PHARMACODYNAMIC EVALUATION OF ADCC-MEDIATED EFFECTS IN HUMANIZED IL2 OR IL15 NOG MICE MODELS

Caroline Mignard[1], Jean-François Mirjolet[1], Olivier Duchamp[1], Yasuyuki Ohnishi[2], Ikumi Katano[3], Mamoru Ito[3]

Oncodesign[1], France In-Vivo Sciences Inc.[2], Japan CIEA[3], Japan

1207

Abstract

Humanized mice (i.e. immunodeficient NOG mice engrafted with human hematopoietic stem cells (HSC)) have demonstrated their usefulness for studying the human immune system. These models should help recapitulating some pathology such as viral infection and then are suitable system to evaluate new human specific therapies. Nevertheless, one issue of these models is the incomplete maturation of natural killer (NK) cell subpopulation.

This lake of NK cell functionality was solved by the Central Institute for Experimental Animals (CIEA) with the second generation of transgenic NOG mice expressing human IL2 or IL15 (NK-NOG). The serum levels of hIL-2 and hIL-15 in mice were 1-2 and 0.1-0.2 ng/mL, respectively. In these mice, human NK cells differentiated from HSC are well distributed in the mouse body (peripheral blood, bone marrow, spleen…). They expressed various NK receptors such as NKp30, NKp44, NKp46, etc., granzyme A and perforin, indicating their maturity in the mice. They are also fully functional as evidenced using an ex vivo 51Cr release assay demonstrating lysis properties of human NK cells from collected spleen of HSC-humanized mice.

In vivo proof of concept studies were successfully performed with humanized NK-NOG mice xenografted with either human Burkitt's disseminated lymphoma cells or with human solid tumors treated with humanized therapeutic antibodies. Prediction of ADCC-mediated antitumor activity of humanized antibodies is now routinely feasible for preclinical evaluation using these new in vivo NK-NOG models.

Material and Methods

Generation of transgenic NOG mice

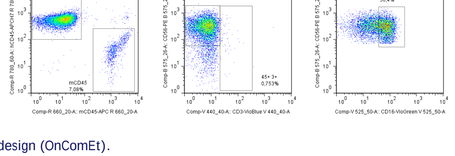
To generate the human IL-2 or IL-15 expressing transgenic NOG mouse, DNA fragments containing human IL-2 cDNA or IL-15 cDNA replaced with hIL-2 signal peptide, under the control of a CMV-promoter, were microinjected into fertilized eggs of NOD-IL-2Rγnull or (NOG x NOD-scid) F1 mice. Founder mouse strains were backcrossed with NOG mice to obtain NOG-IL-2 or NOG–IL-15 Tg mice. The average IL-2 and IL-15 concentrations in the serum of NOG-IL-2 and NOG–IL-15 Tg mice were 1,000–2,000 pg/ml and 50-100 pg/ml, respectively, and none was detected in the serum of non-transgenic mice.

Kinetic evaluation of human cell engraftment and antitumor efficacy of Herceptin®(1)

- Whole-body irradiation of adult NOG-Tg mice (hIL-2).
- IV injection of freshly prepared CD3+ T cell-depleted HSCs from umbilical cord blood (CD34+).
- Examination of hematopoietic chimerism in target tissues at different timepoints post-engraftment using flow cytometry.
- Calculation of human immune cells as absolute cell number and percentage.
- SC injection of human BT-474 tumor cells into HSC-humanized mice.
- Treatment of tumor bearing mice with Xolair® or Herceptin®.

Ex vivo evaluation of human NK cell functionality (^{51}Cr release assay)

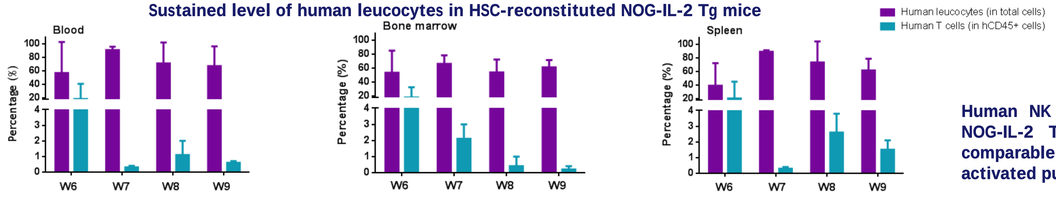
- Target cells: Raji lymphoma cells.
- Effector cells : human NK cells (mouse spleen, ratio 10:1).
- ^{51}Cr release assay : 45 µCi/10^6 Raji cells.
- Treatment : IgG or Rituximab (0, 100, 1000 ng/mL).
- Each condition in quadruplicate.



