Alloimmune Defense Receptor Redirects Host Immune Cell Alloreactivity to Potentiate Functional Persistence and Anti-Tumor Activity of Off-the-Shelf Cell-Based Cancer Therapy

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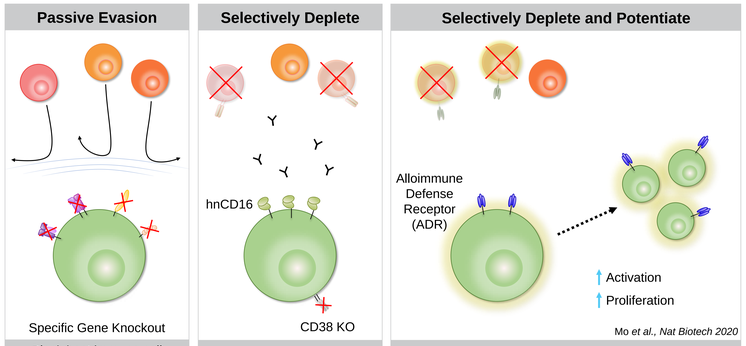
INTRODUCTION

Chimeric antigen receptor (CAR) T-cell therapies hold great promise as a potentially curative therapeutic option for many cancer types. However, complexities associated with patient-specific CAR T-cell therapies often limit broad accessibility. Many of these challenges can be overcome with an allogeneic cellular product, but the perceived lack of functional persistence and the potential for immune cell-mediated rejection in an allogeneic setting remain significant concerns. Both allogeneic and autologous cell therapies currently rely on lymphodepleting conditioning to modulate the immune system and create greater access to homeostatic cytokines. However, protracted lympho-conditioning has been associated with poor immune reconstitution and increased susceptibility to opportunistic infections. Therefore, an ideal allogeneic cell therapy would be able to maintain functional persistence and anti-tumor activity while reducing or eliminating the need for chemotherapeutic conditioning to deplete host lymphocytes.

To address many of these challenges, we combined our alloimmune defense receptor (ADR) that targets 4-1BB$^+$ alloreactive immune cells while providing a CD3ζ signaling boost with our anti-CD19 CAR (CAR19) that has been fine-tuned for NK cell biology and effectively targets B cell malignancies. These two unique modalities were then engineered into an iPSC master cell line to serve as a renewable starting material for the derivation of iPSC-derived NK (iNK) cells, uniformly expressing ADR and CAR19.

STRATEGIES TO REDUCE LYMPHO-CONDITIONING



Mo *et al.*, Engineered off-the-shelf therapeutic T cells resist host immune rejection. Nature Biotechnology 2020
doi: 10.1038/s41587-020-0601-5

CONCLUSIONS

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

- ADR potentiates CAR19 iNK cells, increases activity and extends functional persistence by selectively targeting 4-1BB-positive alloreactive immune cells.
- ADR-armed CAR19 iNK cells can also utilize ADCC to further enhance anti-tumor efficacy during alloreactive T cell responses *in vivo*.
- Uniquely, ADR-armed CAR19 iNK cells control tumor growth in an *in vitro* cytotoxicity assay with rigorous daily dosed tumor cell challenge and in an aggressive disseminated *in vivo* xenograft model of leukemia, both in the presence of alloreactive T cells.

Collectively, this dataset support the development of off-the-shelf, iPSC-derived CAR 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

Multiplexed-engineered iPSC-derived CAR NK cells have a uniform profile of five edits and are armed with a novel synthetic alloimmune defense receptor (ADR) targeting 4-1BB expressed on alloreactive immune cells

