Journal for ImmunoTherapy of Cancer

Basic rules to respond to PD-1 blockade cancer immunotherapy

Antoni Ribas

To cite: Ribas A. Basic rules to respond to PD-1 blockade cancer immunotherapy. *Journal for ImmunoTherapy of Cancer* 2025;**13**:e012096. doi:10.1136/jitc-2025-012096

Accepted 12 May 2025

Medicine, University of California Los Angeles, Los Angeles, California, USA

Correspondence to
Dr Antoni Ribas;
aribas@mednet.ucla.edu

ABSTRACT

After 15 years of clinical testing and analyses of biopsies from patients with cancers treated with antibodies blocking the programmed death receptor 1 (PD-1) pathway, several requirements for inducing durable clinical responses have become evident. These basic rules for a response to anti-PD-1 include: (1) the cancer must be immunogenic and differentially recognizable by antitumor T cells, (2) there must be pre-existing antitumor T cells that have the ability to recognize the cancer, which had been activated and had received costimulation, but then are kept in a dysfunctional state due to the reactive cancer expression of the PD-1 ligand 1, (3) on PD-1 blockade, T cells are reinvigorated and produce increased amounts of interferon gamma, which forces the cancer cells into becoming enablers of the antitumor immune response, directly increasing the cancer cell immunogenicity and changing the tumor microenvironment from an unfriendly environment to a friendly environment for the antitumor T cells, and (4) reactivating antitumor T cells before surgery using neoadjuvant anti-PD-1 therapy improves patient outcomes, as the surgery would otherwise take away the majority of antitumor T cells. Collectively, these features are the basis of clinical responses and durable benefit of anti-PD-1 therapy in patients with cancer.

Programmed death receptor 1 (PD-1) blockade therapy is a targeted immunotherapy approach that can only work if a series of events have happened, leading to the PD-1 checkpoint being the only limiting step that separates an effective antitumor immune response to clear up the cancer cells locally and distantly. It is analogous to the use of oncogene-targeted therapies, where the presence of a specific mutation is the requirement for a specific targeted drug to result in antitumor responses. However, as the key interaction between the antitumor T cells and the cancer cells is mediated by the most polymorphic genes in the human genome, the T cell receptor (TCR) and the major histocompatibility complex (MHC), it has been harder to study and specifically define the requirements for response to PD-1 blockade therapy. With the study of biopsies from patients with cancer who responded to PD-1 blockade therapy, and biopsies from patients who initially responded and later progressed (acquired resistance), several general rules have emerged:

1. THE CANCER MUST BE IMMUNOGENIC AND DIFFERENTIALLY RECOGNIZABLE BY THE ANTITUMOR T CELLS

Cancer therapies aim at killing cancer cells and sparing normal cells. The immune system achieves this goal through the T cells, which have specific TCRs that recognize peptide (shorter sequences from a protein) antigens presented on the surface of cancer cells that are not expressed by normal somatic cells. The antigenic peptide presentation process is through the MHC, which in humans is called the human leukocyte antigens (HLA). This is a basket-like molecule that samples peptides of certain sizes inside the cell and presents them on the cell surface. This system has evolutionarily developed to allow the clearance of cells that are infected by viruses and bacteria, presenting abnormal intracellular peptides on the surface of the infected cell surface, thereby allowing the T cells that are outside to find out which cells have been infected, while sparing the non-infected cells that do not express the abnormal peptides from the infectious agent. Many cancer cells have abnormal gene sequences from a carcinogenic event, from failure to correct DNA damage, or from viral insertions, which result in the expression of altered peptide sequences that are different from normal cells, which when presented on the cancer cell surface by an HLA molecule, these serve as neoantigens to be differentially recognized by antitumor T cells. The commonality between the cancers with the highest response rates to single-agent anti-PD-1 therapy is the presence of strong cancer-specific antigens, either as a result of oncogenic viral infections in the case of Epstein-Barr virus for Hodgkin's lymphoma and Merkel cell polyoma virus for Merkel cell carcinoma, or from very high tumor mutational burden (TMB) from DNA mismatch repair deficiency in microsatellite instability (MSI) high cancers, and from the

