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POSTER ABSTRACTS

802.CHEMICAL BIOLOGY AND EXPERIMENTAL THERAPEUTICS

High-Throughput Screens Identify NEDD8 Inhibition As a Strategy to Augment Natural Killer Cell Cytotoxicity Against Blood Cancers

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Natural killer (NK) cell-based therapies are amongst the emerging immunotherapeutic approaches that aim to target malignant cells refractory to standard therapies. Currently used strategies to augment the NK cell anti-cancer function of NK cells include cytokine treatments and the use of feeder expansion protocols for culturing NK cells. Recent studies have indicated that NK cell cytotoxicity and function may also be enhanced by certain anti-cancer drugs. However, the effects of the hundreds of available oncology drugs on NK cell function have not been systematically evaluated. Here, we perform large-scale high-throughput testing of NK cell-drug combinations using co-culture models with diverse blood cancer cells, identifying the NEDD8-activating enzyme inhibitor pevonedistat as a promising candidate for augmenting NK cell anti-cancer responses.
To identify oncology drugs synergizing with NK cell-based immunotherapy, we established a high-throughput drug sensitivity and resistance testing (DSRT) platform to investigate effects of 527 approved drugs and investigational compounds on NK cell cytotoxicity against blood cancer cell lines. Overall, 10 cell lines, including acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL), diffuse large B-cell lymphoma (DLBCL), and multiple myeloma (MM), were screened in combination with primary expanded NK cells. Target cells were treated for 24 hours with drugs at five different concentrations both with and without NK cells. In NK cell co-culture, an effector-target (ET) cell ratio at which half of the target cells were killed was selected for experiments. Most effective compounds were then selected for further testing using multiplexed single-cell RNA sequencing (scRNA-seq) and analysis of secreted cytokines (Figure 1).
Overall, the results from our DSRT platform demonstrated varying effects of different drug classes on NK cell cytotoxicity against blood cancer cell lines. In general, 5-10% of drugs had NK cell cytotoxicity-enhancing effects, 30-40% inhibited NK function and 35-50% of drugs had no effect. Similarities in results between cell lines highlighted the dependence of NK cell-drug synergistic effects on disease pathobiology. Across multiple disease models, pevonedistat (MLN4924), a selective NEDD8 activating enzyme inhibitor that has been studied as treatment for relapsed/refractory AML, showed strong synergistic effects with NK cells. For example, in OCI-AML-3 (AML), pevonedistat alone showed no effect on target cell viability, but in combination with NK cells at a 1:4 ET ratio, it resulted in 98% target cell killing at 100 nM. Similar effects were also seen in MM1.S (MM), although a higher concentration (10000 nM) of pevonedistat was required (Figure 2).
Results from scRNA-seq after combination treatment with NK cells and pevonedistat revealed distinct transcriptomic changes in both NK cells and target cells when compared to controls. In both NK cells and target cells, pevonedistat caused upregulation of *TXN*, *TALDO1*, *TXNRD1*, *NQO1*, *SRXN1* and *SLC7A11*,collectively indicating effects on the NRF2 signaling pathway. The upregulation of these genes correlated with the tested concentration of pevonedistat, with notably higher expression

when treated with 1000 nM pevonedistat compared to 100 nM. Changes were also observed in the secretion of cytokines detected from the cell culture media, where concentrations of IL-1β, IL-2, IL-6, IL-8, IL-10, IFN-γ and TNF-α all increased up to 300% compared to controls.

In HEL (AML) and LAMA-84 (CML), where pevonedistat had only minor effects on NK cell cytotoxicity, the expression *TNF* and *TNFRSF1B* at baseline and after pevonedistat treatment were notably lower, when compared to cell lines where the combination therapy led to stronger responses. This suggests a possible mechanism for pevonedistat-NK cell synergy, given that previous reports have demonstrated a synergy between pevonedistat and TNF-α to activate apoptosis.

In conclusion, we discovered a cell context-specific synergy between pevonedistat and NK cells, which increased NK cell cytotoxicity against malignant cells *in vitro*. In addition, our results demonstrate the potential of using a high-throughput platform for studying NK cell-drug interactions against malignant cells, while aiming to improve treatment for hematological malignancies through combination immunotherapies.

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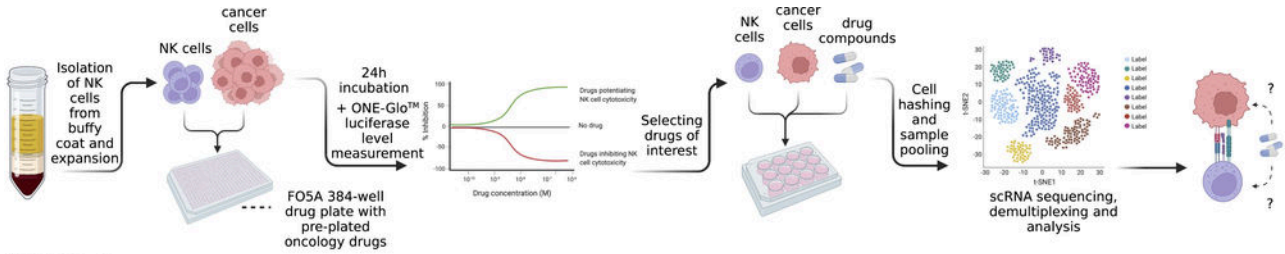

Figure 1.

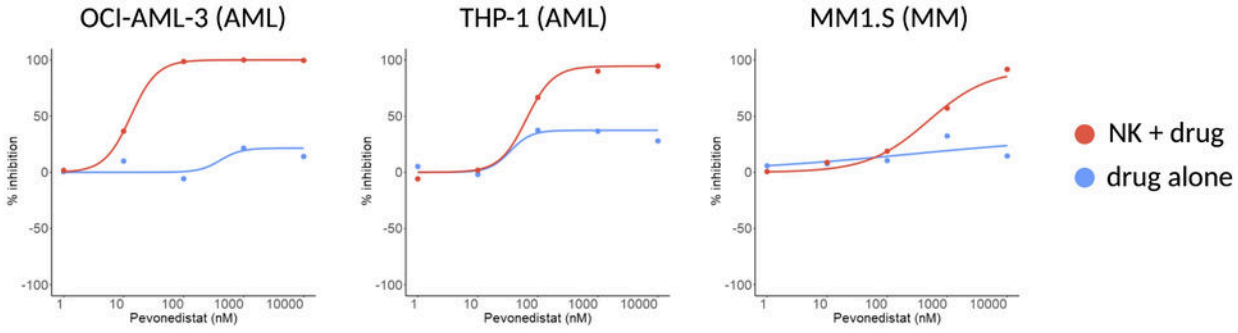

Figure 2.

Figure 1

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