Novel Combination of iPSC-derived Dual-CAR NK Cells with CD16 Fc Receptor for Multi-antigen Targeting of Multiple Myeloma to Overcome Clonal Resistance and Antigen Escape

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Dual CAR Targeting

Resistance to targeted cell therapy can arise from antigen loss and clonal heterogeneity within the tumor. A dual CAR approach targeting two tumor associated antigens (TAA) was tested in primary CAR-T cells and iPSC-derived NK cells to demonstrate an effective combination targeting B cell maturation antigen (BCMA) and major histocompatibility complex class I chain-related A/B (MICA/B) in multiple myeloma (MM).

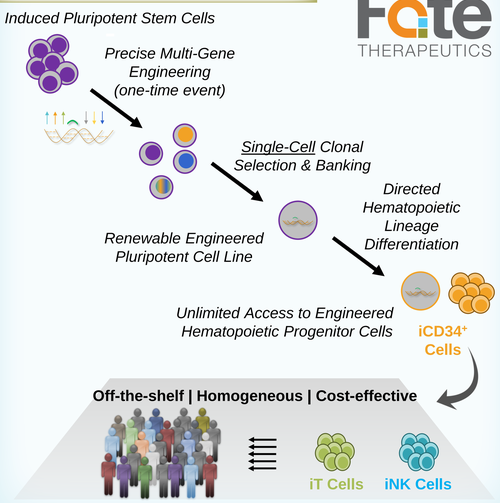
BCMA CAR

BCMA is a highly expressed TAA in MM. To develop the BCMA CAR motif, we utilized our previously published high-affinity binding sequence that was shown to exhibit high selectivity of BCMA+ targets and enhanced recognition of low-BCMA expressing myeloma cells (Bluhm *et al.*, Molec Ther 2018).

MICA CAR

MICA and MICB are pan-TAAs expressed on MM plasma cells. Our MICA/B CAR binding sequence targets the conserved α3 domain of MICA/MICB, which we have previously shown to inhibit MICA/B shedding and drive anti-tumor immunity (Andrade *et al.*, Science 2018).

Off-The-Shelf Application



Conclusions

- iPSCs were uniformly engineered with several anti-cancer modalities including non-cleavable CD16, an IL15 receptor fusion, CD38 knockout and two unique CARs, anti-BCMA and anti-MICA/B-α3, for enhanced multi-antigen targeting and prevention of antigen escape.
- Co-expression of BCMA and MICA/B CARs in primary T cells enhanced avidity to target cells relative to single CAR controls, and enabled killing of MM target cells.
- Dual CAR-iNK cells:
  - exhibit anti-tumor responses *in vitro* against engineered and heterogenous MM lines
  - retain potency against multiple myeloma in the presence of soluble BCMA
  - display superior control of a heterogeneous, aggressive tumor model *in vivo* over single-antigen targeting
- These data highlight the applicability of a multi-targeted approach in MM patients, whereby dual CAR-iNK cells maintain responsiveness to malignant cells that shed or downregulate individual tumor antigens.

Results

Multiple myeloma tumor lines express BCMA and MICA/B

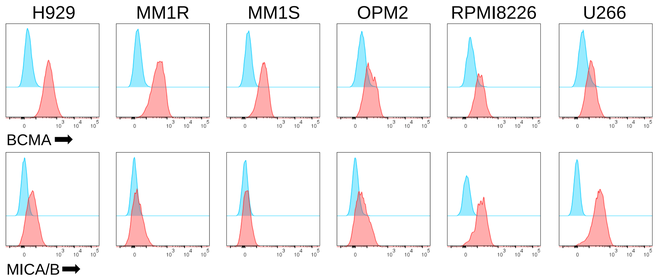


Figure 1. Multiple myeloma lines were screened for BCMA and MICA/B expression by flow cytometry. Variable expression of each antigen was observed across tumor lines, and all lines were positive for at least one antigen.

