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## Commentary

## CXCR3: Here to stay to enhance cancer immunotherapy?

Meri Rogava<sup>a</sup>, Benjamin Izar<sup>a,b,c,\*</sup><sup>a</sup> Laboratory for Systems Pharmacology, Harvard Medical School, Boston, MA, United States<sup>b</sup> Department of Medical Oncology and Center for Immunology and Virology, Dana-Farber Cancer Institute, Boston, MA, United States<sup>c</sup> Broad Institute of MIT and Harvard, Cambridge, MA, United States

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Antibodies directed against immune checkpoints, such as programmed cell death-1 (PD-1) and Cytotoxic T-Lymphocyte Associated Protein 4 (CTLA-4), produce previously unseen and potentially durable responses in patients with melanoma, non-small cell lung cancer, kidney cancer, tumors with mismatch-repair deficiency and other cancers. However, most patients never respond (intrinsic resistance) to these therapies or experience disease progression after an initial response (acquired resistance) [1]. A fundamental challenge in the field is the identification of biomarkers that predict response or resistance to these immune checkpoint inhibitors (ICI) to better stratify patients to potentially efficacious therapies. While expression of PD-L1 (by immunohistochemistry), tumor mutational burden (TMB) [2], pre-existing T cell infiltration [3], and gene signatures [4] have emerged as helpful predictors in specific contexts, better biomarkers are needed. Blood-based biomarkers provide easy access and would be preferable in particular for sequential sampling. In this issue of *EBioMedicine*, Han et al., performed mass cytometry and flow-cytometry analysis of peripheral blood mononuclear cells (PBMCs) sequentially sampled in cancer patients who received anti-PD-1 therapy and propose a predictive role of CXCR3 on T cells [5].

Using a 34-marker 'cytometry by time of flight' (CyTOF) panel, the authors first find significant changes of multiple immune subsets when comparing sequential on-treatment specimens of two melanoma patients receiving anti-PD-1 therapy, including a several-fold increase of CD4+CXCR3+ and CD8+CXCR3+ T cells. Interestingly, a sharp increase in these populations was followed by a marked reduction by the third ICI infusion, and this pattern was not observed for other immune cell subsets. Such dy-

namic changes in CXCR3+ T cells were also observed in patients with esophageal, rhinopharyngeal and gallbladder cancer. To further interrogate the relevance of this observation, the authors performed flow-cytometry in 40 blood samples, including 29 from responders (defined as either stable disease [SD] or partial response [PR]) and 11 non-responders (progressive disease [PD]) to ICI, and find that non-responders had an increased level of CD4+CXCR3+ and CD8+CXCR3+ T cells. In a previous study, Krieg et al. performed CyTOF on PBMCs of 20 melanoma patients before and 12 weeks following initiation of therapy [6]. They found that a higher frequency of classical monocytes (CD14+CD16–HLA-DR<sup>hi</sup>) was a predictor for response to ICI, highlighting the value of using this approach to define subsets of patients that may or may not respond. In the same study, CXCR3 was also measured, although only at a later time point compared to Han et al., and appeared to be enriched in the non-responders, consistent with the current study.

In Han et al., sequential PBMC collections were available for a subset of patients treated with anti-PD-1 therapy. Persistence of CD4+CXCR3+ and CD8+CXCR3+ T cells was associated with drug resistance, while an initial increase followed by a drop in this subset was found in patients with clinical response to anti-PD-1 therapy. How might this dynamic abundance of CXCR3-expressing T cells predict ICI response?

CXCR3 is a highly expressed chemokine receptor that is activated by interferon-(IFN)- $\gamma$  inducible ligands CXCL9, CXCL10 and CXCL11. CXCR3 is rapidly induced and expressed in activated CD8 effector T cells and CD4 T helper cells (Th1) and plays an essential role in the migration into lymphoid and peripheral tissues [7,8]. The authors therefore speculate that while initially induced, the fraction of CXCR3-expressing T cells may drop because cells migrate into the tumor, and the inability to do so may result in persistence in circulation. To begin addressing this hypothesis, the authors treated B16F10 tumor bearing animals (which are typi-

\* Corresponding author at: Columbia Center for Translational Immunology (CCTI), 650 West 168th St. William Black Building, 17th floor, New York, NY 10032  
E-mail address: [bi2175@cumc.columbia.edu](mailto:bi2175@cumc.columbia.edu) (B. Izar).

