A Tri-specific Killer Engager (TriKE®) against B7-H3 enhances NK cell mediated killing of multiple myeloma

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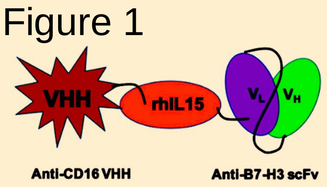
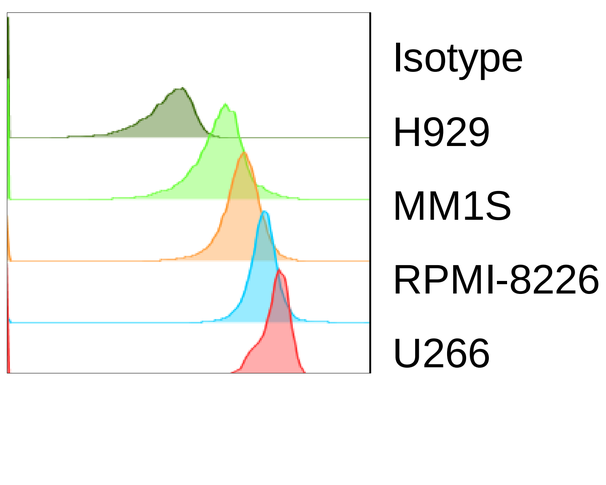
Background: Natural Killer (NK) cell-based therapies hold great promise in treating multiple myeloma. One method to enhance NK cell specificity against myeloma is antibody dependent cellular cytotoxicity through CD16 receptor. We targeted B7-H3 (CD276) because its expression in myeloma is associated with decreased progression free survival, it exhibits low expression on healthy tissue, and it is expressed on myeloid derived suppressor cells (MDSC), which promote myeloma growth.

Methods: We developed a tri-specific killer engager (TriKE) with camelid single-chain Fv fragments against B7-H3 and CD16 linked by IL-15 to enhance NK cell killing of myeloma (Figure 1).

