Humanized SCID mouse as tool for evaluation of B cell targeting therapies

Jean-François Mirjolet, Zina Koob, Francis Bichat, Caroline Mignard
Oncodesign®, Dijon, France

715

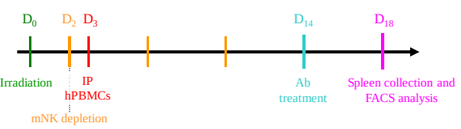
Abstract

Background: Antibody strategies are of huge importance in targeted therapies drug development. However, most of the time, antigen is specific of human species with bad cross-reactivity with mouse antigen, leading to difficulties to apply regular mouse models. The "gold" model for the preclinical evaluation of antibody strategy should have cells expressing human antigen, to analyze Fab binding activity but should also contain human immune effector cells in order to be able to test the antibody Fc mediated activity (Antibody Dependent Cell Cytotoxicity, ADCC). We here propose the use of CB17-SCID mouse strain having the ability to exhibit complement activation, useful to test the CDC (Complement Dependent Cytotoxicity) activity of antibody. This strain of mice supports the engraftment of human peripheral blood mononuclear cells (hPBMCs), containing human effector cells as NK cells and moncoytes, responsible of ADCC activity. In our case, the target of interest is expressed on human B lymphocytes, whatever the tumoral or healthy origin. The engrafted hPBMCs contained both the NKs as effector cells but also the human B cells as target cells.
Methods: Whole body irradiated (D-3) female CB17-SCID mice were treated twice (Q7Dx2) (starting at D-2) with subcutaneous injections of mouse NK cell-depleting antibody TM-Beta 1 and received an intraperitoneal injection of freshly prepared hPBMCs at D0. At D11 mice received a single intravenous injection of trastuzumab used as negative control, of new B cell targeting antibody or rituximab used as positive control (10 mice *per* group). At D15, mice were sacrificed to collect spleen. Single cell suspensions were prepared from each collected spleen, labeled with mCD45, hCD45, hCD19 and hCD20 antibodies before FACS analysis. Absolute cell number as well as percentage of human B cells were calculated. Statistical comparisons were performed using Mann-Whitney U test after having discarded outlier values by Dixon Q test.
Results: All mice injected with hPBMCs were found humanized at the time of sacrifice. Percentage of human leukocytes in mouse spleen ranged from 0.6 to 59.4% with a mean value of 12.9 ± 12.5 % and a median value of 8.3 %. Considering the human B-cell percentage, only 3 out of 80 values were considered as outliers. In the trastuzumab treated group (10 mg/kg), the spleen contained about 6 % of human B cells (within the human leukocytes, i.e. hCD45+). When the mice were treated with rituximab at 2 mg/kg, a significant decrease in the percentage of human B cells was evidenced in the mouse spleen, with about 1.5% of human B cells. A dose-dependent decrease in human B cells was also observed in mouse spleen when mice were treated with the new B-cell targeting antibody (1.4 ± 1.1 % and 3.8 ± 2.2 % of human B cells for 10 mg/kg and 0.02 mg/kg respectively). The decrease starts to be significant from 0.1 mg/kg of the new B-cell targeting antibody.
Conclusions: These results show that this humanized mouse model is suitable to evaluate B-cell targeting therapies. Humanization level as well as sensitivity to treatment are compatible with dose-response analysis and then offer the possibility to use this model for benchmarking of human B-cell targeting therapy tested on human target in the presence human effectors. Moreover, this model could be applied to other targets expressed on human immune cells.

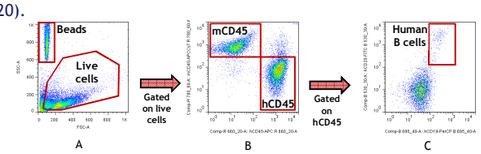
Material and Methods

- Whole-body irradiation of female adult SCID mice.
- Repeated SC injections of mouse NK cell-depleting antibody TM-Beta 1.
- IP injection of freshly prepared hPBMCs (hCD45+).
- Treatment with new B cell targeting antibody (γ B-Ab), rituximab or trastuzumab.
- Characterization of human leucocytes and B cells (hCD45, hCD19, hCD20) by flow cytometry in target tissues.
- Calculation of human leucocytes and human B cells as absolute cell number and percentage.
- Statistical comparisons using Mann-Whitney U test after having discarded outlier values by Dixon Q test.

