

# In vivo Model Development for Genome-Edited T Cell Therapeutics

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## INTRODUCTION

The implementation of various mouse models is critical to assess safety, efficacy, and short- and long-term persistence of therapeutic modalities, especially for cell-based therapies. To increase our repertoire of viable humanized murine models, we developed two *in vivo* models by taking advantage of Taconic's immunodeficient mice: one to monitor graft versus host disease (GvHD) and the other to evaluate human natural killer (NK) cell cytotoxicity. To develop a model of GvHD, we transplanted NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Sug</sup>/JicTac (NOG) mice from Taconic with varying doses of human peripheral blood mononuclear cells (PBMCs) and monitored mice for changes in body weight over time. When NOG mice were co-injected with human natural T regulatory cells (nTregs) and PBMCs, we observed a prolonged survival rate and a slower loss of body weight, as compared to mice transplanted with PBMCs alone. Additionally, whereas NOG mice co-injected with antigen presenting cells (APCs) and wild type T cells were moribund within 3 weeks, 80% survival for 90 days was observed in mice receiving APCs plus T cells in which the TRAC locus was knocked out by CRISPR/Cas9 editing. To create an NK cell cytotoxicity model, we transplanted primary human NK cells into NOG-hIL15 mice from Taconic, which constitutively produce human IL-15. We observed successful engraftment and proliferation of NK cells, with peak engraftment occurring 4–5 weeks post injection, and no signs of xenogeneic GvHD. Utilizing K562 tumor cells expressing luciferase and IVIS imaging, we found that the engrafted primary human NK cells have fast and potent cytotoxic activity, resulting in elimination of tumor cells, as compared to tumor survival in non-NK cell engrafted mice. Our results collectively suggest that the two *in vivo* models developed here will be valuable pharmacology models to support the development of adoptive cell therapies.

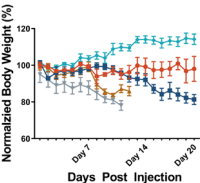
## GvHD MODEL

### Titrate PBMC Dose to Induce GvHD in NOG Mice

#### A Workflow of GvHD Study



#### B Weight Loss



#### C Survival

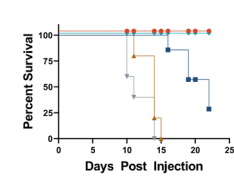
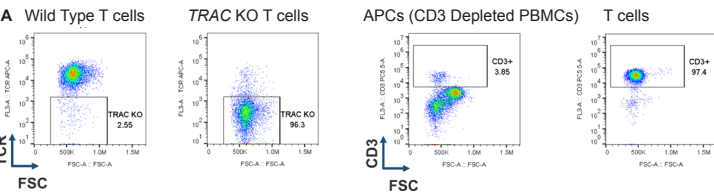


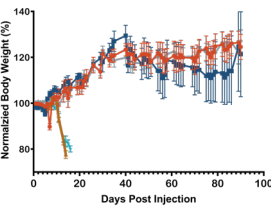
Figure 1. Established an *in vivo* GvHD model.

To induce xenogeneic GvHD in NOG mice, mice were irradiated with 200 rads prior to inoculation of donor PBMC in varying amounts (A). Body weight (B) and survival rate (C) were determined over the time.

### Test the Effect of TCR KO T Cells in GvHD Model



#### B Weight Loss



#### C Survival

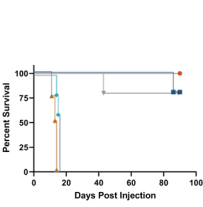
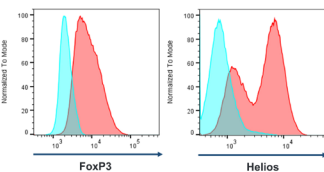


Figure 2. TCR KO T cells did not induce GvHD.

