Screening and identification of optimal costimulatory domains for iPSC derived CAR-NK cells targeting GPC3 expressing hepatocellular carcinoma

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Introduction & Methods

Introduction: Given the distinct expression profiles of costimulatory receptors in NK and T, cells we set out to identify optimal costimulatory domains for our iPSC-derived CAR (chimeric antigen receptor) NK cell platform for targeting GPC3-expressing Hepatocellular Carcinoma (HCC). To this end, we designed both 2nd and 3rd generation CAR constructs containing costimulatory domains that are expressed in NK cells or shared with T cells and expressed them in NK cells. We then evaluated their NK cell cytotoxic function against GPC3 expressing HCC tumors.

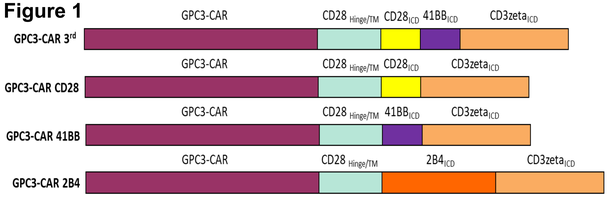
Methods:

The following 2nd and 3rd generation CAR constructs containing GPC3 scFV binder (YP7 mAb) and CD28 hinge and transmembrane domains were expressed in tandem with the following costimulatory domains: CD28+CD3 zeta (2nd generation), 41BB+CD3 zeta (2nd generation), 2B4+CD3 zeta (2nd generation) and CD28+41BB+CD3 zeta (3rd generation). The CAR constructs were expressed in either an EF-1a promoter containing mammalian expression vector for electroporation into NK-92 CD16-erIL2 cells or in a pAAV shuttle vector encoding donor DNA template and Rep Cap plasmids for AAV6 production and targeted knock-in by homology directed repair (HDR) at the Beta 2 microglobulin locus using sgRNA and Cas9 or in a retrovirus shuttle vector for production of retroviral particles containing BaEV envelope (Baboon envelope) proteins for transduction of PBNK cells. Transduced PBNK cells were expanded in K562 feeder cells expressing 41BB ligand and membrane-bound IL-21. PBNK and NK cells expressing CARs were phenotyped and sorted for CAR expression using soluble GPC3 PE and tested for cytotoxicity against HCC tumors (Hep3B tumors) by flow cytometry or in tumor spheroid killing assays using special U-bottom adhesive plates and in serial tumor killing assays using the Incucyte™ Live Cell Analysis System.

Results

Schematic of GPC3 CAR costimulatory domain constructs

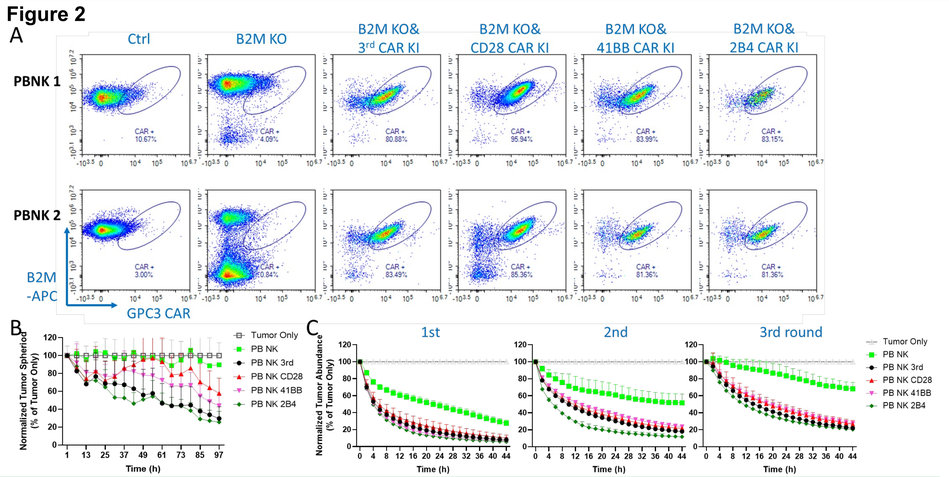
Figure 1



Results

Targeted knock-in of GPC3-CAR costim domain constructs show comparable CAR expression but different potencies for NK cell cytolysis of HCC tumors

