Mulinyawe, S.B., Ponnuswami, A., et al. (2021). NF1 mutation drives neuronal activity-dependent initiation of optic glioma. Nature *594*, 277–282. https://doi.org/10.1038/s41586-021-03580-6.

Varn, F.S., Johnson, K.C., Martinek, J., Huse, J.T., Nasrallah, M.P., Wesseling, P., Cooper, L.A., Malta, T.M., Wade, T.E., Sabedot, T.S., et al. (2022). Glioma progression is shaped by genetic evolution and microenvironment interactions. Cell *185*, 2184–2199.e16. https://doi.org/10.1016/j.cell.2022.04.038.

Venkataramani, V., Tanev, D.I., Strahle, C., Studier-Fischer, A., Fankhauser, L., Kessler, T., Korber, C., Kardorff, M., Ratliff, M., Xie, R., et al. (2019). Glutamatergic synaptic input to glioma cells drives brain tumour progression. Nature *573*, 532–538. https://doi.org/10.1038/s41586-019-1564-x.

Venkataramani, V., Yang, Y., Schubert, M.C., Reyhan, E., Tetzlaff, S.K., Wißmann, N., Botz, M., Soyka, S.J., Beretta, C.A., Pramatarov, R.L., Fankhauser, L., Garofano, L., Freudenberg, A., Wagner, J., Tanev, D.I., Ratliff, M., Xie, R., Kessler, T., Hoffmann, D.C., Hai, L., Dörflinger, Y., Hoppe, S., Yabo, Y.A., Golebiewska, A., Niclou, S.P., Sahm, F., Lasorella, A., Slowik, M., Döring, L., Iavarone, A., Wick, W., Kuner, T., and Winkler, F. (2022). Glioblastoma hijacks neuronal mechanisms for brain invasion. Cell *185*, 2899–2917. https://doi.org/10.1016/j.cell.2022.06.054.

Venkatesh, H., Johung, T., Caretti, V., Noll, A., Tang, Y., Nagaraja, S., Gibson, E., Mount, C., Polepalli, J., Mitra, S., et al. (2015). Neuronal activity promotes glioma growth through Neuroligin-3 Secretion. Cell *161*, 803–816. https://doi.org/10.1016/j.cell.2015.04.012.

Venkatesh, H.S., Morishita, W., Geraghty, A.C., Silverbush, D., Gillespie, S.M., Arzt, M., Tam, L.T., Espenel, C., Ponnuswami, A., Ni, L., et al. (2019). Electrical and synaptic integration of glioma into neural circuits. Nature *573*, 539–545. https://doi.org/10.1038/s41586-019-1563-y.

Immune checkpoint blockade provokes resident memory T cells to eliminate head and neck cancer

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Immune checkpoint blockade is effective in treating many human cancers. In this issue of *Cell*, Luoma et al. show that tissue-resident memory T cells in head and neck cancers rapidly respond to immune checkpoint blockade, and they identify specific CD8$^+$ T cells in pretreatment blood that predict pathologic tumor regression.

Tumors are often infiltrated by various subsets of T cells, including T cells specific for antigens expressed by cancer cells. Many of these T cells express immune checkpoint molecules such as PD-1 and CTLA-4 that inhibit T cell function, thereby allowing tumors to progress. Immune checkpoint blocking antibodies that target PD-1 and CTLA-4 can unleash T cell effector functions and have impressive antitumor activity in many tumor types. Elucidating the differentiation state, phenotype, location, and specificities of T cells that respond to immune checkpoint blockade (ICB) and mediate tumor regression has been a focus of research to identify biomarkers of response and improve treatment of patients that fail to respond. In this issue of *Cell*, Luoma et al. address these issues in a study in which they administered ICB to patients with head and neck squamous cell carcinoma (HNSCC) as neoadjuvant treatment (meaning prior to any surgical tumor resection or cytotoxic therapy) and performed single-cell RNA and T cell receptor (TCR) sequencing of T cells present in pre- and post-treatment tumor tissue and peripheral blood (Luoma et al., 2022). This powerful experimental design enabled a temporal characterization of how T cell subsets in tumors and blood respond to PD-1 and CTLA-4 blocking antibodies in patients not previously treated with surgery or cytotoxic chemotherapy, which can disrupt lymphatic drainage and/or damage immune cells.