cally resistant to anti-PD-1 monotherapy) with an anti-CXCR3 antibody with or without concurrent anti-PD-1 therapy, which resulted in accelerated tumor growth. In contrast, intra-tumoral injection of recombinant CXCR3-ligands CXCL9/CXCL10 and concurrent anti-PD-1 therapy resulted in (moderately) reduced tumor growth. While this experiment did not conclusively prove the point of CXCL9/10-CXCR3 axis dependent T cell migration *into* the tumor, it indicates that the presence of CXCR3-expressing T cells *within* the tumor may be important for promoting a response to anti-PD-1 therapy. Indeed, a recent study by Chow et al., using MC38 (a murine colorectal cancer cell line sensitive to anti-PD-1 therapy) in CXCR3<sup>-/-</sup> mice, demonstrates that expression of CXCR3 on T cells is not necessary for tumor infiltration, but important for promoting a tumor specific response when treated with anti-PD-1 therapy [9]. This effect was mediated by CXCL9 secretion by CD103+ dendritic cells; in line with prior studies, an increase of (serum) CXCL9/CXCL10 correlated with response to ICI in an independent set of melanoma patients. In contrast, adoptively transferred T cells require CXCR3 to enter the established tumor [10]. While different experimental models and stages of tumor development may explain some of these differences, the presence of CXCR3-expressing T cells may improve response to immunotherapies through one or more mechanisms.

Overall, this study by Han et al. is limited by the relatively small patient numbers (in particular those with sequential specimens) and the need for further mechanistic evaluation, but it supports previous work highlighting the role of CXCR3 (and its ligands) in modulating response to ICI. It furthermore contributes a translational experience using blood biomarkers for predicting response to ICI. In light of previously published work, this study further supports a potential therapeutic role for augmentation of CXCR3 signaling in cancer immunotherapy.

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## References

- [1] Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell* 2017;168:707–23. doi:10.1016/j.cell.2017.01.017.
- [2] Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015;348:124–8. doi:10.1126/science.aaa1348.
- [3] Tumei PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJM, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014;515:568–71. doi:10.1038/nature13954.
- [4] Jerby-Aronson L, Shah P, Cuoco MS, Rodman C, Su M-J, Melms JC, et al. A cancer cell program promotes T cell exclusion and resistance to checkpoint blockade. *Cell* 2018;175 984–997.e24. doi:10.1016/j.cell.2018.09.006.
- [5] Han X, Wang Y, Sun J, Tan T, Cai X, Lin P, et al. Role of CXCR3 signaling in response to anti-PD-1 therapy. *EBioMedicine* 2019. doi:10.1016/j.ebiom.2019.08.067.
- [6] Krieg C, Nowicka M, Guglietta S, Schindler S, Hartmann FJ, Weber LM, et al. High-dimensional single-cell analysis predicts response to anti-PD-1 immunotherapy. *Nat Med* 2018;24:144–53. doi:10.1038/nm.4466.
- [7] Groom JR, Luster AD. CXCR3 in T cell function. *Exp Cell Res* 2011;317:620–31. doi:10.1016/j.yexcr.2010.12.017.
- [8] Kastenmüller W, Brandes M, Wang Z, Herz J, Egen JG, Germain RN. Peripheral prepositioning and local CXCL9 chemokine-mediated guidance orchestrate rapid memory CD8+ T cell responses in the lymph node. *Immunity* 2013;38:502–13. doi:10.1016/j.immuni.2012.11.012.
- [9] Chow MT, Ozga AJ, Servis RL, Frederick DT, Lo JA, Fisher DE, et al. Intratumoral activity of the CXCR3 chemokine system is required for the efficacy of anti-PD-1 therapy. *Immunity* 2019;50 1498–1512.e5. doi:10.1016/j.immuni.2019.04.010.
- [10] Mikucki ME, Fisher DT, Matsuzaki J, Skitzki JJ, Gaulin NB, Muhitch JB, et al. Non-redundant requirement for CXCR3 signalling during tumoricidal T-cell trafficking across tumour vascular checkpoints. *Nat Commun* 2015;6:7458. doi:10.1038/ncomms8458.