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Classifying cancers based on T cell infiltration and PD-L1

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Abstract

Cancer immunotherapy may become a major treatment backbone in many cancers over the next decade. There are numerous immune cell types found in cancers and many components of an immune reaction to cancer. Thus the tumor has many strategies to evade an immune response. It has been proposed that four different types of tumor microenvironment exist based on the presence or absence of tumor infiltrating lymphocytes and programmed death-ligand 1 (PD-L1) expression. We review this stratification and the latest in a series of results that shed light on new approaches for rationally designing ideal combination cancer therapies based on tumor immunology.

Keywords

T cell checkpoint; antibodies; tumor microenvironment; immunotherapy

Introduction

After years of controversy, it is now recognized that the immune system can play a role in the control of tumor growth and progression (1), a process known as cancer immunoediting (2). The host immune system can also contribute to the efficacy of some cancer therapies where the tumor death induced may be “immunogenic” (3). While the principles of cancer immunoediting have largely been defined in mice with immunogenic tumors, it has now been demonstrated that an immune reaction against cancer can also occur in humans (4). In tumors, there are all types of immune cells that can have various effects on tumor

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progression, and a spectrum of soluble cytokines and chemokines that regulate the entry of different types of infiltrating immune cells. These cells can be located in the tumor centre (CT), in the invasive margin (IM) or in the adjacent tertiary lymphoid structures (TLS). Notably, immune infiltrates are highly heterogeneous, not only between tumor types, but also within one patient or between different patients with the same cancer types.

A majority of studies using human samples have reported a T_H1 -type signature to be associated with good clinical outcome in many different tumor types including colorectal cancer, melanoma, head and neck, breast, bladder, urothelial, ovarian, renal, prostate and lung cancer (4,5). In general, high densities of myeloid cells, i.e. macrophages and myeloid-derived suppressor cells (MDSCs), correlate with poor prognosis (6). When it has been characterized, it appears that the negatively impacting macrophages are of the M2 phenotype (7). In any case, the correlation between macrophage density and patient survival is less significant than that of T cells, particularly $CD8^+$ T cells (8).

Furthermore, the field of cancer immunotherapy has experienced a resurgence in recent years, due in part to the remarkable clinical efficacy observed with immune checkpoint inhibitors against a number of cancer types such as melanoma, renal cell carcinoma, bladder cancer, non small cell lung carcinoma (NSCLC) and Hodgkin's disease (9–12) (13). Immune checkpoint receptors on immune cells, when engaged by their ligands, transmit an inhibitory signal, maintain self-tolerance and regulate the duration and amplitude of immune responses in peripheral tissues in order to minimize tissue pathology (14). We now appreciate cancer can use these pathways to suppress tumor immunity. In the clinic, three immune checkpoint inhibitor antibodies have been approved by the US FDA for the treatment of advanced melanoma, the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) blocking antibody ipilimumab, and two antibodies blocking programmed death 1 (PD-1), pembrolizumab and nivolumab. Anti-CTLA-4 and anti-PD-1 are thought to mediate their anti-tumor activity by blocking CTLA-4 or PD-1 on effector immune cells (such as $CD8^+$ T cells) from interacting with their ligands CD80/CD86 or PD-L1/PD-L2 (program death ligand 1/2), respectively (9,10). This release of suppression on effector cells thus allows their full anti-tumor function to be exerted. Central to the efficacy of immune checkpoint blockade is the requirement for immune cells to infiltrate into tumors.

In this perspective, we discuss the current effort to predict patients who will respond to checkpoint blockade, particularly anti-PD-1 or anti-PD-L1, according to a framework previously proposed to stratify the tumor microenvironment into different types based on the presence or absence of tumor infiltrating lymphocytes (TILs) and PD-L1 expression (15) (16). The strengths and weaknesses of this stratification are raised. We conclude by discussing which immunotherapeutic strategies are best suited to treat different tumors based on this proposed stratification and how the framework may be refined.

Success of immune checkpoint blockade defines adaptive immune resistance

Excitement regarding immune checkpoint inhibitor therapies such as anti-CTLA-4 and anti-PD-1/PD-L1, has resulted from the unprecedented number of durable clinical responses

(measured in years) obtained in patients with a variety of advanced cancer types (10,17–20). This new survival profile now raises questions about how to increase the number of patients who receive long-term clinical benefit from immune checkpoint inhibitor therapy, and how to predict the patients that will respond. An earlier study in biopsies of patients with melanoma demonstrated that TILs were strongly associated with local PD-L1 expression on the tumor (primary or metastases) (15). PD-L1 is generally not detectable in normal tissues but inflammatory cytokines, particularly IFN- γ , can up-regulate its expression in various cell types including tumors. This indicates that tumors up regulate PD-L1 in response to IFN- γ released by TILs as an adaptive immune-resistance mechanism (14) to suppress local effector T-cell function, implying that immunosurveillance exists even in advanced cancers. PD-L1 can also be expressed constitutively on cancer cells through poorly characterized oncogenic signaling pathways (21,22). Indeed, PD-L1 expression has been observed in various solid human malignancies, including melanoma, breast, lung, kidney cancer as well as Hodgkin's disease, and is a major factor in evaluating responses to anti-PD-1/PD-L1 therapies (11,23,24). Given the responses observed with anti-PD-1/L1 and its better safety profile compared to ipilimumab, the identification and characterization of factors in the tumor microenvironment that predicts which patients will respond to anti-PD-1/L1 is a top priority in cancer medicine (25).

