Background Natural killer (NK) cell effector function is suppressed in the tumor microenvironment (TME) of metastatic castration-resistant prostate cancer (mCRPC), the lethal form of prostate cancer. This is largely due to the inherently 'cold' nature of the TME of mCRPC that is immunosuppressive, hypoxic and lacks cytolytic lymphocyte infiltration. To improve NK cell anti-tumor responses against mCRPC in the TME, we designed a novel tri-specific killer engager (TriKE®) molecule that consists of three parts: an arm that engages with CD16, an activating receptor of NK cells, an arm that binds to prostate-specific membrane antigen (PSMA) that is highly and specifically expressed on mCRPC, and an interleukin (IL)-15 moiety that is essential for NK cell survival, proliferation, priming and motility (figure 1).

Methods Flow cytometry-based functional and dye dilution proliferation assays were used to compare activation and proliferation of NK cells treated with PSMA TriKE or IL-15. NK cell cytolytic capacity against C4-2, a PSMA-expressing prostate cancer cell line, was measured using IncuCyte live cell imaging. In various assays, hypoxic (1% oxygen) culture condition and cytokine-induced myeloid-derived suppressor cells (MDSC) were incorporated to better examine PSMA TriKE function in the physiological setting of mCRPC.

Results PSMA TriKE significantly enhanced expansion of peripheral blood NK cells derived from healthy donors up to 10 folds (N=9). This effect was specific to NK cells and not T cells. Additionally, PSMA TriKE induced NK cell degranulation (up to 60%) and intracellular IFNγ and TNFα buildup (up to 50%) when compared to IL-15 and no treatment groups after incubation with C4-2 (N=6). This result was not observed when PSMA knockout C4-2 were used as target cells. NK cell incubation in hypoxia for 7 days severely impacts cytotoxicity, some of which can be improved with IL-15. However, PSMA TriKE treatment markedly improved NK cell cytolytic capacity against C4-2 in hypoxia (N=5) (figure 2). Similarly, MDSCs suppressed NK cell degranulation in the presence of IL-15 alone but not with PSMA-TriKE treatment (N=4) (figure 3).

Conclusions PSMA-TriKE induces specific NK cell proliferation and activation against PSMA+ tumor cells. Efficient delivery of IL-15 to NK cells by PSMA-TriKE robustly relieves NK cells from suppression induced by hypoxia and MDSCs. These results demonstrate promising potential of PSMA TriKE in overcoming suppression of NK cells in the TME of mCRPC.

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PSMA TriKE overcomes suppressed NK cell degranulation induced by MDSCs. NK cells were cultured alone or co-cultured with allogeneic monocytes or MDSCs at 1:1 ratio with no treatment, 0.06nM IL-15 or 3nM PSMA TriKE (at equifunctional concentrations) for 5 days. Flow cytometry was used to assess NK cell degranulation (measured by CD107a) after 5 hours incubation with C4-2 as target cells with no treatment, 0.06nM IL-15 or 3nM PSMA TriKE. Monocytes were CD14+ selected from peripheral blood mononuclear cells and MDSCs were differentiated from monocytes for 7 days using 10ng/mL of IL-6 and granulocyte monocyte-colony stimulating factor (GM-CSF). N=4; mean ± SEM (Two-way ANOVA with Tukey’s multiple comparison test); * p< 0.05, ** p<0.01. IL-15, interleukin-15; MDSC, myeloid-derived suppressor cells; PSMA, prostate-specific membrane antigen; TriKE, tri-specific killer engager