Humanized mouse models for the preclinical evaluation of novel cancer immunotherapy options

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Background

The preclinical evaluation of many novel immune therapies requires the use mouse models with a functional human immune system. In previous studies, we have demonstrated that either peripheral blood mononuclear cells (PBMCs) or subpopulations of PBMCs such as T- and NK-cells or hematopoietic stem cells (HSC) can be used to establish a humanized immune system with functional T-, B-, and NK cells in immunodeficient mice. By transplanting either cell-line or patient-derived tumor xenografts into humanized mice, we successfully generated a fully human tumor-immune-cell model for several tumor entities. Finally, we validated the functionality of these models using either immune-checkpoint inhibitors, cell therapies or immune cell engagers.

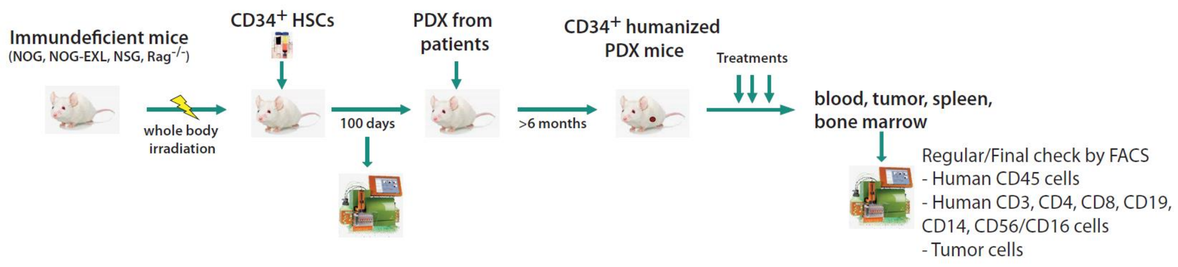
Methods

HSC-humanized mice were generated by i.v. injection of CD34+ stem cells into immunodeficient NOG mice. PBMCs or enriched T- or NK-cell populations from a curated set of blood donors were used to humanize mice by either single or multiple i.v. injections. CDX and PDX models from different entities (i.e. colon cancer, HNSCC, breast cancer and lymphoma) were transplanted into these humanized mice which were used to evaluate novel immune therapy options. The presence of immune cells and their activation status was analyzed by flow cytometry in blood and tumor samples.

