Solid tumor cytotoxicity by natural killer cells expressing a HER2-directed chimeric antigen receptor enhanced by MyD88/CD40 (MC)

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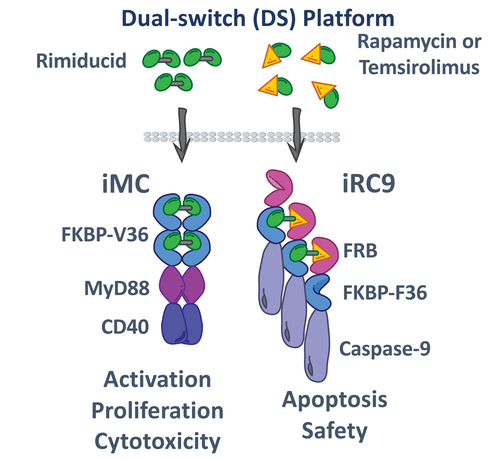
Background

The potent, innate anti-tumor cytotoxicity of natural killer (NK) cells combined with their low risk of inducing graft-versus-host disease have made NK cells an emerging platform for allogeneic, off-the-shelf CAR-based cell therapies. However, adoptive transfers of NK cells have shown limited expansion and persistence which may impact their ability to induce durable anti-tumor responses. Signals from TLR and IL-18 receptors, through MyD88, activate NK cells. Here, we demonstrate that constitutive expression of a novel chimeric costimulatory protein, comprised of the signaling domains from MyD88 and CD40 (MC) and secreted IL-15 dramatically improves the proliferation and anti-tumor efficacy of CAR-redirected NK cells. An orthogonally-regulated Caspase-9 switch was included to provide safety.

Technology

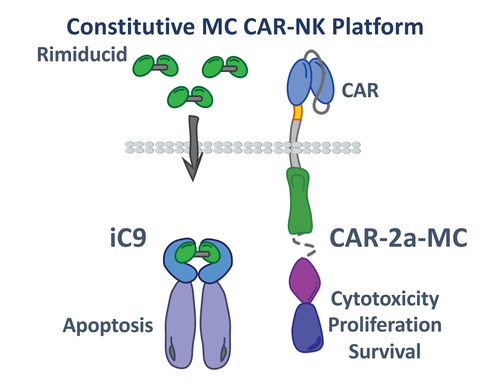


iMC ACTIVATION SWITCH

"On demand" NK cell activation via rimiducid-induced MyD88/CD40 protein dimerization enhances NK cell proliferation and anti-tumor activity.

iRC9 APOPTOTIC SWITCH

Orthogonally-regulated, rapid and efficient clearance of NK cells follows administration of dimerizing drug rapamycin (Rap).



CONSTITUTIVE MC

Fusion of MC with a CAR provides membrane bound, constant MC signaling and enhances NK cell proliferation, activation, and anti-tumor activity.

Rimiducid-inducible Caspase-9 (iC9) provides a rapid proapoptotic switch for safety.

