IN VIVO ADCC-MEDIATED ANTITUMOR ACTIVITY STUDY WITH NEW PRECLINICAL HUMANIZED MODELS

 # B11

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Introduction

Experimental systems to effectively evaluate therapeutic antibodies are urgently needed. One of the mechanisms used by antibodies to kill tumor cells is antibody-dependent cellular cytotoxicity (ADCC) in which natural killer cells (NK) are the main mediator. The generation of humanized mice as immunodeficient mice (such as NOG) engrafted with hematopoietic stem cells (HSC) demonstrated their usefulness for studying the human immune system. Nevertheless, one issue of this humanized model is the incomplete maturation of NK cells. To circumvent this lack of functionality, the Central Institute for Experimental Animals (CIEA) has developed two severe immunodeficient NOD/Shi-scid-IL2Rγnull (NOG) mouse strains expressing the human interleukin 2 (IL-2) (NOG-IL-2 Tg) or IL-15 (NOG-IL-15 Tg) trangene. After transfer of human cord blood derived hematopoietic stem cells (HSCs), CD3-CD56high CD16+/- human activated NK cells were observed predominantly in both NOG-IL-2 and NOG-IL-15 Tg mice (Katano et al., unpublished data). They expressed various natural killer (NK) receptors such as NKp30, NKp44, NKp46, etc., granzyme A and perforin, indicating their maturity in the mice (data not shown). Moreover, the human NK cell function was evidenced using an ex vivo ^{51}Cr assay demonstrating lysis properties of NK cells from collected spleen.
Genetic manipulation of the host continue to improve the ability of humanized mice to more accurately recapitulate the *in vivo* function of human immune cells such as NK cells.

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(1)

- Whole-body irradiation of adult NOG-Tg mice (hIL-2 and hIL-15).
- 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.
- Quantification of human IL-2 in plasma from NOG-IL-2 Tg mice using ELISA assay.

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.

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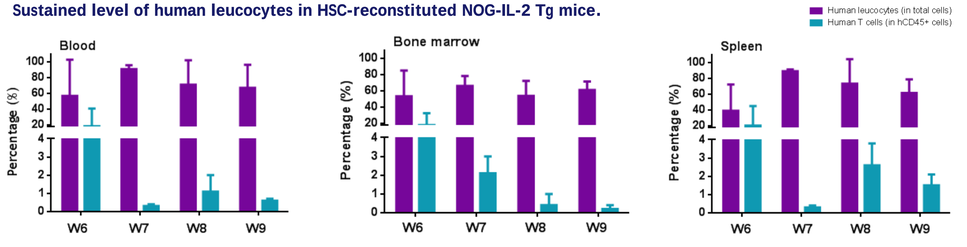
In 2013, Oncodesign has obtained accreditation from international AAALAC organism.
All procedures with animals were submitted to the Animal Care and Use Committee of Oncodesign (OnComEt).
Collection of human biological resources by registered Oncodesign BRC and establishment of *in vivo* human immune system reconstituted mouse models.
(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.

Conclusions and Perspectives

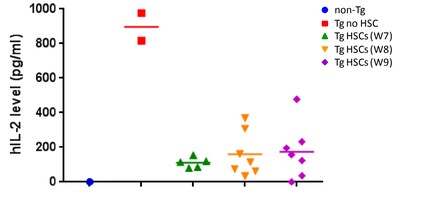
- NOG-IL-2 and NOG-IL-15 Tg mice were successfully reconstituted with HSCs and high levels of human activated NK cells were detected in peripheral blood and central lymphoid organs.
- The ADCC mediated lysis through human activated NK cells was demonstrated using an *ex vivo* ^{51}Cr release assay.
- Evaluation of *in vivo* NK-mediated ADCC of Rituximab in these Tg mice simultaneously engrafted with human immune system and human Burkitt's lymphoma is on going.

Results

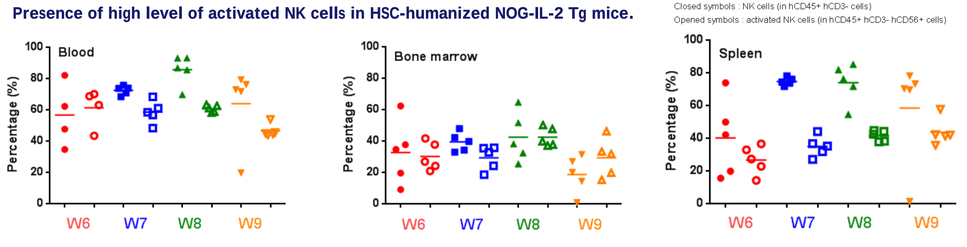
Sustained level of human leucocytes in HSC-reconstituted NOG-IL-2 Tg mice.



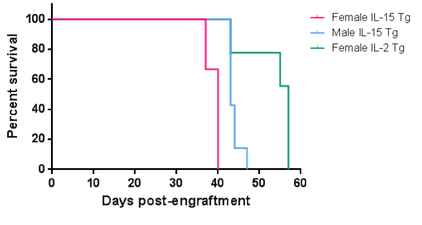
High human IL-2 plasma level in NOG-IL-2 mice.



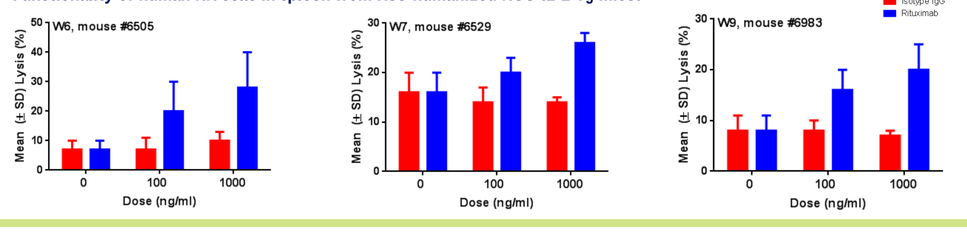
Presence of high level of activated NK cells in HSC-humanized NOG-IL-2 Tg mice.



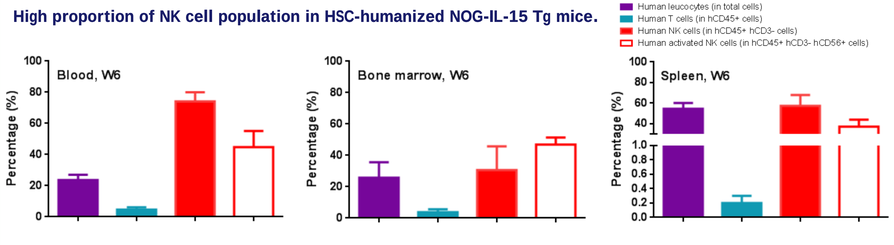
HSC-humanized NOG-IL-2 and NOG-IL-15 mice survive during 40 to 60 days.



Functionality of human NK cells in spleen from HSC-humanized NOG-IL-2 Tg mice.



High proportion of NK cell population in HSC-humanized NOG-IL-15 Tg mice.



Functionality of human NK cells in spleen from humanized NOG-IL-15 Tg mice.