Development of large-scale expansion protocol for NKG2C-positive NK cells for treatment of glioblastoma

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Introduction

Glioblastoma, a WHO grade IV glioma and the most common primary adult brain tumor, express elevated levels of HLA-E and therefore block NK cells with expression of the inhibitory NKG2A receptor. Of note, glioblastoma cells also overexpress non-classical HLA-G, which provides a nonameric peptide that, in complex with HLA-E, has the highest affinity for inhibitory NKG2A receptor described so far. However, these HLA-E-peptide complexes are recognized by a small NK cell subset expressing the activating NKG2C receptor, preferentially found in human cytomegalovirus (HCMV)-seropositive donors. The NKG2C+ NK cell subset in peripheral blood mostly represent differentiated NK cells with high lytic capacity as well as less cytokine production and is therefore a potential candidate for immunotherapy of glioblastoma. However, low frequencies of NKG2C+ NK cells and therefore their low absolute cell numbers in peripheral blood limits further clinical use. In this project, we aim to establish a large-scale *ex vivo* expansion of functional NKG2C+ NK cells from peripheral blood employing bottom-gas permeable membrane bioreactors (G-Rex®) and using a recently developed PC-3 feeder cell line genetically engineered with IL-2, membrane-bound IL-15, as well as a first generation single chain trimeric HLA-E molecule.

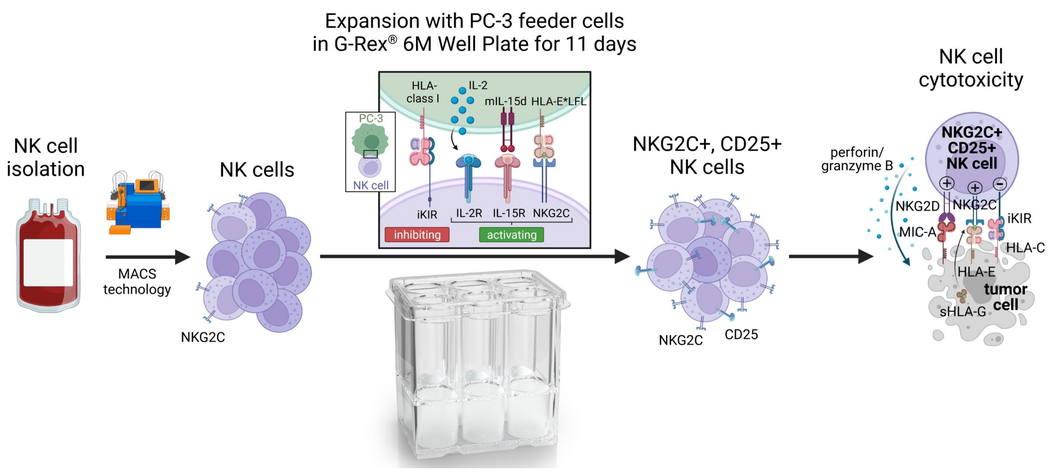

Fig. 1: Work flow of large-scale expansion of NKG2C+ NK cells with PC-3 feeder cells in a ratio of 10:1 in a G-Rex® 6M Well Plate. 5 x 10^5 PC-3 feeder cells were fed on day of isolation, day 3 and 7. (created by BioRender.com)

