Humanized immune-PDX mouse models for 10 different tumor indications in translational immune-oncology research

Stecklum, Maria[1], Wulf-Goldenberg, Annika[1]; Paterka, Magdalena[1]; Brzezicha, Bernadette[1]; Fichtner, Iduna[1]; Hoffmann, Jens[1]

[1] Experimental Pharmacology & Oncology GmbH, Berlin, Germany

EUROPÄISCHE UNION
Europäischer Fonds für regionale Entwicklung
Investition in Ihre Zukunft

Background and Aim

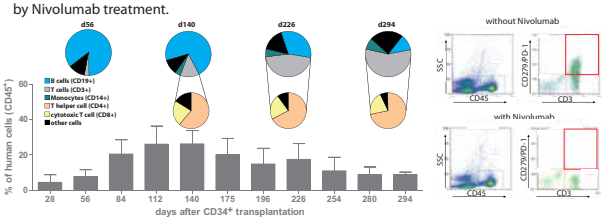
The preclinical evaluation of novel immune checkpoint modulators is dependent on models with functional human immune cells. In previous experiments, we established humanized mouse models by transplantation of hematopoietic (stem) cells to immunodeficient mice.
Transplantation results in the engraftment of a functional human immune system. By co-transplantation of human patient-derived xenografts (PDX) we successfully generated a fully human tumor-immune-cell model in mice. These humanized immune-PDX models for melanoma and lung cancer were further characterized by treatment with imunotherapeutic drugs like the CTLA-4 inhibitor Ipilimumab (Ipi) and the PD-1 inhibitor Nivolumab (Nivo). In our current studies we investigated the functionality of the human immune cells and evaluated concepts for combination therapies i.e. with chemotherapy or radiation. We further established new human immune-PDX models from other tumors entities like lymphoma, pancreatic or breast cancer.

Summary and Outlook

In summary, HSC transplantation into immunodeficient mice generates a humane immune system. PDX from different entities are characterized by a differential expression of PD-L1 comparable to the clinical situation. Depending on PD-L1 expression PDX showed a tumor growth delayed on humanized mice compared to non-humanized mice. Our humanized models enable preclinical studies on tumor immunology, evaluation of new immune therapies and combinations, as well as the identification and validation of biomarkers for tumor immune therapy.

Humanization of mice with CD34+ stem cells (HSC) from cord blood

1x10^5 HSC isolated from cord blood by MACS separation were transplanted intravenously (i.v.) into three week old irradiated nod scid gamma mice. Every 4 weeks, blood was collected and screened by FACS for human immune cells (huCD45$^+$ marker expression) for long time monitoring of engraftment of HSC. In the next step, functionality of developed human T cells was investigated by Nivolumab treatment.

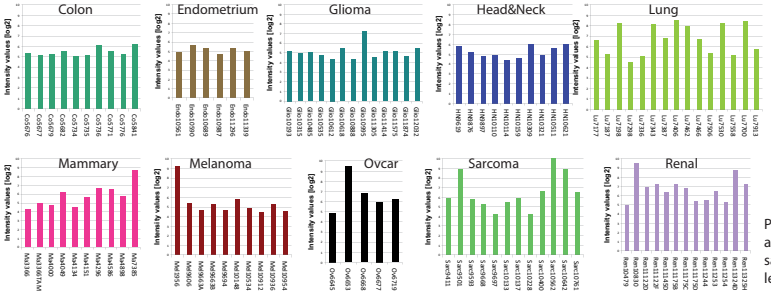


CD34$^+$ stem cells from cord blood induced an humanized immune system in mice and long time engraftment. Engraftment potential is in some cases donor dependent. Irradiation influences the early steps of humanization (data not shown). Functionality of T cells was determined by inhibition of PD-1 expression of these cells through treatment with Nivolumab.

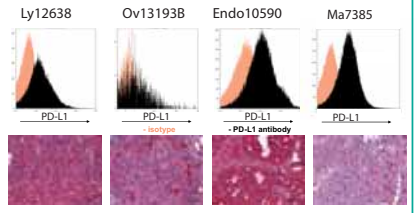
PD-L1 expression of patient-derived xenografts (PDX)

A comparative mRNA expression analysis of PD-L1 with Affymetrix chips was performed for different PDX entities (selective panel is shown, more are available on www.epo-berlin.com). On protein level, PD-L1 expression of PDX was determined by flow cytometry and immunohistochemistry.