carcinogenic effect of ultraviolet (UV) light exposure in desmoplastic melanoma and Merkel cell carcinoma.[1] Response rates to anti-PD-1 immunotherapy are lower in cancers with lower mutational burden, like cigarette smoking-induced head and neck, lung, gastroesophageal and bladder cancers, and cutaneous melanomas, which have lower TMB compared with desmoplastic melanoma, as cutaneous melanoma is most frequently related to intermittent UV light carcinogenesis.[1] There are infrequent responses to anti-PD-1 immunotherapy in cancers that have gene sequences that are very similar to the normal cellular counterparts, for example, most prostate cancers and estrogen receptor-positive breast cancers, as the lack of genetic differentiation between the cancer and the normal cells does not allow T cells to differentially recognize the cancer. Together, these clinical data highlight the key importance of the cancer cell inherent immunogenicity for patients to be able to respond to anti-PD-1 therapy.

The evidence that acquired resistance (progression after a period of response) to anti-PD-1 immunotherapy can happen through loss of function mutations in *HLA* or *beta-2-microglobulin* (*B2M*), which is the required fourth subunit of the HLA class I molecule, reflects that it is not only important to express neoantigens but also presenting them on the cancer cell surface for T cell recognition.[2 3] Cancer cell-intrinsic genetic resistance through *HLA* loss is uncommon, as the immune system has overlapping mechanisms to avoid the presence of somatic cells that are not recognizable by T cells and may carry pathogens or mutations that would be detrimental if not presented by HLA molecules. In the absence of surface HLA, natural killer (NK) cells and gamma-delta T cells can be activated and eliminate cancer cells due to missing self and the expression of stress molecules that activate these alternate cytotoxic immune effectors.[4] NK cells spare cells with matched HLA expression while promoting the elimination of cells lacking this expression, thus targeting cancer cells with reduced or absent surface HLA molecules. These overlapping mechanisms of recognition of cancer cells explain why in some cases patients with cancers with evidence of genetic loss of *B2M* and lack of HLA surface expression can still respond to anti-PD-1 immunotherapy.[5 6]

2. THERE MUST BE PRE-EXISTING POLYCLONAL ANTITUMOR T CELLS

The relationship between cancer immunogenicity and response to anti-PD-1 immunotherapy is not completely linear, as there is the important variable of the receptor on T cells that recognize the cancer. The diverse recognition of the adaptive immune system is based on the random somatic recombination of immune receptor recognition genes in developing lymphocytes, leading to the diversity of antibodies produced by B cells and TCRs on the surface of T cells, which combine to prepare our organism to protect itself from pathogens and cancers it has not been previously exposed to. Thymocytes recombine the TCR genes while developing in the thymus, and this random creation of new TCRs predates by several decades the development of most cancers. If T cell clones in a person had not randomly generated TCRs with high affinity and functionality for future cancer neoantigens, then it would be futile attempting to release immune checkpoints as the T cells with cancer specificity do not exist in this case. The chances of having good TCR clonotypes to recognize a cancer increase with the number of potential targets in the cancer, which provides an association between a higher TMB and a higher frequency of clinical responses to anti-PD-1 immunotherapy, but this relationship is not linear. It has been described as having tickets to a lottery, where the more tickets someone has it increases the chances of winning, but does not guarantee winning, and having fewer tickets may still include a winning one.

