Preclinical model of patient-derived tumor xenograft in humanized mice

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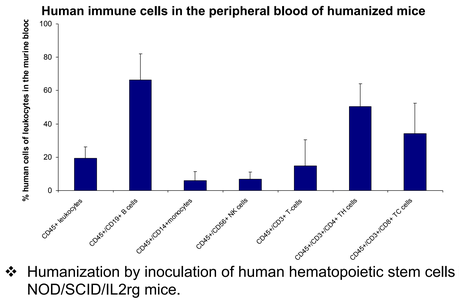
Background and Aim

The stimulation of the endogenous antitumor immunity has the potential to achieve clinically significant tumor regression. The recent clinical success with antibodies interfering with immune checkpoints on T cells (PD1 and CTLA-4) has motivated oncology research world wide to focuses on new immunotherapy approaches.
The identification and validation of new targets for antitumor immune therapy is still a challenge for the preclinical research as the classical syngeneic tumor models are of limited translational value and the patient-derived human tumor xenograft models (PDX) are growing on immunodeficient animals.
To overcome these constraints our aim is the development of PDX models on mice with a functional human immune system to improve predictability of drug efficacy and safety.

Summary and Outlook

- We reconstituted a functional human immune system by engrafting human hematopoietic stem cells in immunodeficient mice.
- Repopulation of mouse organs with human hematopoietic cells and maturation of human T and B cells has been demonstrated.
- Fragments from patient-derived melanoma were successfully engrafted on our humanized mouse models
- No evidence for tumor rejection was observed, although engraftment was accompanied by an increase of human T cells in the peripheral blood.
- Treatment with the checkpoint-inhibitor ipilimumab decelerate the tumor growth in one of two tested humanized mouse models.
- Accumulation of cytotoxic T cells were found in ipilimumab responsive tumors compared to ipilimumab non-sensitive tumors.
- Treatment with the checkpoint-inhibitor nivolumab in combination with ipilimumab decelerate the tumor growth in the humanized PDX-model Mel10936 and decreased the RNA-expression of PD-1 and PD-L1 in the tumor.
- Our humanized mouse models enable a appropriate preclinical assessment of immune-based therapeutic antitumor strategies especially when combining the humanized mouse with patient-derived tumor xenografts.

Engraftment and differentiation of hematopoietic cells and detection of human immune cells in the peripheral blood

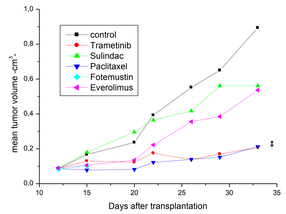


- Humanization by inoculation of human hematopoietic stem cells in NOD/SCID/IL2rg mice.
- Efficient engraftment of human stem cells and differentiation in all types of immune cells can be shown.
- Cytokine treatment resulted in no significant differences in the frequency of human leucocytes (CD45⁺ cells). Human IL-2 and IL-15 treatment resulted in a higher number of human CD14⁺ monocytes and CD56⁺ NK cells and in a lower number of CD19⁺ B cells (data not shown).

Development and characterization of patient-derived melanoma xenograft models

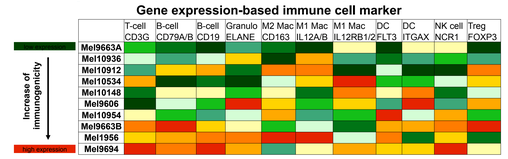
- More than 10 patient-derived xenografts from skin cancer surgery have been established on immunodeficient mice .
- The tumor growth of patient-derived melanoma xenografts were monitored and showed differential growth behavior which demonstrates the heterogeneous tumor growth of skin cancer.

Tumour ID	Histology	Patient gender	Relevant mutations found
1956	Malignant melanoma	male	BRAF (V600E), TP53
2092	Malignant melanoma	male	BRAF (V600E)
4988	Malignant melanoma	not available	no mutations detected
9363	Uveal melanoma	female	GNAQ
9606	Malignant melanoma	male	NRAS, ATM
9663A	Acral lentiginous melanoma (ALM)	female	no mutations detected
9663B	Acral lentiginous melanoma (ALM)	female	no mutations detected
9694	Malignant melanoma	male	BRAF (V600E), NOTCH1
10148	Malignant melanoma	female	BRAF (V600E)
10534	Malignant melanoma	not available	NRAS
10912	Acral lentiginous melanoma (ALM)	not available	NRAS
10936	Malignant melanoma	not available	BRAF (V600E), TP53
10954	Acral lentiginous melanoma (ALM)	female	no mutations detected

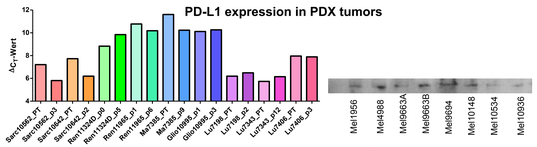


- The PDX models were characterized for their response to a panel of standard of care drugs at dose close to the respective clinical treatment schedules. A differential in vivo sensitivity of the melanoma PDX to these drugs was observed.
- The histology and tissue architecture of the xenografts were found similar to those of the original patient sample, with an increase of malignant cell content in the xenografts (data not shown).

