USE OF HUMANIZED NOG® MICE WITHIN THE PRE-CLINICAL DEVELOPMENT OF NEW HUMAN SPECIFIC THERAPIES

Caroline Mignard[1], Jean-François Mirjolet[1], Holger Kissel[2], Francis Bichat[1], Olivier Duchamp[1]
Oncodesign[1], France and Taconic[2], Germany

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Abstract

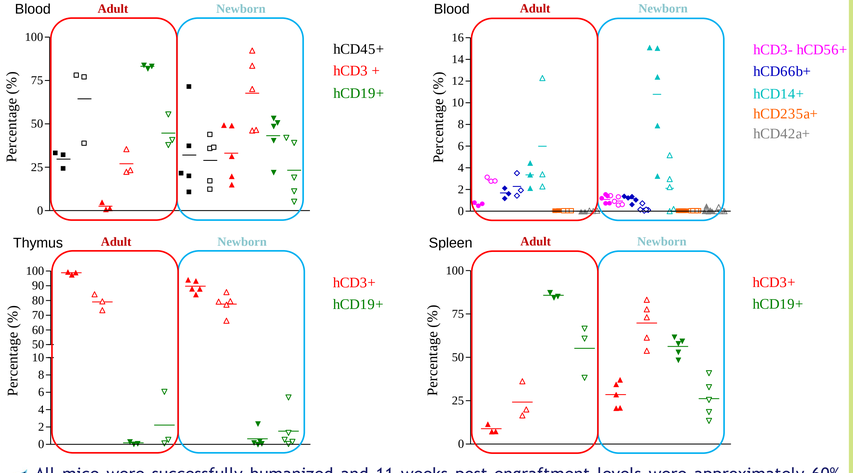
Immune cells within the tumor microenvironment are now well recognized as to be targets of interest for cancer treatment. However there are no available pre-clinical models that will accurately support the pre-clinical development of such therapeutic approaches. Humanized NOG® (NOD/shi-scid/IL-2Rγ^{null}) mice, bearing human immune cells with or without human target tumor cells, are relevant models to test various therapeutic strategies (e.g. antibody dependent cell cytotoxicity, Treg targeting antibodies, TLR agonists, vaccines, adoptive T cells transfer, …) in various pathologies (e.g. oncology, autoimmune disease, inflammation, transplantation, …). Humanization of NOG® mice was already characterized and validated using freshly collected human PBMCs or hematopoietic stem cells (HSCs) in both newborn and adult NOG® mice.
The complete characterization of the reconstituted NOG® mice was done in peripheral blood as well as central lymphoid organs such as bone marrow, spleen and thymus, using FACS analysis. Moreover, the human immune reconstitution and function in NOG® mice was also evidenced with human cytokine and immunoglobulin quantification in mouse plasma samples. The injection of mature human PBMC in adult NOG® mice leads to the consistent development of a xenogeneic graft-versus-host disease (GvHD), which mimics human GvHD (modification of CD4/CD8 ratio, expression of T cells activation markers and cytokine production).
Growth characteristics of human tumors models on humanized mice will be presented either as subcutaneous or as disseminated intravenous model. Randomization parameters were selected regarding both tumor and immune cell sides. Required readouts to understand immune cell modulation (Cell phenotyping and functionality) and related antitumor efficacy will be described. Finally, such models, i.e. humanized NOG mice bearing or not human tumor cells in the context of evaluation of new therapies will be detailed in various pathologies.

Material and Methods

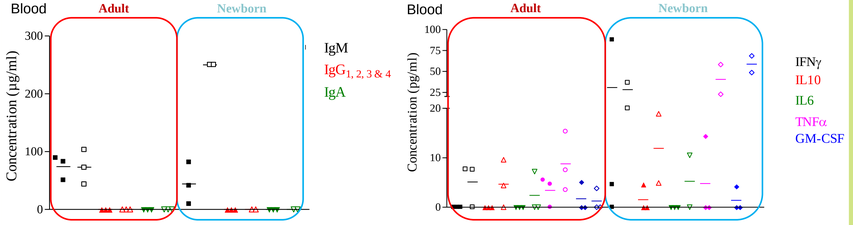
- Whole-body irradiation of NOG® mice (TACONIC).
- Injection of freshly prepared human PBMCs (hCD45+) or CD3+ T cell-depleted HSC from umbilical cord blood (hCD34+).
- Examination of hematopoietic chimerism in target tissues using flow cytometry at different time points.
- Calculation of human hematopoietic cells as absolute cell number and percentage (% hCD45 + cells in total cells, % hCD3+, hCD19+, hCD3- hCD56+, hCD14+, hCD235a+ and hCD42a+ cells in hCD45+ cells).
- Quantification of human cytokines and human immunoglobulins using a multiplex assay system (Bio-Plex).

