Humanized mouse model for *in vivo* antibody-dependent cell-mediated cytotoxicity evaluation

4138

J.F. Mirjolet, C. Mignard, M. Hillairet de Boisferon, O. Duchamp, F. Bichat, P. Genne

Oncodesign Biotechnology, Dijon, France

ABSTRACT

Background: Importance of human immune system is needed to be evaluated early in preclinical relevant model in various therapeutic areas as cancer biology, hematopoiesis, innate and adaptive immunity, autoimmunity, allergy, infection diseases, vaccine development and transplantation. Humanized mice, i.e. immunodeficient mice engrafted with human hematopoietic cells, could be an appropriated powerful tool. However, the "optimal" humanized mouse is not fully defined and many parameters such as mouse strain, preconditioning treatment and human cell selection are of importance for quality and relevance of this preclinical model. We mainly focused on humanized mice in cancer therapy area and on the development of this tool as relevant model for preclinical evaluation of antibody and in particular as a way to highlight the *in vivo* antibody-dependent cell-mediated cytotoxicity (ADCC). Aims of this study were first to define optimal conditions for the engraftment of human NK cells in immunodeficient mice and second to validate a tumor-bearing humanized mouse model.

Methods: Various SCID background, patent-free strains of mice (CB17-SCID, NOD-SCID, SCID-Bg), various preconditioning regimens (whole body irradiation, mouse NK cell-depleting antibody), various route of transplantation (IP, IV) as well as various criteria to select human immune cells (PBMCs, Stem cells, FcγRIIIa 158 V/V) were tested. Absolute circulating human cell number using multicolor flow cytometry as well as quantification of human IgG by ELISA were performed on mouse blood and used as endpoint to validate the mouse humanization. Human BT-474 breast subcutaneous (SC) tumor-bearing NOD-SCID humanized mice were developed using trastuzumab (Herceptin®) as therapeutic antibody.

Results and conclusions: When preconditioned, i.e. whole-body irradiated and treated with mouse NK cell-depleting antibody, NOD-SCID mouse is a suitable mouse background. Both IV and IP transplantations of human PBMCs, selected based on NK proportion and/or phenotype are appropriated. Engraftment of human cells is more rapidly achieved by the IV route and as a consequence graft-versus-host disease appeared also more rapidly. Source of human hematopoietic cells, method of selection as well as phenotype of human cells were key factors for the humanization process. Growth of BT-474 SC tumor was validated in humanized NOD-SCID mice whatever phenotype of human PBMCs donor. Herceptin® antitumor activity was improved according to the FcγRIIIa phenotype i.e. FcγRIIIa V/V NK cells being more potent than F/F.

PRELIMINARY REQUIRED CONDITIONS

Mouse Strain

IMMUNOSUFFICIENCY

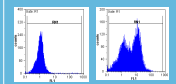
***Nude* Mouse**
Athymic -No T cells
Humoral immunity intact
High NK cell activity

CB17-SCID Mouse
No T cells or B cells
Moderate NK cell activity
Radiosensitive

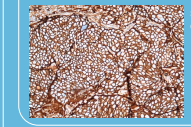
NOD-SCID Mouse
Defects in Innate Immunity
Reduced NK cell function
Impaired macrophage activation
Defective DC maturation
Lack of hemolytic complement

NOG / NSG Mouse
Defects in Innate & Adaptative Immunity
No NK cell function
Impaired macrophage activation
Lack of hemolytic complement

Target Expression



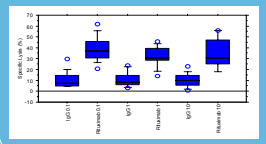
BT-474 tumor cells expressing Her-2



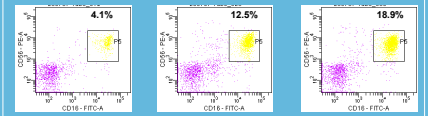
SC BT-474 tumor expressing Her-2

In vitro ADCC- mediated activity of antibody (^{51}Cr release)

Raji + Rituximab + Purified IL-2 activated hNKs



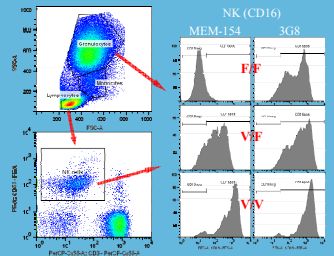
Selection of PBMCs donors based on the huNK level