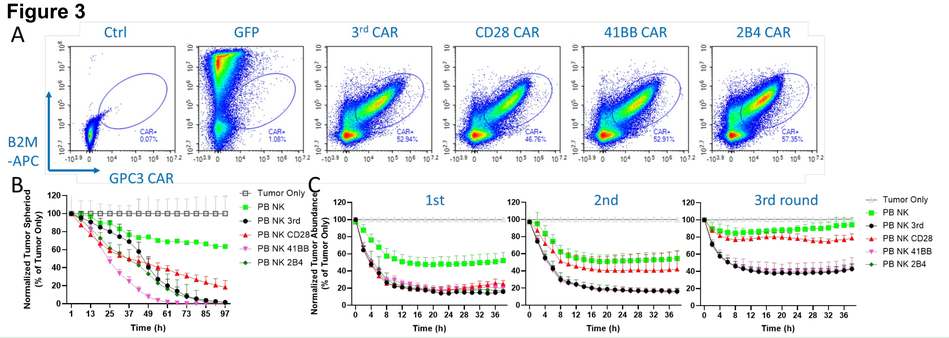
Figure 2



Phenotype of AAV6 transduced GPC3 CAR-NK cells (A), Tumor spheroid (B), and serial killing (C) of HCC tumors

Randomly integrated GPC3-CAR costim domain constructs show comparable CAR expression but different potencies for NK cell cytolysis of HCC tumors

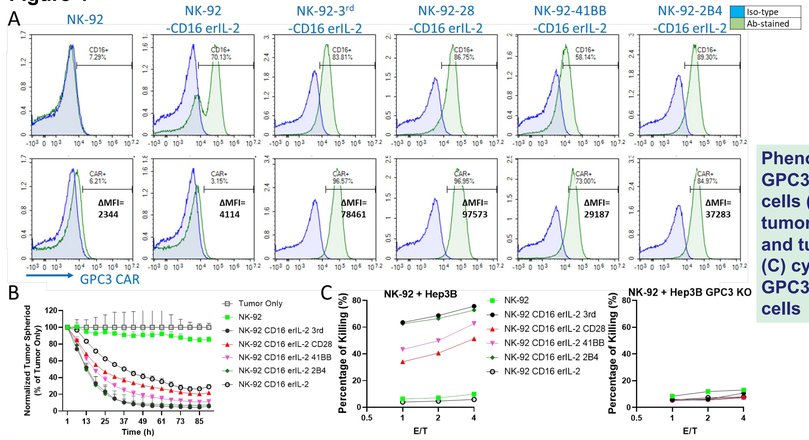
Figure 3



Phenotype of Retroviral transduced GPC3 CAR-NK cells (A), tumor spheroid (B), and serial killing (C) of HCC tumors

NK-92 stably expressed GPC3-CAR costim domain constructs show comparable CAR expression but different potencies for NK cell cytolysis of HCC tumors

Figure 4



Phenotype of GPC3 CAR NK-92 cells (A), HCC tumor spheroid (B) and tumor cell line (C) cytolysis by GPC3 CAR-NK92 cells

SUMMARY & CONCLUSIONS

- Using a targeted and non-disruptive HDR gene knock-in approach, we generated AAV6 transduced PBNK cells where CARs containing different costimulatory domains were knocked into the B2M locus. PBNK donors showed comparable GPC3 CAR and B2M expression (80-90%). In both HCC tumor spheroid and serial killing assays, the second generation 2B4 CAR showed the highest potency for HCC tumor lysis, while CD28 CAR showed the least activity.
- Although the transduction efficiency in retroviral transduced PBNKs was lower (45-50%), CAR expression was comparable among the different costimulatory domain constructs. In both HCC tumor spheroid and serial killing assays, the second generation 2B4 and 41BB and 3rd generation CARs in general showed the highest potency for HCC tumor lysis. CD28 2nd generation CAR showed the least activity.
- A similar trend for CAR costimulatory domain constructs was observed in NK-92 transduced cells for both CAR expression and HCC tumor lysis.
- Collectively, these results suggest that 2B4 or 41BB co-stimulatory domains expressed in NK cells are desirable for second generation CAR-NK cells. These results also imply the feasibility of creating a highly potent 3rd generation CAR-NK where the CD28 costimulatory domain could be replaced with 2B4.
- The above data enabled the selection of optimal CAR co-costimulatory domain constructs for iPSC derived gene edited CAR-NK cells against HCC tumors.

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