508 IL-15-STIMULATED NK CELL PROLIFERATION IS GREATLY AUGMENTED BY THE ANTI-ADAM17 MAB MEDI-1 AND REQUIRES CD137

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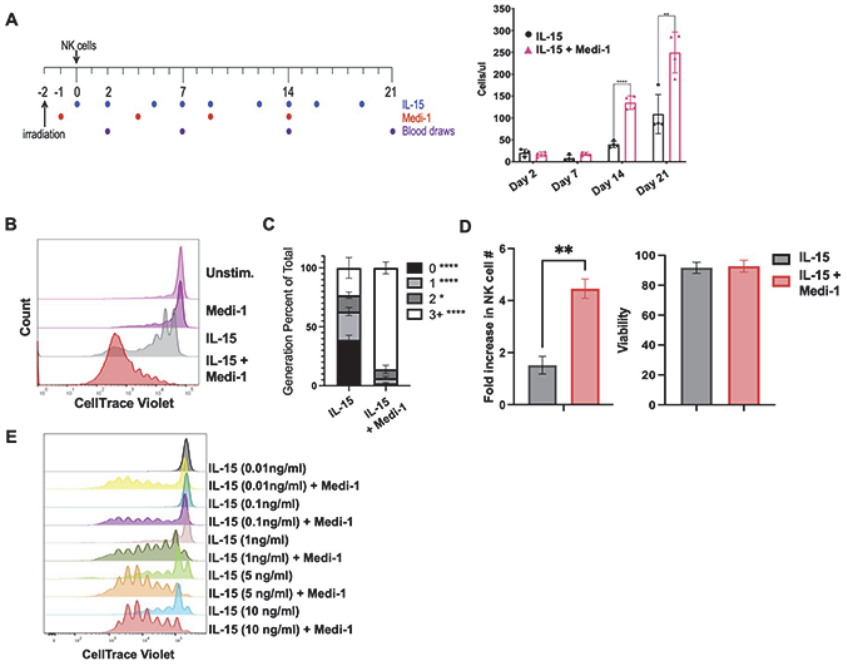
Background Natural killer (NK) cells are innate cytolytic lymphocytes that can directly and rapidly kill malignantly transformed cells without clonal expansion. While NK cell adoptive transfer has shown clinical benefits in cancer patients, this approach requires cytokine support for the NK cells to persist, which IL-15 is commonly used. Cytokine stimulation of NK cells activates ADAM17 (a disintegrin and metalloproteinase-17). This membrane-associated protease then rapidly cleaves a number of cell surface receptors, serving as a regulatory checkpoint that restrains NK cells in a polyfunctional manner. Medi-1 is a human IgG1 mAb utilized by us to block ADAM17 function.

Methods Human NK cell expansion in vivo was performed using NSG mice administered enriched NK cells and rhIL-15 ± Medi-1. For ex vivo NK cell expansion, PBMCs or enriched NK cells were labeled with CellTrace Violet dye and cultured for 7 days ± rhIL-15 ± Medi-1.

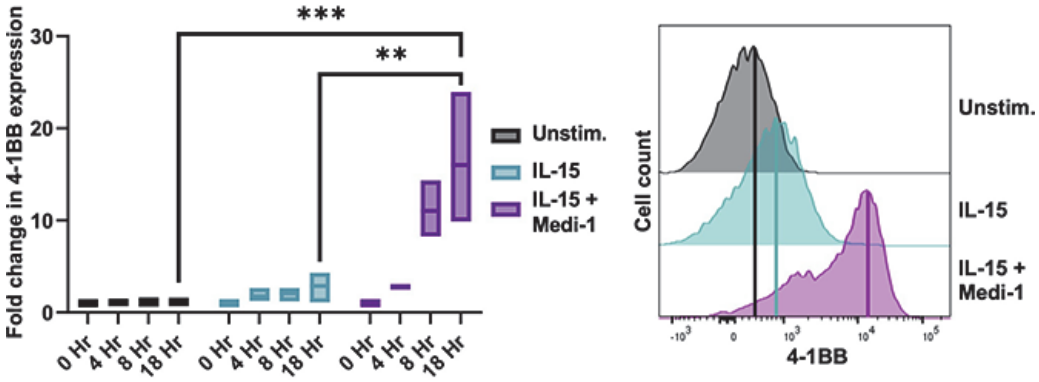
Results We show that IL-15-driven NK cell expansion is markedly enhanced by Medi-1 treatment ex vivo, including under IL-15 limiting conditions, and in a xenograft mouse model (figure 1). This did not cause NK cell exhaustion based on phenotypic profiling and functional assays. Here, we focus on the mechanism underlying enhanced IL-15-driven NK cell proliferation by Medi-1. Various markers of lymphocyte activation were examined on NK cells stimulated with IL-15 ± Medi-1. The expression rate and level of CD69, CD25 (IL-2Rα), and CD215 (IL-15Rα) were significantly increased on NK cells stimulated with IL-15 + Medi-1, as was CD137 (4–1BB), which demonstrated a dramatic upregulation (figure 2). It is well established that CD137 is a key co-activation receptor on T cells and its stimulation enhances their proliferation. Inhibiting CD137 on NK cells in various manners blocked their enhanced proliferation by IL-15 + Medi-1 (figure 3). Of interest is that 4–1BB inhibition also blocked NK cell proliferation by IL-15 alone (figure 3). Currently there is little information on the physiological role of CD137 in NK cell proliferation by IL-15. Our data reveal that CD137 is necessary for this process and the augmented proliferation of NK cells by Medi-1 treatment. Moreover, the anti-4–1BB agonistic mAb urelumab further enhanced NK cell proliferation by IL-15 in the presence of Medi-1.