When a cancer first develops, T cells with a TCR that recognizes tumor neoantigens try to eliminate it, but the cancer cells can protect themselves with the reactive expression of the PD-1 ligand 1 (PD-L1). Naïve T cells become antigen-experienced, with the involvement of dendritic cells and other antigen-presenting cells in the tumor or in lymphoid structures to get past the costimulatory immune checkpoint, but then the antitumor T cells become chronically dysfunctional T cells limited by the PD-1 receptor signaling engaged by PD-L1 expressed by cancer cells. Once the immune response is unleashed by blocking the PD-1 checkpoint, it depends on the strength of the antitumor T cell response, which is a reflection of the precursor frequency of pre-existing T cells with the ability to recognize the cancer and with how many different TCR clonotypes recognize cancer neoantigens.[7] Patients who have a response to anti-PD-1 therapy start with higher numbers of T cells infiltrating the tumor, and these T cells represent multiple clones with different TCRs recognizing a limited set of immunodominant cancer antigens.[7 8] The stronger the T cell response to tumor antigens that differentiate cancer from normal cells results in higher interferon gamma (IFNg) signaling in the cancer, changing the tumor microenvironment (TME) and helping the antitumor immune response.[9]

3. THE CANCER NEEDS TO HELP THE ANTITUMOR T CELLS THROUGH IFNG SIGNALING AND THE EXPRESSION OF THOUSANDS OF IFNG-STIMULATED GENES

At steady state, few tumor-specific T cells recognize cognate neoantigens presented by cancer cells, but they are inhibited by the reactive expression of PD-L1 by cancer cells. PD-L1 is an IFNg response gene. When cancer cells recognize the presence of IFNg, they sense that they are under attack, and they then reactively express PD-L1, which allows the cancer cells to specifically turn off the T cells that recognize them.[8 10] If the cancer is immunogenic enough and the host has a diverse set of T cells with TCRs recognizing tumor antigens, then blockade of PD-1

leads to a productive antitumor response.[7] On blockade of PD-1, the pre-existing rare tumor-specific T cells that had been kept in check and dysfunctional by PD-L1 then start to kill the cancer cells that they are physically in contact with. The antitumor T cells also secrete IFNg, leading to the forced expression of a conserved set of~1500 IFNg-stimulated genes by the cancer cells that change the TME, turning the cancer cells more immunogenic and the TME from a hostile environment for T cells to enabling the antitumor immune response.[9] Multiple cells in the TME, including dendritic cells, macrophages and fibroblasts, also express IFNg-stimulated genes as the cancer cells do, which together help orchestrate the antitumor immune response. It should be noted that the sources of IFNg are very limited, mainly activated T cells and NK cells, while the receptors for IFNg are widely distributed on most cells of the human body, and the majority of cancer cells express IFNg receptors. The bystander effects of IFNg production go well beyond the T cell-cancer cell physical interaction, as the expression of IFNg-stimulated genes can be traced up to 30–40 layers of cells in a tumor beyond where antitumor T cells produce IFNg.[11] Therefore, the few initial T cells that are unleashed by PD-1 blockade therapy induce a field effect, where cancer cells and other cells in the TME make IFNg-stimulated genes that help the T cells expand, better recognize cancer cells and kill them.

Cancer cells can escape anti-PD-1 therapy through cancer cell-intrinsic genetic alterations in the IFNg receptor pathway, in particular with loss of function mutations in the Janus kinases 1 or 2 (*JAK1* or *JAK2*).[2 12] These are infrequent events in patient samples but have a clear biological meaning. Loss of IFNg signaling provides an advantage to cancer cells, as they avoid the amplification of the antitumor immune response mediated by the~1500+ IFNg-stimulated genes. Without IFNg signaling, cancer cells stop the increased expression of antigen presentation genes (including HLA class I and II, proteasome subunits and transporters associated with antigen presentation), expression of IFNg signaling molecules that provide a forward feedback loop that enhances IFNg signaling (including IFNg itself, the IFNg receptor chains, JAKs, signal transducers and activators of transcription, and interferon regulatory factors), the production of chemokines that attract other immune cells, and being sensitive to the direct antitumor cytotoxic effects of IFNg.[8 9 13] Cytotoxicity induced by perforin and granzyme, and by triggering the tumor necrosis factor (TNF) family of receptors (TNF, Fas, TRAIL), is limited to one-on-one interactions between T cells and cancer cells and only functional within the tight constraints of the immunological synapse. Meanwhile, IFNg-induced growth inhibition and cell death is the main cytotoxic mechanism induced by T cells that goes beyond the immunological synapse between antitumor T cells and cancer cells.[11 14] Therefore, mutations in IFNg signaling molecules allow cancer cells to escape from IFNg-induced gene expression and direct cytotoxic effects, highlighting the critical role of IFNg in orchestrating an effective antitumor immune response.[9]