Affymetrix chip analysis for PD-L1 expression in PDX



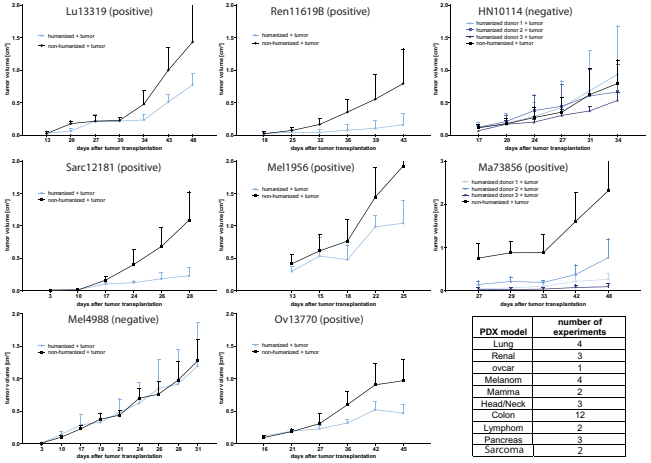
PD-L1 expression by immunohistochemistry and flow cytometry



PDX of different entities with differential expression of PD-L1 are available. PD-L1 expression of PDX correlated between patient samples and passages of PDX (high expression of PD-L1 on RNA level and on protein level in investigated PDX).

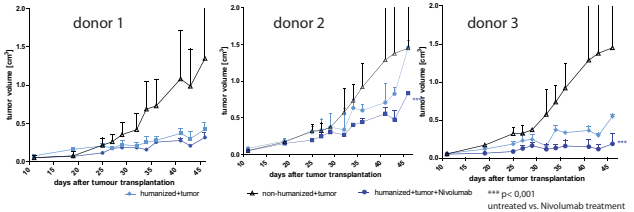
Humanized patient-derived xenograft models on humanized mice

Tumor growth of PDX models from different entities and with different PD-L1 epxression on humanized mice

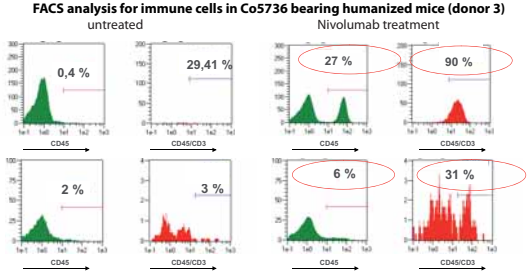


PDX model	number of experiments
Lung	4
Renal	3
ovcar	1
Melanom	4
Mamma	2
Head/Neck	3
Colon	12
Lymphom	2
Pancreas	3
Sarcoma	2

Tumor growth of Co5736 (high PD-L1 expression) in humanized mice from three different donors under immunoterhapy treatment

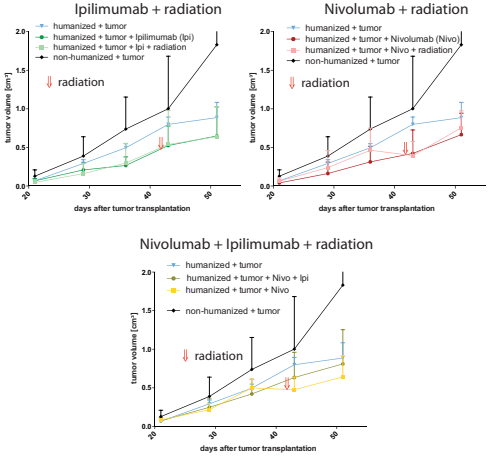


FACS analysis for immune cells in Co5736 bearing humanized mice (donor 3)



Tumor growth of PDX models under immunotherapy treatment in combination with radiation

Panc12559 (medium PD-L1 expression)



Growth of human PDX from 10 different entities was confirmed on humanized mice. Engraftment delay seems to be dependent on PD-L1 expression of PDX (the higher PD-L1, the higher growth delay). Lymphocyte and T cell population in blood was increased in tumor bearing humanized mice and could be enhanced by Nivolumab treatment. Efficacy of immunotherpy treatment seemed to independent from stem cell donor. Radiation therapy showed a positive efffect in combination with immunotherapy treatment.

Corresponding address: maria.stecklum@epo-berlin.com; jens.hoffmann@epo-berlin.com