Classification of tumor microenvironments based on TIL and PD-L1 expression

Strengths

Classification of tumors into 4 groups on the basis of their PD-L1 status and presence or absence of TILs has already been proposed (Fig. 1 adapted from (15)). These include type I (PD-L1 positive with TILs driving adaptive immune resistance), type II (PD-L1 negative with no TIL indicating immune ignorance), type III (PD-L1 positive with no TIL indicating intrinsic induction), and type IV (PD-L1 negative with TIL indicating the role of other suppressor(s) in promoting immune tolerance). The proportions of various human tumors that fit into each of these types, as defined by TILs/PD-L1 status, likely depends on the genetic aberrations and oncogene drivers of the cancer as well as the tissue they arise in. In human melanoma—where the data is most mature, a high proportion of type I (~38%) and type II (~41%) tumors are observed, with the former having considerably the best prognosis. Good analogous frequencies of tumor type generated by the same methodologies are not yet available for most other cancers. Yet at this stage, it's fair to assume that type I cancer microenvironments are not as prevalent as observed in melanoma. Indeed, in some cancers like NSCLC, oncogenes may be more important drivers of tumor PD-L1 expression and thus the frequency of type III tumors may be higher than observed in melanoma. Other cancers like pancreatic cancer have a lower level of PD-L1 expressed on tumor and intratumor immune cells as measured by IHC (11). In one recent IHC study of NSCLC, PD-1 positivity was significantly associated with current smoking status and with the presence of KRAS mutations, whereas PD-L1 was significantly associated to adenocarcinoma histology and with presence of EGFR mutations (26). Increased levels of CD3 and CD8⁺ TILs were associated with better outcome in a large series of NSCLC, but only CD8 was independent from other prognostic variables (27).

Favorably, this simple initial stratification of human tumors into 4 types based on their immune reactions sets a framework to identify which pathways should be targeted to elicit the best response for each tumor type. We will briefly describe how different types of immunotherapeutic approaches can be applied to this classification below. Even within each tumor type, we envisage that further stratification correlating with outcome can be made as the patient cohort treated with anti-PD-1/PD-L1 increases and the data becomes mature for different cancer types. For example, further stratification might be based on whether the tumor is primary or metastatic and sub-stratified based on spatial distribution of immune infiltration (immune contexture) as demonstrated in Erdag *et al.*(28).

Caveats

From the outset it is clear that this simplistic and pragmatic definition of tumor environments merely forms a framework to begin discussions of how best to tailor combination therapies to the tumor microenvironment. TIL density, location, and tumor PD-L1 status will not necessarily define whether tumor-specific T cells and M1 macrophage effectors can be reactivated by therapeutic intervention; instead, tumor origin, genetics, histopathology, and other factors will all probably contribute. Although PD-L1 appears to enrich for response to anti-PD-1/L1 therapy, it has been documented that patients with PD-L1 negative tumors can also respond to treatment, raising concerns that excluding the “marker negative” patient population from treatment might exclude potential responders (29,30). As discussed by Taube *et al.*,(23), this may be due to the differences in staining for PD-L1 and definition of positivity (tumor cells only or expression on other cells in the various studies). In addition, given the focal nature of PD-L1 expression within many tumors and emerging information about intratumoral genetic heterogeneity (31), if very small needle biopsies or dispersed single-cell cytology specimens are evaluated, a false-negative evaluation could potentially result (23). From a recent study it is clear that consideration also has to be given to the PD-L1 expression on various leukocytes in tumors such as myeloid cells and even the T cells themselves (11). Expression of PD-L1 is clearly dynamic where adaptive immune resistance is concerned and thus a static picture of one or few biopsies may not accurately reflect the potential complexity, nor predict outcome. Immune expression of PD-L1 may also be therapeutically relevant and must be seriously considered in the stratification of tumor types. Finally it is likely that PD-L1 expression must be put within the context of additional variables such as the pre-existence of PD-1 positive CD8⁺ T cells with tumor antigen specificity at the invasive tumor margin (25,32).