Single-cell RNA sequencing (scRNA-seq) can be used to describe immune-cell differentiation states and match cellular phenotype with the unique TCR gene rearrangements that define clonal T cell populations and antigen specificity. When examining tumor-infiltrating immune cells before and shortly after ICB therapy, Luoma et al. identified distinct subsets of CD8$^+$ T cells within tumors; these included two subsets that express a gene signature corresponding to tissue-resident memory (T_{RM}) cells, a specific subset of T cells that provide a surveillance function in peripheral tissues (Masopust et al., 2001). The two T_{RM} clusters contained individual TCR clonotypes that were present in higher frequency, indicative of local antigen-driven clonal expansion. In post-ICB treatment samples, a greater fraction of T_{RM} expressed genes associated with cell proliferation, most notably in patients that received the combination of anti-PD-1 and anti CTLA-4. Integration of scRNA- and TCR-seq data identified TCR clonotypes that were present in pretreatment samples and expanded post ICB treatment, but also a substantial fraction of new or emergent clonotypes, suggesting either the priming of *de novo* antigen-specific T cells or substantial expansion of rare pre-existing responses. By performing a genome-wide screen of candidate tumor

antigens in a subset of patients, some TCR clonotypes that expanded during treatment were shown to be specific for tumor antigens, including the cancer-testes antigen MAGE A1. This work adds to recent observations across multiple tumor types, including non-small cell lung cancer, melanoma, and gastrointestinal cancer, suggesting that only a subset of tumor-infiltrating $CD8^+$ T cells have specificity to self or mutated tumor antigens (Caushi et al., 2021; Lowery et al., 2022; Oliveira et al., 2021; Simoni et al., 2018), with the tumor-specific subsets showing properties of T_{RM} cells and expression of coinhibitory receptors. Whether these T_{RM} are present in the tissue prior to tumor development as a component of the immunosurveillance provided by tissue resident immune cells or, more likely, infiltrate and differentiate into T_{RM} in the tumor microenvironment after tumor development remains an open question. Luoma et al. also found that tumor-infiltrating $CD4^+$ T cells expressing high levels of CXCL13, a chemokine involved in recruiting immune cells to the tumor microenvironment, expand after ICB treatment. This phenotype of $CD4^+$ T cells has been observed in other tumors and shown to contain T cells specific for tumor antigens (Bassez et al., 2021; Lowery et al., 2022; Veatch et al., 2022).

The observations of Luoma et al. demonstrate that tumor-reactive $CD8^+$ and $CD4^+$ T cells are functionally impaired in the tumor microenvironment prior to ICB, and they identify the subsets of T cells that are capable of rapidly proliferating and mediating effector functions in response to ICB. Clinical tumor regression with ICB often lags histologic tumor destruction by weeks to months, so it would be ideal if blood sampling could identify those patients destined to respond, as repeated examination of tumor samples is not always feasible. Luoma et al. identify a proliferating $CD38^+$ HLA-DR^+ $CD8^+$ T cell population in blood that transiently expands early after ICB treatment and includes a repertoire of TCR clonotypes that are similar to those that expand in the tumor, suggesting that tumor-specific T cells are mobilized from the tumor into the peripheral blood during ICB. This systemic response may prove important for surveillance and elimination of disseminated tumor cells that could later form metastases. Within the $CD38^+$ HLA-DR^+ $CD8^+$ T cell subset, Luoma et al. identified that the presence of PD-1^+ KLRG1^- $CD8^+$ T cells in blood samples obtained pretreatment and during treatment is associated with pathologic tumor regression. If validated in additional cohorts of patients, the presence of PD-1^+ KLRG1^- $CD8^+$ T cells would provide a clinically useful predictive blood biomarker to identify patients destined to benefit from ICB.