4. REACTIVATING ANTITUMOR T CELLS BEFORE SURGERY IMPROVES PATIENT OUTCOMES IN PATIENTS WITH LOCALLY ADVANCED CANCERS

The mechanism of action of PD-1 blocking antibodies relies on the presence of pre-existing antitumor T cells attempting to attack cancer cells inside a tumor, with the reactive expression of PD-L1 by the cancer cells inhibiting the antitumor immune response at steady state.[1 8] T cells are attracted to and accumulate in the sites where there is expression of their cognate antigen. Even though T cells are highly mobile and circulate through blood and in and out of lymphoid organs, when their TCR recognizes antigen, they decrease motility and stay local for extended periods of time.[15] This results in the accumulation of tumor antigen-specific T cells in cognate antigen-expressing cancers. Based on the understanding of this mechanism of action, resection of the bulk of the cancer, along with the tumor-infiltrating lymphocytes contained in the surgical specimen, is likely to take away most of the pre-existing antitumor T cells with high affinity and diverse TCRs, as well as other cells in the TME that would support an antitumor immune response. If they were in place when first being treated with anti-PD-1, the tumor-resident antitumor T cells would be better able to be reactivated and proliferate on PD-1 blockade therapy, resulting in the induction of a systemic antitumor immune response that decreases the rates of cancer relapse. Therefore, administering anti-PD-1 blocking therapy before surgery, termed neoadjuvant immunotherapy, activates more antitumor T cells and improves clinical outcomes compared with the same amount of anti-PD-1 delivered postoperatively.[16 17]

In conclusion, PD-1 blockade therapy only works when a patient's immune system had already orchestrated an antitumor immune response, but the cancer cells found a way to specifically stop it by the reactive expression of PD-L1. On PD-1 blockade, the pre-existing and polyclonal T cells with cancer specificity can proceed with the antitumor immune response as the PD-1 receptor no longer inhibits the TCR signaling induced by cognate antigen recognition. PD-1 blockade allows the T cells to induce direct cytotoxic effects on the cancer cells that they are physically recognizing, and at the same time also produces a field effect mediated by the expression of IFNg-stimulated genes by multiple cells in the TME. A logical consequence of recognizing this biology is that anti-PD-1 therapy should work better before surgically resecting the cancer, the TME and the containing cancer-specific T cells accumulated in the cancer specimen. The dominance of these basic rules for a response to anti-PD-1 immunotherapy is provided by the evidence that genetic resistance to PD-1 blockade therapy can develop through mutations in genes involved in antigen presentation and IFNg signaling. The central role of IFNg signaling

in response to anti-PD-1 therapy is highlighted by the combined effects of increased antigen presentation and expression of IFNg signaling molecules that amplify the antitumor response, chemokine production to attract more immune cells, and direct IFNg antitumor effects that result in antiproliferative and apoptotic cancer cell death.

Acknowledgements AR is funded in part by the Parker Institute for Cancer Immunotherapy (PICI), NIH grants R35 CA197633 and P01 CA168585, the Ressler Family Fund, the Grimaldi Tanner Giving Fund, and the support from Ken and Donna Schultz, Todd and Donna Jones, Robert (Bob) and Mary Jean Rumer, Karen and James Witemyre, and Thomas Stutz through the Jonsson Cancer Center Foundation.

Contributors AR is the sole contributor.

