A novel synthetic stealth receptor that redirects host immune cell alloreactivity and potentiates functional persistence of adoptively transferred off-the-shelf cell-based cancer therapy

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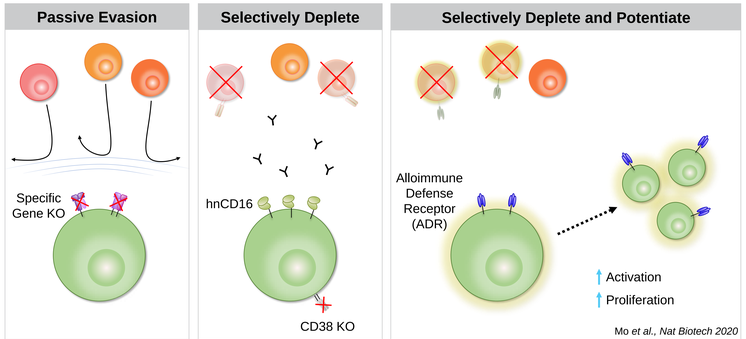
INTRODUCTION

Chimeric antigen receptor (CAR) T-cell therapies have revolutionized the treatment of hematologic malignancies and have shown significant potential in solid tumor indications. However, complexities associated with patient-specific CAR T-cell therapies often limit broad patient accessibility. Many of these challenges can be overcome with an allogeneic cellular product.

Both autologous and allogeneic cell therapies currently rely on lympho-conditioning of the patient, which induces a cytokine-rich environment for potentiation of adoptively-transferred cells and modulates the host immune system. Lympho-conditioning, however, has been associated with hematologic toxicities, including increased susceptibility to severe infections. Therefore, next-generation cell therapies should seek to significantly reduce the requirement for chemotherapy conditioning while maintaining the anti-tumor activity of adoptively-transferred cells.

To address these challenges, we developed a novel alloimmune defense receptor (ADR) that selectively targets host immune cells and significantly boosts the functional activity of adoptively-transferred cells. In preclinical studies, off-the-shelf iPSC-derived NK (CAR-iNK) cells armed with a novel ADR exhibited functional persistence and maintained anti-tumor activity in an immuno-competent host system.

STRATEGIES TO REDUCE LYMPHO-CONDITIONING



Mo *et al.*, Engineered off-the-shelf therapeutic T cells resist host immune rejection. Nature Biotechnology 2020
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CONCLUSIONS

Our preclinical work provides a framework to eliminate the need for patient lympho-conditioning in the field of cell-based cancer immunotherapy by arming adoptively-transferred cells with a novel alloimmune defense receptor (ADR) targeting 4-1BB expressed on host NK and T cells.

- 4-1BB is upregulated on activated effector cells, including allo-reactive NK and T cells.
- ADR+ CAR-iNK cells uniquely demonstrate high levels of functional persistence *in vitro* in the presence of allo-reactive lymphocytes.
- Unlike ADR- controls, anti-tumor efficacy of ADR+ CAR-iNK cells is uncompromised *in vivo* in the presence of a competent allo-reactive immune system.

These data support the development of off-the-shelf, iPSC-derived CAR NK cell therapies which, when armed with a novel ADR targeting 4-1BB, have the potential to maintain potent anti-tumor activity without requiring chemotherapy conditioning.

RESULTS

Off-the-shelf, iPSC-derived CAR NK cells armed with a novel synthetic alloimmune defense receptor (ADR) targeting 4-1BB expressed on allo-reactive immune cells

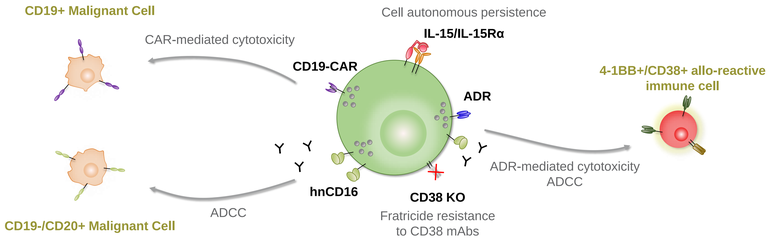

Figure 1 iNK cells are engineered to express: (1) high-affinity, non-cleavable CD16 (hnCD16) to augment antibody dependent cellular cytotoxicity (ADCC); (2) an IL-15/IL-15Rα fusion to enhance NK functionality and persistence; (3) CD38 knockout to eliminate anti-CD38 monoclonal antibody-induced fratricide; (4) CD19-CAR to selectively target CD19 antigen expressed on B-cell lymphomas; (5) ADR to selectively target 4-1BB expressed on allo-reactive NK and T cells.

ADR-armed CAR-iNK cells demonstrate functional persistence *in vitro* in the presence of allo-reactive T and NK cells

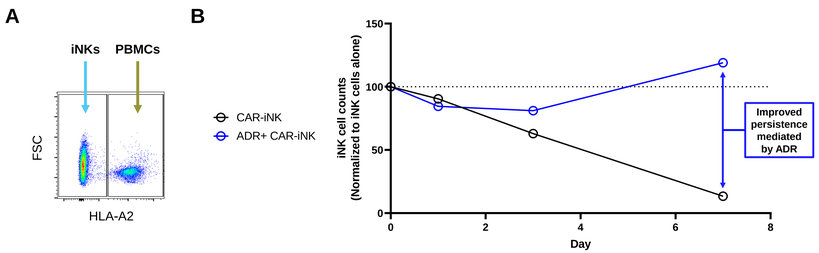

Figure 2 ADR+ CAR-iNK cells demonstrate enhanced functional persistence in mixed lymphocyte reactions (MLR). (A) Representative FACS plot depicting gating schematic to quantify iNK cells against HLA-A2$^+$ PBMCs. (B) CAR-iNK ± ADR cells were co-cultured at a 4:1 iNK cell to PBMC ratio. iNK cell counts from the co-cultures were plotted at the indicated timepoints. Data are normalized to iNK cells cultured without PBMCs.

