LB-088

Cytokine-Transgenic NOG Mice Engrafted with Human Peripheral Blood Cells Support Natural Killer Cell Expansion

Paul Volden[1], Azusa Tanaka[1], Dimitri Gimnopoulos[1], Magdalena Paterka[2], Iduna Fichtner[2]; Taconic Biosciences, Inc., Rensselaer, NY[1], EPO Berlin GmbH, Berlin, Germany[2]

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ABSTRACT

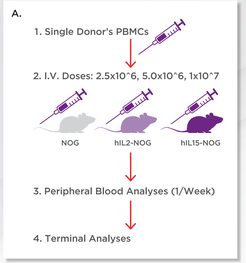
Harnessing natural killer (NK) cells and their ability to mediate antibody-dependent cellular cytotoxicity (ADCC) forms the basis for many ongoing immuno-oncology efforts. Currently, most preclinical *in vivo* studies of ADCC-dependent efficacy rely on syngeneic models, which mandate the generation and use of surrogate therapies to overcome differences between mouse and human immune systems. These steps add time and cost, and can be particularly challenging for advanced antibody therapeutics (e.g. bispecific antibodies and antibody-drug conjugates). A humanized immune system (HIS) model that supports human NK cells could improve evaluations of human NK cell-dependent cancer therapies. However, HIS models have largely failed to support human NK cell-engraftment, rendering them inadequate for ADCC studies. Prior work has shown that human cytokine-transgenic hIL-2 NOG and hIL-15 NOG mice support the development of human NK cells from engrafted human hematopoietic stem cells (HSCs). Furthermore, the hIL-15 NOG model has been shown to support *in vivo* expansion and long-term engraftment of isolated human NK cells. These properties suggest hIL-2 NOG and/or hIL-15 NOG might also sufficiently support NK cells following engraftment of peripheral blood mononuclear cells (PBMCs). Such a model could enable cost-effective and efficient *in vivo* studies of human NK cells and therapeutic human antibodies. To test this hypothesis, the CIEA NOG mouse® (NOG), hIL-2 NOG, and hIL-15 NOG mice were engrafted with PBMCs from a single donor and at one of three increasing cell doses. The onset of graft vs host disease and peripheral blood human immune cell subsets were monitored through the duration of this study. Results showed that hIL-15 NOG mice had a slightly diminished survival compared to conventional NOG. Nevertheless, hIL-15 NOG survived up to 7 weeks after PBMC engraftment without any signs of graft vs host disease. Although hIL-2 NOG mice showed the best engraftment rate for NK cells and other immune cell subpopulations, the overall survival was severely decreased post engraftment. Comparing NK cell-enagraftment in hIL-15 NOG and conventional NOG mice, we observed a tenfold increase of NK cell numbers in hIL-15 NOG mice independent of engrafted PBMC numbers making it a highly suitable HIS model for studying NK cells.

BACKGROUND

Table 1: Human NK cell engraftment properties reported in NOG, hIL-2 NOG, and hIL-15 NOG mice.

Engrafted-Cell Type	NOG[1-3]	hIL-2 NOG[4,5]	hIL-15 NOG[4,5]
Human CD34+ HSCs	Prohibitively low human NK cell reconstitution (<1% total hCD45)	Predominant development of human NK cells by six weeks post-HSC transplantation, mice succumb to wasting disease within 60 days post engraftment (25x10^3 HSCs)	Predominant development of human NK cells, mice succumb to wasting disease within 40 days post engraftment (25x10^3 HSCs)
NK cells isolated from human PBMCs	NK cells diminish within one week post engraftment	Not yet reported	NK cells proliferate over initial four weeks and are maintained up to 24 weeks post engraftment
Human PBMCs	NK cells not supported. Mice succumb to GvHD within four to six weeks, depending on number of cells injected and donor-specific properties	Not yet reported	Not yet reported

METHODS



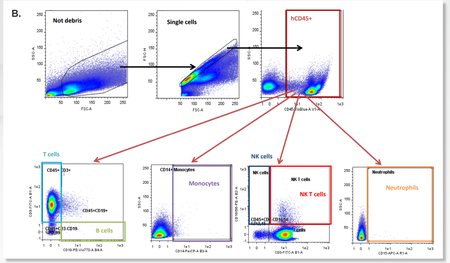


Figure 1A: NOG, hIL-15 NOG, and hIL-2 NOG mice were engrafted (i.v.) with 2.5, 5.0, or 10x10^6 PBMCs (n=6/group) from the same donor.
Figure 1B: Mice were bled once a week to determine the human immune cell engraftment (hCD45+) and human T cell, B cell, NK cell, NKT cell, and neutrophil counts. Single cell solutions of spleen, bone marrow, and thymus were analyzed similarly. No irradiation was performed prior to PBMC engraftment. PBMC-engrafted mice were produced at Taconic Biosciences. All downstream analyses were directed and/or performed by EPO GmbH.