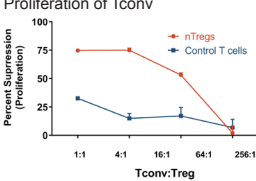
(A) The endogenous TCR was removed via CRISPR/Cas9 targeting of the *TRAC* locus with 97% efficiency. (B) Injection of TCR KO T cells into NOG mice did not induce GvHD, as no mice lost >20% of body weight as compared to baseline (B) and induce similar survival as compared to controls (C).

### Test the Effect of Natural Treg Cells in GvHD Model

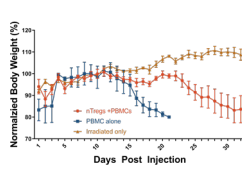
#### A Natural Tregs Express Helios and FoxP3



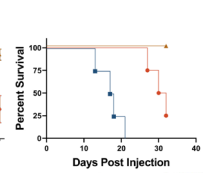
#### Natural Tregs Can Suppress the Proliferation of Tconv



#### B Weight Loss



#### C Survival



#### D Different Cell Types in Splenocytes from NOG Mice with Different Treatment

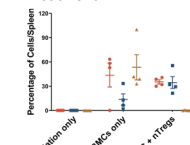


Figure 3. Natural Tregs can suppress GvHD *in vivo*.

(A) Natural Tregs, which express FoxP3 and Helios, were mixed with conventional T cells (Tconv) at various ratios in a mixed lymphocyte reaction and their suppression activity was determined. (B) To test the suppressive effect of nTregs *in vivo*, NOG mice were irradiated with 200 rads one day prior to engraftment of PBMCs +/- Treg at 1:1. nTreg were able to suppress GvHD, as determined by monitoring body weight loss (B) and prolong survival (C). nTreg suppressed the number of CD19<sup>+</sup> B cells in spleen potentially impacting the slowed onset of GvHD (D).

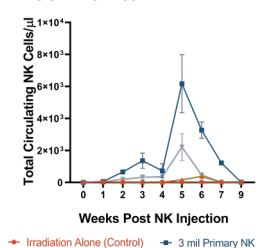
## CONCLUSIONS

- Two *in vivo* models developed here will be useful tools for testing human T cells or NK cells
- Established an *in vivo* GvHD model using human PBMCs and NOG mice
- Human primary T cells without TCR did not induce GvHD
- Human primary nTregs suppressed GvHD
- Developed a humanized NK cytotoxicity model using NOG-IL-15 mice

## NK CYTOTOXICITY MODEL

### Explore the Kinetics of Human NK cell Engraftment in NOG-huIL-15 Mice

#### A Time Course of NK Engraftment in NOG-IL-15 Mice



#### B Weight Loss

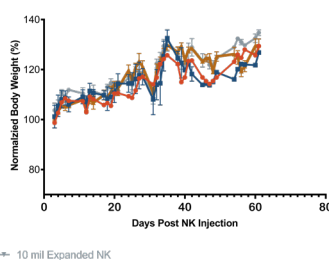
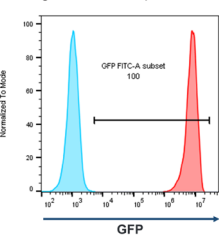


Figure 4. Primary and expanded NK cells engraft in NOG-huIL-15 mice.

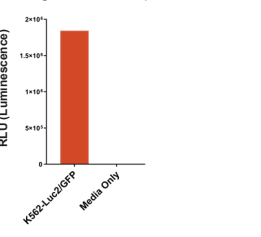
NOG-huIL-15 mice were injected intravenously with primary or expanded NK cells. Weekly bleeds via tail nick were carried out to determine the total number of circulating NK cells per ml of blood (A). Primary NK engraftment was well tolerated and did not induce body weight loss (B).