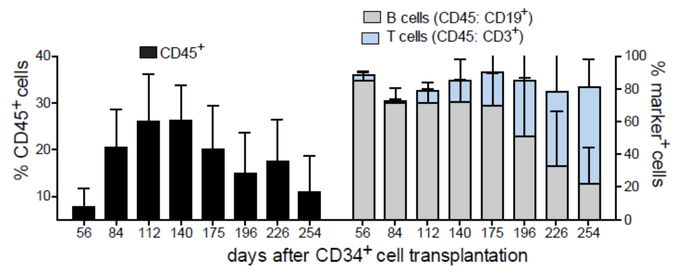
Results

CD34+ Humanization

❖ **Experimental set-up:**

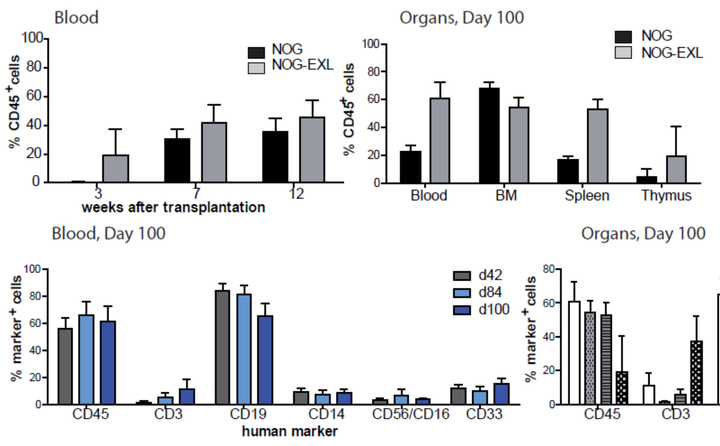


❖ **Immune cell development in CD34+ humanized NOG mice:**



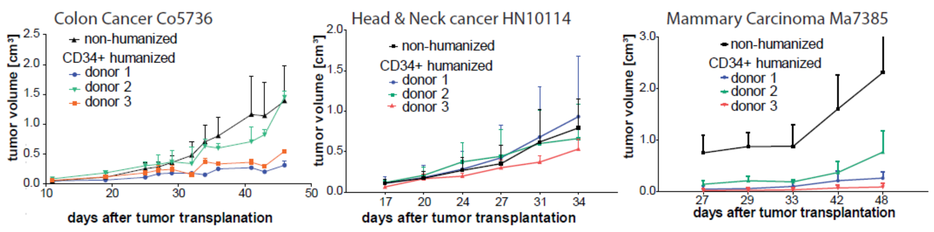
- From day 84 (12 weeks) on: hCD45+ cells above 20% until day ~200
- B cell development after 8 weeks (56 days); B cell compartment decreases over time
- T cell development after 100 days
- Disadvantage: rarely NK cell or myeloid cell development

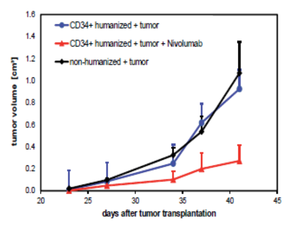
❖ **Immune cell development in CD34+ humanized NOG-EXL (hGM-CSF/hIL-3) mice compared to NOG:**



- Faster and better engraftment of human immune cells (CD45+) in NOG-EXL mice in blood and spleen
- Development of different immune cell subsets:
  - CD14+ monocytes
  - CD56/CD16+ NK cells
  - CD33+ myeloid cells

❖ **PDX tumor models on CD34+ humanized mice & evaluation of a checkpoint inhibitor in lymphoma PDX model:**

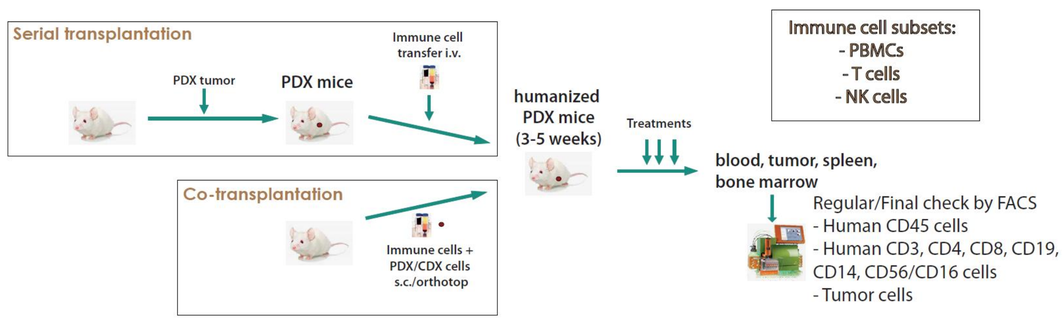




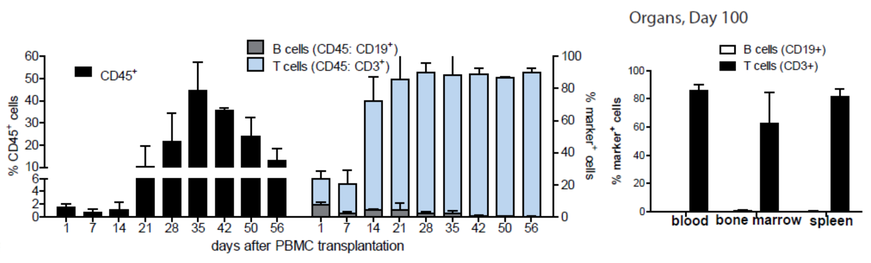
- PDX tumor engraftment can be influenced by HSC donor
- Lymphoma growth delay in the humanized PDX model by immune checkpoint inhibitor Nivolumab
- Exhausted T cells can be reactivated by checkpoint inhibition in humanized PDX model