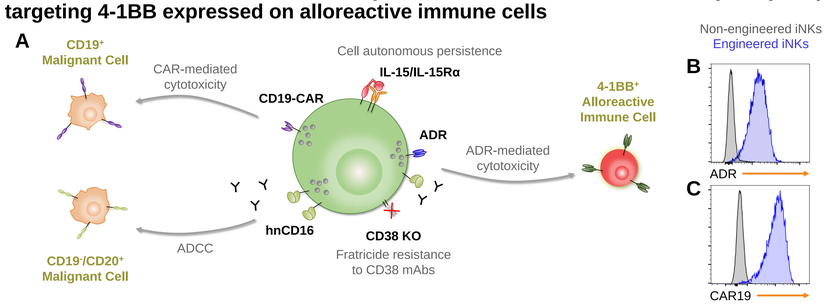


Figure 1 ***Combinatorial edits for iPSC-derived NK cells.*** (A) iNK cells are engineered to express: (1) high-affinity, non-cleavable CD16 (hnCD16) to augment antibody dependent cellular cytotoxicity (ADCC); (2) an IL-15 receptor fusion to enhance NK functionality and persistence; (3) CD38 knockout to prevent anti-CD38 mAb-induced fratricide; (4) CD19-CAR (CAR19) to selectively target CD19 antigen expressed on B-cell lymphomas; and (5) ADR to selectively target 4-1BB expressed on alloreactive cells. (B) Representative FACS plots examining engineering of (B) ADR and (C) CAR19 on iNK cells.

ADR potentiates iPSC-derived NK cells by extending functional persistence and proliferation through selective targeting of 4-1BB-positive alloreactive immune cells

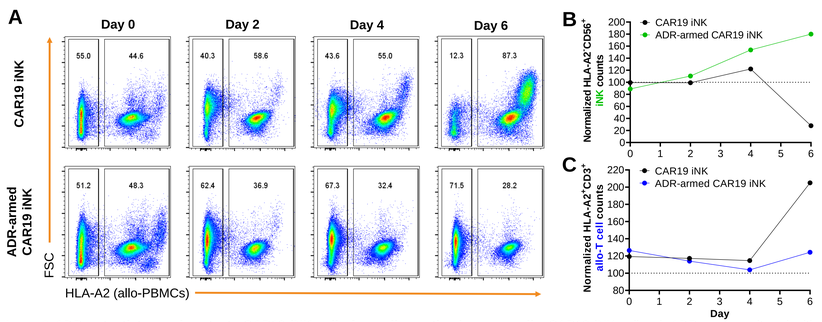


Figure 2 ***ADR potentiates and protects CAR19 iNK cells from alloreactive immune cells***. CAR19 iNK cells +/- ADR are co-cultured with allogeneic PBMCs (allo-PBMCs) at a 1:1 ratio in a mixed lymphocyte reaction (MLR). (A) Representative FACS plots depicting gating schematic to quantify CAR19 iNK cells +/- ADR and HLA-A2$^+$ allo-PBMCs during 6 days of co-culture. (B) CD56$^+$ iNK cell counts are normalized to alone wells. (C) CD3$^+$ allogeneic T (allo-T) cell counts from co-cultures with iNK cells are normalized to alone wells.

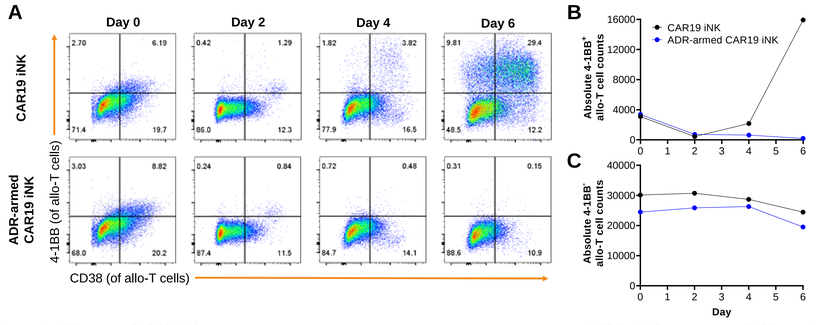


Figure 3 ***ADR-armed CAR19 iNK cells selectively deplete alloreactive immune cells expressing 4-1BB.*** (A) Representative FACS plot of CD3$^+$ allo-T cells examining expression of CD38 and 4-1BB during 6 days of co-culture with CAR19 iNK cells +/- ADR. (B-C) Absolute cell counts of CD3$^+$ allo-T cells that either (B) express 4-1BB or (C) do not express 4-1BB.