Immunophenotype and PD-L1 expression



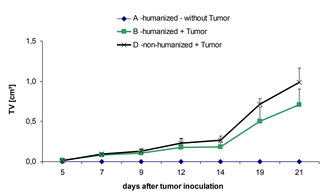
- Gene expression-based immune cells markers were used to generate an immunophenotype of PDX
- The calculated immunogenicity did not correlate with the load of mutations (number of SNPs)



- PD-L1 expression in tumor samples from patients (PT) and in xenografted tumors were determined. Most of the models remained a similar PD-L1 RNA-expression over passaging except sarcoma samples. The analyzed lung PDX samples had the highest expression of PD-L1.
- Protein analysis of PD-L1 showed a differential expression of PD-L1 in PDX melanoma tumors. The immunogenic model Mel9663B had a high content of PD-L1.

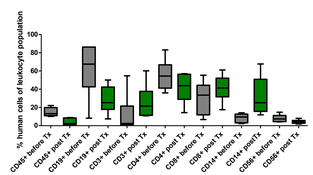
Humanized patient-derived melanoma models

Tumor growth curve of Mel1956



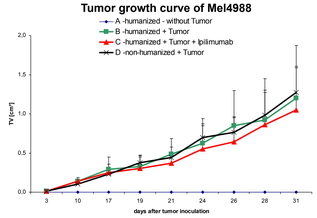
- Humanized mice received patient-derived melanoma fragments s.c. (Mel1956). Growth of human melanomas was confirmed on all humanized mice without evidence for rejection.

Proportional distribution of human white blood cells in the blood of Mel1956-bearing humanized mice



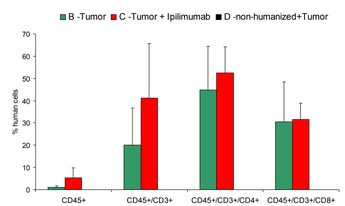
- Humanized melanoma (Mel1956) bearing mice have decreased overall numbers of human leukocytes in the peripheral blood. Tumor growth seems to have an effect on the proportional distribution of the human immune cells in this model: whereas the numbers of B cells and NK cells decreased, an increase of T cells, monocytes and CD8+ T cells was observed.

Tumor growth curve of Mel4988



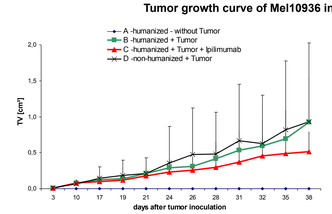
- Humanized mice received patient-derived melanoma (Mel4988) fragments s.c. Group C was treated with Ipilimumab weekly from day 21 on.
- Treatment with Ipilimumab had no effect on tumor growth

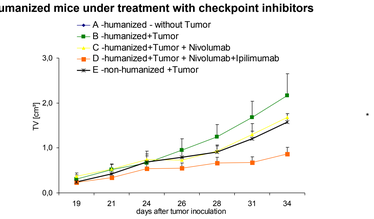
Proportional distribution of human cells in Mel4988 tumors



- In contrast to the Mel1956 model, transplantation of Mel4988 melanoma resulted in a decrease of human T cells in the blood (data not shown).
- Ipilimumab treatment increased the number of T cells in the blood and resulted in an accumulation of human leukocytes and T cells in the tumor, however obviously without relevant T-cell activation

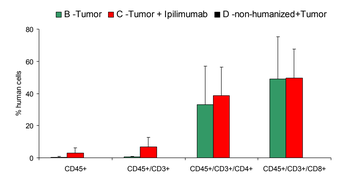
Tumor growth curve of Mel10936 in humanized and non-humanized mice under treatment with checkpoint inhibitors



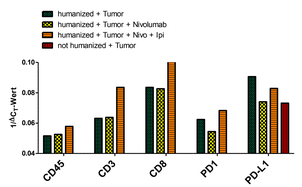


- Growth of human melanomas was confirmed on all humanized mice without evidence for rejection.
- Humanized mice received patient-derived melanoma (Mel10936) fragments s.c. Group C was treated with Ipilimumab weekly from day 25 on.
- Ipilimumab treatment induced a minor tumor growth delay.
- Nivolumab reduced the tumor growth and in combination with Ipilimumab significantly compared to untreated humanized mice.

Proportional distribution of human leukocytes in treated Mel10936 tumors



Differential expression of checkpoint marker under treatment in the tumor



- An increase of human T cells in the blood was observed after tumor inoculation (data not shown)
- Treatment with Ipilimumab increased the portion of human leukocytes and T cells in the tumor and in the blood compared to un-treated humanized tumor-bearing mice. The ratio between CD4/CD8 remained unchanged.
- Nivolumab decreased the PD-1 and PD-L1 RNA-expression in the tumor.
- The combination Ipilimumab+Nivolumab resulted in an high RNA-expression of CD3, CD8, PD-1 and PD-L1 in treated tumor.

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