Title
The "cancer immunogram"

Permalink
https://escholarship.org/uc/item/1n83k1hm

Journal
SCIENCE, 352(6286)

ISSN
0036-8075

Authors
Blank, CU
Haanen, JB
Ribas, A
et al.

Publication Date
2016-05-06

DOI
10.1126/science.aaf2834

Peer reviewed
The “cancer immunogram”

Visualizing the state of cancer–immune system interactions may spur personalized therapy

By Christian U. Blank,1,2 John B. Haanen,1,2 Antoni Ribas,3 Ton N. Schumacher2

The impact of cancer immunotherapy on clinical cancer care is growing rapidly. However, different immunotherapies remedy distinct problems in cancer–immune system interactions. What would be the most effective therapy for an individual patient? Here, a framework is proposed for describing the different interactions between cancer and the immune system in individual cases, with the aim to focus biomarker research and to help guide treatment choice.

This “cancer immunogram” (see the figure) builds on two key observations. The outcome of cancer-immune interactions is based on a number of largely unrelated parameters such as tumor “foreignness” and T cell–inhibitory mechanisms. Furthermore, the “value” of these parameters can differ greatly between patients. For example, in some patients, intratumoral inhibition of tumor-specific T cells will be the sole defect that needs to be addressed, whereas in other patients, the tumor may simply be insufficiently foreign to elicit a clinically relevant T cell response in the first place. Because of the multifactorial nature of cancer-immune interactions, combinations of biomarker assays will by definition be required.

The proposed cancer immunogram assumes that T cell activity is the ultimate effector mechanism in human tumors. This by no means implies that inhibition of, for instance, tumor-associated macrophages, or modulation of the microbiome, is without value. Rather, the effects of such therapies are assumed to ultimately involve enhanced T cell activity. Future research will reveal whether this presumption is correct. We also acknowledge that our

REFERENCES

understanding of cancer-immune interaction is still too fragmented to consider the cancer immunogram a static entity. Thus, new biomarkers are expected to be added while other biomarkers may be removed over time. Seven parameter classes may constitute a reasonable initial framework for building such an immunogram, and a brief description of these classes is provided below (I).

TUMOR FOREIGNNESS. The induction of T cell responses by antigen-presenting cells requires the presentation of an altered repertoire of major histocompatibility complex (MHC)-associated peptides. Such a repertoire may be formed either by tumor-derived self peptides from aberrantly expressed proteins, or by presentation of neoantigens derived from viral or mutated gene products. The outcome of a T cell–antigen encounter is modulated by T cell checkpoint markers such as cytotoxic T lymphocyte–associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1).

Recent data suggest that the foreignness of human cancers may in large part be determined by their expression of neoantigens. Specifically, a correlation between mutational load—a surrogate marker for tumor neoantigen load—and outcome upon the blockage of T cell checkpoint inhibitors has been observed in melanoma and nonsmall cell lung cancer. The activity of PD-1 blockade in DNA mismatch repair–deficient cancers is also consistent with tumor foreignness as a determinant of anti–PD-1 immunotherapy. In addition, low intratumoral genetic heterogeneity appears associated with response to T cell checkpoint blockade, providing indirect support for a dominant role of clonal neoantigens.

Mutational load is, however, an imperfect marker for tumor foreignness, as it does not take into account a possible contribution of self antigen recognition to tumor control. Also, the formation of neoantigens from individual mutations is a probabilistic process, with each mutation representing an additional ticket in a “neoantigen lottery.” Thus, although tumor foreignness can likely be guaranteed for tumors with very high mutational loads, the odds of tumor foreignness can only be inferred for tumors with an intermediate or low mutational load; more sophisticated readouts are required.