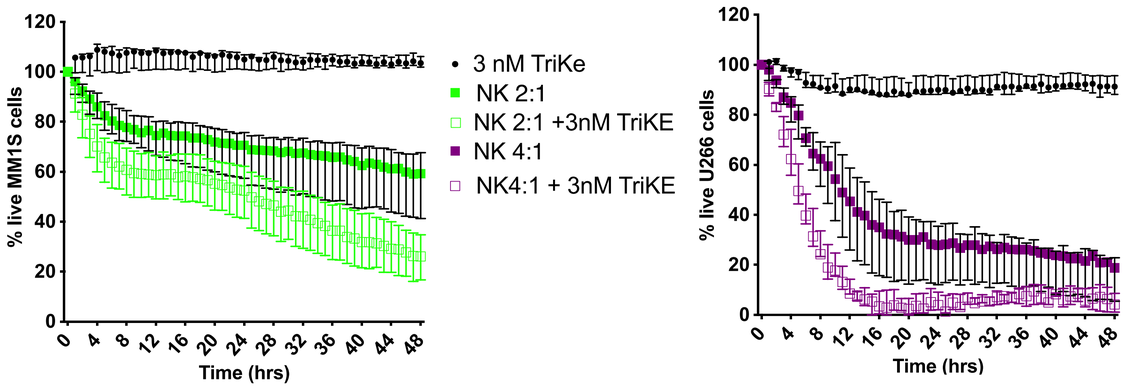
Figure 1

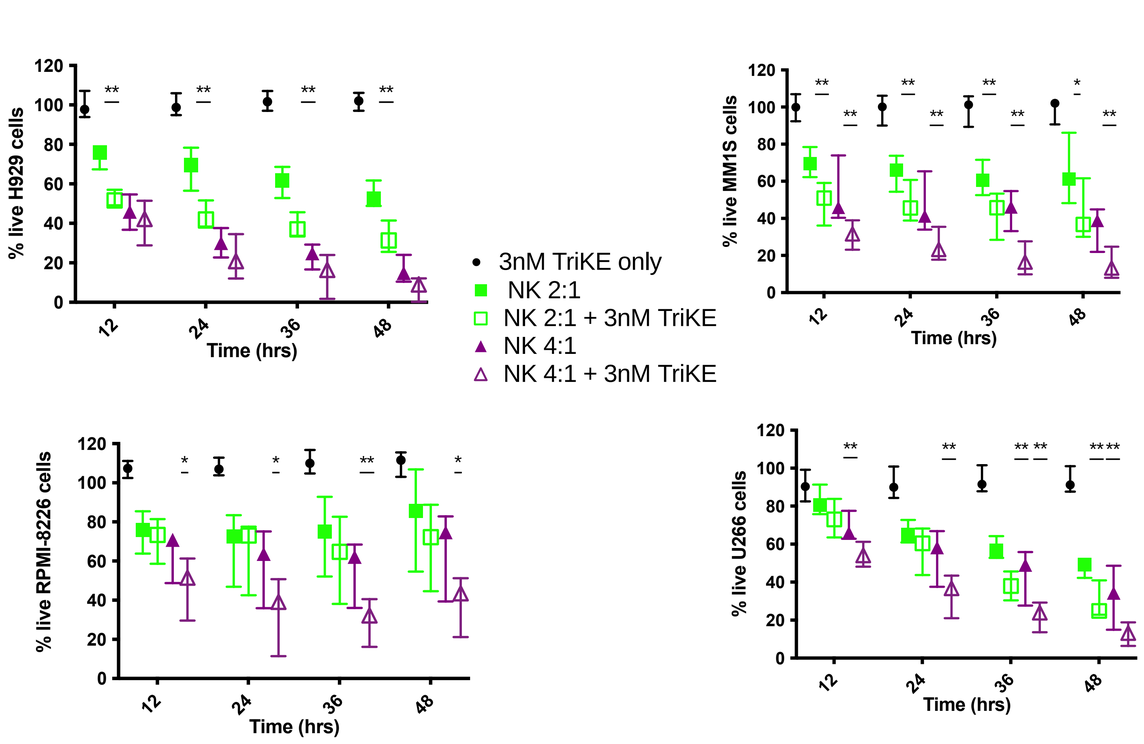


Results: We found high expression of B7-H3 on the myeloma lines RPMI-8226, U266, and MM1S and relatively low expression on H929 by flow cytometry (Figure 2).

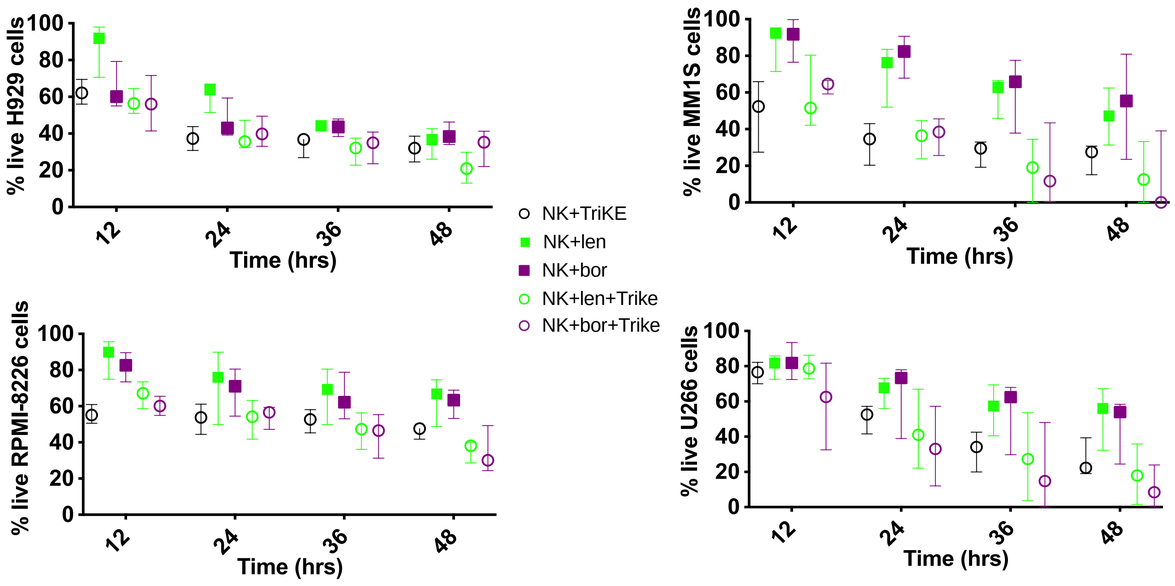


We compared the ability of peripheral blood NK cells with or without B7-H3-TriKE to kill myeloma cells in live imaging IncuCyte Zoom assays with escalating doses of TriKE. Maximal killing occurred with 3 nM concentration. We found a statistically significant increase in NK cell mediated killing of all four myeloma lines when 3nM B7-H3-TriKE was added. Against U266 and MM1S, B7-H3-TriKE significantly enhanced killing at effector:target (E:T) ratios of 2:1 and 4:1. RPMI-8226 showed relatively high resistance to NK cell cytotoxicity but B7-H3-TriKE enhanced killing at E:T of 4:1. H929 cells were more potently killed in the presence of B7-H3-TriKE at E:T of 2:1 but there was no difference in killing at E:T 4:1 likely due to high natural cytotoxicity in both groups (Figure 3).

