333 NOVEL PHYTOCHEMICALS-BASED CELL CULTURE MEDIUM MITIGATES NATURAL KILLER (NK) CELLULAR EXHAUSTION AND ENHANCES MATURE SUBPOPULATIONS, LEADING TO HIGHER ANTICANCER CYTOTOXIC ACTIVITY

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Background The biological significance of the mitigation of NK cell dysfunction (exhaustion and/or anergy and/or senescence) is increased potential for solid tumor infiltration by NK cells, as well as decreased dysfunction of NK cells in the tumor microenvironment.[1,2] Enable-NK, a novel phytochemicals-based culture medium that increases the cytotoxic activity of NK cells, has been previously demonstrated to mitigate cellular exhaustion in the NK cell line KHYG-1, as illustrated by the consistent downregulation of several exhaustion biomarkers (e.g. Tim-3, TIGIT, NKG2A). We evaluated the mitigation of exhaustion in human primary NK cells by culturing them in Enable-NK.

Methods Peripheral Blood Mononuclear Cells (PBMCs) were isolated from fresh buffy coats using density gradient centrifugation, and NK cells were enriched to ~90% purity using magnetic separation. Media conditions used were Enable-NK (DMEM/F12 supplemented with proprietary ingredients) or control (DMEM/F12). Cocultures were set up with K562-GFP target cells, undisturbed for several days using a 24-well G-Rex plate. Propidium iodide (PI) staining was used for cytotoxicity determination, and a panel of 12 antibodies for cellular exhaustion and subpopulation determination using surface biomarkers.

Results Several endpoints illustrate mitigation of cellular exhaustion when NK cells are cultured in Enable-NK media. Cell surface exhaustion markers (e.g. Tim-3, TIGIT, NKG2A) are consistently lower, and cytotoxicity-activating receptors (e.g. NKp46) trend higher with the use of Enable-NK media (figure 1). The CD56dimCD16+CD57+ subpopulation is doubled in Enable-NK monocultures compared to control monocultures (figure 2), and also greatly expanded in cocultures. Finally, increased cytotoxic performance of Enable-NK-cultured NK cells, predicted based on the phenotypic differences and enrichment of the highly cytotoxic subpopulation, was confirmed by assessing the death of target cells in the cocultures by staining with propidium iodide (figure 3). Cytotoxic activity was initially equivalent to the control on Day 1, but it was boosted by over 40% in the Enable-NK group by Day 4 while remaining at the same level in the control group. Greatly enhanced cytotoxic activity was maintained through Day 7.

Conclusions The phenotypic and functional data indicate that cellular exhaustion in NK cells is significantly mitigated by culturing NK cells in the phytochemicals-based Enable-NK medium. By inducing downregulation of several inhibitory cell surface molecules, simultaneously inducing upregulation of cytotoxicity-activating receptors, and expanding cytotoxic subpopulations such as CD56dimCD16+CD57+, Enable-NK-cultured NK cells are better equipped to kill cancer cells.

