PD-L1 KNOCKOUT NATURAL KILLER CELLS AS A CELLULAR PRODUCT FOR THERAPEUTIC USE IN COMBINATION WITH HUMANIZED ADCC-COMPETENT ANTI-PD-L1

Background Immunotherapeutic strategies, such as checkpoint blockade of PD-1/PD-L1, have become a focal point of immunotherapy in oncology. Recent studies highlight the importance of Natural Killer (NK) cells in the success of these immunotherapies and adoptive NK cellular therapy is being explored to enhance response to these treatments. Antibodies targeting PD-L1 are mostly Fc silent but some, such as Avelumab, can engage FcγR (CD16) receptor on NK cells resulting in killing cancer cells via antibody-dependent cellular cytotoxicity (ADCC). PM21-particle expanded NK (PM21-NK) cells are an optimal NK cell product to consider for a combination strategy as these NK cells lack PD-L1 but can induce PD-L1 on tumors cells. Previous reports, however, have shown that PD-L1 can be induced on NK cells. This could potentially lead to fratricide of NK cells in the presence of Fc-competent, PD-L1 targeting antibodies and mitigate their cytotoxic response. This study determined if PD-L1 can be induced on PM21-NK cells and what effect PD-L1 engagement had on their activity and potential for fratricide in both WT and PD-L1 knockout PM21-NK cells.

Methods CRISPR-based Knockout (KO) of PD-L1 in PM21-NK cells was performed and efficiency was determined after overnight incubation of WT or PD-L1 KO PM21-NK cells with cytokines to induce PD-L1 expression. Cancer cells were incubated with NK cells in the presence of non-competent or Fc-competent α-PD-L1 and cytotoxicity was measured using a kinetic live-cell imaging assay. NK cell fratricide was measured in cultures of WT or PD-L1 KO PM21-NK cells induced for PD-L1 expression with non-competent or Fc-competent α-PD-L1.

Results PM21-NK cells were found to express low levels of PD-L1 (< 20%) after exposure to various cancer cell line monolayers or K562 co-culture. Exposure to SKOV-3 spheroids or to a cytokine combination of IL12, IL15, and IL18 led to a significant induction in PD-L1 in WT PM21-NK cells (> 80%). PD-L1 knockout prevented the induction of PD-L1 on NK cells after cytokine exposure and enhanced cytotoxicity. Fratricide was decreased and cytotoxicity increased in PD-L1 KO PM21-NK cells in combination with Fc-competent α-PD-L1.

Conclusions Knockout of PD-L1 in ex vivo expanded PM21-NK cells prevented the induction of PD-L1 on NK cells without negative effects on cytotoxicity. PD-L1 KO PM21-NK cell cytotoxicity was further enhanced in combination with Fc-competent α-PD-L1 compared to WT NK cells. PD-L1 knockout PM21-NK cells are a potential cell product for therapeutic use in combination with PD-L1 targeting antibodies.

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