Regulated Natural Killer Cell Expansion and Anti-Tumor Activity with Inducible MyD88/CD40

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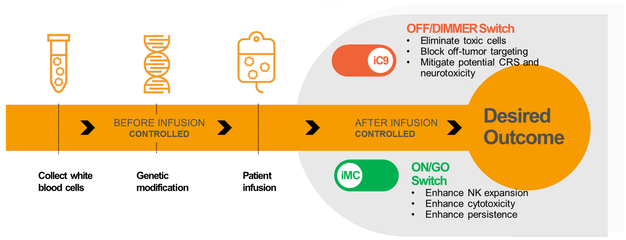
Background

Natural Killer (NK) lymphocytes possess innate anti-tumor activity that has the potential to be used as an allogeneic cell therapy due to reduced GvHD risk relative to αβ T cells. Despite their potential, adoptive NK cell immunotherapies have been limited by poor expansion *in vivo* and potency loss following cryogenic storage.

Using our previously developed Chimeric Antigen Receptor-T cell (CAR-T) strategy that relies on rimiducid-based dimerization of inducible MyD88/CD40 (iMC) to regulate T cell expansion and survival, we demonstrate that iMC can also be applied to NK cell growth and anti-tumor efficacy *in vitro* and *in vivo*. Moreover, a rapamycin-inducible Caspase-9 (iRC9) was used to provide an orthogonally regulated safety switch.

Technology



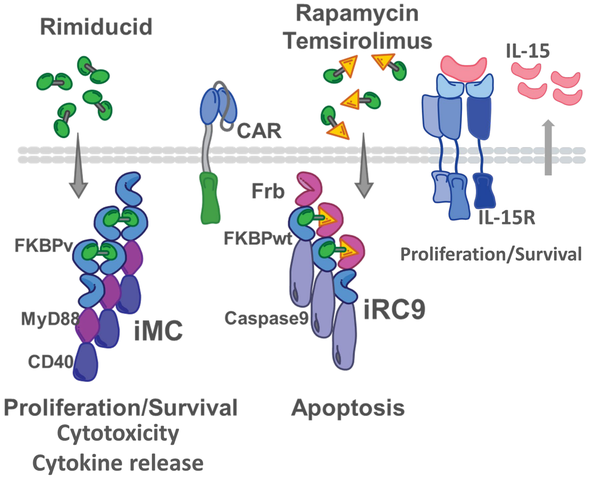
ACTIVATION SWITCH

"On demand" stimulation via drug administration enhances NK cell proliferation, activation, and anti-tumor activity.

Paired with autocrine IL-15 expression, this platform drives NK expansion and efficacy *in vivo*.

APOPTOTIC SWITCH

Rapid and efficient clearance of NK cells follows administration of dimerizing drug rapamycin or the rapalog temsirolimus (Rap or Tem).



- The allele specificity of Rim to the $FKBP_V$ mutant, and not to wild-type FKBP, prevents cross-talk between the ON and OFF switches.
- While Rap and Tem are mechanistically heterodimerizing agents, the fusion of FRB and FKBP directs homodimerization of Caspase-9

Conclusion

Inducible MyD88/CD40 is an activation switch that supports NK cell expansion, persistence and anti-tumor activity. When paired with autocrine IL-15 expression, this platform supports NK expansion and persistence *in vivo*, as well as AML tumoricidal activity that can be further activated by target-specific CAR expression. Moreover, the fast-acting, orthogonally regulated proapoptotic switch, iRC9, mitigates the risk of off-tumor targeting. Therefore, we describe a novel, regulated NK cell platform that solves many of the challenges of NK cell-based therapy and should be amenable to a readily translatable off-the-shelf cellular therapy for malignancies.