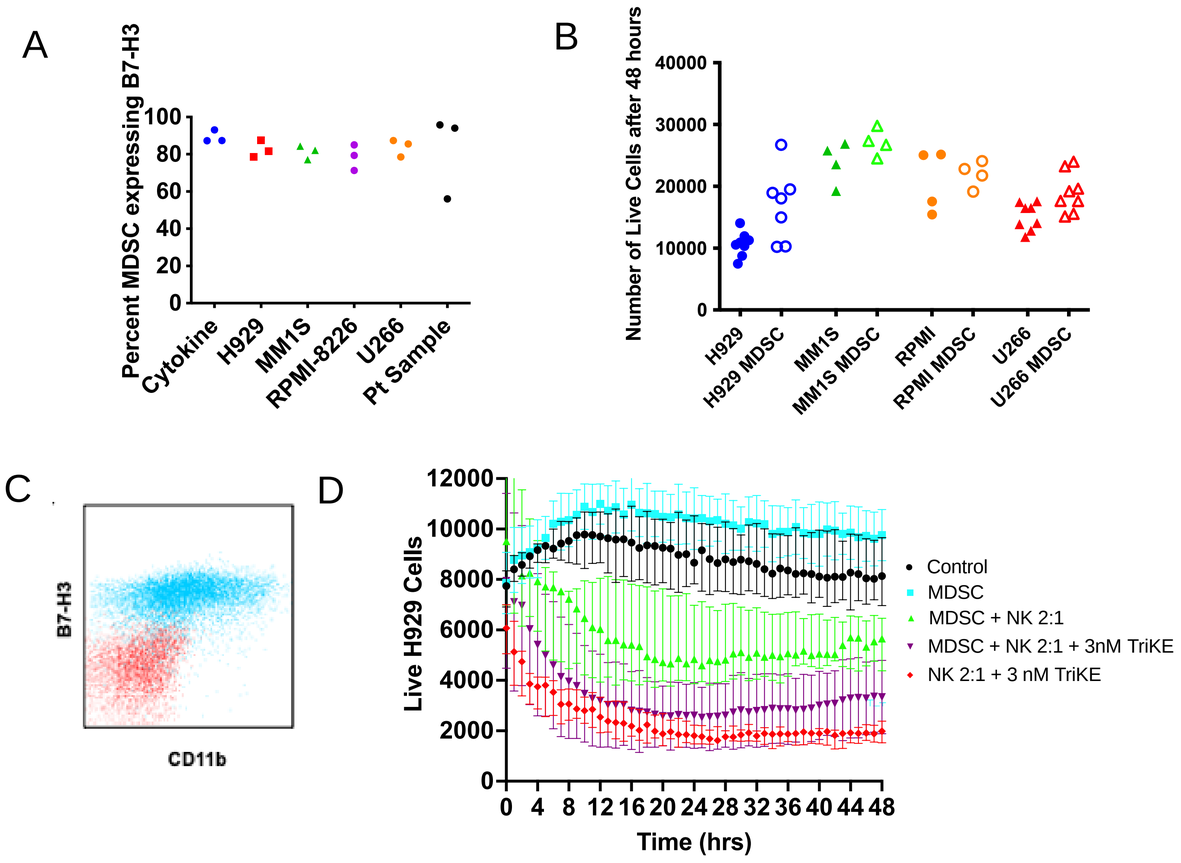




We also tested the efficacy of B7-H3-TriKE with the proteasome inhibitor bortezomib (10nM) and the immunomodulatory drug lenalidomide (5μM). Cytotoxicity curves were compared by repeated measures ANOVA and performed in triplicate. Combination therapy with B7-H3-TriKE, NK cells, and lenalidomide showed synergistic killing of H929 cells after 48 hours of live cell imaging (p=0.047) but combination with bortezomib did not further enhance killing compared to NK cells and TriKE alone (Figure 4). Both lenalidomide and bortezomib showed a trend toward improved killing against MM1S when given with NK cells and B7-H3 TriKE but it did not reach statistical significance. Combination therapy with B7-H3-TriKE, NK cells, and lenalidomide or bortezomib showed synergistic killing of RPMI-8226 cells after 48 hours of live cell imaging (p<0.001 and 0.015 respectively). Bortezomib combined with with B7-H3-TriKE and NK cells enhanced killing in U266 cells (p=0.037) (Figure 4)



We developed MDSC from $CD33^+$ myeloid cells from healthy donors using IL-6 and GM-CSF or incubating them with myeloma cells at 1:100 ratio for seven days. MDSC ($CD14^+CD11b^+$) exhibited high expression of B7-H3 (Figure 5A). MDSC were also isolated from the bone marrow aspirates of three newly diagnosed myeloma patients and exhibited 56-95 (Aspirates were processed with lysis buffer and stained for CD14, CD11b, and B7-H3. Shown is a flow cytometry plot of live, CD14+ cells (Figure 5C).MDSC were incubated with myeloma cells and growth was measured over 48 hours by live cell imaging (Figure 5B). Addition of MDSC to cytotoxicity assays enhanced myeloma cell growth but was overcome by B7-H3 TriKE and NK cells (Figure 5D).



Conclusions: B7-H3-TriKE significantly enhances NK cell mediated killing of myeloma cells, even in the relatively low B7-H3-expressing H929 line. Our data also shows it can reverse MDSC-induced myeloma growth. Commercial manufacturing of B7-H3 TriKE (GTB-5550) has begun and a phase I trial will begin enrollment in 2022.