Evaluation of a human peripheral blood NK humanized hIL-15 mouse model lacking murine Fc gamma receptors

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Abstract

Fc gamma receptors (FcγRs) on residual murine immune cells can interact with human IgG-based therapeutics and confound preclinical results. This is critical for therapeutics with human NK cell mediated antibody-dependent cellular cytotoxicity (ADCC) as their primary mode of action. We compared humanization, kinetics, and persistence of NK cell reconstitution in human IL-15-expressing mice with or without FcγRs using intraperitoneal (IP) or intravenous (IV) administration of cells from multiple donors.

Methods: Negatively selected human NK cells were engrafted into hIL-15 NOG or FcResolv™ hIL-15 NOG (FcγR knockout) with identical protocols. Human reconstitution, immune profile and persistence was evaluated via serial blood sampling and terminal collections for blood, spleen, and bone marrow.

Results: Human NK cell reconstitution and persistence was equivalent between strains. There was a significant difference in the overall chimerism in IV vs. IP administration (IV > IP). There was no significant difference in % body weight, body condition scores, morbidity or mortality across study duration. NK cell persistence and kinetics were consistent between strains, administration, and donors, with a peak at 4-5 WPE and persistence to 12+ WPE in the blood. The dominant circulating NK phenotype was CD56+CD16+.

Conclusions: huNK-engrafted hIL-15 NOG and FcResolv™ hIL-15 NOG mice provide a 12+ week study window, with equivalent reconstitution, immune profiling and kinetics.

Background

The NOG Portfolio

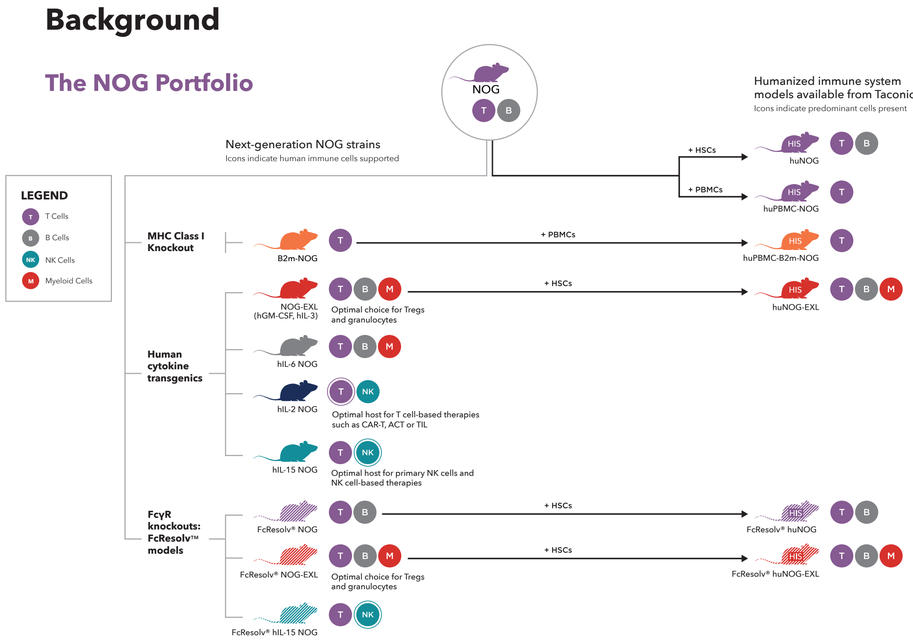

Fig 1. The Taconic NOG Portfolio of super immunodeficient and humanized immune system mice

Mouse Fcγ Receptors are Absent in FcResolv™ NOG Models

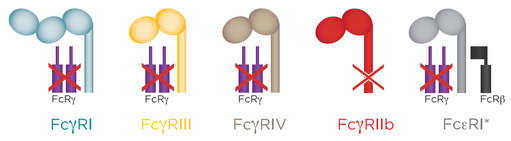

Fig 2. The FcResolv™ NOG model portfolio is based on the super immunodeficient NOG mouse. This highly versatile strain lacks adaptive immune cells and has an attenuated innate immune response, yet still retains some residual mouse immune cells that can interact with therapeutic antibodies. FcResolv™ NOG models eliminate this interaction by knocking out the activity of all murine FcγRs, including the FcγRI, IIB, III and IV types, along with the high affinity FcεRI receptor. The low affinity FcεRII receptor remains present.

Specifically detect human NK cell-mediated ADCC with FcResolv™ hIL-15 NOG

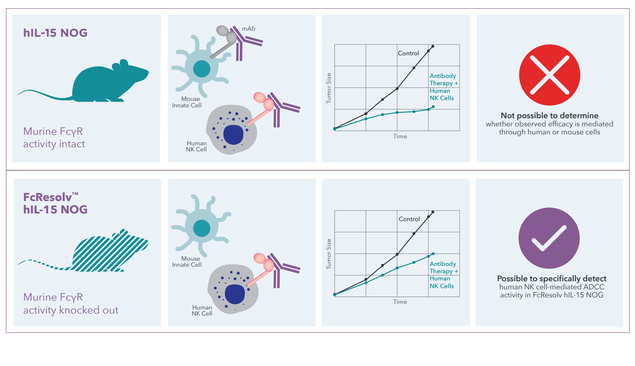

Fig 3. FcResolv™ NOG models eliminate various confounding effects attributable to mouse Fc gamma receptors on residual immune cells, and the FcResolv™ hIL-15 NOG mouse enables specific detection of ADCC mediated by human NK cells.