Results

Rim-directed iMC activation enhances NK cytotoxicity

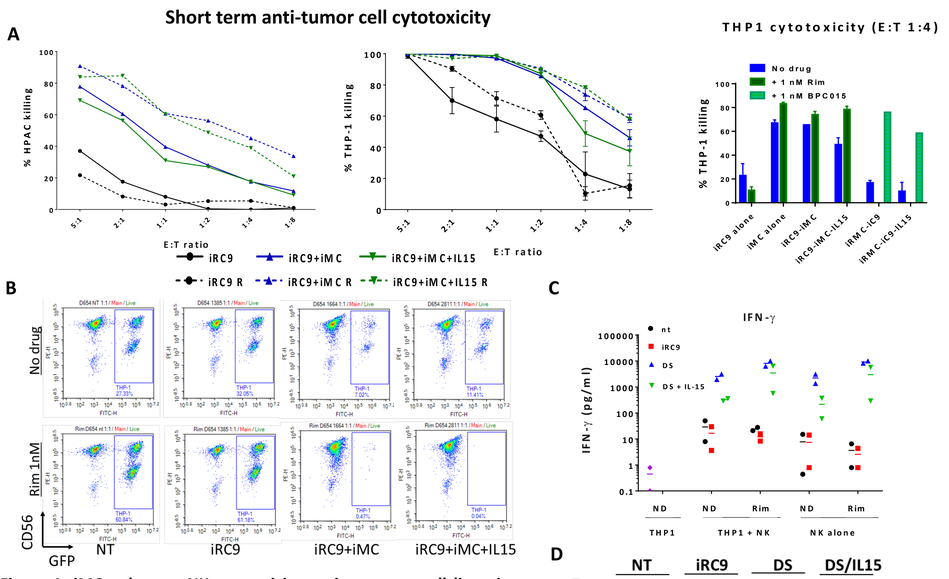
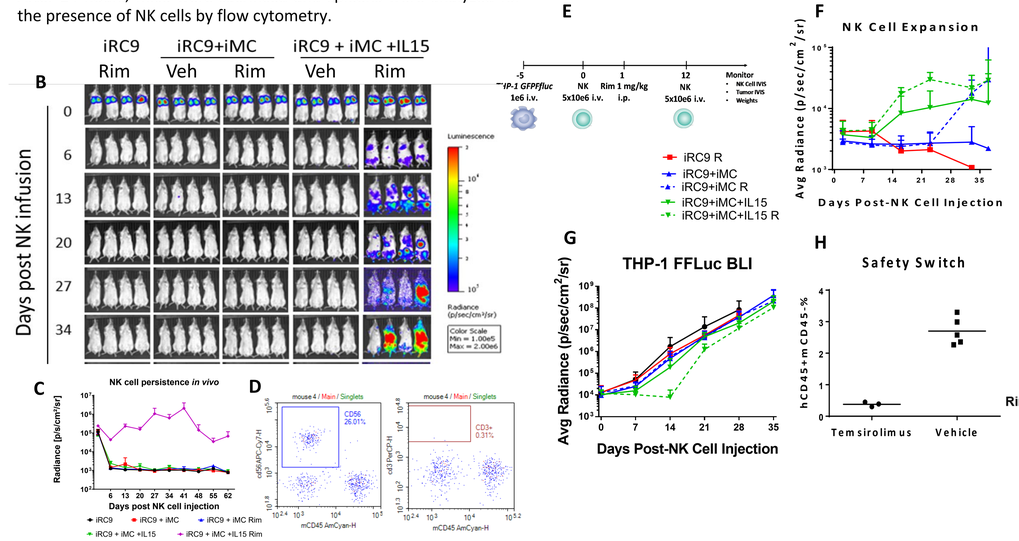
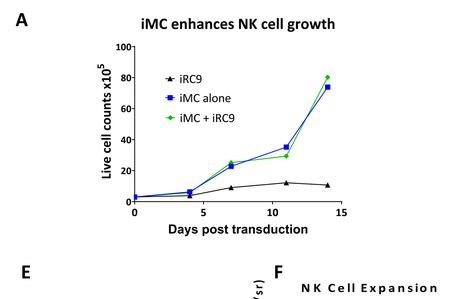


Figure 1. iMC enhances NK cytotoxicity against tumor cell lines *in vitro*. Activated NK cells (3 donors) were transduced with γ-RV encoding the indicated iMC ± the iRC9. (**A**) 10 days after transduction, NKs were co-cultured with HPAC-eGFPFluc or THP-1-eGFPFluc, tumor cell lines at different E:T ratios in the presence of 0 or 1nM Rimiducid (R) for 1 day. Short term NK cytotoxicity was accessed by loss of luciferase activity. (right) Basal MC activity was reduced with a rapalog inducible MC platform, but cytotoxicity was enhanced by activation with the non-immunosuppressive rapalog BPC015. (**B**) GFP-labeled THP1 cells were cocultured with transduced NK cells derived from three donors for 72 hours. Live THP1 cells were determined by flow cytometry. Donor 654 is represented. (**C, D**) Supernatants of NK cultures with/without THP-1 were analyzed by ELISA (C) or for a 29-plex of cytokines and chemokines (D) expressed as base 10 log relative to NT controls. n=2.

