Background Glioblastoma (GBM) is the most common primary malignant brain tumor and shows poor outcomes, with a median survival of 12–18 months using the current standard-of-care therapy. GBM exhibits sex differences in incidence and overall survival, with males experiencing a higher incidence and worse prognosis compared to females. Emerging evidence suggests that these differences extend to genetic/epigenetic and cellular levels, including immune responses. However, the mechanisms driving immunological sex differences are not fully understood.

Methods To investigate the underlying mechanisms of sex differences in GBM, we used orthotopic GBM mouse models. Male and female mice received intracranial injections of murine syngeneic GBM cells (SB28 and GL261) and underwent survival analysis or immune cell profiling. To interrogate the immune cell-intrinsic and -extrinsic mechanisms, bone marrow chimeras were generated by reconstituting immune system of male or female recipient mice with male or female donor bone marrow cells. Additionally, adoptive transfer of T cells to tumor-bearing mice was performed in a sex-matched or mismatched manner.

Results Sex differences in survival were recapitulated in immune-competent B6 mice, but not in immune-deficient NSG or RAG1KO mice. By depleting CD8+ T cells, we further confirmed that CD8+ T cells play a critical role in driving sex differences in survival. Flow cytometry analysis revealed that male CD8+ T cells from tumors expressed higher levels of exhaustion markers such as PD-1, CTLA-4, and Tox, with a higher frequency of progenitor exhausted T cell subsets, whereas female tumors contained more effector-like CD8+ T cells expressing elevated cytokine production. Treatment with anti-PD-1 antibodies exclusively extended the survival of male mice by inducing decreased exhausted CD8+ T cell subsets and increased effector function and proliferation. Survival analysis using the bone marrow chimera model and adoptive transfer model indicated that T cell-mediated tumor control was predominantly regulated in a cell-intrinsic manner, as the sex of donor cells was a critical contributing factor. Furthermore, we confirmed these observations in GBM patient tumor samples, with male tumors having a higher frequency of progenitor exhausted T cells with increased TOX expression. Lastly, we found that an X chromosome inactivation escape gene, Kdm6a, is associated with sex-biased T cell exhaustion.

Conclusions Taken together, these findings demonstrate sex-biased pre-determined behavior of T cells is critical in inducing sex differences in GBM progression and immunotherapy responses (figure 1).