iMC activates innate NK cytotoxicity and growth

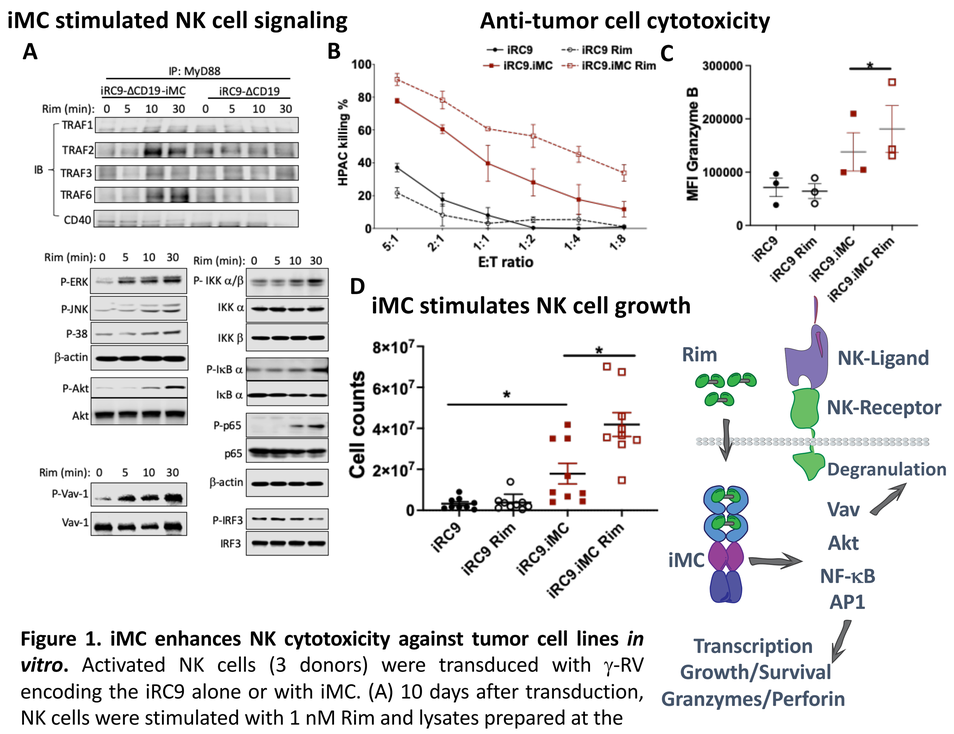


Figure 1. iMC enhances NK cytotoxicity against tumor cell lines *in vitro*. Activated NK cells (3 donors) were transduced with γ-RV encoding the iRC9 alone or with iMC. (A) 10 days after transduction, NK cells were stimulated with 1 nM Rim and lysates prepared at the indicated timepoints. **(B)** Transduced NK cells were co-cultured with HPAC-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 luciferase activity. (**C**) Granzyme B expression was determined by intracellular flow staining. **(D)** Growth of 50000 transduced NK cells from 9 donors over 10 days.

iMC synergizes with IL-15 for NK cell growth *in vivo*

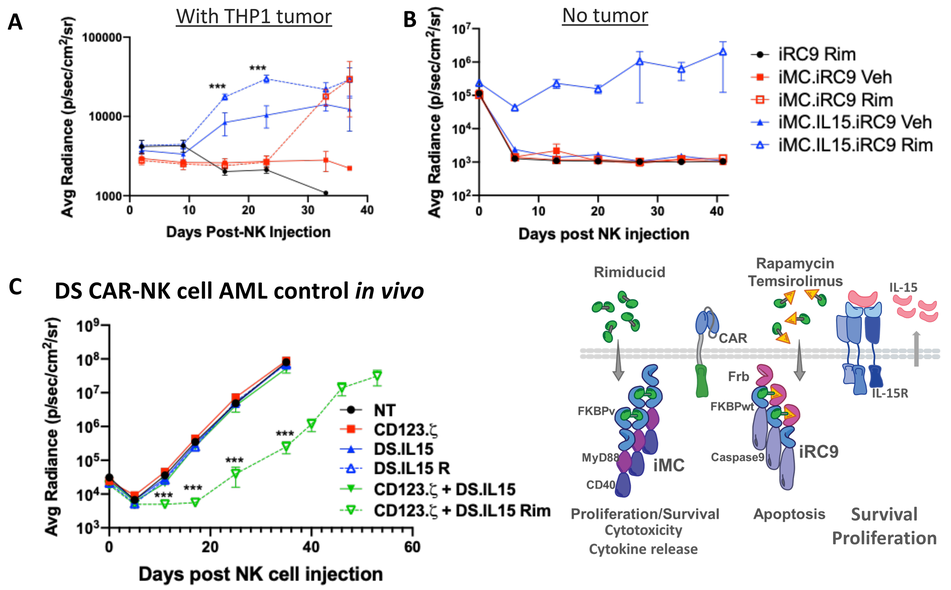


Figure 2. iMC and autocrine IL-15 synergize to stimulate NK cell growth, persistence and CAR-directed anti-tumor activity in NSG mouse xenografts. (A, B) Biologic Imaging (BLI) of NSG mice were engrafted with 5x10^6 NK cells transduced with RV encoding iRC9 ± iMC ± IL-15 and oNL.R*luc.* Rimiducid or vehicle was administered i.p. weekly **(A)** when pre-engrafted with THP1 tumor cells or **(B)** in the absence of activating tumor targets. (**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.

