter study. *Cancer Res.* 78(13 Suppl.), CT139–CT139 (2018).
15. Diab A, Rahimian S, Haymaker CL *et al.* A Phase 2 study to evaluate the safety and efficacy of intratumoral (IT) injection of the TLR9 agonist IMO-2125 (IMO) in combination with ipilimumab (ipi) in PD-1 inhibitor refractory melanoma. *J. Clin. Oncol.* 36(15), 9515 (2018).
16. Scutti JAB, Vence LM, Royal RE *et al.* Abstract 614: resiquimod, a Toll-like receptor agonist promotes melanoma regression by enhancing plasmacytoid dendritic cells and T cytotoxic activity as a vaccination adjuvant and by direct tumor application. *Cancer Res.* 78(13 Suppl.), 614–614 (2018).
17. Corrales L, McWhirter SM, Dubensky TW Jr., Gajewski TF. The host STING pathway at the interface of cancer and immunity. *J. Clin. Invest.* 126(7), 2404–2411 (2016).

18 . Weichselbaum RR, Liang H, Deng L, Fu Y-X. Radiotherapy and immunotherapy: a beneficial liaison? *Nature*14(6), 365–379 (2017).

19. Burnette BC, Liang H, Lee Y *et al.* The efficacy of radiotherapy relies upon induction of type i interferon-dependent innate and adaptive immunity. *Cancer Res.* 71(7), 2488–2496 (2011).

20. Luke JJ, Lemons JM, Karrison TG *et al.* Safety and clinical activity of pembrolizumab and multisite stereotactic body radiotherapy in patients with advanced solid tumors. *J. Clin. Oncol.* 36(16), 1611–1618 (2018).