Induced innate CD8+T cells (BKO, BCKO) engineered to express a new class of anti-GD2 chimeric antigen receptors for neuroblastoma immunotherapy

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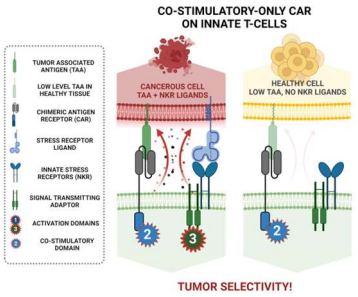
Background

- Innate effector cells are important for immunotherapy of neuroblastoma (NK cells; anti-GD2 antibodies)
- Cells generated by BCL11B knock out (**B-ko**) and in combination with CISH (**BC-ko**) have innate characteristics and are able to effectively kill chemotherapy-resistant neuroblastoma cells in vitro (1)
- CAR, only bearing a costimulatory domain (co-CAR), combined with innate stress receptors could provide higher specificity and reduce toxic side effects in vivo
- Here we evaluated the use of CAR constructs expressed by BC-ko cells, lacking the CD3 zeta stimulatory domain and determined the most effective costimulatory domain



Methods

- BCL11B or in combination with CISH was deleted in human CD8+ T cells using CRISPR/Cas9, followed by culturing with IL-7 and IL-15
- Innate receptors were detected by flow cytometry and function was determined using CD107a/IFN-γ assay
- Co-CAR constructs were generated using the GD2-specific variable domain of ch14.18 linked to CD28, OX40, 41BB, DAP10, DAP12 costimulatory domains
- A conventional 4th generation CAR with 41BB as costimulatory domain was used as a control
- Cytotoxicity assays were performed using GD2-positive neuroblastoma cells (LAN-1, CHLA-136), expressing near infrared protein (iRFP), for live-cell viability analysis (IncuCyte®)
- Selectivity towards tumor cells was shown by deletion of the B7H6 stress ligand in CHLA-136 cells using CRISPR/Cas9

Results

T cells express NK-specific stress receptors upon BCL11B deletion

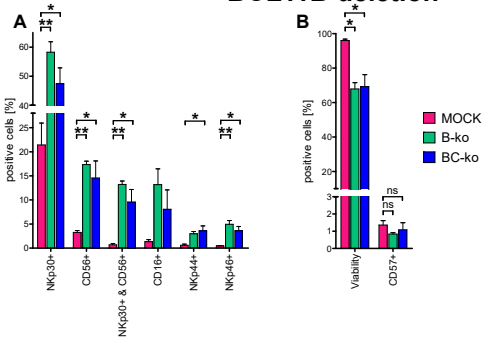


Fig1. Cell phenotype reprogramming after knock-out following two weeks of in-vitro expansion with IL-7 and IL-15 of each effector cell. (A) Expression of activating innate receptors and surface markers and (B) viability and exhaustion for each effector cell as percentage of positive cells. Mean ± SEM, n = 3. MOCK: mock gRNA transfected control, B-ko: BCL11B knock-out, BC-ko: combined BCL11B and CISH knock-out. Statistical significance was calculated by the One-way ANOVA with Tukey's multiple comparisons test correction. *P ≤ 0.05, **P ≤ 0.01.

