HUMANIZED MOUSE MODELS

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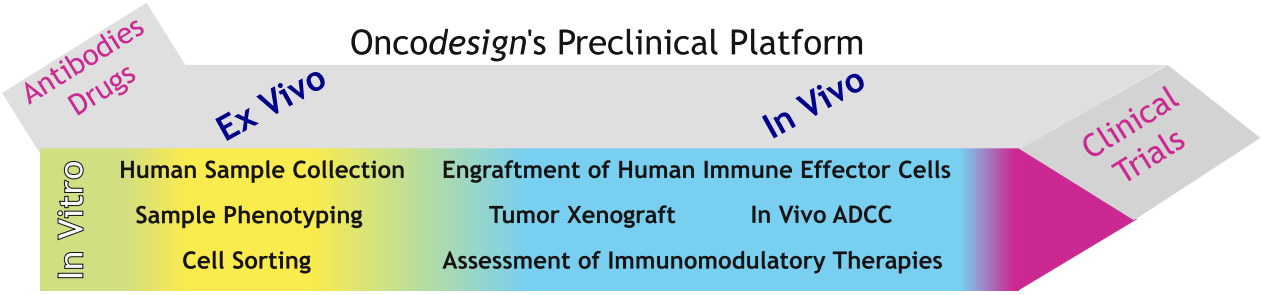
How to evaluate the contribution of the human immune system in a preclinical protocol?



The immune system is a key component for therapeutic efficacy and much research focuses on the activation of immune effector cells. The evaluation of immunomodulatory therapies in a preclinical setting requires the engraftment of human immune effector cells into immunodepleted mice. Oncodesign's humanized mouse models enable the translation of immunotherapies to human.

Key Benefits

- Profiling the immunomodulatory activities of compounds or antibodies
- Evaluation of antitumor activity related to antibody dependent cell cytotoxicity (ADCC)
- Study of various human diseases (autoimmune, infectious disorders...) or therapies (vaccines...)

Protocol

- Mouse strain selection (levels of immunodeficiency, use of transgenic animals)
- Murine immune effector cell depletion by irradiation, cell-depleting antibodies...
- Selection of human blood donors, immunophenotyping and sorting of human immune effector cells (PBLs, PBMCs, NK, T or B-cells, monocytes, macrophages, HSC, CBMC...)
- Selection of the human tumor model to be xenografted (antitumor activity)
- Inoculation (IP, IV...) of human immune effector cell population
- Flow cytometry analysis of engrafted human immune effector cells
- Evaluation of the antitumor activity and the in vivo ADCC contribution

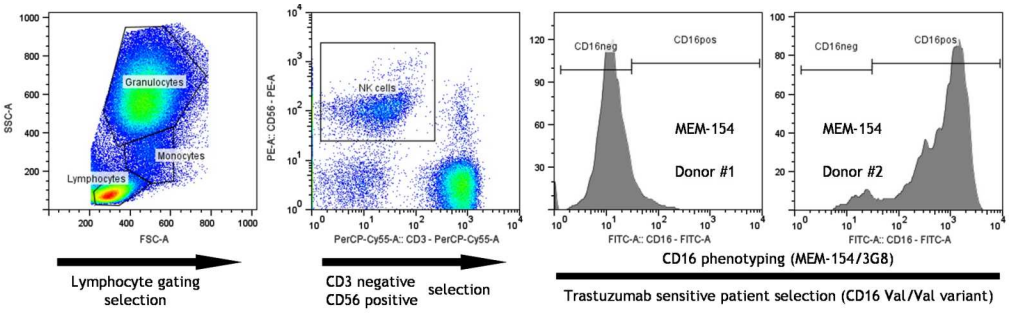
Human Sample Phenotyping and Cell Sorting

Human sample selection:

- Flow cytometry phenotyping
  Total hPBMC/sample
  $CD56^+/CD16^+$ NK cells/sample
- Negative or positive selection
- Magnetic beads or cell sorter selection

Readout:

- Selection of human samples
  (i.e. % of NK, phenotype of NK...)
- Cell subpopulation enrichment



Discrimination of NK cells harboring CD16 (Val/Val) variant antigen versus CD16 (Phe/Phe) from human donors using multiple staining flow cytometry analysis.

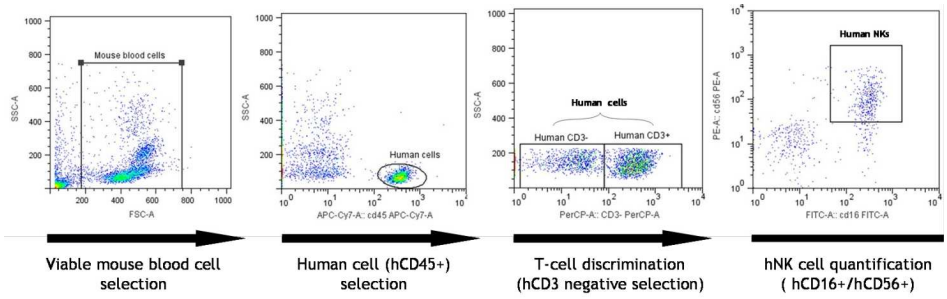
- CD16 (Val/Val) variant NK cells enhance trastuzumab antitumor activity in the clinic and can be used for the proof of concept of humanized mouse models.