GENERAL IMMUNE STATUS. Analysis of general immune status seems mundane but will likely be of relevance in many clinical settings. A decrease in lymphocyte counts has been associated with poor outcome upon CTLA-4 blockade in melanoma patient cohorts. Furthermore, neutrophil/lymphocyte ratio has been correlated with poor patient outcome after immunotherapy, whereas elevated eosinophil counts may be associated with improved outcome in melanoma patients treated with anti–CTLA-4 antibody. In addition, myeloid-derived suppressor cell counts in circulating blood seem a negative predictor of immunotherapy outcome. Thus, simple blood analyses could characterize an immune status that is associated with poor outcome upon checkpoint modulation. Mechanistically, these correlations may reflect a reduced ability to mount or maintain a systemic tumor-specific T cell response. Alternatively, systemic immune dysfunction may simply indicate a more profound intratumoral immune inhibition. Regardless, therapies that reverse general immune dysfunction should be tested for the ability to enhance the activity of checkpoint blockade. New technologies for multidimensional measurement of immune cells and proteins are likely to yield additional parameters to gauge immune status in humans, and thereby predict capacity to respond to immunotherapeutic intervention.

IMMUNE CELL INFILTRATION. An obvious requirement for T cell–mediated tumor control is the infiltration of tumor-reactive T cells into the tumor. Absence of such T cell infiltration into an intrinsically foreign tumor may reflect a defect at the level of T cell priming (the activation of T cells within lymphoid organs that leads to T cell proliferation), a mechanical barrier by cancer-associated fibrosis, impermeable tumor-associated vasculature, or the absence of T cell–attracting chemokines. In support of the latter, CXCL9 and CXCL10 (C-X-C motif ligands 9 and 10)—two chemokines for the receptor CXCR3—are part of a gene signature associated with improved outcome upon PD-1 blockade. More directly, a brisk preexisting CD8+ T cell infiltrate is associated with improved outcome in melanoma upon anti–PD-1 immunotherapy.

The strength of the intratumoral T cell infiltrate may be a secondary consequence of other parameters of the cancer immunogram. For example, the interferon-γ (IFN-γ)–induced production of CXCL9 and CXCL10 that occurs upon recognition of tumor cells by infiltrating T cells is expected to enhance T cell recruitment in a positive feedback loop. Thus, absence of a T cell infiltrate may reflect a lack of foreignness, inefficient T cell priming, or lack of T cell attraction. Assays that can distinguish among these possibilities should be of value to guide therapy choice. In this regard, factors such as the presence of stabilized β-catenin (a transcriptional regulator) and the subset of CD103+ dendritic cells deserve further attention.

ABSENCE OF CHECKPOINTS. The expression profile of both T cell checkpoints and their ligands is likely to be a valuable biomarker in many settings because it reports on the presence of specific therapeutic targets and provides information on more general aspects of the tumor-specific T cell response. In melanoma, programmed death ligand 1 (PD-L1) expression has been associated with improved outcome upon both PD-1 and CTLA-4 blockade. In the former case, the correlation may simply reflect presence of the therapeutic target. In the latter case, PD-L1 expression is likely to provide a crude measure of an ongoing tumor-specific immune response, as expression of PD-L1 can be induced by IFN-α and -γ. However, PD-L1 expression on tumor cells can also occur in an interferon-independent fashion. To further increase the value of PD-L1 as a biomarker, it will be useful to assess T cell–induced and tumor cell–intrinsic expression in clinical samples. A straightforward way to achieve this may be to combine analyses of PD-L1 expression on tumor cells with interferon expression, or with the expression of markers of the activation-exhaustion cascade in tumor-resident T cells.