DAP12 co-CAR shows highest costimulatory activity

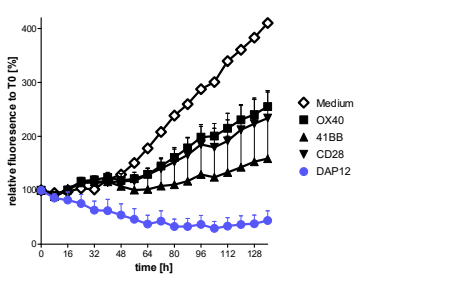
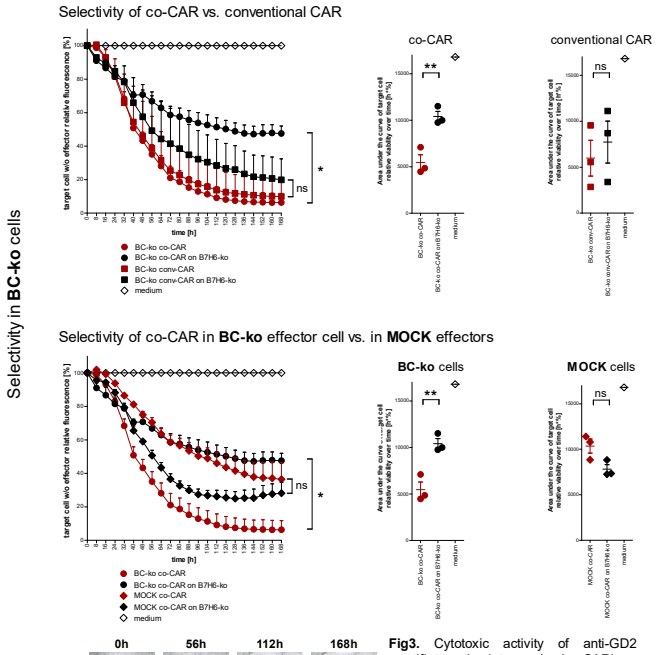


Fig2. Cytotoxic activity of anti-GD2 specific costimulatory-only CAR constructs containing either the OX40, 41BB, CD28 or DAP12 costimulatory domain expressed by BCL11B knock-out CD8+ T cells against CHLA-136 neuroblastoma tumor cell line. The left panel shows the viability of the tumor cell line over time, while the right panel shows the corresponding area under the curve from the left panel. Mean ± SEM, n = 3. Medium: CHLA-136 tumor cells without effector cells. Statistical significance was calculated by the One-way ANOVA with Dunnett's multiple comparisons test. *P ≤ 0.05.

BC-ko cells expressing co-CARs provide increased specificity against stress ligand positive neuroblastoma



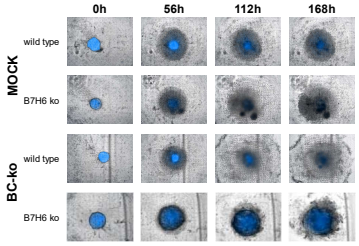


Fig4. Fluorescence microscopy images of CHLA-136 wild-type or B7H6 knock-out tumor spheroids from selected time points in co-culture with co-CAR bearing MOCK or BC-ko effector cells from one representative donor. wild type: wild type CHLA-136, B7H6-ko: CHLA-136 with B7H6 knock-out.

Fig3. Cytotoxic activity of anti-GD2 specific costimulatory-only (co-CAR) or conventional 4th generation CAR constructs expressed by MOCK or combined BCL11B and CISH knock-out CD8+ T cells against CHLA-136 wild type or B7H6 knock-out neuroblastoma tumor cell lines. The left panel shows the relative reduction in viability of the tumor cell lines by different effector cells versus the corresponding medium control of tumor cells without effector cells over time. The right panel shows the corresponding area under the curve from the left panel. Mean ± SEM, n = 3. B7H6-ko: CHLA-136 with B7H6 knock-out. Statistical significance was calculated for viability curves by the One-way ANOVA with Tukey's multiple comparisons test correction and for the AUC values by unpaired t test. *P ≤ 0.05, **P ≤ 0.01.

Conclusion

The BC-ko cells expressing DAP12-co-CAR promises superior specificity against GD2 and stress ligand positive tumor cells and thus, could reduce side effects in vivo.

(1.) Forkel H, Grabarczyk P, Depke M, Troschke-Meurer S, Simm S, Hammer E, et al. BCL11B depletion induces the development of highly cytotoxic innate T cells out of IL-15 stimulated peripheral blood alphabeta CD8+ T cells. Oncoimmunology 2022; 11:2148850.

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