00321 Intrinsic Functional Potential of NK-cell Subsets Constrains Retargeting Driven by Chimeric Antigen Receptors

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Aims: Natural killer (NK) cells are potential source of allogeneic cytotoxic effectors for chimeric antigen receptor (CAR)-mediated therapies. We explored the feasibility of transfecting CAR-encoding mRNA into primary NK cells and investigated how the intrinsic potential of discrete NK-cell subsets affects retargeting efficiency.

Methodology: Mononuclear cells isolated from peripheral blood were stimulated in CellGro SCGM with 5% human serum and 20ng/ml IL-15 for various lengths of time. NK cells were magnetically isolated by negative selection, electroporated with 100ug/mL of various anti-CD19 CAR mRNA and incubated overnight. CAR expression was assessed by flow cytometry staining for human IgG antigen on CH_3 domain. Functional assessments were performed against various CD19+/- targets and analysed by flow cytometry.

Result: A third-generation, anti-CD19 CAR without CH_2-domain was selected after screening expression and functional profiles of five second-/third-generation constructs with different signalling-domains and spacer-regions. Kinetics experiments revealed optimal (over 80% expression consistently) CAR expression after 3 days of IL15 stimulation prior to transfection. CAR-engineered NK cells acquired increased degranulation toward CD19+ targets while maintaining their intrinsic response toward CD19- targets.

Retargeting response of NK-cell subsets was dependent upon their intrinsic thresholds for activation determined through both differentiation and education by killer cell immunoglobulin-like receptors (KIR) and/or CD94/NKG2A binding to self-HLA class I and HLA-E, respectively. Redirected NK cells were insensitive to inhibition through NKG2A/HLA-E interactions but remained sensitive to inhibition through KIR depending on the amount of HLA class I expressed on target cells. Adaptive (NKG2C+/CD57+/self-HLA-specific KIR+) NK cells, displayed superior ability to kill CD19+, HLA low, or mismatched tumour cells. These results have been replicated on a different anti-CD19 CAR construct.

Conclusion: These findings support the feasibility of primary allogeneic NK cells for CAR engineering and highlight a need to consider NK-cell diversity when optimizing efficacy of cancer immunotherapies based on CAR-expressing NK cells.