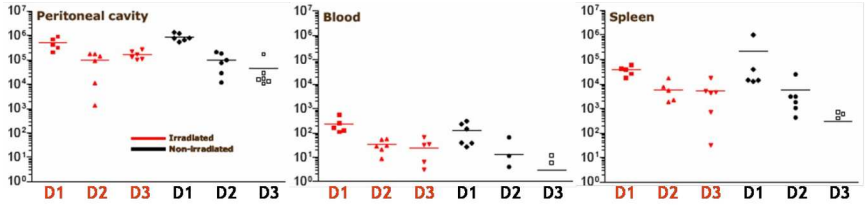
Characterization of Human Immune Effector Cell Populations

Engraftment follow-up:

- Identification of human immune cells in mouse blood or tissues by flow cytometry
- Persistence of the engrafted human immune effector cells for at least 2 months

Quantification of hNK cells in mouse blood using a 4-color flow cytometry analysis.





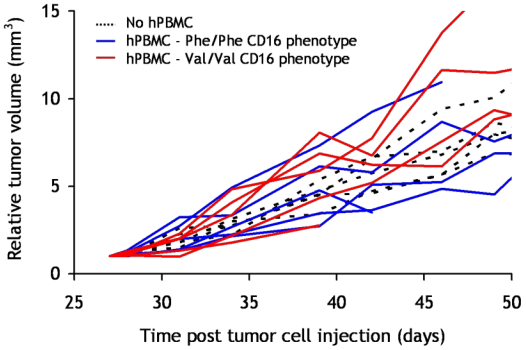
Absolute number of hNK cells from irradiated (red) and non-irradiated (black) mice injected with hPBMCs from 3 different donors (D1, D2, D3).

Assessment of Antitumor Activity and In Vivo ADCC Contribution

3-step study protocol:

- 1) Verification of the tumor growth in the presence of immune effector cells
- 2) Determination of the suboptimal drug dose in the selected tumor model
- 3) Run of the tumor model with immune effector cells and drug treatment

1) BT-474 tumor growth with hPBMCs

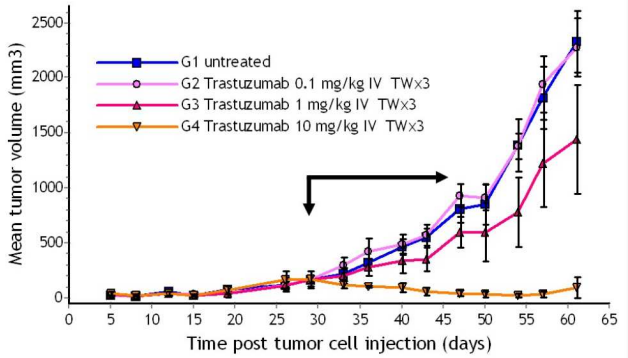


Relative individual tumor volume growth curves in NOD.*Scid* mice bearing subcutaneously BT-474 breast adenocarcima in the presence (blue and red curves) or absence (dashed black curves) of fresh hPBMCs. The hPBMCs were intraperitonealy injected after selection from healthy donors according to the Phe/Phe (blue) or Val/Val (red) phenotype of CD16 antigen of hNK cells.

Readout:

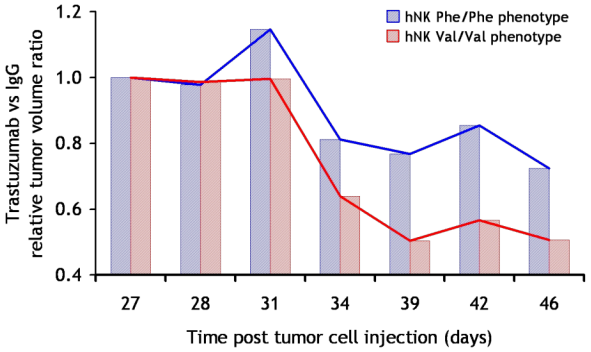
- Proof of concept of the translational use of humanized mouse models
- Assessment of drug antitumor activity in humanized mouse models
- Determination of in vivo ADCC
- Evaluation of the immune effector cell contribution in the therapeutic efficacy

2) BT-474 tumor growth treated with trastuzumab



Mean tumor volume growth curves in NOD.*Scid* mice bearing BT-474 human breast adenocarcima SC injected at D0 and treated from D29 with IV injections of trastuzumab twice weekly for 3 consecutive weeks.

3) BT-474 tumor growth with hPBMCs and treated with trastuzumab



Ratio of relative mean BT-474 tumor volumes between mice treated with the suboptimal dose of trastuzumab and control IgG. These ratios show that the decrease of tumor growth in trastuzumab-treated mice is more pronounced in presence of hNK cells exhibiting a Val/Val (red) than for the Phe/Phe (blue) CD16 antigen phenotype.

References Available on our Website in the Media Section

- 2010 AACR Poster, #394
- 2008 AACR Poster, #1039

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