Funding This study was funded by the Ressler Family Fund, the Grimaldi Tanner Giving Fund, and the support from Ken and Donna Schultz, Todd and Donna Jones, Robert (Bob) and Mary Jean Rumer, Karen and James Witemyre, and Thomas Stutz through the Jonsson Cancer Center Foundation (Donations), Parker Institute for Cancer Immunotherapy, National Cancer Institute (P01 CA168585, R35 CA197633).

Competing interests AR has received honoraria from consulting with Amgen, Jazz, Merck, Roche-Genentech, is or has been a member of the scientific advisory board and holds stock in Apricity, Arcus, Compugen, CytomX, Highlight, Kite-Gilead, Larkspur, Lutris, Lyell, Merus, PACT, Synthekine and Tango, has received research funding from Agilent and from Bristol-Myers Squibb through Stand Up to Cancer (SU2C), and patent royalties from Arsenal Bio.

Patient consent for publication Not applicable.

Ethics approval Not applicable.

Provenance and peer review Commissioned; internally peer reviewed.

ORCID iD

Antoni Ribas http://orcid.org/0000-0003-3669-8458

REFERENCES

1 Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science* 2018;359:1350–5.
2 Zaretsky JM, Garcia-Diaz A, Shin DS, *et al*. Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma. *N Engl J Med* 2016;375:819–29.
3 Sade-Feldman M, Jiao YJ, Chen JH, *et al*. Resistance to checkpoint blockade therapy through inactivation of antigen presentation. *Nat Commun* 2017;8:1136.
4 Kärre K. NK cells, MHC class I molecules and the missing self. *Scand J Immunol* 2002;55:221–8.
5 Germano G, Lu S, Rospo G, *et al*. CD4 T Cell-Dependent Rejection of Beta-2 Microglobulin Null Mismatch Repair-Deficient Tumors. *Cancer Discov* 2021;11:1844–59.
6 de Vries NL, van de Haar J, Veninga V, *et al*. γδ T cells are effectors of immunotherapy in cancers with HLA class I defects. *Nature* 2023;613:743–50.
7 Puig-Saus C, Sennino B, Peng S, *et al*. Neoantigen-targeted $CD8^+$ T cell responses with PD-1 blockade therapy. *Nature* 2023;615:697–704.
8 Tumeh PC, Harview CL, Yearley JH, *et al*. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014;515:568–71.
9 Ribas A, Haining WN, Schumacher TNM. When Cancer Cells Become the Enablers of an Antitumor Immune Response. *Cancer Discov* 2022;12:2244–8.
10 Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012;12:252–64.
11 Hoekstra ME, Bornes L, Dijkgraaf FE, *et al*. Long-distance modulation of bystander tumor cells by CD8+ T-cell-secreted IFN-γ. *Nat Cancer* 2020;1:291–301.
12 Sade-Feldman M, Yizhak K, Bjorgaard SL, *et al*. Defining T Cell States Associated with Response to Checkpoint Immunotherapy in Melanoma. *Cell* 2018;175:998–1013.
13 Ayers M, Lunceford J, Nebozhyn M, *et al*. IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade. *J Clin Invest* 2017;127:91190:2930–40:.
14 Alspach E, Lussier DM, Schreiber RD. Interferon γ and Its Important Roles in Promoting and Inhibiting Spontaneous and Therapeutic Cancer Immunity. *Cold Spring Harb Perspect Biol* 2019;11:a028480.
15 Miller MJ, Wei SH, Parker I, *et al*. Two-photon imaging of lymphocyte motility and antigen response in intact lymph node. *Science* 2002;296:1869–73.
16 Patel SP, Othus M, Chen Y, *et al*. Neoadjuvant-Adjuvant or Adjuvant-Only Pembrolizumab in Advanced Melanoma. *N Engl J Med* 2023;388:813–23.
17 Blank CU, Lucas MW, Scolyer RA, *et al*. Neoadjuvant Nivolumab and Ipilimumab in Resectable Stage III Melanoma. *N Engl J Med* 2024;391:1696–708.