➢ Human biological resources were provided by registered Oncodesign BRC.
➢ Research projects using human biological resources were approved by an ethics committee.
➢ Animals experiments were performed according to animal guidelines of animal experimentation(1). All procedures with animals were submitted to the Animal Care and Use Committee of Oncodesign (OnComEt).
(1)Principe d'éthique de l'expérimentation animale. Directive n°86/609 CEE du 24 Nov. 1986, Décrêt n°87/848 du 19 Oct. 1987, Arrêté d'Application du 19 Avril 1988, Directive 2010/63/EU.

Results



- All mice were successfully humanized and 11 weeks post-engraftment levels were approximately 60% and 30% hCD45+ cells in the spleen and the peripheral blood, respectively.
- The majority of cells in the spleen were CD19+ B cells, while in the thymus, the majority of cells were composed of hCD3+ T cells.
- While, T cells constituted only a minor leucocyte subtype on week 11 in blood and spleen, they were detected at higher levels at week 20.
- Very low levels of hCD45+ cells expressing markers of granulocytes, monocytes/macrophages and NK cells were detected and surprisingly circulating hCD235a+ erythrocytes and hCD42a+ platelets were not detected.

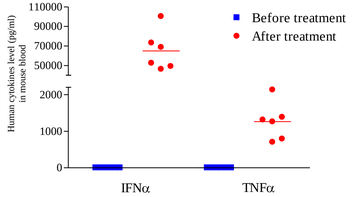


Eleven and 20 weeks after injection of human HSC, high levels of IgM were observed in the plasma of engrafted mice, suggesting the maturation of naïve B cells.

NOG® mice transfered with human HSC can produce T-helper 1 (IFNγ), T-helper 2 (IL10) and inflammatory cytokine IL6 as well as the cytokine GM-CSF that functions as a white blood cell growth factor. TNFα, mainly produced by human macrophages after T-helper 1 activation was also produced in HSC engrafted NOG® mice.

Closed symbol = W11 and Open symbol = W20

Results

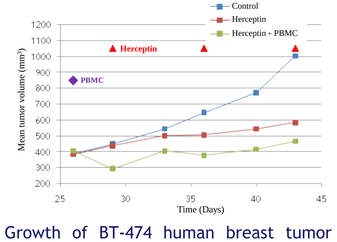


Measurement of human cytokines level using Luminex analysis. Figure represents individual and mean level of IFNα and TNFα in plasma samples from mice reconstituted with hematopoietic stem cells and receiving one injection of immuno modulating agent 12 week post-engraftment.

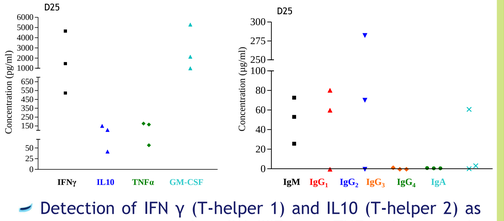
NOG® mice transplanted with 2x10^7 PBMCs died from GvHD within 23 ± 4 days (range: 18-28 days) after cell transfer. When NOG® mice received 10^7 PBMCs, the mice died between 48 ± 20 days (range D29 and D74).

D18		human Leucocytes	human T lymphocytes			Activated T lymphocytes				ratio CD4+/CD8+
		CD45+	CD3+	CD8+	CD4+	CD25+	CD134+	CD25+	CD134+	
		(% of live cells)	(% of CD45+)	(% of CD3+)		(% of CD8+)		(% of CD4+)		
Blood	mean	27.76	98.65	52.49	19.83	11.49	7.76	34.23	35.61	0.38
	SD	21.28	0.42	13.57	10.70	7.28	3.04	3.44	7.35	0.20
Spleen	mean	39.05	92.22	52.50	34.65	11.66	10.55	24.06	42.87	0.73
	SD	29.66	5.66	12.34	14.08	2.50	2.69	7.37	15.81	0.45

The results evidenced the presence of human activated T lymphocytes in blood and spleen. The T human cells consisted mainly of CD3+ T lymphocytes (>90%) containing both CD4 and CD8 subsets (CD8+ cells at higher proportions than CD4+ cells).



Growth of BT-474 human breast tumor xenografted into mice reconstituted with hPBMC. Tumor growth inhibition of Herceptin therapy appears higher in mice reconstituted with hPBMC.



- Detection of IFN γ (T-helper 1) and IL10 (T-helper 2) as well as the cytokine GM-CSF and TNFα, involved in development of GvHD.
- Presence of plasma IgG and IgM suggesting that human B cells were fonctionnal for Ig secretion.

Conclusions

- PBMCs engrafted NOG® mice constitute a relevant model of GvHD associated with activation of T lymphocytes and cytokine release.
- HSCs engrafted NOG® mice leads to multi-lineage differentiation with presence of functional T-Helper (1-2) cells and activation of B lymphocytes.
- Multi-lineage differentiation from HSC in NOG® mice was not significantly different between adults and new borns.
- Functionality of human immune system could be completely demonstrated through antigen exposure and LPS response.
- Mice reconstituted with human HSCs are considered as a tool to investigate the human immune-hematopoietic system *in vivo*.