Dual CAR Expression Increases Cell Contact Through Antigen Avidity

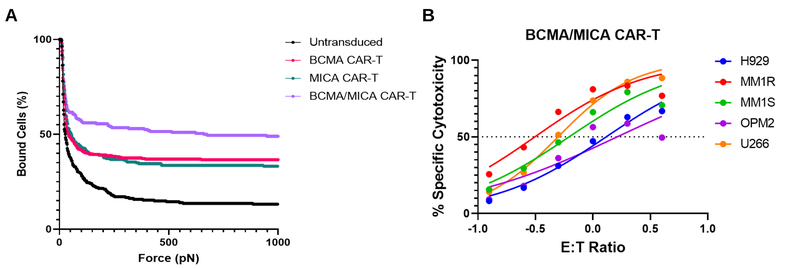


Figure 2. Dual CAR-T cells show greater avidity relative to single CAR-T cells and kill multiple myeloma cancer lines. (A) Nalm6-BCMA-MICA cells were incubated with the indicated CAR-T cells and exposed to an acoustic force ramp ranging from 0 to 1000 pN using the Lumicks z-Movi. The percentage of bound cells over this ramp is shown. **(B)** Dual CAR-T cells were co-cultured with MM lines for 4 hours before being stained for caspase 3/7 and analyzed by flow cytometry.

Characterization of BCMA and MICA/B Dual CAR-iNK cells

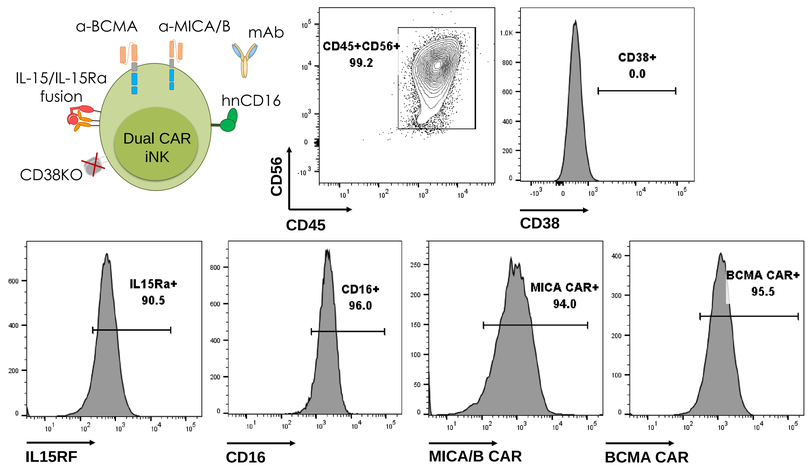


Figure 3. Sequential engineering of iPSC-derived NK (iNK) cells with BCMA and MICA/B CARs. iPSCs lacking CD38 and containing BCMA CAR, an IL15 receptor fusion (IL15RF) and a non-cleavable Fc receptor (hnCD16) were differentiated to mature CD45+CD56+ NK cells. A second round of engineering and differentiation was performed to introduce a MICA/B CAR to create dual CAR-iNK cells.

Dual Expression of BCMA & MICA/B Specific CARs Increases Breadth of Killing by CAR-iNK cells

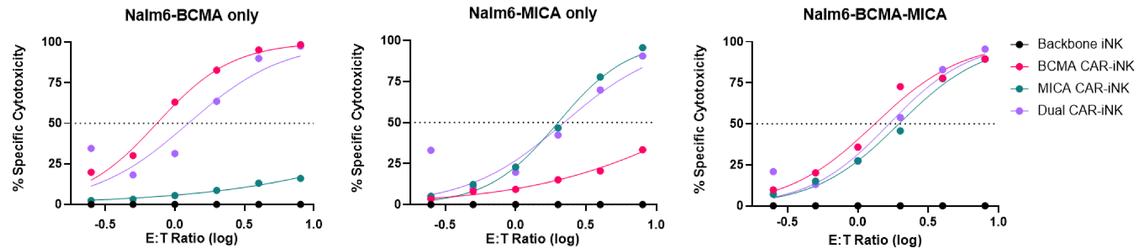


Figure 4. Dual CAR-iNK cells exhibit broader cytotoxicity over single CAR-iNKs. Nalm6-BCMA, Nalm6-MICA or Nalm6-BCMA-MICA target cells expressing luciferase were incubated with the indicated iNKs at effector:target ratios from 0.25:1 to 8:1. Luciferase activity of co-cultures was evaluated 6 hours later.