Requirements for TIL infiltration – Neoantigens and Tumor Vasculature

The availability of germline DNA sequences has allowed exploration of the relationship between host genetics and the development of a favorable immune phenotype. Many somatic tumor mutations may create neo-antigens with the potential to be recognized by the immune system and these can also be identified by high throughput genetics (33,34). Evidence also supports the correlation between genomic instability, density of T cell infiltration, and favorable prognosis in CRC patients (35,36). Interestingly, a number of studies have reported that the hierarchy of PD-L1 expression prevalence correlated with the prevalence of DNA mutations among various cancer types which melanoma, squamous cell carcinoma of the lung, and adenocarcinoma of the lung heading the list of cancers bearing

the highest mutation rate and complexity (37). This suggests that the degree of mutagenesis may directly or indirectly correlate with the degree of immunogenicity of any given tumor (37). Intriguingly, in recent phase 1a clinical trials, responses to anti-PD-L1 (MPDL3280A) were more frequent in patients with smoking-induced NSCLC than in those who did not smoke (38). More recently, Brown *et al.*, performed RNA-seq analysis on 6 different tumor types (colorectal, ovary, breast, brain, kidney, and lung) obtained from 515 patients to identify mutations that were predicted to be immunogenic (39). Their studies demonstrated that mutated epitopes were associated with increased patient survival. Moreover, these corresponding tumors had higher CTL content, and elevated expression of the CTL exhaustion markers *PDCDI* and *CTLA4*. In contrast, mutated epitopes were very scarce in tumors without evidence of CTL infiltration (39). But the correlation between predicted tumor neo-antigen levels and TIL infiltration in tumors is sometimes negligible and other factors are more critical in regulating TIL infiltration.

Tumors disrupt antigen presentation and T/NK-cell activation and homing, through soluble and cell-surface mediators, the vasculature, low levels of innate immune activation and appropriate chemokines, and immunosuppressive cells such as myeloid-derived suppressor cells and regulatory T cells (40)(41). Despite the presence of neo-antigens, there may be a lack of appropriate innate immune activation or chemokines required to promote T cell infiltration (40). In many instances effector T cells do not gain entry into the tumor bed because they are physically blocked by dense stroma or the tumor vasculature. Endothelial cells lining the vessels can suppress T cell activity, target them for destruction, and block them from gaining entry into the tumor in the first place through the deregulation of adhesion molecules (42). T cell extravasation is dependent upon endothelial cell expression of vasculature cell adhesion molecule-1 (VCAM-1) and intracellular cell adhesion molecule-1 (ICAM-1). Tumor-derived growth factors such as VEGF and endothelin-1 (ET-1) signal through VEGFR and ET_BR, respectively, to block the expression of adhesion molecules and inhibit T cell infiltration into the tumor mass. The endothelium regulated by tumor-derived VEGF can inhibit T cell activation by upregulating inhibitory molecules, such as PD-L1, IL-6, IL-10 and IDO. Tumor endothelial cells can also express FasL that selectively leads to apoptosis of Fas expressing effector T cells (43).

Tailoring cancer immunotherapy based on type of tumor microenvironment

Type I cancers (PD-L1⁺TILs⁺)

In advanced melanoma, ~38% of patients present with a type I tumor microenvironment and are thought to be the group that are largely responding to checkpoint blockade (15,23). Type I tumors are most likely to benefit from single agent anti-PD-1/L1 blockade, as these tumors have evidence of pre-existing intratumor T cells that are turned off by PD-L1 engagement. Therefore, being able to correctly define this subset may allow the benefit of anti-PD-1/L1 therapy avoiding the additional potential toxicities and costs from using combined immunotherapy approaches.

However, the presence of TIL is not a dichotomous variable, and both density and location of TIL and their interaction with PD-L1 positive tumor microenvironment will need to be considered (32). When T cells are present in sufficient numbers inside the tumor, and these

T cells are inducing an adaptive expression of PD-L1, then patients may be most likely to respond to PD-1/L1 blockade. Therefore, there is a need for a quantitative assessment of TIL and PD-L1 presence in biopsies to derive the desired predictive information. This quantitation may need to be quite sophisticated since the precise level of PD-1 on T cells may correlate strongly with the state of differentiation and level of dysfunction of T cells in other biological models like chronic virus infection (44). Initial responses to single agent PD-1/L1 blocking antibodies will need to be evaluated long term, as it remains unclear what proportion of patients with type I melanoma will survive long term following therapy, and indeed whether patients with type I cancers of other histologies will perform as favorably with single agent therapy.