MyD88/CD40 stimulates NK cell cytokine production

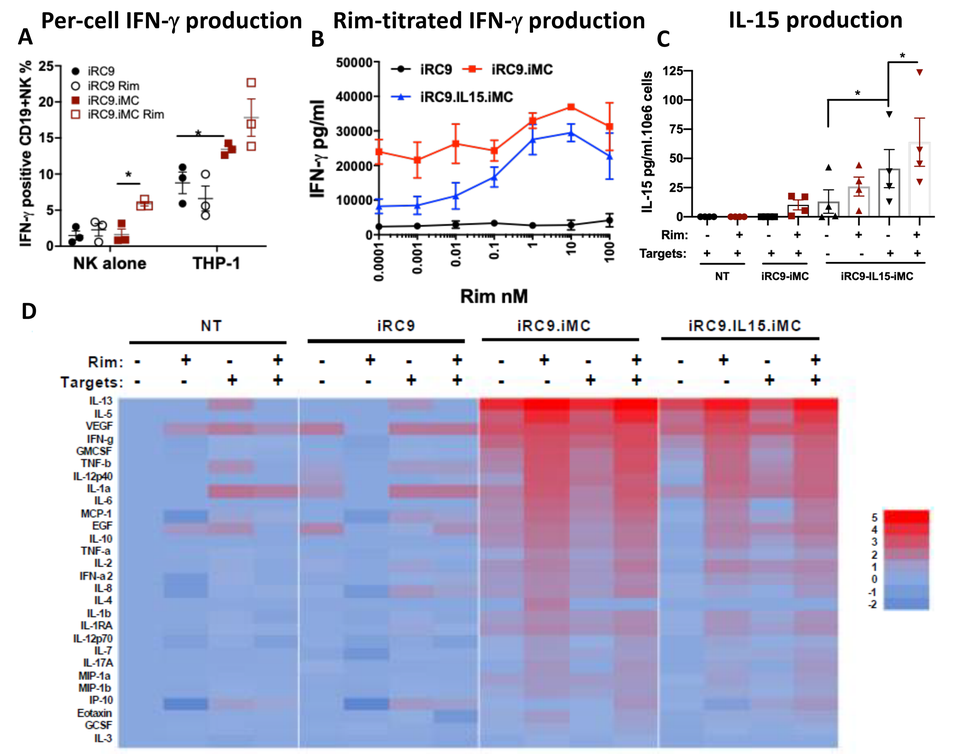


Figure 3. iMC signaling stimulates NK cell cytokine production. (A) NK cells transduced to express the indicated transgenes were cultured alone or in the presence of THP1 tumor targets with or without 1 nM Rim. IFN-γ producing cells were identified by intracellular flow cytometry. **(B)** IFN-γ production with titration of Rim in iMC and iMC/IL-15-expressing NK cells. **(C)** Interleukin-15 secretion upon iMC activation and target presence. **(D)** Base 10 exponential increased production of 20 cytokines relative to non-transduced NK cells determined by multiplex (Bio-Rad).

Conclusions and Perspectives

- Signaling by MyD88/CD40 enhances innate NK cell cytotoxicity and cytokine production. This activity may eliminate targets cells with low CAR-antigen levels.
- NK cell growth *in vitro* is stimulated by MC signaling and synergizes with IL-15 signals to promote NK cell expansion and persistence *in vivo.*
- MC/IL-15 drives CAR-directed responses against hematological and solid tumor targets *in vivo.* Robust cytokine and chemokine production from iMC-NK cells may have agonist effects to generate host-derived anti-tumor inflammation.