ABSENCE OF SOLUBLE INHIBITORS. Tumor inflammation-associated factors can promote tumor progression. Such inflammation is characterized by the presence of subtypes of neutrophils, γδ cells, and macrophages that secrete proinflammatory factors, such as vascular endothelial growth factor A, colony-stimulating factors, the interleukins IL-1, IL-6, and IL-17, and CXCL1. IL-1 and IL-6 induce C-reactive protein (CRP), a clinical marker for tumor-associated inflammation. Mouse model data have shown that tumor-derived prostaglandin E2 can promote an inflammatory response characterized by IL-6, CXCL1, and granulocyte colony-stimulating factor secretion. Blockade of melanoma prostaglandin E2 production shifted the local environment to a type I IFN-dominated “T cell inflamed” state, resulting in improved T cell–medi-
Absence of inhibitory tumor metabolism. In healthy cells, glycolysis generally results in entry of pyruvate into the Krebs cycle in the mitochondria. Under conditions of hypoxia (e.g., in muscles during exercise), pyruvate is converted to lactate by lactate dehydrogenase (LDH) and pumped out of the cell. In cancer cells, however, the conversion of pyruvate into lactate takes place even in the presence of sufficient oxygen. High serum LDH concentrations correlate strongly with poor outcome upon CTLA-4 and PD-1 blockade, and phase 3 clinical trial data have corroborated these results prospectively. Lactic acid and low local pH can impair crucial T cell functions, such as cytokine production (IL-2, IFN-γ), proliferation, and lytic activity, perhaps providing a mechanistic explanation for the strength of LDH as a biomarker. On the basis of mouse model results, intratumoral hypoxia and glucose depletion also deserve attention as potential biomarkers in this class.

Tumor sensitivity to immune effectors. Reduced “visibility” for the immune system and resistance to T cell killing are accepted mechanisms of cancer immune evasion in preclinical models, and inactivation of components of the antigen presentation machinery has been observed in human cancer. Upon inactivation of antigen presentation machinery components, tumors may still be perceived as foreign through cross-presentation of tumor cell-derived antigens by antigen-presenting cells, but the final stage of tumor cell recognition will be affected. No studies have yet linked MHC expression or defects in apoptosis mechanisms to clinical outcome upon CTLA-4 or PD-1 blockade. Analysis of immunotherapy resistance at the level of tumor cell sensitivity to immune effectors will not only be useful to identify patients who are less likely to respond to T cell–activating therapies, but should also point to the T cell effector mechanisms that exert the greatest Darwinian pressure in human cancers. In particular, although tumor control by T cells is often interpreted as classical perforin- and granzyme-mediated lysis, mouse model data also suggest a role of T cell effector cytokines such as IFN-γ and tumor necrosis factor–α on either tumor stroma or cancer cells themselves.

OUTLOOK. The described cancer immunogram suggests that it may be valuable to ask the following questions: Can the immune system see this tumor as foreign? Is the immune status of the patient likely to be sufficient? Is there evidence for infiltration of effector T cells into the tumor site? Are there checkpoints, soluble mediators, or metabolic factors that may hamper the activity of these cells? Would the tumor cells be sensitive to an unleashed T cell response? The information required for this analysis may be obtained from the combination of tumor genomics, immunohistochemistry, and standard assays on the peripheral blood compartment. Such measurements will be useful to determine which states of the cancer immunogram are most commonly inhabited, both during natural cancer-immune interaction and upon immunotherapy.

Certainly, a cancer immunogram should evolve, incorporating new biomarkers that reflect, for example, the capacity for T cell priming. Nonetheless, even a cancer immunogram based on present-day knowledge does make it possible to visualize the state of cancer-immune interactions in individual patients, and thereby discuss treatment options in a more refined and personalized manner (2).

REFERENCES AND NOTES
1. Examples of hypothetical patient cases and potential treatment options can be found in the supplementary materials.

SUPPLEMENTARY MATERIALS
www.sciencemag.org/content/352/6286/658/suppl/DC1

10.1126/science.aaf2834

The described cancer immunogram suggests that it may be valuable to ask the following questions: Can the immune system see this tumor as foreign? Is the immune status of the patient likely to be sufficient? Is there evidence for infiltration of effector T cells into the tumor site? Are there checkpoints, soluble mediators, or metabolic factors that may hamper the activity of these cells? Would the tumor cells be sensitive to an unleashed T cell response? The information required for this analysis may be obtained from the combination of tumor genomics, immunohistochemistry, and standard assays on the peripheral blood compartment. Such measurements will be useful to determine which states of the cancer immunogram are most commonly inhabited, both during natural cancer-immune interaction and upon immunotherapy.