Anti-PD-1 may also be either substituted or combined with various anti-PD-L1 mAbs (MPDL3280A, BMS 936559, MSB0010718C), which are currently being evaluated in clinical trials (11,12,45). An anti-PD-1 antibody should prevent PD-1 from interacting with both PD-L1 and PD-L2, but not the known interaction between PD-L1 and the co-stimulatory molecule CD80 (B7-1). By contrast, most anti-PD-L1 antibodies would block interactions with both CD80 and PD-1, but not PD-L2:PD-1 which would still allow the function of PD-L2 to be preserved while relieving PD-1 mediated suppression (46). Furthermore, some tumors have been reported to express PD-L2 (47). Thus, it is possible that, depending upon which interactions dominate in a particular cancer, PD-1 and PD-L1 antibodies might not have redundant activity, suggesting their use in combination may be a potential avenue to increase anti-tumor efficacy. Notably, PD-1 blockade will also inhibit interactions of T cells with PD-L2 expressed on antigen presenting cells, especially in the lung, which could increase the chances for toxicity, as shown in patients treated with nivolumab who show increased risk of pneumonitis (10). In contrast, the preservation of the PD-L2 and PD-1 pathway would maintain immune tolerance in the lymphoid organs and may explain the relatively infrequent immune related adverse events in patients treated with anti-PD-L1 (37,48). The diversity of interactions amongst these three ligands (which belong to the so called B7 family) with PD-1 and other receptors underscores the complexity of the crosstalk between T cells, surrounding immune cells and tumor. In addition to T cells, PD-1 is also expressed on other immune cell types such as B cells, NK cells, DCs and activated monocytes, although it is not known how PD-1 blockade impacts on the anti-tumor function of these cell types.

Other targets have been associated with inhibition of lymphocyte activity. PD-1, LAG-3 (Lymphocyte-activation gene 3), TIGIT (T cell immunoreceptor with Ig and ITIM domains) and TIM-3 (T-cell immunoglobulin domain and mucin domain 3) are commonly co-expressed on activated and potentially exhausted T cells in the tumor microenvironment, their targeting using specific antibodies—either alone, together, or in combination with other immunotherapies—has been already shown to enhance anti-tumor immunity in mouse models of cancer (49–52). Although human blocking antibodies that are specific for a number of these inhibitory receptors are under development, very few have yet entered the clinic. These make good candidates for testing in type I tumors and perhaps other types of cancers where TILs are present, but anti-PD1/PD-L1 are ineffective (eg. type IV). Not only inhibiting checkpoints, but also agonizing T and antigen presenting cell function via co-

stimulatory molecules and Toll-like receptors has great merit in these cancers where TILs are present and potentially functional.

Type II cancers (PD-L1⁻TIL⁻)

A large fraction of melanoma patients (~41%) present with a type II tumor microenvironment and are predicted to have very poor prognosis based on their lack of detectable immune reaction. In this group of patients, single agent checkpoint blockade would most likely not to be successful given the lack of pre-existing T cell infiltrates. Combination therapy that is designed to bring T cells into tumors and then avoid them being turned off, such as the combination of anti-CTLA-4 and anti-PD-1, would be considered in this scenario. CTLA-4 blockade induces frequent T cell responses beyond its rate of clinical responses (53). A recent trial combining the checkpoint inhibitors ipilimumab and nivolumab reported 45–50% response rates characterized by rapid and deep tumor regression in a substantial proportion of advanced melanoma patients (54). Importantly, the 2 year overall survival rate was ~70%. This trial demonstrates that combination approaches are the way forward for increasing anti-tumor efficacy in the clinic although this has to be balanced by the potential increase risk in toxicity (45). As this combination was shown to be active both in patients with PD-L1 positive and negative tumors, it is logical to think that it could reverse the immune ignorance of type II tumors.

Another approach to attract T cell infiltrates into tumors would be to induce a type I IFN response. Recently, Bald *et al.*, utilized a mouse model of melanoma which had a type II tumor microenvironment and demonstrated that peritumoral injections of immunostimulatory RNA (poly:IC) initiated a cytotoxic inflammatory response (55). They further showed that this infiltration resulted in upregulation of PD-L1 gene expression and importantly showed that anti-PD-1 therapy could synergize with poly:IC to induce regression of established tumors and improved survival compared to single agent treatment alone. Other approaches to attract tumor-specific T cells into these tumors by vaccination or adoptive transfer (eg. chimeric antibody receptor (CAR)-specific T cells (56), if there are known tumor-associated antigens present to target) may be useful approaches in this type of tumor. Certain chemotherapies, small molecule targeted therapies and radiotherapy that all debulk tumors, but at the same time promote “immunogenic” cell death (3), may also be promising strategies for type II tumors.

Type III cancers (PD-L1⁺ TIL⁻)

Only 1% of melanoma patients display a type III tumor microenvironment, although this group may be higher in other cancers such as NSCLC. This may happen when PD-L1 is expressed constitutively on cancer cells through oncogenic signaling. This group highlights that PD-L1 positivity alone cannot be taken as a predictive factor for response to anti-PD-1 or anti-PD-L1 therapies, as without TIL in the tumor it is unlikely that blocking PD-1 or PD-L1 will lead to a T cell response to cancer. For this group of patients, a similar approach for type II patients (as discussed above) might be employed to try to recruit lymphocytes into tumors. Radiotherapy to induce immunogenic cell death to liberate neo-antigens has been used to induce T cell responses in combination with anti-PD-1(57).