SURVIVAL, WEIGHTS, & HISTOLOGY

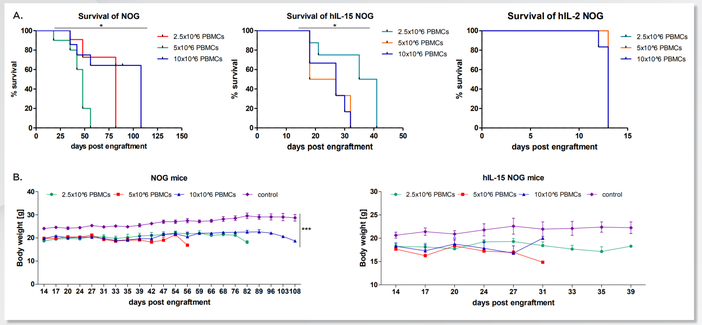


Figure 2A: Survival of NOG, hIL-15 NOG, and hIL-2 NOG mice post engraftment. Data shown from one experiment (n=6 in each group) and analyzed by Log-rank (Mantel-Cox) test, p<0.05 was considered significant, *p<0.05.
Figure 2B: Body weight of NOG and hIL-15 NOG mice post engraftment over the whole observation period. Data shown from one experiment (n=6 in each group that received PBMCs; n=2 for control) and analyzed by Kruskal-Wallis test and Dunn's Multiple Comparison post test: p<0.05 was considered significant, *** p<0.001.

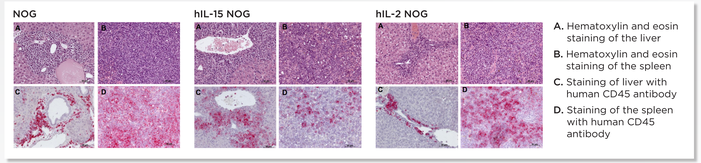


Figure 3: Histology of representative animals engrafted with human PBMCs sacrificed on day 18 (day 13 for hIL-2 NOG) after PBMC engraftment.

- **A.** Hematoxylin and eosin staining of the liver
- **B.** Hematoxylin and eosin staining of the spleen
- **C.** Staining of liver with human CD45 antibody
- **D.** Staining of the spleen with human CD45 antibody

hIL-2 NOG PERIPHERAL BLOOD ANALYSES

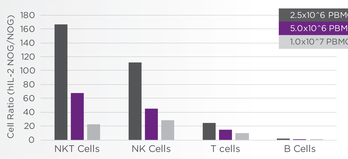


Figure 4: Relative human immune cells in hIL-2 NOG vs NOG mice. Peripheral blood analyses were performed at day 13 (hIL-2 NOG) or day 18 (NOG) following engraftment of PBMCs. Absolute numbers of immune cell subpopulations within the human CD45+ cells in the blood were used to calculate relative ratio (hIL-2 NOG:NOG). With the exception of high-dose (1.0x10^7 PBMCs) hIL-2 NOG (n = 2), ratios were quantified from averages of cell counts from six mice per cell-dose group.

hIL-15 NOG PERIPHERAL BLOOD ANALYSES

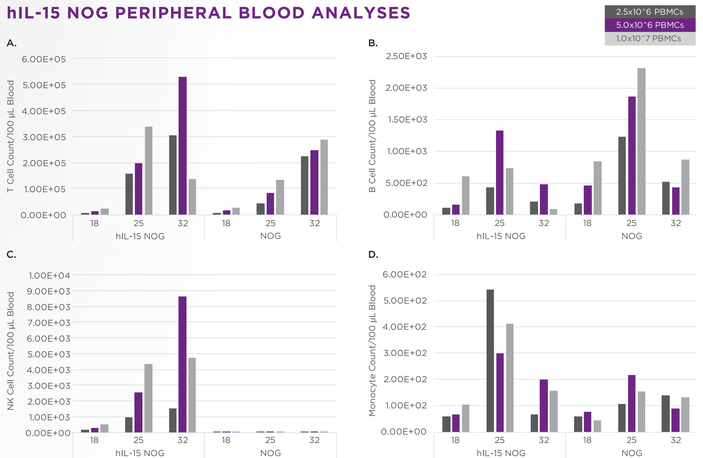


Figure 5: Human immune cell engraftment in hIL-15 NOG vs NOG mice. Data from peripheral blood analysis of human T cells **(A)**, B cells **(B)**, NK cells **(C)**, and monocytes **(D)**, performed at 18, 25, and 32 days post PBMC engraftment. Single donor's cells were engrafted into both models at the color-code indicated cell doses. NOG: n=6 per group; hIL-15 NOG: n=5–6, 3–5, and 1–4 per group at day 18, 25, or 32 post engraftment, respectively.

SUMMARY

- Compared to conventional NOG mice, survival was severely decreased in hIL-2 NOG mice and slightly diminished in hIL-15 NOG mice
- PBMC-engrafted hIL-2 NOG mice showed the highest engraftment rate early (day 13 vs day 18) for NK cells
- NK cell numbers in hIL-15-NOG vs conventional NOG mice were 10-fold or more higher, independent of engrafted PBMC numbers

CONCLUSION

- PBMC-engrafted hIL-15 NOG mice support efficient engraftment of human NK cells, making hIL-15 NOG mice highly suitable for studying human NK cell properties. Because of the severely reduced survival timeline following PBMC engraftment, alternative engraftment strategies (e.g. using purified human NK cells) are necessary to enable human NK-cell studies in hIL-2 NOG mice. Both models hold promise as cost-effective and efficient *in vivo* tools for studying human NK cells, therapeutic human antibodies, and NK-cell engaging bispecific therapies.

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