Background The effectiveness of natural killer (NK) cell-based immunotherapy against solid tumors is limited by the inadequate infiltration of NK cells into the tumors and the heterogeneous immunosuppressive tumor microenvironment (TME), suggesting that a multi-specificity of targeting mechanisms is needed to achieve durable responses.

Methods We isolated and phenotyped tumor-infiltrating NK cells and peripheral blood (PB) NK cells from glioblastoma multiforme (GBM) patients for the expression of chemokine receptor CXCR3. The effect of exogenous CXCL10 on NK cell migration and functions was also established. We further investigated the regulation of the CXCR3-CXCL10 axis on NK cells and tumor cells by establishing CXCR3-knockdown primary NK cells and CXCL10-overexpressing GBM cells. Based on these findings, we designed and synthesized a novel natural killer cell engager (NKCE), which not only targets NKp46 on NK cells and IL13Ra2 on tumor cells but also specifically releases CXCL10 at the tumor sites while sustaining NK cell activation via interleukin (IL)-15.

Results CXCR3 was highly expressed on NK cells in PB from either GBM patients or healthy donors. Although low numbers of tumor-infiltrating NK cells in patients were observed, CXCR3 expression was up-regulated on these cells. CXCL10 induced NK cell migration via CXCR3 but did not affect NK cell functions. Simultaneously, CXCL10 overexpression showed no effect on tumor growth but resulted in enhanced NK cell migration into tumor sites and, in turn, improved the anti-tumor activity of NK cells. Furthermore, our novel NKCE activated NK cells by binding NKp46 and recognized GBM tumor cells via IL13Ra2, thus promoting the contact between NK cells and tumor cells. The engager’s CXCL10-releasing domain, which is activated in the local TME, promotes the specific increase in CXCL10 concentration and, in turn, NK cell homing in the TME.

Conclusions The CXCR3-CXCL10 axis contributes to the recruitment of NK cells to GBM. Our novel NKCE within a locally-cleavable CXCL10 domain induced NK cell migration and boosted NK cell anti-tumor activity against solid tumors. Such a multi-specific approach not only activates NK cells locally but promotes their recruitment and retention in the TME.