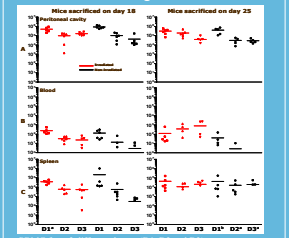
% CD56+CD16+ from 4.1 to 18.9 % mean 8.3 ± 4.3% (n=11)

Selection of PBMCs donors based on the CD16 (FcγRIII) polymorphism

ratio MFI FITC MEM-154/3G8	Granulocytes			NK			NK corrected		
	median	range -	range +	median	range -	range +	median	range -	range +
F/F (n=4)	1.031	0.789	1.754	**0.043**	0.030	0.055	**0.041**	0.024	0.057
V/F (n=5)	1.188	0.467	1.318	**0.476**	0.406	0.589	**0.447**	0.381	0.529
V/V (n=1)	0.901	-	-	**0.719**	-	-	**0.798**	-	-

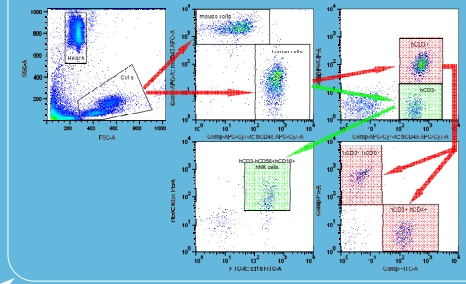


Humanization of mice proportional to huNK level in original PBMCs [1]

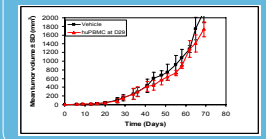


PBMC from 3 different donors (D1, D2 and D3) and sacrifice at day 18 or day 25 after irradiation.
*One dead mouse *per* group. ^{3}One mouse discarded *per* group.

Selection of humanized mice based on the huNK level

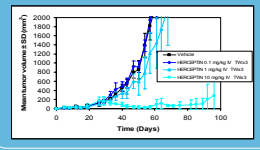


No effect of PBMCs on BT-474 tumor growth

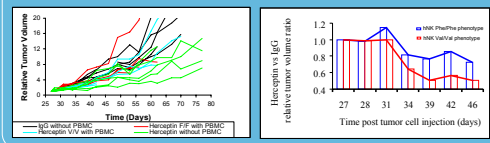


Whole body irradiated NOD-SCID mice
Estradiol supplementation in drinking water
Tumor cells:BT-474 SC (matrigel)
Randomisation parameters: TV, circulating hNKs, circulating hu IgG...
First treatment:100-200 mm^3
hPBMCs IP:5x10^7 cells/500 µl/mouse (V/V or F/F)
Herceptin® / IgG:0.1, **1** and 10 mg/kg/inj
Treatment Schedule:(Q3Dx2)x3

Choice of suboptimal dose of Herceptin® in BT-474 tumor model

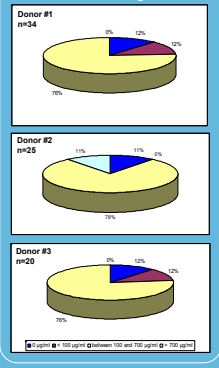


In vivo proof of concept



HUMANIZED MODEL CHARACTERIZATION

Selection of humanized mice based on the huIgG level



Animal experiments were performed according to ethical guidelines of animal experimentation and the English guidelines for welfare of animals in experimental neoplasia. All procedures with animals were submitted to the Animal Care and Use Committee of Pharmacy and Medicine University (Dijon)
(1) 2008 AACR poster #1039, Oncodesign/UCB-Celltech

CONCLUSIONS

- The latest developments in antibody engineering are allowed the generation of superior antibody therapeutics, with strategies ranging from complement-mediated and ADCC enhancement. Consequently, the development of a small animal model, with a human immune system, is needed for evaluating these agents.
- Host mouse strain, PBMC source, selection of human donor cells, irradiation, tumor expressing target are several factors to consider.
- Based on our experience, we optimized a panel of experimental conditions and acceptance criteria to succeed in the preclinical evaluation of these new therapeutics antibodies.