



Resistance mechanisms to PD-1 inhibition in melanoma

Raphael Brandao, Patrick A. Ott

Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, USA

Correspondence to: Patrick A. Ott. Department of Medical Oncology, Dana Farber Cancer Institute, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA, USA. Email: Patrick_Ott@dfci.harvard.edu.

Comment on: 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.

Submitted Oct 22, 2016. Accepted for publication Oct 31, 2016.

doi: 10.21037/tcr.2016.11.03

View this article at: <http://dx.doi.org/10.21037/tcr.2016.11.03>

Immune checkpoint inhibition using antibodies against cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed death 1 (PD-1) have substantially improved treatment outcomes for patients with advanced melanoma. (1-3). However, despite the remarkable durability of objective responses induced by these treatments, progressive disease occurs in about a quarter of the patients within the first 3 years (4). Understanding the molecular mechanisms of acquired resistance by focused comparison of biopsy samples from paired baseline and relapsing lesions should open strategies for the rational design of salvage therapies and may guide mechanistic biomarker studies for the identification of patients, before the initiation of treatment, who are unlikely to have a response.

A recent study (5) has identified two likely mechanisms for the development of acquired resistance to PD-1 blockade in patients with melanoma. Clonal evolution of tumors to silence effector signaling pathways involved gamma interferon activation by mutations in the *JAK1* and *JAK2* genes, or by affecting antigen presentation through the *B2M* gene.

The report focuses on 4 advanced melanoma patients treated with pembrolizumab who had tumor progression after an initial confirmed objective response that lasted for at least 6 months; these patients were selected from 15 initial responders who later progressed and from whom serial tumor biopsies were available prior to treatment and at the time of progression. In order to identify resistance mechanisms to PD-1 blockade, analyses of the serial tumor samples were performed and included pathological analysis as well as genomic studies of DNA and RNA. Immunohistochemical studies in pre-treatment and relapse

tumor samples of patients 1, 2, and 3 demonstrated CD8 T-cell infiltration at the invasive margin that colocalized with PD-L1 expression on surrounding macrophages and melanoma cells. In two of the 4 patients studied, resistance-associated loss-of-function mutations in genes for interferon receptor-associated JAK1 or JAK2 were identified, concurrent with suppression of the wild-type allele. In a third patient, a truncating mutation in the gene for the antigen-presenting protein beta-2-microglobulin (B2M) was found. The JAK1 and JAK2 truncating mutations resulted in a lack of response to interferon-gamma and loss of its antiproliferative effects, and the B2M truncating mutation led to loss of surface expression of major histocompatibility complex (MHC) class I (6).

Using whole genome sequencing, the authors identified loss-of-function mutations in kinases linked to the interferon receptor pathway. Comparing the response of the primary cell lines that were derived from the tumor of the patients—at baseline and at the time of relapse—the authors found an absence of JAK1 and JAK2 protein expression, respectively, following relapse, and a consequent lack of response to interferon gamma. The third patient's relapsed tumor showed a mutation in B2M, the gene for B2M, which has been previously implicated in resistance to immunotherapy (7-10). Without this protein, tumor cells are no longer able to present antigens to T cells, a critical function of immune recognition and an effective anti-tumor T cell response. Therefore, while mutations in JAK1 and JAK2 suggest that the tumor is insensitive to T cell mediated cytotoxicity, a mutation in B2M means that the T-cells cannot identify the tumor in the first place. The fourth patient did not exhibit any of these mutations,

suggesting that other genetic variations may be involved in the acquired resistance to immunotherapy.

Two pathways were identified in the functional inactivation of CD8 T cells during the development of acquired immune resistance. The first pathway—identified in relapsed patients 1 and 2—leads to a decrease of tumor cell response to interferon gamma that was mediated by loss of function mutations in the genes encoding JAK1 or JAK2. Previous *in vitro* studies had already shown that the tumor cells could still be identified by appropriate T cells. Those produce interferon- γ , but the JAK mutations resulted in a complete lack of tumor cell sensitivity to interferon- γ evidenced by a lack of phosphorylation of signal transducer and activator of transcription 1 (STAT1) in the interferon- γ signaling pathway, lack of interferon gamma-induced expression of MHC class I and PD-L1, and insensitivity to the antiproliferative effects of interferon- γ (5,11-13). Using the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 approach, melanoma cell lines expressing the cancer testis antigen NY-ESO-1 that lacked JAK1 or JAK2 were created. T cells expressing an NY-ESO-1 transgenic T cell receptor were able to produce IFN- γ when exposed to the JAK1 and JAK2 deficient melanoma cell lines however the cell lines were insensitive to IFN- γ mediated growth arrest. This finding indicates that the resistance to IFN- γ induced cytolysis in the JAK deficient cell lines was due to deficient IFN- γ pathway signaling and not a lack of recognition by the T cells. The second pathway—identified in patient 3—linked to a previously identified mechanism for cytotoxic T-cell escape—loss of expression of MHC class I on the tumor cell surface, that in this case was due to a truncating mutation in the gene encoding B2M (8). Consequently, whereas mutations in JAK1 and JAK2 suggest that the tumor is insensitive to T cell inhibition, a mutation in B2M means the T-cells cannot recognize the tumor in the first place.