Objectives

- Characterize the kinetics and persistence of human NK cells in human IL-15-expressing NOG mice with or without FcγRs
- Determine the optimal route of administration for human NK cells and the tolerability of NK cell engraftment

Methods

- Mice used: hIL-15 NOG (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug} Tg(CMV-IL2/IL15)1-1Jic/JicTac) and FcResolv™ hIL-15 NOG (NOD.Cg-*Fcgr2b*^{tm1Ttk} *Fcer1g*^{tm1Rav} *Prkdc*^{scid} *Il2rg*^{tm1Sug} Tg(CMV-IL2/IL15)1-1Jic/JicTac) were from Taconic Biosciences (Rensselaer, NY)
- Mice were injected with previously-frozen primary NK cells from human donors (huNK; 2 x 10^6 cells/mouse; negatively selected for hCD3)
- NK cell engraftment was determined by serial bleeds between 3 and 13 weeks post-engraftment (WPE) via flow cytometry using either hCD45 or a validated human NK cell panel (mCD45, hCD45, hCD3, hCD16 and hCD56)

Results

Human NK cell kinetics in hIL-15 NOG mice

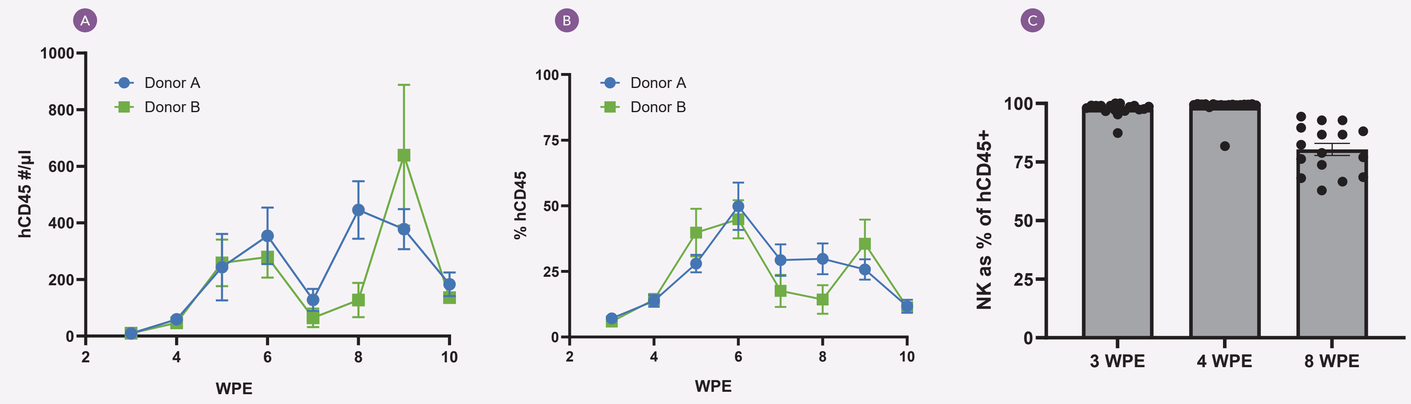

Fig 4. huNK cells from two different donors were administered IP and peripheral blood was analyzed via flow cytometry. (A) Absolute NK cell counts and (B) % hCD45 in hIL-15 NOG mice over time (mean±SEM, n=3-20). No morbidity or mortality was observed during the study. (C) The human CD45 fraction was evaluated for NK cells by flow cytometry (mean±SEM) gated on mCD45-, hCD45+, hCD3-, HCD16+, hCD56+.

Equivalent human NK cell engraftment between hIL-15 NOG and FcResolv™ hIL-15 NOG mouse strains

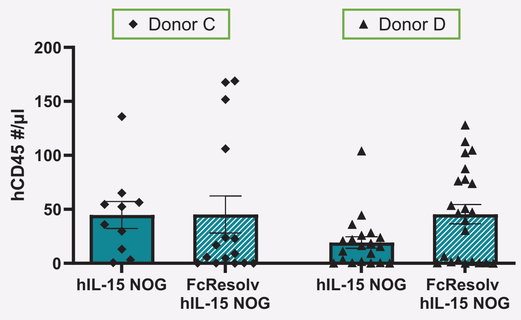

Fig 5. Human cells in the peripheral blood of hIL-15 NOG and FcResolv™ hIL-15 NOG 4-6 WPE with human NK cells from two different donors (mean±SEM, n=10-23). There was no significant difference between the two strains within a given donor (unpaired t-test).

