Unique Immunodeficient Murine Host Strains Impact Expansion and Engraftment of T cells in PBMC Humanized Mice

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ABSTRACT

The development of animal models capable of mimicking human immune responses and functions are crucial to study the safety and efficacy of immune checkpoint inhibitors, functionality of CAR-T therapies, and immuno-oncology treatments including gene and cell therapies. Mice reconstituted with human hematopoietic stem cells (HSC) are able to differentiate and repopulate human immune cells sufficient to model many aspects of tumor immunity. In partnership with the Central Institute for Experimental Animals (CIEA), Taconic Biosciences has developed the CIEA NOG mouse® (NOG) portfolio which is a powerful tool, capable of accepting human HSC that develop into multiple immune cells including T cells. The NOG mouse is the ideal host for human tumor xenografts due to its hyper-immune deficiency caused by the IL-2Rg mutation and NOD/scid background.

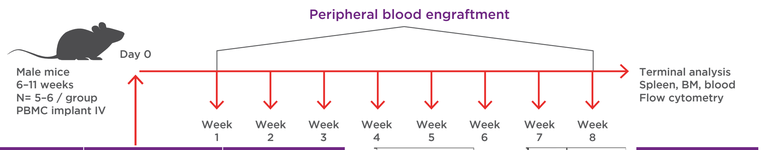
Taconic and the CIEA have continued to expand the NOG portfolio with a variety of next generation immune-deficient NOG mice that are suitable and highly effective at modeling the diverse mechanistic functions of human immunity as well as being the host for human tumor xenografts including patient derived xenografts, (PDX). This presentation will focus on the current state of human immune system engraftment models (huNOG and huNOG-EXL) and the utilization of the wide range of the NOG mouse portfolio, as the next generation model that diversifies the mechanistic functionality of immune engraftments and recent advances in modeling human immunity in immuno-oncology applications.

OBJECTIVES OF THE STUDY

Evaluate and compare various transgenic NOG mice strains for ability to support:

- Humanization using normal donor PBMC
- Engraftment level and kinetics
- Types of immune cell subsets engrafting
- Susceptibility to xGVHD

Figure 1. Study design for evaluation of PBMC engraftment in different mice strains



Mice strain	Transgene/ knockout	PBMC dose (IV)
NOG	N/A	2.5 x 10^6, 5 x 10^6, 1 x 10^7
NOG-EXL	Human GM-CSF, Human IL-3	2.5 x 10^6, 5 x 10^6, 1 x 10^7
NOG-IL-6	Human IL-6	2.5 x 10^6, 5 x 10^6, 1 x 10^7
NOG-B2M	B2m knockout (MHC Class I deficient)	2.5 x 10^6, 5 x 10^6, 1 x 10^7

Flow panel
Mu CD45
Hu CD3
Hu CD33
Hu CD4
Hu CD8
Live/dead

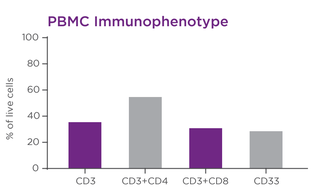


Figure 2. Evaluation of the immunophenotype for normal donor PBMC used for engraftment

PBMC were isolated via ficoll gradient density separation from a normal donor leukopak. PBMC were immunophenotyped for presence of T cells (CD3), T cell subsets (CD4,CD8) and myeloid cells (CD33) by flow cytometry and analyzed with a Miltenyi MACSquant cytometer. Data represent frequency of gated live, singlet cells.

3A

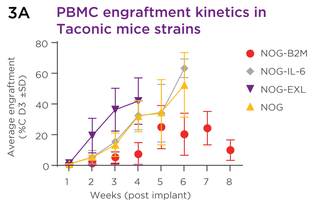


Figure 3. Engraftment kinetics and chimerism after PBMC implantation is dependent on mouse host strain and cell dose

Mice of various strains (6–11 weeks of age) were implanted IV from a single donor via tail vein injection on day 0 with 1 x 10^7 PBMC (A) or various doses of PBMC (B). Blood draws were taken weekly and circulating immune cells were assessed by flow cytometry for up to 8 weeks. N=5-6 mice/cohort. Engraftment is expressed as human CD3/mouse CD45 as described in Figure 1. No CD33 expressing cells were detected in circulation. Engraftment was most rapid in NOG-EXL, and slowest in NOG-B2M.

3B

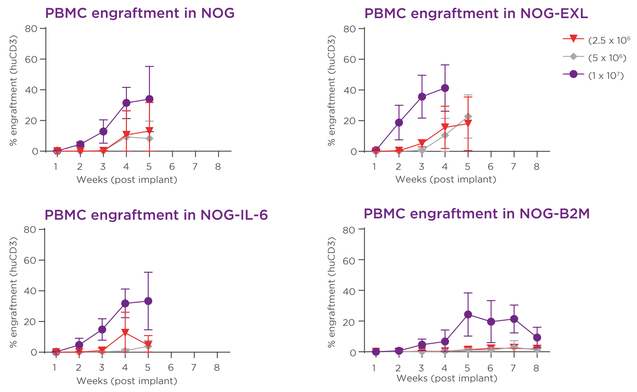


Table 1. Rates of engraftment per cohort is shown as a proportion of total mice implanted. Engrafted = 1% or greater human CD3+ cells in circulation

