Deep immune profiling reveals targetable mechanisms of immune evasion in immune checkpoint inhibitor-refractory glioblastoma.

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**Public Summary:**
Background: Glioblastoma (GBM) is refractory to immune checkpoint inhibitor (ICI) therapy. We sought to determine to what extent this immune evasion is due to intrinsic properties of the tumor cells versus the specialized immune context of the brain, and if it can be reversed. Methods: We used CyTOF mass cytometry to compare the tumor immune microenvironments (TIME) of human tumors that are generally ICI-refractory (GBM and sarcoma) or ICI-responsive (renal cell carcinoma), as well as mouse models of GBM that are ICI-responsive (GL261) or ICI-refractory (SB28). We further compared SB28 tumors grown intracerebrally versus subcutaneously to determine how tumor site affects TIME and responsiveness to dual CTLA-4/PD-1 blockade. Informed by these data, we explored rational immunotherapeutic combinations. Results: ICI-sensitivity in human and mouse tumors was associated with increased T cells and dendritic cells (DCs), and fewer myeloid cells, in particular PD-L1+ tumor-associated macrophages. The SB28 mouse model of GBM responded to ICI when grown subcutaneously but not intracerebrally, providing a system to explore mechanisms underlying ICI resistance in GBM. The response to ICI in the subcutaneous SB28 model required CD4 T cells and NK cells, but not CD8 T cells. Recombinant FLT3L expanded DCs, improved antigen-specific T cell priming, and prolonged survival of mice with intracerebral SB28 tumors, but at the cost of increased Tregs. Targeting PD-L1 also prolonged survival, especially when combined with stereotactic radiation. Conclusions: Our data suggest that a major obstacle for effective immunotherapy of GBM is poor antigen presentation in the brain, rather than intrinsic immunosuppressive properties of GBM tumor cells. Deep immune profiling identified DCs and PD-L1+ tumor-associated macrophages as promising targetable cell populations, which was confirmed using therapeutic interventions in vivo.

**Scientific Abstract:**
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