Activation of iMC enhances intrinsic NK cell growth

Figure 2. NK growth and persistence with iMC. (A) NK transduced with iRC9 alone, iMC alone, or iMC + iRC9 were cultured in SCGM NK medium supplemented with IL-2 for 2 weeks. Donor 080 is representative of three donors. (**B,C**) NSG mice were injected i.v. with $1x10^7$ NKs transduced with iRC9, iRC9+iMC, or iRC9+iMC+IL15. NKs were also cotransduced with GFPFfluc. Rim (1 mg/kg) or vehicle control were administrated i.p. weekly. BLI was assessed to monitor NKs persistence in vivo. (**D**) Human NK (left) and T cells (right) were identified from peripheral blood of mouse 5-4. **(D) iMC enhances NK anti-tumor efficacy *in vivo*.** NSG mice were injected i.v. with $5x10^6$ NKs transduced with RV encoding iRC9 ± iMC and oNL.R*luc* , 5 and 12 days following i.v. implantation of 10^6 THP-1.GFP*luc* tumor cells. Rimiducid or vehicle was administered i.p. weekly. **(F)** NKs expansion and persistence (ONLRluc) and **(G)** tumor cells growth (D-luciferin substrate) were monitored by IVIS. **(H)** Activation of the proapoptotic safety switch. At day 37, all 8 mice with NK cell Rluc signal were pooled and temsirolimus (1 mg/kg) was administered to one group. After 24 hours, mice were sacrificed and spleens were analyzed for the presence of NK cells by flow cytometry.





Dual-switch CAR-NK cells have potent inducible anti-tumor efficacy

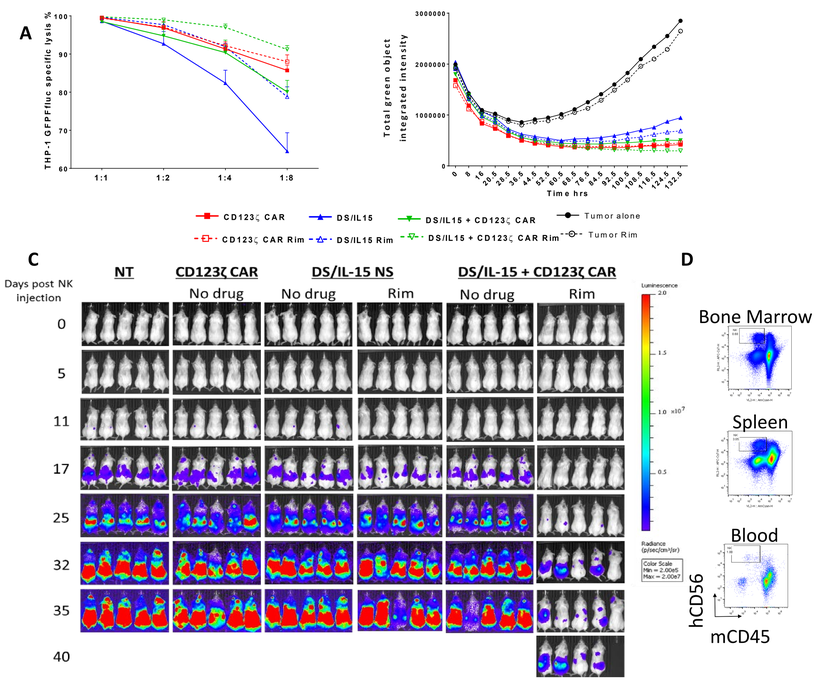
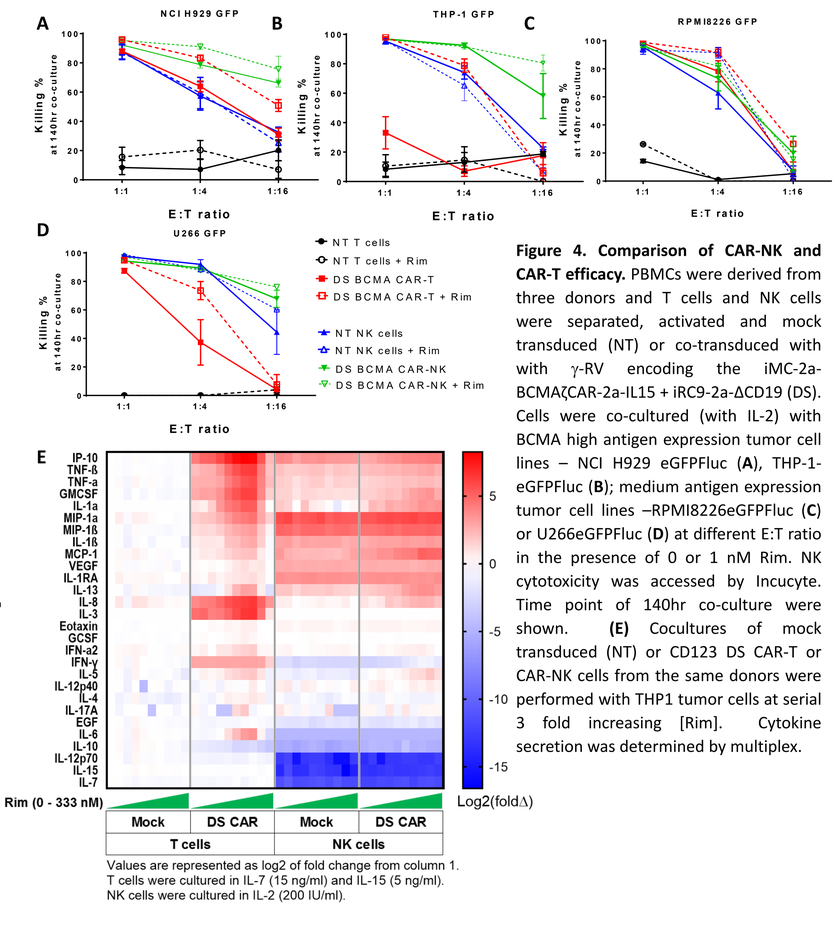