Type IV cancers (PD-L1⁻ TIL⁺)

For the ~20% of melanoma patients with a type IV (immune tolerance) tumor microenvironment, other suppressive pathways might be dominant given that many tumors are heterogeneous with respect to the proportion of lymphoid and myeloid cells. A substantial number of M2 polarized macrophages that can be switched to M1 phenotype may control or reduce tumor growth. Certainly, type IV tumors containing TIL, but no obvious adaptive resistance, may also be amenable to targeting of other non-PD-1/PD-L1 checkpoint receptors, other immunosuppressive pathways such as metabolites (eg. adenosine, IDO), and non-T cell effector strategies. These types of therapeutic approaches are mostly still in their infancy, but many will probably enter the clinic in the near future.

Conclusion

Despite advances in the description of immune gene signatures in tumors, no pretreatment biomarker has been validated to date to be included in part of the standard-of-care decision making (although a number of biomarkers have been suggested for anti-CTLA-4 mAb treatment in melanoma patients (58)). The stratification proposed forms a starting framework to consider various combination cancer therapy approaches. The tumor stratification based on the presence of T cells and PD-L1 will likely be more complex than the initial morphological studies performed in melanoma using IHC analyses (15,16,32), and will likely require quantitative and special determination to be used as highly predictive tools to define optimal therapy for patients with advanced cancers. With the ability to perform multiparameter analyses by immunofluorescence or histo-cytology (59) (60), it is likely that in the near future the single or double staining by IHC will be substituted by techniques that allow further T cell, myeloid-macrophage, stromal cell and cancer cell characterization and still maintain the morphology information of the structure of the tumor microenvironment. Imaging technologies should play a central role in non-invasively determining tumor infiltrating leukocytes and the temporal expression of immunosuppressive pathways including PD-L1/PD-1. Furthermore, it is likely that other variables will need to be incorporated, including tumor genomic studies of mutational load, studies of TCR usage and clonality in tumors, and transcriptome studies detecting interferon-inflammatory signatures in tumors. Pre-clinical mouse models generally support the importance of TIL infiltrates and an active PD-1/PD-L1 axis for response to immune checkpoint blockade, but it is clear that every tumor transplant and model is distinct and even some cancers that contain T cells expressing PD-1 maybe resistant to anti-PD-1 therapy. It is early in our understanding of the PD-1/PD-L1 pathway in tumors and both pre-clinical models and more interrogation of patient tumors pre- and post-therapy will greatly accelerate our understanding.

New checkpoint blockade pathways that complement PD-1/PD-L1 interactions hold great promise to improve responses in type I tumors displaying adaptive resistance. Expression of tumor PD-L1 (and other ligands), TIL infiltration and certain genetic signatures of tumor cells will help stratify patients and inform about the best combination strategy to utilize for treatment of each tumor type. The very large fraction of tumors with an immune ignorant phenotype (type II) have very poor prognosis regardless of any treatment intervention, but

being able to define this at baseline would help in deciding to treat with combination immunotherapies that may reverse this situation in certain cases (54). The fraction of immune ignorant tumors may be very high in some non-melanoma cancer types and they will require a completely new strategy of treatment. One could assume that these tumors have strong simple genetic drivers creating no or few neoantigens or that any tumor antigens that were originally present have since been immunoedited. To apply immunotherapy to patients bearing such tumors, effective vaccination of some type is required or neoantigens may have to be introduced into the tumor initiating population, or immune infiltrates engineered. Alternatively, T cells are actively excluded from some of these tumors and manipulation of the vasculature or chemokine axes may allow T cells to infiltrate lesions they could otherwise recognize. Although personalized medicine has the potential to bring the best outcome for any individual cancer patient, to ensure economical development of combination therapies that increasingly incorporate immunology, it is crucial a simple rational stratification is initially employed.