Mice strain	Average weekly engraftment in periphery								
	Dose	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
NOG	1 x 10^7	0/6	6/6	6/6	6/6	6/6			
	5 x 10^6	0/6	0/6	1/6	4/6	6/6			
	2.5 x 10^6	0/6	0/6	1/6	4/6	6/6			
NOG-EXL	1 x 10^7	4/6	6/6	6/6	5/5				
	5 x 10^6	0/6	0/6	1/6	6/6	6/6			
	2.5 x 10^6	0/6	3/6	6/6	6/6	5/6			
NOG-IL-6	1 x 10^7	0/6	6/6	6/6	6/6	6/6			
	5 x 10^6	0/6	0/6	1/6	4/6	4/6			
	2.5 x 10^6	0/6	4/6	4/6	4/6	4/6			
NOG-B2M	1 x 10^7	0/6	1/6	5/6	6/6	6/6	5/6	6/6	6/6
	5 x 10^6	0/6	0/6	0/5	1/5	3/5	2/5	3/5	3/5
	2.5 x 10^6	0/6	0/6	1/6	2/6	3/6	4/6	3/6	2/6

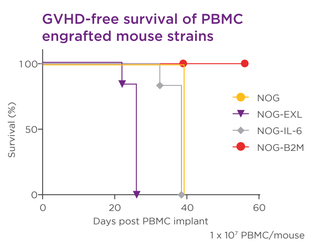


Figure 4. NOG-B2M mice strain is resistant to onset of xGVHD after PBMC implantation

Mice from various strains were implanted with were implanted with 1 x 10^7 PBMC. For up to 8 weeks post-transfer, mice were monitored for signs of severe xGVHD (poor body condition, extreme lethargy, and/or weight loss) and were euthanized when signs were observed. In some instances, mice were found dead prior to the next observation, both FD and euthanized mice were recorded as a death due to xGVHD. Data is shown in the Kaplan-Meier survival curve to represents proportion of mice exhibiting xGVHD-free survival for the study duration by group (N=6/cohort). (P<0.001, Log rank Mantel-Cox test).

Figure 5. T cell subset engraftment kinetics is dependent on mouse host strain

Mice implanted with 1 x 10^7 PBMC were bleed weekly and immunophenotyped for T cells (CD3) and T cell subsets (CD4, CD8) to assess of T cell subset development and kinetics. Flow cytometry was used to assess CD4 and CD8 T cell subsets across groups. Results represent the average ± SD (N=5-6/cohort). Enhanced CD8 expansion was observed in NOG-EXL and NOG-IL-6, whereas increased CD4 expansion was observed in NOG-B2M.

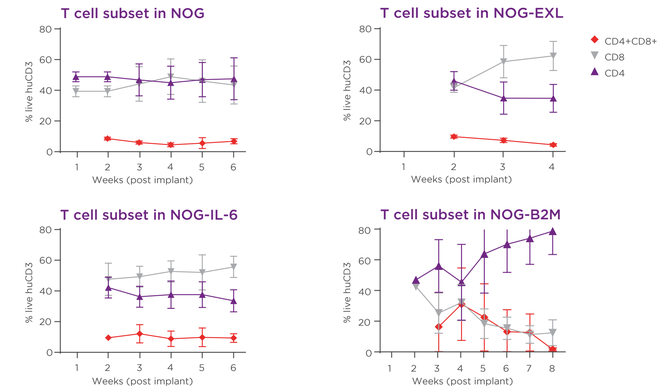
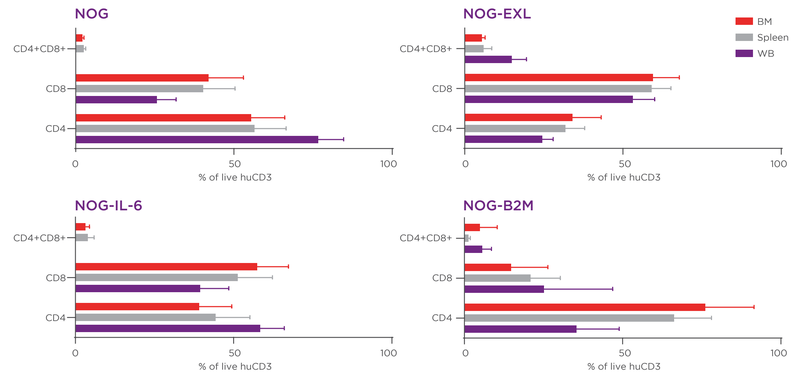


Figure 6. Spleen and bone marrow engraftment of CD4 and CD8 T cell subsets and generally reflect levels observed in circulation

Whole blood (WB), spleen and bone marrow (BM) were collected at terminal time points from mice of various strains implanted with 1 x 10^7 PBMC. After preparation of single cell suspensions, flow cytometry was used to analyze CD4 and CD8 subsets and results represent the average ± SD of 5-6 mice/cohort.



CONCLUSIONS

- T cells efficiently engraft and expand in transgenic NOG mouse strains after IV implantation with PBMC
- CD3 engraftment and expansion was time- and cell dose-dependent
- NOG-EXL mice strain had the most rapid *in vivo* expansion of CD3 T cells levels with the highest cell dose (1 x 10^7 mouse) and had the fastest onset of xGVHD
- NOG-B2M mice, which lack MHC Class I, had the most prolonged survival after PBMC implantation, with no signs of xGVHD observed for the duration of the study (8 weeks)
- NOG-B2M mice had slower kinetics of engraftment and lower maximal levels of circulating T cells as compared to the parental NOG mice recipients of PBMC
- NOG-B2M may be a suitable host for Immuno-Oncology studies with PBMC engrafted tumor-bearing mice due to the longer window for therapeutic interventions with engraftment in the absence of xGVHD

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