Results

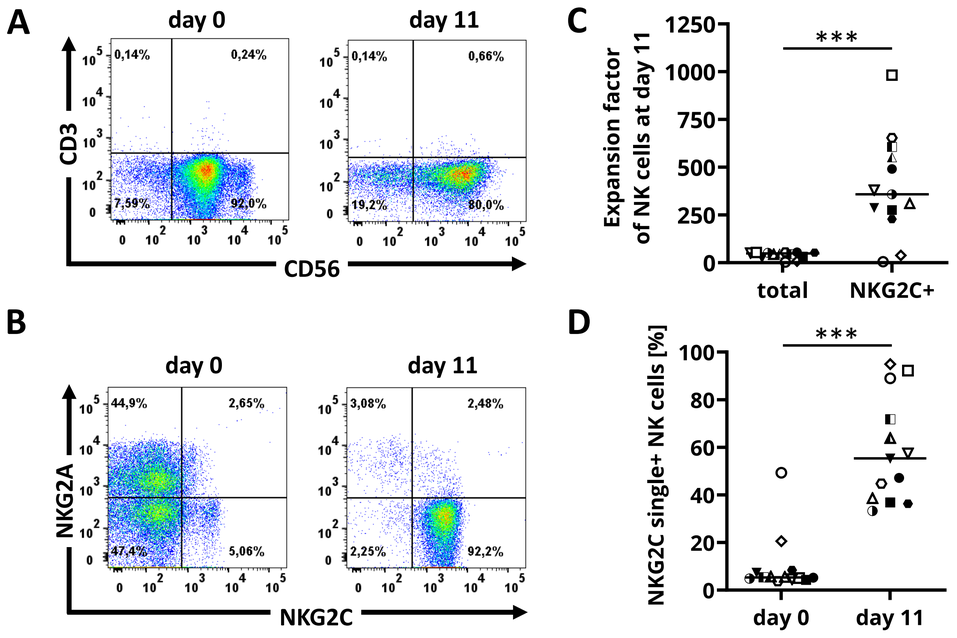

Fig. 2: Selective expansion of NKG2C single+ NK cells from 13 HCMV-seropositive donors with PC-3-IL-2-mIL-15d-HLA-E*LFL feeder cells. Flow cytometry analysis of (**A**) purity of NK cell population and (**B**) percentage of NKG2C+ NK cells at day of isolation and day 11 of expansion using one donor as an example. (**C**) Co-cultivation of NK cells with PC-3 feeder cells resulted in efficient NKG2C single+ NK cell expansion with a median expansion factor of 358.5 in comparison of total NK cell expansion (median: 49.1). (**D**) Furthermore, the percentage of NKG2C+ NK cells increased highly significant (day 0: median 5.4% vs. day 11: median 55.3%).

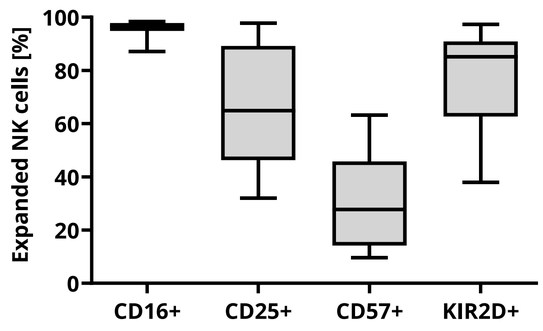

Fig. 3: Investigation of NK cell activation and exhaustion marker expression by flow cytometry. 10 days expanded NK cells from 13 HCMV-seropositive donors exhibited increased numbers of CD16+ (median 96.6%), CD25+ (median 64.8%), CD57+ (median 27.8%) and KIR2D+ (median 85.1%) cells indicating maturation and a shift to memory-like NK cells.

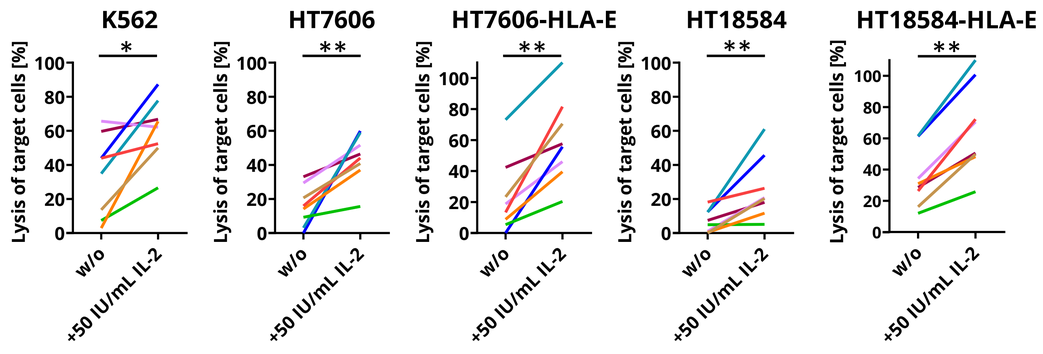

Fig. 4: Investigation of cytotoxicity of expanded NKG2C+ NK cells by chromium release assays with a target to effector ratio of 10:1. Expanded NKG2C+ NK cells of eight donors showed cytolytic activity against K562 and two HLA-E low-expressing primary glioblastoma cell lines HT7606 (KIR:HLA mismatch) and HT18584 (KIR:HLA match). Cytotoxicity was significantly increased by HLA-E overexpression (HT7606-HLA-E and HT18584-HLA-E) and when boosted by treatment with 50 IU/mL IL-2.

Conclusion

Our results furthermore demonstrate an efficient cytotoxicity of NKG2C+ NK cells primed with low doses of IL-2 towards primary glioblastoma cells. Further *in vivo* studies are warranted for future translation into the clinics.

Acknowledgements

The project is funded by a grant from the BMBF to A. T. and kindly supported by ScaleReady, a joint venture between Bio-Techne, Fresenius Kabi, and Wilson Wolf.

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