Study design for engraftment of mice with hPBMCs and evaluation of human immune cell targeting therapies .

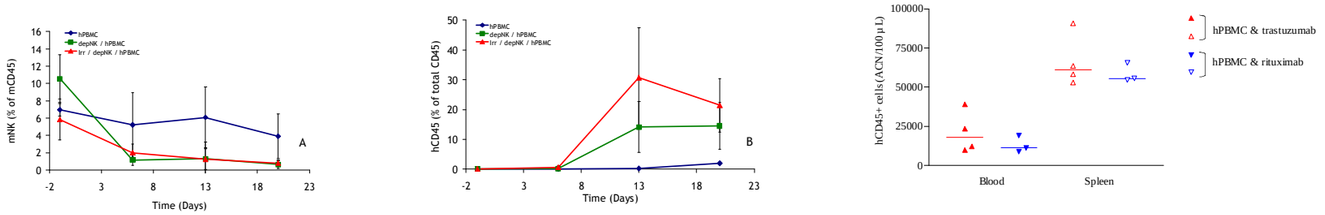


(A) Forward scatter by side scatter plot of all collected events from the spleen of a SCID mouse 15 days after hPBMC engraftment, showing gating strategy. (B) Contour plot of gated events from panel A, showing human hematopoietic chimerism by plotting mCD45 by hCD45. (C) Contour plot gated on human CD45+ cells from panel B plotted to show human B cells (hCD19 & hCD20).

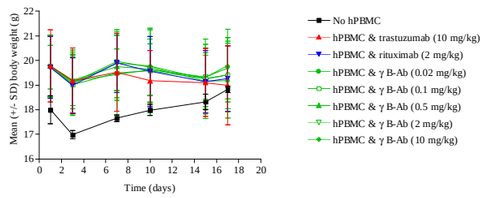


Results

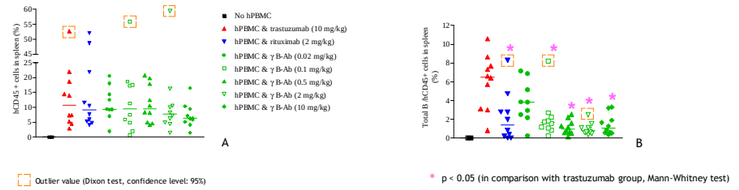
Validation of the study design for humanization of SCID mice. Protocols including depletion of mouse immune system with whole-body irradiation and/or injection of mouse NK depleting antibody prior to process engraftment (A) show an improved percentage of human CD45+ cells in peripheral blood (B). The level of human CD45+ cell engraftment after IP hPBMC injection is depending on the lymphoid tissue examined (C). Blood contains human cells at lower proportion than in spleen.

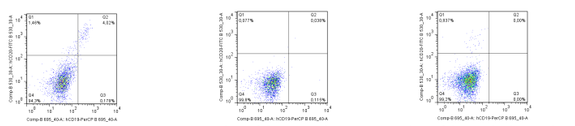


Body weight of mice over the time. The experiment reach an end point within 14 days post-transplantation and before the onset of xenogeneic graft-versus-host disease (GVHD) symptoms that can be monitored by weight loss.



(A) Human leucocytes after hPBMC transplantation into SCID mice. The individual percentages of hCD45+ cells is shown with median (n = 10). Treatment of mice with B cell targeting antibodies did not influence human engraftment level. (B) B cell population in humanized SCID mice after treatment with trastuzumab, rituximab or new B cell targeting antibody at the indicated doses. The individual percentages of human B cells is shown with median (n = 10). As expected rituximab and new B cell targeting therapy resulted in a marked B cell depletion in spleen from engrafted mice.





Representative example of human B cells analysis from mice humanized with hPBMC and treated with different antibodies (trastuzumab, rituximab or new B cell targeting antibody) when gating on leucocytes in spleen sample.

Conclusions

- We have set up the whole process to reconstitute the immunodeficient SCID mice with mature human PBMCs.
- The protocol outlines the steps needed to verify engraftment levels in humanized mice.
- As an example, we have demonstrated that the humanized SCID mice model is adapted to screen B cell targeting therapies for oncology purpose.
- The use of the model could be applied to all human cells (T,B,…) targeting therapies in multiple therapeutic areas.