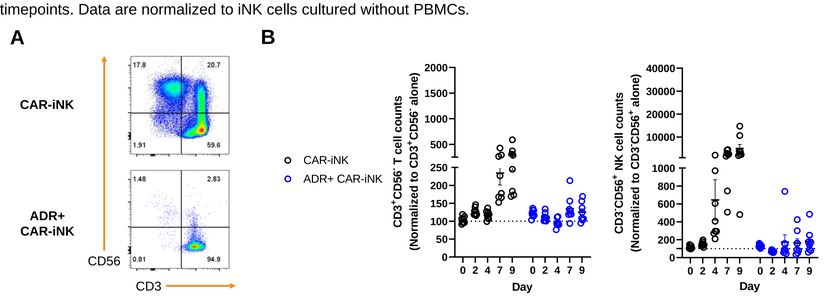

Figure 3 ADR+ CAR-iNK cells stifle expansion of allo-reactive T and NK cells. (A) Representative FACS plots depicting gating schematic of CD3$^+$ T and CD56$^+$ NK cells after nine days of co-culture with iNK cells. (B) Quantification of T- and NK cell counts across eight donors co-cultured with CAR-iNK ± ADR cells at a 2:1 iNK cell to PBMC ratio. Data are normalized to T- and NK cell counts from cultures of eight donor PBMCs maintained in the absence of iNK cells.

ADR-armed CAR-iNK cells selectively target and deplete allo-reactive CD8$^+$ and CD4$^+$ T-cell compartments *in vitro*

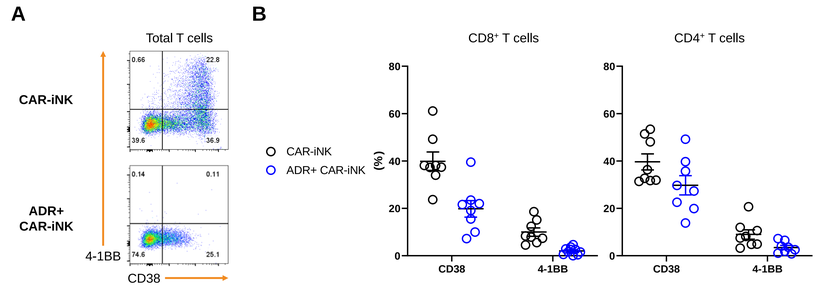

Figure 4 ADR+ CAR-iNK cells selectively target both CD4$^+$ and CD8$^+$ allo-reactive T-cell subsets. (A) Representative FACS plots of T cells after nine days of co-culture with iNK cells and analyzed for expression of CD38 and 4-1BB among CD3$^+$ T cells as depicted in Figure 2. (B) Compilation of %CD38 and %4-1BB expression among CD4$^+$ and CD8$^+$ T cells from the eight donors in CAR-iNK ± ADR co-cultures.

ADR-armed CAR-iNK cells exhibit uncompromised tumor control *in vivo* in the presence of host allo-reactive T-cell system

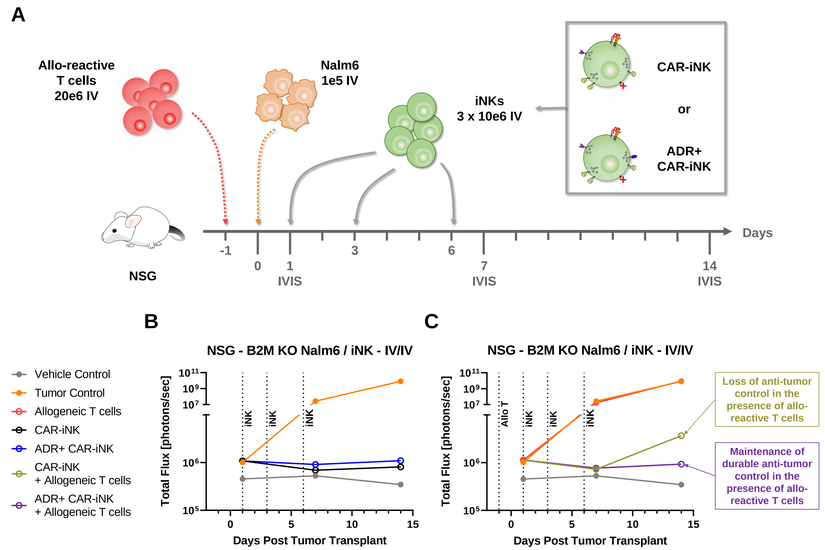

Figure 5 *In vivo* schematics. (A) NSG mice were injected with iNK cells, luciferized B2M KO Nalm6 and allo-reactive T cells. Allo-reactive T cells were previously expanded *in vitro* for 10 days with 100IU/ml IL-2 and Dynabeads^{TM} Human T-Activator CD3/CD28 as indicated by manufacturer protocol. (B) Bioluminescence-based tumor quantification by total flux was measured to monitor tumor growth in mice. (C) Total flux analysis of mice injected with allo-reactive T cells.