Equivalent human NK cell persistence between hIL-15 NOG and FcResolv™ hIL-15 NOG mouse strains

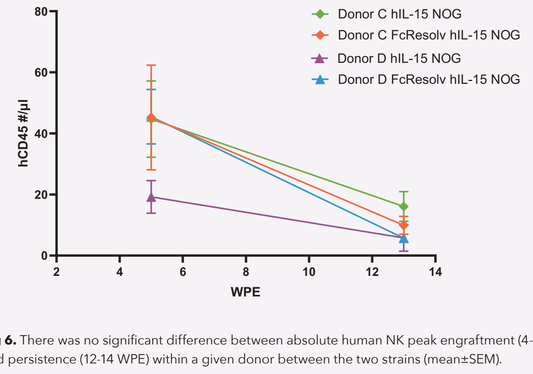

Fig 6. There was no significant difference between absolute human NK peak engraftment (4-6 WPE) and persistence (12-14 WPE) within a given donor between the two strains (mean±SEM).

Superior human NK cell engraftment following intravenous vs. intraperitoneal injection in FcResolv™ hIL-15 NOG mice

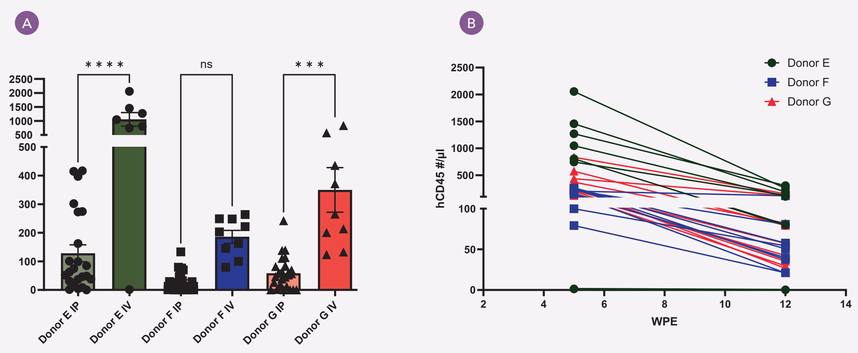

Fig 7. (A) Increased human NK cell engraftment (at 5 WPE) in FcResolv™ hIL-15 NOG in 2 of 3 donors (mean±SEM; ns=p>0.05, ***p<0.001,****p<0.0001). **(B)** Human NK cell persistence following IV administration (lines show repeated bleeds of the same animal at each timepoint).

No significant difference in tolerability or survival following human NK cell engraftment between hIL-15 NOG and FcResolv™ hIL-15 NOG mouse strains

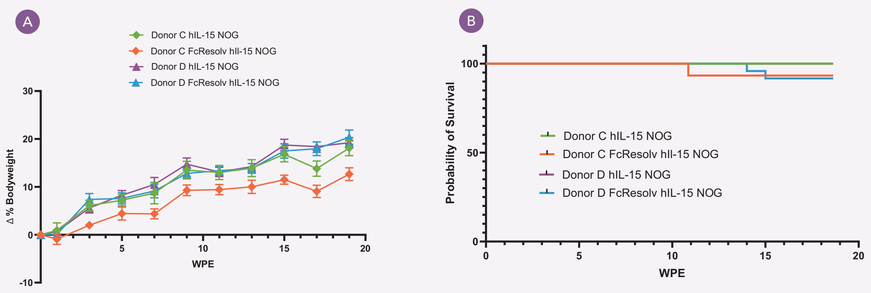

Fig 8. (A) Body weights of FcResolv™ hIL-15 NOG and hIL-15 NOG following huNK engraftment (mean±SEM). There was no significant difference between strains within a given donor (multiple unpaired t-test). All study animals were clinically observed across the entire study duration; there was no significant difference in body conditioning scores (not shown) nor mortality. **(B)** Kaplan-Meier survival curves.

NK cell subtypes present in FcResolv™ hIL-15 NOG mice following human NK cell engraftment

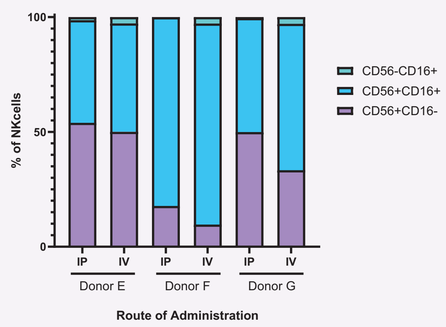

Fig 9. Peripheral blood was analyzed by flow cytometry across three different donors (E, F, G) using hCD56 and hCD16 (gated on mCD45-hCD45+hCD3-) at 5 WPE.

Conclusions

- hIL-15 NOG and FcResolv™ hIL-15 NOG mice support equivalent engraftment of primary human NK cells with a peak at 4-6 WPE and persistence to 12+ WPE
- Engrafted human NK cells were primarily of CD56+CD16+ and CD56+CD16- subsets, with subset frequency varying by donor
- Route of administration impacts chimerism levels, with higher engraftment via IV versus IP route
- huNK-engraftment is well-tolerated, with 92%+ survival to study limit (19 weeks post-engraftment)
- huNK-engrafted hIL-15 NOG and FcResolv™ hIL-15 NOG mice are important tools for research directed at NK cell-modulating therapies

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