Certainly, a cancer immunogram should evolve, incorporating new biomarkers that reflect, for example, the capacity for T cell priming. Nonetheless, even a cancer immunogram based on present-day knowledge does make it possible to visualize the state of cancer-immune interactions in individual patients, and thereby discuss treatment options in a more refined and personalized manner (2).
Supplementary Materials for

The “cancer immunogram”

Christian U. Blank,* John B. Haanen, Antoni Ribas, Ton N. Schumacher*

*Corresponding author. Email: c.blank@nki.nl; t.schumacher@nki.nl

Published 6 May 2016, Science 352, 658 (2016)
DOI: 10.1126/science.aaf2834

This PDF file includes:

Fig. S1
References
Supplementary Materials

References for the main text (by subsection)

TUMOR FOREIGNNESS


GENERAL IMMUNE STATUS


IMMUNE CELL INFILTRATION


ABSENCE OF CHECKPOINTS


ABSENCE OF SOLUBLE INHIBITORS


ABSENCE OF INHIBITORY TUMOR METABOLISM


32. J. Larkin *et al*., Efficacy and safety in key patient subgroups of nivolumab (NIVO) alone or combined with ipilimumab (IPI) versus IPI alone in treatment-naïve patients with advanced melanoma (MEL) (CheckMate 067). *The European Cancer Congress 2015* (abstract 3003).


TUMOR SENSITIVITY TO IMMUNE EFFECTORS


**Figure S1. Hypothetical patient cases and therapies that modulate different cancer immunogram parameters.** The radar plots shown in A, B, and C highlight possible use of the cancer immunogram by describing three simple example states that may be encountered in clinical practice. The radar plots D to F show how acquired resistance may be visualized.

(A) Case 1 is a patient with melanoma with a high mutational load who also scores well with respect to all other parameters in the cancer immunogram, except for strong expression of PD-L1 at the tumor site, limiting what would otherwise have been a productive tumor-specific T cell response. Based on this analysis, single agent PD-1 blockade, rather than combined PD-1 and CTLA-4 blockade, could be a first treatment of choice. [This is an extended description of Figure 1 in the main text.]

(B) Case 2 is a patient who scores unfavorably concerning inhibitory factors (soluble, checkpoint, and tumor metabolism); e.g., a patient with a *BRAF* mutant PD-L1 positive melanoma, with high CRP/ESR and LDH serum levels. As discussed in the main text, single PD-1 blockade is less likely to be effective in this situation, and pretreatment with targeted therapy (e.g., BRAF and MEK inhibition), with the aim to reverse immune dysfunction, could be attractive.

(C) Case 3 is a patient with a tumor with low mutational load and, potentially because of this, absence of a significant CD8$^+$ T cell infiltrate. If infusion of TCR- or CAR-modified T cells is feasible for this malignancy, this may be a preferred clinical option.

(D to F) Case 4 is a patient with a favorable cancer immunogram for response to PD-1/PD-L1 blockade (D). The patient experiences a clinical response after restoring this parameter by anti-PD-1 antibody treatment (E). However, selection by immune pressure leads to relapse of a tumor that is insensitive to T cell effector mechanisms (F).
General immune status

Tumor foreignness

Mutational load

Lymphocyte count

Tumor sensitivity to immune effector

MHC expression, IFN-γ sensitivity

Immune cell infiltration

Intratumoral T cells

Absence of inhibitory tumor metabolism

LDH, glucose utilization

Absence of soluble inhibitors

IL-6, CRP

Absence of checkpoints

PD-L1

Case 1

Case 2

Case 3

Case 4