### Test the Killing Activity of Engrafted Human NKs Using K562 Cells as Target Cells

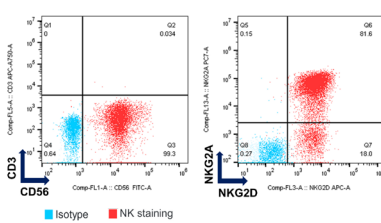
#### A K562 Tumor Cells Were Engineered to Express GFP



#### B K562 Tumor Cells Were Engineered to Express Luciferase



#### C Characterization of Primary NK Cells



#### D Primary NK Cell Lysis of K562-Luc Tumor Cells

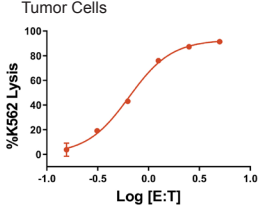


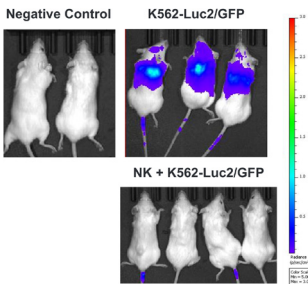
Figure 5. *In vitro* validation of NK cell cytotoxicity.

K562 tumor cells were transduced with lentivirus expressing Luciferase and GFP, allowing for selection of GFP<sup>+</sup> cells with reciprocally high luciferase expression (A–B). Purified primary NK cells that expressed both NKG2A and NKG2D (C) were able to effectively lyse K562 tumor cells engineered to express GFP/Luciferase (D).

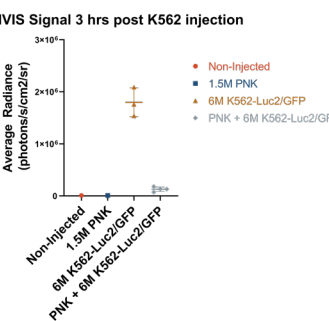
#### A Workflow of NK *In vivo* Cytotoxicity Model



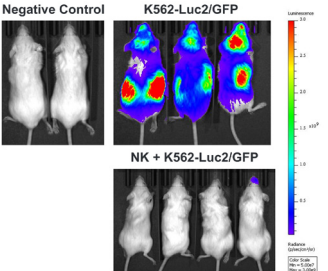
#### B IVIS Images (3 Hrs Post K562 Injection)



#### C IVIS Signal 3 Hrs Post K562 Injection



#### D IVIS Images (24 Days Post K562 Injection)



#### E IVIS Signal 24 Days Post K562 Injection

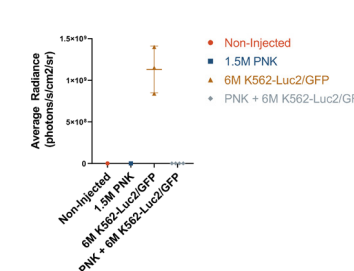
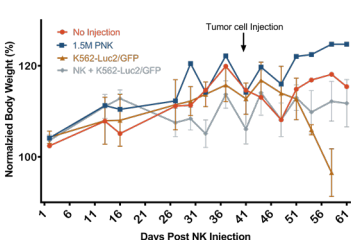


Figure 6. Engrafted NK cells can effectively limit the outgrowth of K562 tumor cells.

NOG-huIL-15 mice were injected with 1.5 million primary NK (PNK) cells 4 weeks prior to inoculation of mice with 6 million K562 cells expressing Luc2/GFP (A). After K562 injection, IVIS images were captured between 3 hours post injection (B) to 24 days post injection (D), and IVIS signals were quantified (C & E). These data revealed that NK cells can effectively lyse K562 tumor cells *in vivo* quickly (B,C) and can efficiently limit the outgrowth of tumor cells at 24 days post-injection (D,E).

#### A Weight Loss



#### B Survival

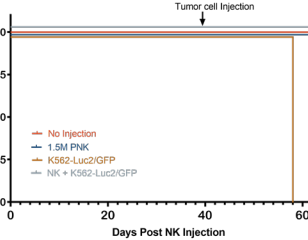


Figure 7. NK cell lysis of K562 tumor cells does not induce GvHD.

Engraftment of primary NK cells did not induce a change in body weight (A) or impact the long-term survival (B) of NOG-huIL-15 mice over the course of the study. Furthermore, the effective clearance of tumor cells by engrafted NK cells did not induce GvHD or impact survival.