Figure 3. CAR-directed NK anti-tumor efficacy. (A) Activated NK cells were transduced with γ-RV encoding the indicated CD123ζ CAR, dual switch (DS (iMC + iRC9 + IL15)), or DS (iMC + iRC9)/IL15 + CD123ζ CAR. NKs were co-cultured with THP-1-eGFPFluc at different E T ratio in the presence of 0 or 1 nM Rimiducid and luciferase activity assessed at 24 hours. (**B**) Long term THP1-eGFPFluc growth control NK cytotoxicity was measured in an Incucyte. **(C)** NSG mice were engrafted i.v. with 10^7 NKs transduced with RV encoding CD123ζ CAR, DS (iMC + iRC9)/IL15, or DS (iMC + iRC9)/IL15 + CD123ζ CAR; 3days following i.v. implantation of 10^6 THP-1.GFP*luc* tumor cells. Rimiducid or vehicle was administered i.p. weekly. BLI was monitored by IVIS. **(D)** At day 53, DS (iMC + iRC9)/IL15 + CD123ζ CAR with rimiducid group was euthanized. Human NK cells were identified in peripheral blood, spleen, and bone marrow at day 53 of Rim treated DS CAR-NK cells.

Comparison of CAR-NK vs CAR-T



Values are represented as log2 of fold change from column 1. T cells were cultured in IL-7 (15 ng/ml) and IL-15 (5 ng/ml). NK cells were cultured in IL-2 (200 IU/ml).

Figure 4. Comparison of CAR-NK and CAR-T efficacy. PBMCs were derived from three donors and T cells and NK cells were separated, activated and mock transduced (NT) or co-transduced with with γ-RV encoding the iMC-2a-BCMAζCAR-2a-IL15 + iRC9-2a-ΔCD19 (DS). Cells were co-cultured (with IL-2) with BCMA high antigen expression tumor cell lines – NCI H929 eGFPFluc (**A**), THP-1-eGFPFluc (**B**); medium antigen expression tumor cell lines –RPMI8226eGFPFluc (**C**) or U266eGFPFluc (**D**) at different E:T ratio in the presence of 0 or 1 nM Rim. NK cytotoxicity was accessed by Incucyte. Time point of 140hr co-culture were shown. **(E)** Cocultures of mock transduced (NT) or CD123 DS CAR-T or CAR-NK cells from the same donors were performed with THP1 tumor cells at serial 3 fold increasing [Rim]. Cytokine secretion was determined by multiplex.