MC stimulates CAR-NK efficacy against HER2$^+$ tumors

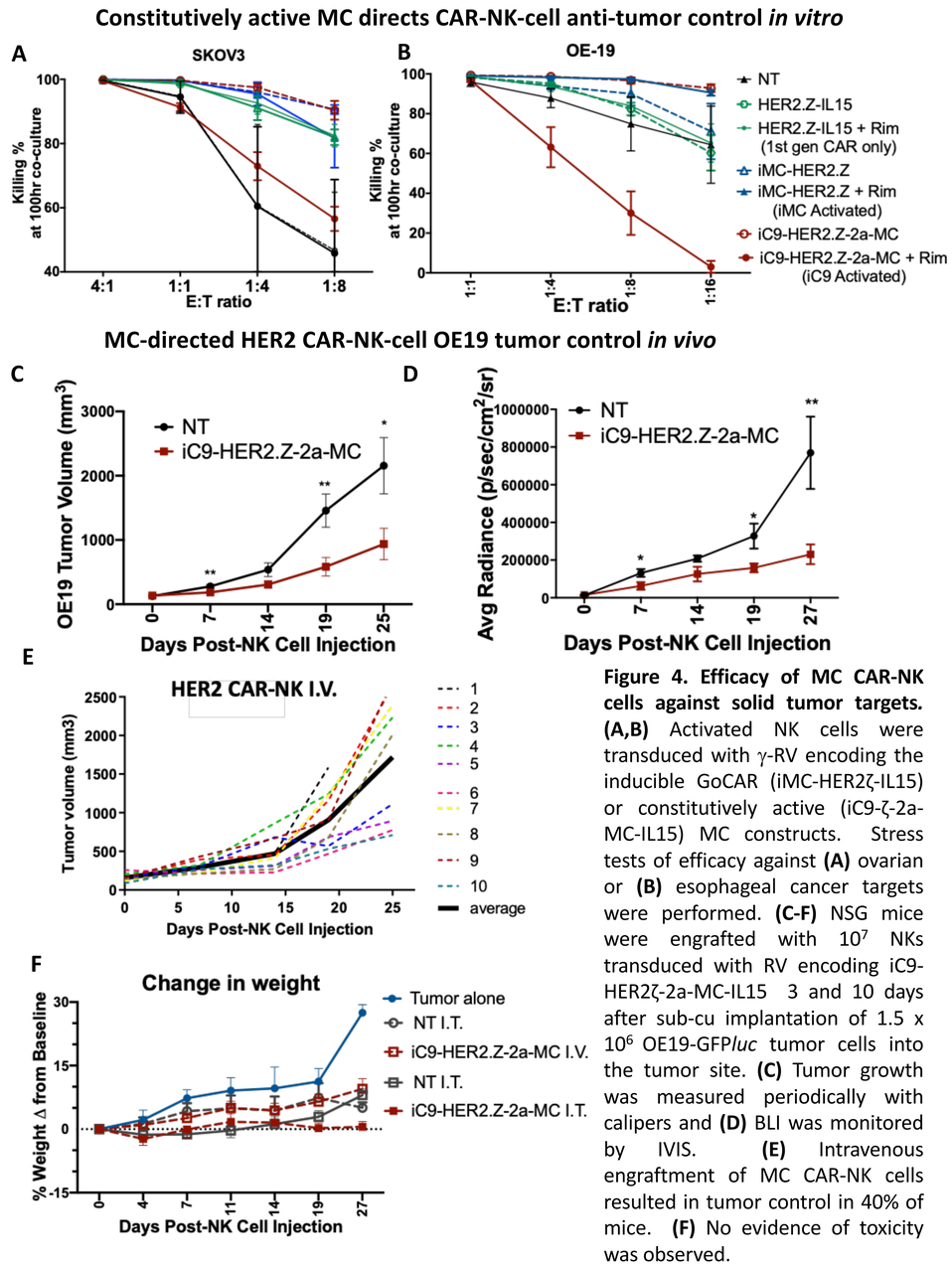


Figure 4. Efficacy of MC CAR-NK cells against solid tumor targets. (A,B) Activated NK cells were transduced with γ-RV encoding the inducible GoCAR (iMC-HER2ζ-IL15) or constitutively active (iC9-ζ-2a-MC-IL15) MC constructs. Stress tests of efficacy against **(A)** ovarian or **(B)** esophageal cancer targets were performed. **(C-F)** NSG mice were engrafted with 10^7 NKs transduced with RV encoding iC9-HER2ζ-2a-MC-IL15 3 and 10 days after sub-cu implantation of 1.5 x 10^6 OE19-GFP/*luc* tumor cells into the tumor site. **(C)** Tumor growth was measured periodically with calipers and **(D)** BLI was monitored by IVIS. **(E)** Intravenous engraftment of MC CAR-NK cells resulted in tumor control in 40% of mice. **(F)** No evidence of toxicity was observed.