REFERENCES

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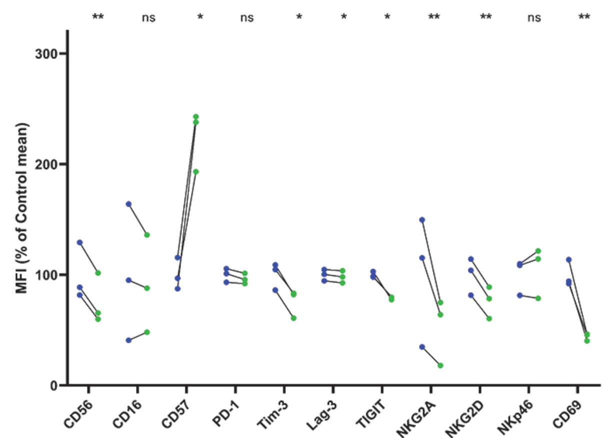
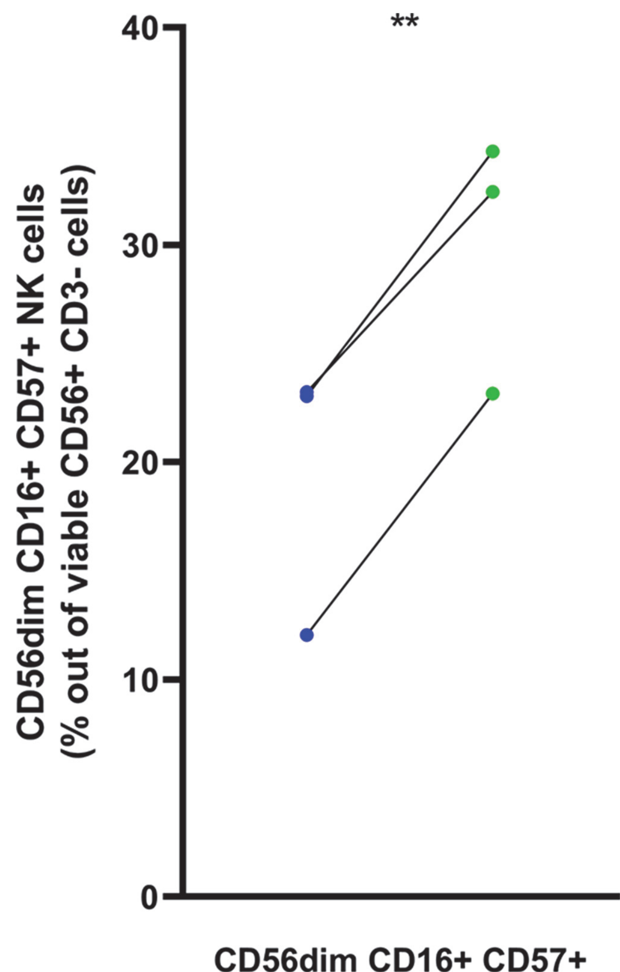
2. Wang J, Matosevic S. Functional and metabolic targeting of natural killer cells to solid tumors. *Cell Oncol*. 2020;**43**(4):577-600.



Abstract 333 Figure 1 Differences in surface marker expression in Enable-NK
The Mean Fluorescence Intensity comparison between surface marker levels on primary human NK cells, on day 3 of culture in control medium (blue) or Enable-NK (green). The represented data is for 3 healthy adult donors of primary NK cells, paired donor-wise between the two media conditions. The notation used for statistical significance is as follows (ratio paired t-test): ns is not significant, *p<0.05, **p<0.01



Abstract 333 Figure 2 Enrichment of a mature cytotoxic subpopulation of NK cells
Comparison of the CD56dimCD16+CD57+ subpopulation between the control culture (blue) and Enable-NK culture (green) at day 3, as a

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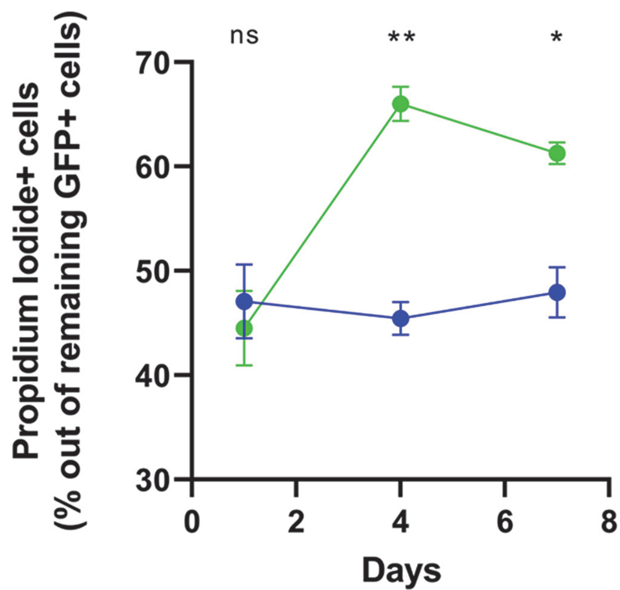
percentage of all NK cells (CD3-CD56+), with data paired donor-wise. The notation used for statistical significance (paired t-test) is as follows: **p<0.01



Abstract 333 Figure 3 Higher cytotoxic activity over time in Enable-NK coculture
Percentage of target cells which are dead, as a function of time, over the course of coculture of primary human NK effector cells with K562-GFP target cells. Cocultures were set up at an Effector:Target ratio of 1:1 on Day 0. Bars indicate Standard Error of Mean. Notation used for statistical significance (paired t-test): ns is not significant, *p<0.05, **p<0.01

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