Two-pronged Cell Therapy: Engineering NK cells to target CD22 and redirect bystander T cells to CD19 for the adoptive immunotherapy of B-cell malignancies

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Abstract

Introduction: T-cell therapy with CD19-CAR T cells has been very successful for the treatment of B-cell derived malignancies in humans. However, cytokine release syndrome, neurotoxicity, and development of CD19-escape variants have emerged as potential limitations. Developing CAR NK-cell based therapies might overcome some of these side effects since NK cells do not rapidly expand or persist long-term after adoptive transfer. However, CAR NK-cell therapies are less effective than CAR T-cell therapies in preclinical models. To overcome these limitations we have devised a strategy to genetically modify NK cells with CD22-CARs and CD19/CD3-bispecific T-cell engager (CD19-ENG) molecules. These NK cells should not only kill CD22+ B cells directly, but also redirect bystander T cells to malignant CD19+ B cells, enhancing antitumor effects and preventing immune escape. Methods: NK cells were generated using K562s expressing 41BBL and membrane bound IL15, and genetically modified with a retroviral vector encoding a CD22-CAR with a 41BB,ξ endodomain and/or a retroviral vector encoding CD19-ENG and mOrange separated by an IRES. To mimic immune escape, CD19 or CD22 knockout (ko) Ph+ leukemia cells (BV173) were generated by CRISPR/cas9. The effector function of genetically modified NK cells was evaluated in vitro and in xenograft models. Results: After transduction 70-80% of NK cells expressed CD22-CARs, and ~50% expressed CD22-CARs and CD19-ENGs as judged by FACS analysis. We performed coculture and cytotoxicity assays using non-transduced (NT), CD22-CAR, CD19-ENG, and CD22-CAR/CD19-ENG NK cells as effectors and BV173 (CD19+/CD22+), BV173.koCD19, BV173.koCD22, Daudi (CD19+/CD22+), and KG1a (CD19-,CD22-) as targets. Cocultures were preformed +/- T cells and after 24 hours IFNγ and IL2 was determined by ELISA. In the absence of T cells, CD22-CAR and CD22-CAR/CD19-ENG NK cells only recognized CD22+ targets as judged by IFNγ production. Moreover, CD22-CAR/CD19-ENG and CD19-ENG NK cells efficiently redirected T cells to secrete IFNγ in the presence of CD19+/CD22- targets. No NK-cell population produced IL2, however CD22-CAR/CD19-ENG and CD19-ENG NK cells induced IL2 production of T cells in the presence of CD19+ targets. No significant cytokine production was observed in the absence of antigen (media, KG1a). Specificity of generated NK cells was confirmed in cytotoxicity assays. In vivo, CD22-CAR/CD19-ENG NK cells recognized tumor cells in an antigen-dependent manner, redirected T cells to tumor cells, and induced significant regression of leukemia in xenograft models in comparison to mice treated with CD22-CAR NK cells or NK cells targeting irrelevant antigens. Conclusions: We have generated for the first time NK cells that kill B-cell malignancies through a CAR (CD22) and simultaneously redirect bystander T cells to a 2nd B-cell antigen (CD19) to enhance antitumor effects and prevent
immune escape. Genetic modification of NK cells to enhance their antitumor activity and redirect bystander T cells may represent a promising addition to current cell therapies for B-cell malignancies.

Disclosure of conflict of interest

None