Immune Cell Subset Humanization

❖ **Experimental set-up:**

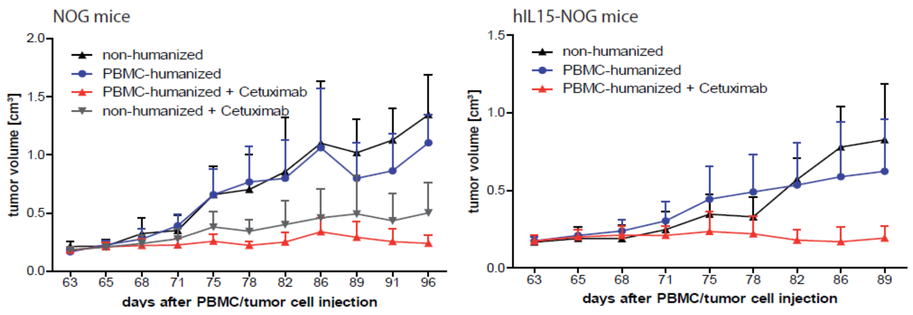


❖ **T cell engraftment in PBMC-humanized NOD/SCID mice:**



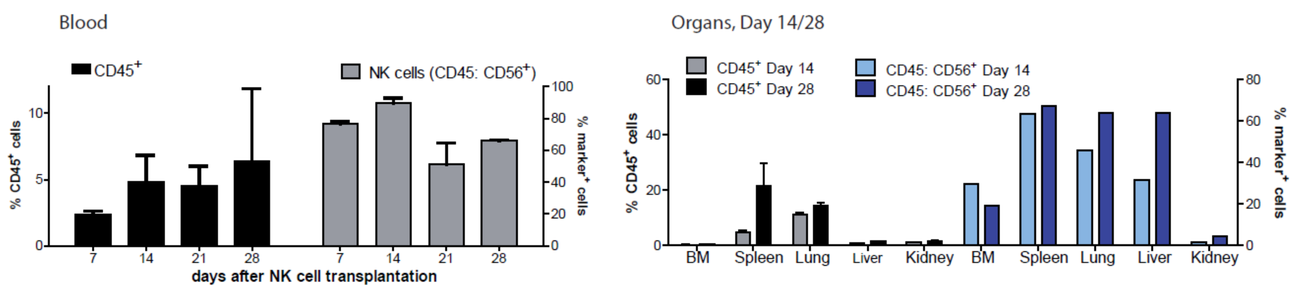
- mainly T cell engraftment
- after 3 weeks above 20% CD45+ cells
  - >90% of CD45+ cells are T cells
- engraftment of T cells in bone marrow and spleen
- disadvantage: rarely NK cell or myeloid cell

❖ **Efficacy of Cetuximab on PBMC-humanized HNSCC PDX (HN11841) tumor bearing mice:**



- Cetuximab in combination with PBMCs shows antibody-dependent celluluar cytoxicity (ADCC)
- In hIL15-NOG mice, the same efficacy of ADCC on tumor growth is observed

❖ **NK cell engraftment of in vitro-expanded NK cells in hIL15-NOG mice**



- NK cells engraft well in hIL15-NOG mice with a proliferation peak on day 14
- NK cells engraft mainly in spleens and lungs

Conclusion

We have demonstrated successful engraftment of HSCs into immunodeficient mouse strains generating mice with a functional human hematopoiesis. Furthermore, we have established human tumor-immune-cell models of different entities using CDXs or PDXs in combination with different donor derived immune cell subsets as effector cells. These models have been used for preclinical evaluation of novel checkpoint inhibitors and immune cell engagers. Our human tumor-immune-cell models allow translational preclinical studies on tumor immune biology as well as evaluation of novel therapy options, drug combinations and biomarker identification and validation.

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