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References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011; 144(5):646–74. [PubMed: 21376230]
2. Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural innate and adaptive immunity to cancer. *Annu Rev Immunol*. 2011; 29:235–71. [PubMed: 21219185]
3. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. *Annu Rev Immunol*. 2013; 31:51–72. [PubMed: 23157435]
4. Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nature Reviews Cancer*. 2012
5. Angell H, Galon J. From the immune contexture to the Immunoscore: the role of prognostic and predictive immune markers in cancer. *Current Opinion in Immunology*. 2013; 25(2):261–7. [PubMed: 23579076]
6. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nature Reviews Immunology*. 2012; 12(4):253–68.
7. Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. *Cancer Res*. 2006; 66(2):605–12. [PubMed: 16423985]
8. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf Anna C, et al. Spatiotemporal Dynamics of Intratumoral Immune Cells Reveal the Immune Landscape in Human Cancer. *Immunity*. 2013; 39(4):782–95. [PubMed: 24138885]
9. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010; 363(8):711–23. [PubMed: 20525992]
10. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012; 366(26):2443–54. [PubMed: 22658127]

11. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature*. 2014; 515(7528): 563–7. [PubMed: 25428504]
12. Powles T, Eder JP, Fine GD, Braiteh FS, Loria Y, Cruz C, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. *Nature*. 2014; 515(7528):558–62. [PubMed: 25428503]
13. Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med*. 2015; 372(4):311–9. [PubMed: 25482239]
14. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nature Reviews Cancer*. 2012; 12(4):252–64.
15. Taube JM, Anders RA, Young GD, Xu H, Sharma R, McMiller TL, et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. *Science Translational Medicine*. 2012; 4(127):127ra37.
16. Sznol M, Chen L. Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer. *Clinical Cancer Research*. 2013; 19(5):1021–34. [PubMed: 23460533]
17. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med*. 2012; 366(26):2455–65. [PubMed: 22658128]
18. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N Engl J Med*. 2013; 369(2):134–44. [PubMed: 23724846]
19. O'Sullivan Coyne G, Madan RA, Gulley JL. Nivolumab: Promising Survival Signal Coupled With Limited Toxicity Raises Expectations. *Journal of Clinical Oncology*. 2014; 32(10):986–88. [PubMed: 24590655]
20. Topalian SL, Sznol M, McDermott DF, Kluger HM, Carvajal RD, Sharfman WH, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. *Journal of Clinical Oncology*. 2014; 32(10):1020–30. [PubMed: 24590637]
21. Parsa AT, Waldron JS, Panner A, Crane CA, Parney IF, Barry JJ, et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. *Nature Medicine*. 2007; 13(1):84–8.
22. Atefi M, Avramis E, Lassen A, Wong DJ, Robert L, Foulad D, et al. Effects of MAPK and PI3K pathways on PD-L1 expression in melanoma. *Clinical Cancer Research*. 2014; 20(13):3446–57. [PubMed: 24812408]
23. Taube JM, Klein AP, Brahmer JR, Xu H, Pan X, Kim JH, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. *Clinical Cancer Research*. 2014; 20(19):5064–74. [PubMed: 24714771]
24. Grosso, JH.; Inzunza, D.; Cardona, D.; Simon, J.; Gupta, A. Association of tumor PD-L1 expression and immune biomarkers with clinical activity in patients with advanced solid tumors treated with nivolumab. ASCO meeting; 2013; p. Abstract: #3016
25. Ribas A, Tumeo PC. The future of cancer therapy: selecting patients likely to respond to PD1/L1 blockade. *Clinical Cancer Research*. 2014; 20(19):4982–4. [PubMed: 24970841]
26. D'Incecco A, Andreozzi M, Ludovini V, Rossi E, Capodanno A, Landi L, et al. PD-1 and PD-L1 expression in molecularly selected non-small-cell lung cancer patients. *British Journal of Cancer*. 2015; 112(1):95–102. [PubMed: 25349974]
27. Schalper KA, Brown J, Carvajal-Hausdorf D, McLaughlin J, Velcheti V, Syrigos KN, et al. Objective measurement and clinical significance of TILs in non-small cell lung cancer. *Journal of the National Cancer Institute*. 2015; 107(3)
28. Erdag G, Schaefer JT, Smolkin ME, Deacon DH, Shea SM, Dengel LT, et al. Immunotype and immunohistologic characteristics of tumor-infiltrating immune cells are associated with clinical outcome in metastatic melanoma. *Cancer Res*. 2012; 72(5):1070–80. [PubMed: 22266112]
29. Gandhi, LBA.; Hui, R. MK-3475 (anti-PD-1 monoclonal antibody) for non-small cell lung cancer (NSCLC)L Antitumor activity and association with tumor PD-L1 expression. AACR Annual Meeting 2014;Abstract CT105. Presented; April 6, 2014;