Similar to targeted therapy, Zaretsky *et al.* show that immunotherapy with anti-PD-1 directed therapy can be subject to acquired resistance that is related to mutations in tumor cells (5). The increase in expression of T-cell immunoglobulin mucin 3 (TIM-3) (14), the presence of somatic aberrations in tumor cells (loss of PTEN) inhibiting the recruitment of T cells (15), and characteristic transcriptional signatures such as regulation of mesenchymal transition, cell adhesion, extracellular matrix remodeling, and angiogenesis (16) in the tumor microenvironment are other recently identified mechanisms for acquired resistance to anti-PD1 therapies.

The study demonstrates impressively how the rapidly emerging tools of precision medicine can be employed to dissect immunotherapy-resistance mechanisms in cancer patients. The elimination of IFN- γ sensitivity through JAK1 or JAK2 inactivation is a particularly compelling resistance mechanism in the context of PD-1 blockade given the adaptive upregulation of PD-L1 by tumors in response IFN- γ secreted by tumor infiltrating T cells. The B2M mutation identified in a 3rd patient is an additional, independent persuasive resistance mechanism. Other mechanisms surely are at play as the lack of a genomic alteration explaining the resistance to PD-1 inhibition in the 4th patient illustrates. The by nature correlative analyses can also not rule out additional, non-genomic mechanisms for resistance, even in the patients with JAK inactivation and B2M mutation. Larger cohorts of patients should be studied to confirm these results and will almost certainly reveal additional mechanisms. This research shows that residual cancer cells continue to fight for survival, even after prolonged inhibition by immunotherapy, through evolution to induce resistance, suggesting that we need new ways to determine and estimate residual disease.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Xia Fang (Department of Hematology, Shanghai Tongji Hospital, Tongji University School of Medicine, Shanghai, China).

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2016.11.03>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-

commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: <https://creativecommons.org/licenses/by-nc-nd/4.0/>.

References

1. Hamid O, Robert C, Daud A, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N Engl J Med* 2013;369:134-44.
2. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010;363:711-23.
3. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med* 2011;364:2517-26.
4. Ribas A, Hamid O, Daud A, et al. Association of Pembrolizumab With Tumor Response and Survival Among Patients With Advanced Melanoma. *JAMA* 2016;315:1600-9.
5. 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.
6. Ribas A. Adaptive Immune Resistance: How Cancer Protects from Immune Attack. *Cancer Discov* 2015;5:915-9.
7. Sucker A, Zhao F, Real B, et al. Genetic evolution of T-cell resistance in the course of melanoma progression. *Clin Cancer Res* 2014;20:6593-604.
8. D'Urso CM, Wang ZG, Cao Y, et al. Lack of HLA class I antigen expression by cultured melanoma cells FO-1 due to a defect in B2m gene expression. *J Clin Invest* 1991;87:284-92.
9. Restifo NP, Marincola FM, Kawakami Y, et al. Loss of functional beta 2-microglobulin in metastatic melanomas from five patients receiving immunotherapy. *J Natl Cancer Inst* 1996;88:100-8.
10. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic β -catenin signalling prevents anti-tumour immunity. *Nature* 2015;523:231-5.
11. Müller M, Briscoe J, Laxton C, et al. The protein tyrosine kinase JAK1 complements defects in interferon- α /beta and - γ signal transduction. *Nature* 1993;366:129-35.
12. Bach EA, Aguet M, Schreiber RD. The IFN gamma receptor: a paradigm for cytokine receptor signaling. *Annu Rev Immunol* 1997;15:563-91.
13. Tumei PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014;515:568-71.
14. Koyama S, Akbay EA, Li YY, et al. Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. *Nat Commun* 2016;7:10501.
15. Peng W, Chen JQ, Liu C, et al. Loss of PTEN Promotes Resistance to T Cell-Mediated Immunotherapy. *Cancer Discov* 2016;6:202-16.
16. Hugo W, Zaretsky JM, Sun L, et al. Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. *Cell* 2016;165:35-44.

Cite this article as: Brandao R, Ott PA. Resistance mechanisms to PD-1 inhibition in melanoma. *Transl Cancer Res* 2016;5(Suppl 6):S1123-S1125. doi: 10.21037/tcr.2016.11.03