The study by Luoma et al. adds to growing evidence suggesting that antitumor immune responses after ICB are mediated by a subset of resident tumor antigen-specific T cells characterized by the expression of inhibitory receptors such as PD-1, chemokines such as CXCL13, and markers of tissue residence. These cells are present also in patients who do not benefit from ICB treatment, and it is unclear what barriers limit their activity or prevent the priming of new T cells specific for tumor antigens in such patients. Recent data showing that high TCR signal strength drives terminal T cell dysfunction might explain the failure of some T_{RM} clonotypes to respond to ICB (Shakiba et al., 2021). Furthermore, spatial arrangement of various T cell subsets in tumors is only beginning to be explored in combination with transcriptional signatures and may identify interactions in the tumor microenvironment that are critical for preserving the capacity of tumor-reactive T cells to respond to ICB. Technologies for simultaneously interrogating transcriptional signatures and spatial distributions of cells are likely to provide this information. These efforts hold promise for identification of barriers to clinical response and could lead to strategies that elicit effective antitumor immunity in a larger fraction of patients.

DECLARATION OF INTERESTS

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REFERENCES

Bassez, A., Vos, H., Van Dyck, L., Floris, G., Arijs, I., Desmedt, C., Boeckx, B., Vanden Bempt, M., Nevelsteen, I., Lambein, K., et al. (2021). A single-cell map of intratumoral changes during anti-PD1 treatment of patients with breast cancer. Nat. Med. *27*, 820–832. https://doi.org/10.1038/s41591-021-01323-8.

Caushi, J.X., Zhang, J., Ji, Z., Vaghasia, A., Zhang, B., Hsiue, E.H.-C., Mog, B.J., Hou, W., Justesen, S., Blosser, R., et al. (2021). Transcriptional programs of neoantigen-specific TIL in anti-PD-1-treated lung cancers. Nature *596*, 126–132. https://doi.org/10.1038/s41586-021-03752-4.

Lowery, F.J., Krishna, S., Yossef, R., Parikh, N.B., Chatani, P.D., Zacharakis, N., Parkhurst, M.R., Levin, N., Sindiri, S., Sachs, A., et al. (2022). Molecular signatures of antitumor neoantigen-reactive T cells from metastatic human cancers. Science *375*, 877–884. https://doi.org/10.1126/science.abl5447.

Luoma, A.M., Suo, S., Wang, Y., Gunasti, L., Porter, C.B.M., Nabilsi, N., Tadros, J., Ferretti, A.P., Liao, S., Gurer, C., et al. (2022). Tissue-resident memory and circulating T cells are early responders to pre-surgical cancer immunotherapy. Cell *185*, 2918–2935. https://doi.org/10.1016/j.cell.2022.06.018.

Masopust, D., Vezys, V., Marzo, A.L., and Lefrançois, L. (2001). Preferential localization of effector memory cells in nonlymphoid tissue. Science *291*, 2413–2417. https://doi.org/10.1126/science.1058867.

Oliveira, G., Stromhaug, K., Klaeger, S., Kula, T., Frederick, D.T., Le, P.M., Forman, J., Huang, T., Li, S., Zhang, W., et al. (2021). Phenotype, specificity and avidity of antitumour CD8+ T cells in melanoma. Nature *596*, 119–125. https://doi.org/10.1038/s41586-021-03704-y.

Shakiba, M., Zumbo, P., Espinosa-Carrasco, G., Menocal, L., Dündar, F., Carson, S.E., Bruno, E.M., Sanchez-Rivera, F.J., Lowe, S.W., Camara, S., et al. (2022). TCR signal strength defines distinct mechanisms of T cell dysfunction and cancer evasion. J. Exp. Med. *219*. e20201966. https://doi.org/10.1084/jem.20201966.

Simoni, Y., Becht, E., Fehlings, M., Loh, C.Y., Koo, S.-L., Teng, K.W.W., Yeong, J.P.S., Nahar, R., Zhang, T., Kared, H., et al. (2018). Bystander CD8+ T cells are abundant and phenotypically distinct in human tumour infiltrates. Nature *557*, 575–579. https://doi.org/10.1038/s41586-018-0130-2.

Veatch, J.R., Lee, S.M., Shasha, C., Singhi, N., Szeto, J.L., Moshiri, A.S., Kim, T.S., Smythe, K., Kong, P., Fitzgibbon, M., et al. (2022). Neoantigen-specific CD4+ T cells in human melanoma have diverse differentiation states and correlate with CD8+ T cell, macrophage, and B cell function. Cancer Cell *40*, 393–409.e9. https://doi.org/10.1016/j.ccell.2022.03.006.