30. Daud, AIHO.; Ribas, A. Antitumor activity of the anti-PD-1 monoclonal antibody MK-3475 in melanoma: Correlation of tumor PD-L1 expression with outcome. AACR Annual Meeting 2014; Abstract CT105. Presented; April 6, 2014;
31. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med.* 2012; 366(10):883–92. [PubMed: 22397650]
32. Tumei PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature.* 2014; 515(7528):568–71. [PubMed: 25428505]
33. Matsushita H, Vesely MD, Koboldt DC, Rickert CG, Uppaluri R, Magrini VJ, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoeediting. *Nature.* 2012; 482(7385):400–4. [PubMed: 22318521]
34. Yadav M, Jhunjhunwala S, Phung QT, Lupardus P, Tanguay J, Bumbaca S, et al. Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. *Nature.* 2014; 515(7528):572–6. [PubMed: 25428506]
35. Guidoboni M, Gafa R, Viel A, Doglioni C, Russo A, Santini A, et al. Microsatellite instability and high content of activated cytotoxic lymphocytes identify colon cancer patients with a favorable prognosis. *The American Journal of Pathology.* 2001; 159(1):297–304. [PubMed: 11438476]
36. Noshu K, Baba Y, Tanaka N, Shima K, Hayashi M, Meyerhardt JA, et al. Tumour-infiltrating T-cell subsets, molecular changes in colorectal cancer, and prognosis: cohort study and literature review. *The Journal of Pathology.* 2010; 222(4):350–66. [PubMed: 20927778]
37. Chen DS, Irving BA, Hodi FS. Molecular pathways: next-generation immunotherapy—inhibiting programmed death-ligand 1 and programmed death-1. *Clinical Cancer Research.* 2012; 18(24): 6580–87. [PubMed: 23087408]
38. Soria, JCCC.; Bahleda, R. Clinical activity, safety and biomarkers of PD-L1 blockade in non small cell lung cancer (NSCLC): Additional analyses from a clinical study of the engineered antibody MPDL3280A (anti-PDL1). European Cancer Congress; 2013; p. Abstract 3408
39. Brown SD, Warren RL, Gibb EA, Martin SD, Spinelli JJ, Nelson BH, et al. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. *Genome Research.* 2014; 24(5):743–50. [PubMed: 24782321]
40. Gajewski TF, Woo SR, Zha Y, Spaapen R, Zheng Y, Corrales L, et al. Cancer immunotherapy strategies based on overcoming barriers within the tumor microenvironment. *Current Opinion in Immunology.* 2013; 25(2):268–76. [PubMed: 23579075]
41. Melero I, Rouzaut A, Motz GT, Coukos G. T-cell and NK-cell infiltration into solid tumors: a key limiting factor for efficacious cancer immunotherapy. *Cancer Discov.* 2014; 4(5):522–6. [PubMed: 24795012]
42. Lanitis E, Irving M, Coukos G. Targeting the tumor vasculature to enhance T cell activity. *Current Opinion in Immunology.* 2015; 33C:55–63. [PubMed: 25665467]
43. Motz GT, Santoro SP, Wang LP, Garrabrant T, Lastra RR, Hagemann IS, et al. Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors. *Nature Medicine.* 2014; 20(6):607–15.
44. Crawford A, Angelosanto JM, Kao C, Doering TA, Odorizzi PM, Barnett BE, et al. Molecular and transcriptional basis of CD4(+) T cell dysfunction during chronic infection. *Immunity.* 2014; 40(2):289–302. [PubMed: 24530057]
45. Page DB, Postow MA, Callahan MK, Allison JP, Wolchok JD. Immune modulation in cancer with antibodies. *Annual review of medicine.* 2014; 65:185–202.
46. Intlekofer AM, Thompson CB. At the bench: preclinical rationale for CTLA-4 and PD-1 blockade as cancer immunotherapy. *J Leukoc Biol.* 2013; 94(1):25–39. [PubMed: 23625198]
47. Rozali EN, Hato SV, Robinson BW, Lake RA, Lesterhuis WJ. Programmed death ligand 2 in cancer-induced immune suppression. *Clinical & Developmental Immunology.* 2012; 2012:656340. [PubMed: 22611421]
48. Callahan MK, Wolchok JD. At the bedside: CTLA-4- and PD-1-blocking antibodies in cancer immunotherapy. *J Leukoc Biol.* 2013; 94(1):41–53. [PubMed: 23667165]

