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NK cells link immune-checkpoint blockade immunotherapy and response in melanoma brain metastases

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ABSTRACT

Melanoma brain metastases (BMs) pose a significant clinical challenge. This commentary highlights the emerging understanding of the mechanisms behind immune-checkpoint blockade (ICB) efficacy in melanoma BMs. Specifically, we focus on a recent study by Fife *et al*, which revealed a non-canonical role for natural killer (NK) cells in shaping the tumor microenvironment following ICB therapy against melanoma BMs. Instead of direct tumor cell killing, this study demonstrates that ICB triggers NK cell chemokine release, CD8 T cell recruitment and enhanced antitumor immunity. The findings from this study highlight that the ICB mechanisms of action are complex and extend beyond the direct interference of inhibitor receptor–ligand interactions between cytotoxic CD8 T cells and tumor cells.

Brain metastases (BMs) are the most common intracranial malignancies in adults, affecting 20%–40% of patients with advanced cancer, and markedly outnumbering the incidence of primary brain tumors. Of these, melanoma is one of the tumor types with a high propensity to spread to the brain. Treatment for BMs remains challenging as surgery may not always be feasible, especially for patients with multiple BMs, and chemotherapeutics have limited brain penetrance. These limitations underscore the urgent need for more effective therapies. Furthermore, the central nervous system (CNS), an immune-specialized organ, influences the tumor microenvironment (TME) and creates additional challenges for therapies, which are usually effective against non-CNS tumors.[1 2]

Immune-checkpoint blockades (ICBs) target crucial regulatory molecules that, under healthy conditions, prevent excessive immune activation. However, many cancers hijack these pathways to inhibit antitumor immunity. While ICBs can lead to remarkable durable responses in some tumor types, in all cases, only a fraction of patients responds. Additionally, different tumor types are associated with variable ICB response rates. In melanoma, ICBs targeting programmed cell death receptor 1 (PD-1) and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) molecules have demonstrated efficacy. Additionally, clinical trials have shown efficacy of these therapies in patients with melanoma BMs. However, response rates were considerably lower in symptomatic patients, opposed to those that are asymptomatic, underscoring the challenges of treating melanoma BMs.[3 4] This shortcoming illustrates a clear need for both understanding the mechanisms behind checkpoint inhibitor efficacy and to identify the predictive biomarkers of ICB response.

Tumor type-specific biomarkers of ICB response have included tumor mutation burden, T cell infiltrate, interferon signatures and checkpoint ligand expression. However, this is clearly only a limited view of the overall scope of what is required for ICB efficacy. Research on the direct actions of ICB has largely focused on checkpoint molecules on T cells. However, it has become increasingly clear that ICBs can directly and indirectly impact a range of immune cell types within the TME and periphery, and that the ICB mechanisms extend beyond blocking the checkpoint ligand to checkpoint receptor interaction between T cells and tumor cells. For example, recent studies have shown that a key mechanism of anti-PD-1 efficacy is mediated through progenitor exhausted T cell (Tpex) expansion in multiple cancer types, including recurrent glioblastoma. Interestingly, however, in BMs, a recent single-cell RNAseq analysis showed that the responding cells were unique and were terminally exhausted CD8 T cells.[5] Therefore, understanding ICB function in these different contexts seems crucial for optimizing ICB efficacy and for determining biomarkers that can be used to predict which patients are most likely to respond to ICB.

Natural killer (NK) cells are key effectors of the innate immune system and play a critical role in antitumor immunity. NK cells directly