ADR-armed CAR19 iNK cells have uncompromised anti-tumor efficacy in both *in vitro* and *in vivo* Nalm6 leukemia models

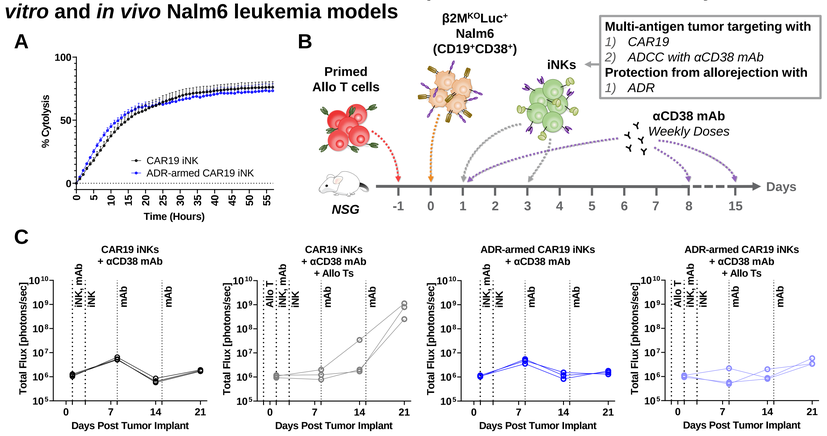


Figure 4 ***ADR-armed CAR19 iNK cells have uncompromised anti-tumor efficacy in an in vitro cytotoxicity assay and an in vivo disseminated Nalm6 leukemia model in alloreactive settings.*** MHC-I (β2M)-deficient Luc$^+$ Nalm6 target cells were utilized due to their expression of CD19, CD38, and their ability to evade alloreactive T cell attack. (A) Killing assay with β2M-deficient Nalm6 target cells on the xCELLigence platform. (B) *In vivo* schematic of disseminated xenograft model of leukemia consisting of β2M-deficient Nalm6 cells, primed alloreactive T cells (Allo Ts), weekly injections of an anti-CD38 monoclonal antibody (αCD38 mAb), and CAR19 iNK cells +/- ADR with three biological replicates per group. (C) Bioluminescence-based tumor quantification by total flux was measured to monitor tumor growth in mice.

ADR protects CAR iNK cells from primed alloreactive immune cells to extend persistence with uncompromised effector function in the presence of daily tumor challenge

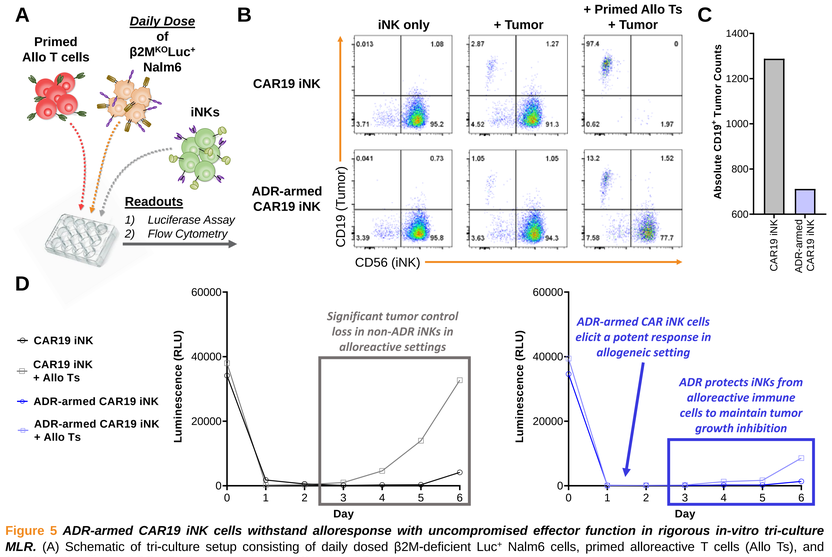


Figure 5 ***ADR-armed CAR19 iNK cells withstand alloresponse with uncompromised effector function in rigorous in-vitro tri-culture MLR.*** (A) Schematic of tri-culture setup consisting of daily dosed β2M-deficient Luc$^+$ Nalm6 cells, primed alloreactive T cells (Allo Ts), and CAR19 iNK cells +/- ADR. (B) Representative FACS plots of Day 6 cell cultures maintained under various conditions including a tri-culture of CAR19 iNK cells +/- ADR, β2M-deficient Luc$^+$ Nalm6 tumor, and Allo Ts. (C) Absolute CD19$^+$ Tumor Counts in tri-cultures on Day 6. (D) Luciferase assay examining β2M-deficient Luc$^+$ Nalm6 tumor growth in tri-cultures maintained with CAR19 iNK cells +/- ADR and Allo Ts.