49. Ngiow SF, von Scheidt B, Akiba H, Yagita H, Teng MWL, Smyth MJ. Anti-TIM3 antibody promotes T cell IFN- γ -mediated antitumor immunity and suppresses established tumors. *Cancer Research*. 2011; 71(10):3540–51. [PubMed: 21430066]
50. Sakuishi K, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK, Anderson AC. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. *The Journal of Experimental Medicine*. 2010; 207(10):2187–94. [PubMed: 20819927]
51. Woo SR, Turnis ME, Goldberg MV, Bankoti J, Selby M, Nirschl CJ, et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res*. 2012; 72(4):917–27. [PubMed: 22186141]
52. Johnston RJ, Comps-Agrar L, Hackney J, Yu X, Huseni M, Yang Y, et al. The immunoreceptor TIGIT regulates antitumor and antiviral CD8(+) T cell effector function. *Cancer Cell*. 2014; 26(6): 923–37. [PubMed: 25465800]
53. Huang RR, Jalil J, Economou JS, Chmielowski B, Koya RC, Mok S, et al. CTLA4 blockade induces frequent tumor infiltration by activated lymphocytes regardless of clinical responses in humans. *Clinical Cancer Research*. 2011; 17(12):4101–9. [PubMed: 21558401]
54. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus Ipilimumab in Advanced Melanoma. *New England Journal of Medicine*. 2013; 369(2):122–33. [PubMed: 23724867]
55. Bald T, Landsberg J, Lopez-Ramos D, Renn M, Glodde N, Jansen P, et al. Immune cell-poor melanomas benefit from PD-1 blockade after targeted type I IFN activation. *Cancer Discov*. 2014; 4(6):674–87. [PubMed: 24589924]
56. Kershaw MH, Westwood JA, Darcy PK. Gene-engineered T cells for cancer therapy. *Nature Reviews Cancer*. 2013; 13(8):525–41.
57. Kalbasi A, June CH, Haas N, Vapiwala N. Radiation and immunotherapy: a synergistic combination. *Journal of Clinical Investigation*. 2013; 123(7):2756–63. [PubMed: 23863633]
58. Ascierto PA, Kalos M, Schaer DA, Callahan MK, Wolchok JD. Biomarkers for Immunostimulatory Monoclonal Antibodies in Combination Strategies for Melanoma and Other Tumor Types. *Clinical Cancer Research*. 2013; 19(5):1009–20. [PubMed: 23460532]
59. Mansfield, JR.; Hoyt, C.; Levenson, RM. Visualization of microscopy-based spectral imaging data from multi-label tissue sections. In: Ausubel, Frederick M., et al., editors. *Current Protocols in Molecular Biology*. Vol. Chapter 14. 2008. p. 19
60. Gerner MY, Kastenmuller W, Ifrim I, Kabat J, Germain RN. Histo-cytometry: a method for highly multiplex quantitative tissue imaging analysis applied to dendritic cell subset microanatomy in lymph nodes. *Immunity*. 2012; 37(2):364–76. [PubMed: 22863836]

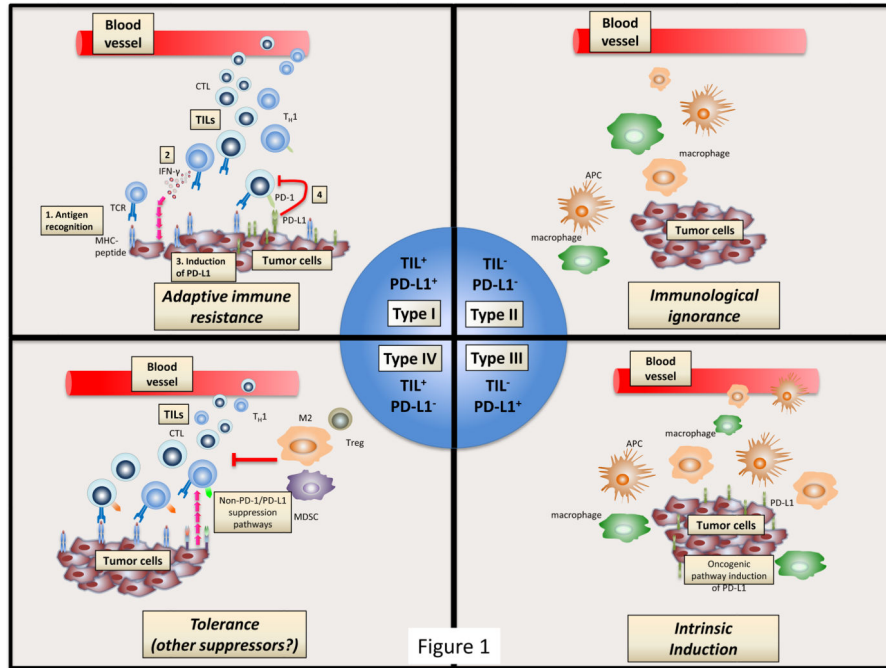


Figure 1

Figure 1. Types of tumor microenvironment to tailoring cancer immunotherapeutic modules
 Cancers have been categorized into 4 different tumor microenvironments based on the presence of TILs and PD-L1 expression (15,16). They are type I (adaptive immune resistance), type II (immunological ignorance), type III (intrinsic induction), and type IV (tolerance). This proposed framework of stratifying tumors is simplistic but allows a platform to discuss the immunotherapeutic strategies best suited to targeting the four different tumor microenvironments. (Abbreviations: APC, antigen presenting cell; MDSC, myeloid derived suppressor cell; M2, M2 macrophage; PD-L1, programmed cell death 1 ligand 1; TCR, T cell receptor; T_H1, T helper 1; TIL, tumor-infiltrating lymphocyte)