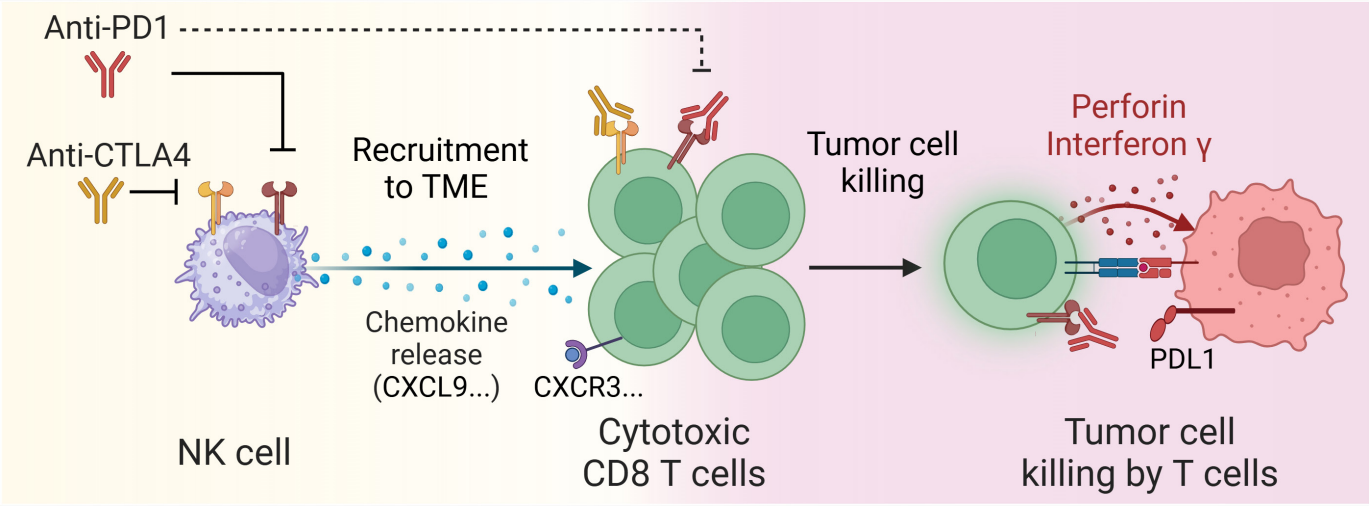

Figure 1 Proposed mechanism of anti-PD-1/CTLA-4 ICB targeting NK cells in melanoma BM. Anti-PD-1 and anti-CTLA-4 antibodies promote NK cell-dependent chemokine release in the TME to enhance the recruitment of CD8 T cells, which kill tumor cells. BM, brain metastasis; ICB, immune-checkpoint blockade; NK, natural killer; TME, tumor microenvironment.

kill tumor cells through the release of cytotoxic molecules, including perforin and granzymes, and can also secrete proinflammatory cytokines to further stimulate antitumor responses. While NK cells have been shown across multiple studies to express checkpoints, including T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT), CTLA-4, lymphocyte activation gene 3 (LAG-3), and T cell immunoglobulin mucin domain-containing protein 3 (TIM-3), the expression and functional relevance of PD-1 on NK cells in cancer appears controversial and likely context dependent. For example, a prior study observed TIGIT expression, but lack of PD-1 expression on mouse and human NK cells under multiple conditions,[6] while another study observed PD-1 expression on tumor-infiltrating NK cells.[7]

Taggart *et al* demonstrated in preclinical studies using a dual tumor melanoma BM model that PD-1/CTLA-4 ICB efficacy is dependent on CD8 T cells and NK cells.[8] However, the mechanism by which NK cells facilitate ICB response in this model remained unestablished. Recently, an eloquent follow-up study by Fife *et al* identified a mechanism by which NK cells mediated the ICB response (summarized graphically in figure 1).[9] Remarkably, ICB triggered a cascade through NK cells to reprogram the TME to recruit cytotoxic CD8 T cells to the TME. In this context, the authors redemonstrated, using a different NK cell depletion strategy than before, that NK cells are necessary to mediate the therapeutic effect of ICB. Despite the clear involvement of NK cells, perforin and interferon-γ in ICB efficacy, the authors found that NK cells isolated from ICB-treated tumors did not exhibit significant direct cytotoxic activity.[9] This unexpected finding led them to explore alternative ways in which NK cells might contribute to the antitumor response. They then observed a marked increase in CD8 T cells within the tumors after ICB treatment, suggesting that these cells are the primary mediators of tumor cell killing. This hypothesis was supported by transcriptomic findings, which revealed that mice with a high percentage of tumor-infiltrating CD8 T cells responded better to ICB and displayed an upregulation of genes associated with antitumor immunity. Critically, NK cell depletion resulted in a molecular profile similar to tumors, which failed to respond to ICB, characterized by low CD8 T cell infiltration.[9]

To further dissect the mechanism behind this NK cell-dependent CD8 T cell accumulation within the tumor, the authors evaluated tumor homing. Blocking chemokine signaling impaired the migration of CD8 T cells into the tumors, while ICB treatment increased the expression of several T cell-attracting chemokines within the BM microenvironment, suggesting that the NK cell-mediated mechanism is the augmentation of CD8 T cell homing to the tumor. Both mouse and human tumor-infiltrating CD8 T cells expressed the corresponding receptors for these chemokines. Importantly, NK cell depletion significantly reduced chemokine expression, and a strong correlation was observed between intratumoral chemokine levels and the abundance of CD8 T cells.[9]