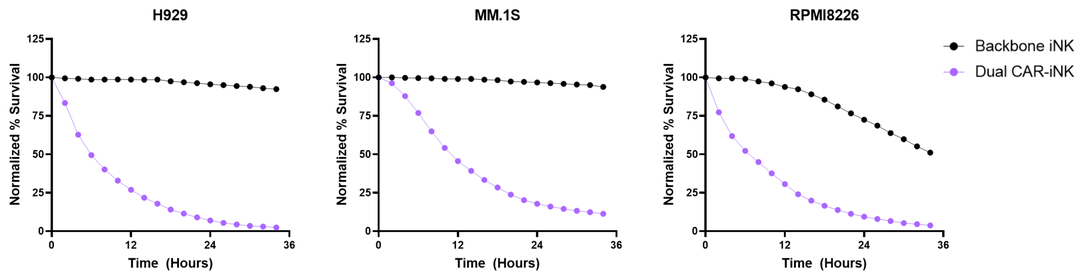
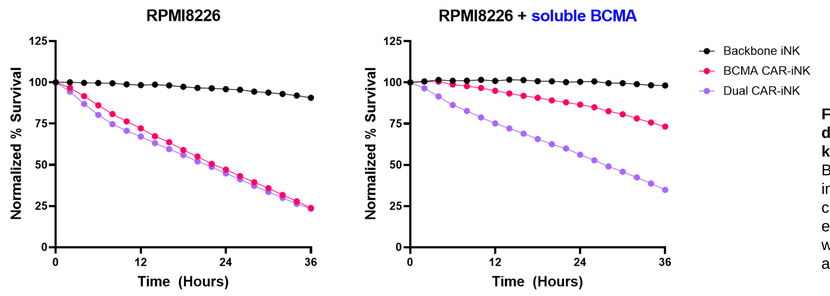


Figure 5. Dual CAR-iNKs recognize and kill multiple myeloma cancer lines. Labelled H929, MM.1S and RPMI8226 targets cells were cultured with backbone or dual CAR-iNKs using an effector:target ratio of 3:1 and monitored for cell killing over 36 hours using an Incucyte.



Dual CAR-iNKs Resist Competition by Soluble BCMA

Figure 6. Single-target recognition by dual CAR-iNK cells is sufficient for killing RPMI8226 cells. Backbone, BCMA CAR- and dual CAR-iNKs were incubated with soluble BCMA and co-cultured with RPMI8226 cells using an effector:target ratio of 1:1. Cell killing was monitored for over 36 hours using an Incucyte.

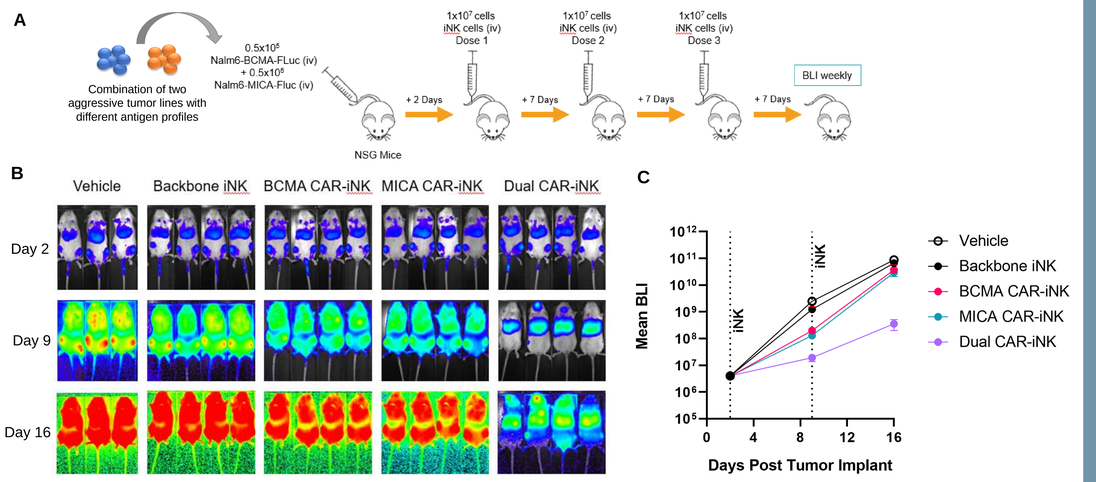


Figure 7. Dual CAR-iNKs show superior control of heterogeneous, ultra-aggressive tumors *in vivo* compared to single-antigen targeting. (A) NSG mice were injected intravenously with a 1:1 mixture of luciferized Nalm6-BCMA and Nalm6-MICA cells. Mice were then dosed with the indicated iNKs without cytokine support. **(B)** Individual IVIS images. **(C)** Mean of total luminescence is shown over 16 days.