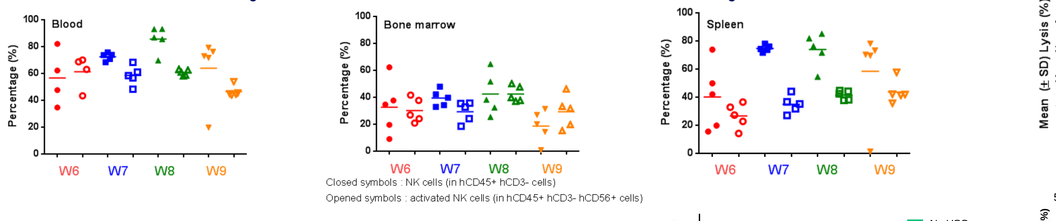
This work was supported by a grant from bpifrance (formerly OSEO) and ANEDO
All procedures with animals were submitted to the Animal Care and Use Committee of Oncodesign (OnComEt).
Collection of human biological resources by registered Oncodesign BRC.
(1)Principe d'éthique de l'expérimentation animale, Directive n°2010/63 CEE du 22 septembre 2010, Décrêt n°2013-118 du 01 février 2013.

Results

Sustained level of human leucocytes in HSC-reconstituted NOG-IL-2 Tg mice



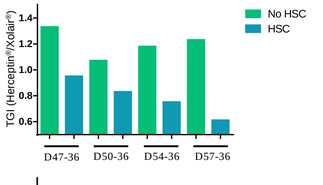
Presence of high level of activated NK cells in HSC-humanized NOG-IL-2 Tg mice

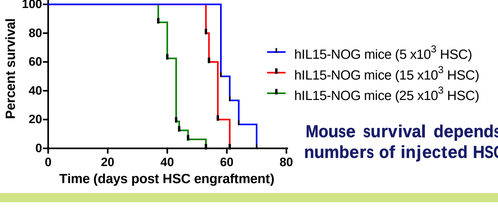


Closed symbols : NK cells (in hCD45+ hCD3- cells)
Opened symbols : activated NK cells (in hCD45+ hCD3- hCD56+ cells)

RTV	no HSCs		HSCs	
	Xolair®	Herceptin®	Xolair®	Herceptin®
Mean	**1.82**	**1.75**	**1.74**	**1.23**
SD	0.70	0.78	1.19	0.37
Median	**1.78**	**1.45**	**1.57**	**1.18**

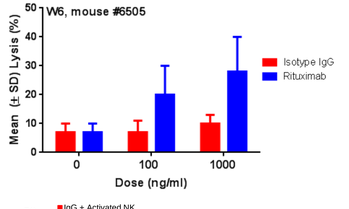
Antitumor effect of Herceptin® was enhanced in HSC-engrafted NOG-IL-2 Tg mice compared to non-humanized mice

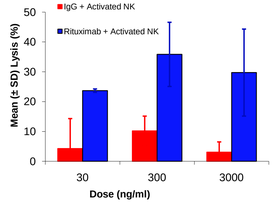


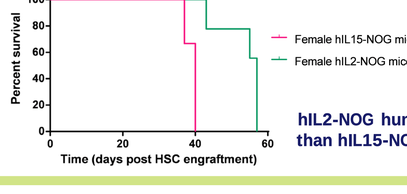


Mouse survival depends on the numbers of injected HSCs

Human NK cells in spleen from HSC-humanized NOG-IL-2 Tg mice are functional (results are comparable to that obtained with human IL-2 activated purified NK cells used as effector cells)







hIL2-NOG humanized mice survive longer than hIL15-NOG humanized mice

Conclusions and perspectives

- NOG-IL-2 Tg mice were successfully reconstituted with HSCs and high levels of human activated NK cells were detected in peripheral blood and central lymphoid organs.
- The ADCC mediated lysis through human activated NK cells was demonstrated using an *ex vivo* ^{51}Cr release assay.
- *In vivo* NK-mediated ADCC of Herceptin® was demonstrated in these Tg mice simultaneously engrafted with human immune system and human breast carcinoma.
- Quantification of infiltrated human NK cells in tumor using immunohistochemistry is on going.
- Further *in vivo* POC studies will be investigated with humanized NK-NOG mice xenografted with other human solid tumors treated with therapeutic antibodies.