Conclusions Our data reveal that Medi-1 may have an important therapeutic use by increasing the expansion, persistence, and effector functions of NK cell therapies in cancer patients, in part by enhancing their sensitivity to IL-15, IL-2, and anti-4–1BB agonist mAb therapies.

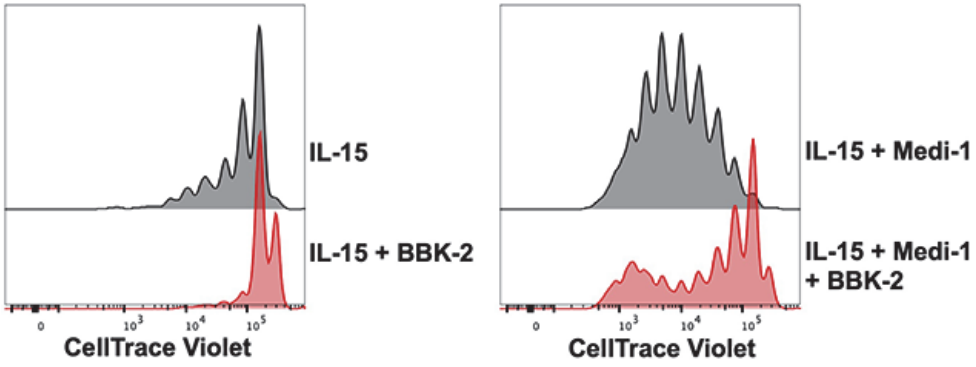
Ethics Approval Peripheral blood was collected with consent from healthy donors. The protocol was approved by the Institutional Review Board of the University of Minnesota (Protocol: 9708M00134). For experiments involving mice, the protocol was approved by the Institutional Animal Care and Use Committee of the University of Minnesota (Protocol: 2112–39650A).



Abstract 508 Figure 1 Medi-1 treatment enhances human NK cell proliferation by IL-15. (A) NSG mice were treated as shown in the schematic. Mice were administered enriched human NK cells (4x106) from a healthy donor and rhIL-15 (5 μg) + Medi-1 (10 mg/kg), as indicated. Circulating human CD45+ CD56+ CD3- NK cells were enumerated by flow cytometry and are shown as cells/ul. n = 4 mice per group. Data are representative of 7 experiments using NK cells from separate donors. (B) PBMCs were labeled with CellTrace Violet dye and cultured for 7 days + rhIL-15 (10ng/ml) + Medi-1 (5μg/ml), as indicated. NK cells were identified by flow cytometry as CD56+ CD3-. (C) Summary results are shown as the mean ± SD of the percentage of cells per generation. (D) Fold expansion and viability of CD56+ CD3- NK cells cultured with rhIL-15 ± Medi-1 for 7 days. n = 4 donors. E. PBMCs were cultured as described in panel B in the presence of rhIL-15 at the indicated concentrations + Medi-1. NK cells were identified by flow cytometry as CD56+ CD3-. Data are mean ± SD. *p < 0.05; **p < 0.01; ****p < 0.0001.



Abstract 508 Figure 2 Medi-1 treatment greatly augments CD137 upregulation by NK cells stimulated with IL-15. Enriched NK cells (>95%) were cultured + rhIL-15 (10ng/ml) + Medi-1 (5µg/ml) for up to 18 hours. At the indicated time points, NK cells were examined for their expression of CD137 by flow cytometry. Fold change in CD137 expression was determined from mean fluorescent intensity. n = 4 donors. **p < 0.01; ***p < 0.005. The histogram plot shows representative CD137 staining at the 18-hour time point. The vertical lines in each histogram indicate mode fluorescent intensity.



Abstract 508 Figure 3 CD137 inhibition diminishes NK cell proliferation by IL-15 in the presence and absence of Medi-1. PBMCs cells were labeled with CellTrace Violet dye and cultured for 7 days with rhIL-15 (10ng/ml) + Medi-1 (5µg/ml) ± the anti-human CD137 function blocking mAb BBK2 (5µg/ml). Data are representative of 3 experiments using NK cells from separate donors.

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