This study expands our understanding of NK cell function in antitumor immunity, highlighting their ability to reprogram the TME and enhance the efficacy of ICB therapy. The authors provided compelling evidence that ICB therapy activates non-canonical NK cell activity, which orchestrates the recruitment of cytotoxic CD8 T cells to the tumor site by promoting chemokine expression.[9] Clinically, this work strongly supports evaluating NK cells and chemokines in the context of ICB, for patients with melanoma BM. Ultimately, monitoring NK cell activity and chemokine levels could provide valuable biomarkers for predicting treatment response and guiding therapeutic decisions. While this study offers valuable insights into ICB therapy in BMs, the use of antibody-mediated NK cell depletion may have pleiotropic effects. For example,

anti-Asialo-GM1 targeting of NK cells has been shown to reduce PD-1 expressing T cells.[6] Therefore, additional depletion strategies, such as genetically engineered mice, have the potential to strengthen the results of the current study.

This work opens new horizons with questions for future research.

I. Can therapeutic strategies be developed to induce an NK cell-mediated ICB response phenotype in patients?
II. How do different types of brain tumors influence NK cell activity and their response to ICB?
III. Is this NK cell-mediated mechanism potent enough to overcome the immunosuppressive phenotype of tumor types, which generally respond poorly to ICB, including primary brain tumors, such as glioblastoma?
IV. Is there a subset of NK cells with a specific phenotype and function that is the most important for CD8 T cell recruitment into the melanoma BM microenvironment?
V. What is the mechanism contributing to NK cell upregulation of immune checkpoints in BMs?
VI. A critical unanswered question is the spatial distribution of ICB effects on NK cells. Does ICB targeting of NK cells occur solely within the TME, in the periphery, or at both sites?

Answers to these questions will be valuable for optimizing ICB therapy for both primary and metastatic brain tumors. Ultimately, this study underscores and expands our knowledge of the complex interplay between different immune cell types in the antitumor response to ICB and highlights the potential of harnessing non-cytolytic non-canonical NK cell activity to improve ICB efficacy.

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REFERENCES

1 Moravan MJ, Fecci PE, Anders CK, *et al*. Current multidisciplinary management of brain metastases. *Cancer* 2020;126:1390–406.
2 Valiente M, Ahluwalia MS, Boire A, *et al*. The Evolving Landscape of Brain Metastasis. *Trends Cancer* 2018;4:176–96.
3 Tawbi HA, Forsyth PA, Algazi A, *et al*. Combined Nivolumab and Ipilimumab in Melanoma Metastatic to the Brain. *N Engl J Med* 2018;379:722–30.
4 Goldberg SB, Gettinger SN, Mahajan A, *et al*. Pembrolizumab for patients with melanoma or non-small-cell lung cancer and untreated brain metastases: early analysis of a non-randomised, open-label, phase 2 trial. *Lancet Oncol* 2016;17:976–83.
5 Sun L, Kienzler JC, Reynoso JG, *et al*. Immune checkpoint blockade induces distinct alterations in the microenvironments of primary and metastatic brain tumors. *J Clin Invest* 2023;133:e169314.
6 Judge SJ, Dunai C, Aguilar EG, *et al*. Minimal PD-1 expression in mouse and human NK cells under diverse conditions. *J Clin Invest* 2020;130:3051–68.
7 Hsu J, Hodgins JJ, Marathe M, *et al*. Contribution of NK cells to immunotherapy mediated by PD-1/PD-L1 blockade. *J Clin Invest* 2018;128:4654–68.
8 Taggart D, Andreou T, Scott KJ, *et al*. Anti-PD-1/anti-CTLA-4 efficacy in melanoma brain metastases depends on extracranial disease and augmentation of $CD8^+$ T cell trafficking. *Proc Natl Acad Sci U S A* 2018;115:E1540–9.
9 Fife C, Williams J, James F, *et al*. Natural killer cells are required for the recruitment of CD8+ T cells and the efficacy of immune checkpoint blockade in melanoma brain metastases. *J Immunother Cancer* 2024;12:e009522.