Preclinical tumor models in humanized mice for translational immuno-oncology research

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Background and Aim

The recent clinical success of immune checkpoint modulators has stimulated immune-oncology research leading to the identification of new tumor immunology targets. However both, target validation and drug development need better preclinical immune oncology models with functional human immune cells. In previous experiments, we have demonstrated, that hematopoietic stem cells (HSC) can proliferate and differentiate in vivo to form a functional humanized immune system. Based on this, we determined PD-L1 expression as a target for immunotherapy on different tumor cell lines (CDX) and patient-derived xenografts (PDX). PD-L1 positive and negative models were selected and co-transplanted on humanized mice. Finally, we evaluated the functionality of the human immune system by the treatment with the checkpoint inhibitors Ipilimumab or Nivolumab and in combination with radiation.

Summary and Outlook

We reconstituted a functional human immune system by engrafting human hematopoetic stem cells in immunodeficient mice.

Repopulation of mouse organs with human hematopoetic cells and maturation of human T and B cells has been demonstrated.

CDX and PDX showed a heterogenous expression of PD-L1 expression comparable to clinical situation in humans.

Fragments from CDX and PDX of different entities were successfully engrafted on our humanized mice.

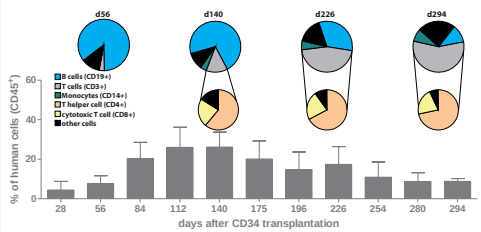
Delayed tumor growth in humanized mice seems to be in correlation with PD-L1 expression.

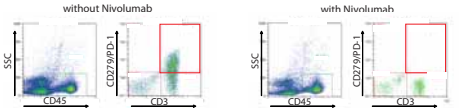
Strong response to checkpoint inhibitors showed a correlation to innate immune response without treatment and to PD-L1 expression in tumors.

Our humanized mouse models enable appropriate preclinical assessment of immune-based therapeutic antitumor strategies plus radiation especially when combining the humanized mouse with cell- and patient-derived tumor xenografts.

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

Long-term engraftment and differentiation of HSC in blood of mice

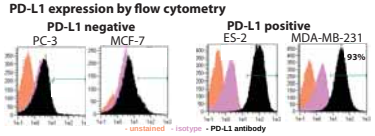




- HSC could reconstitue an humanized immune system in mice
- long-term engraftment could be observed over 300 days
- functionality of T cells was determined by inhibition of PD-1 expression through treatment with Nivolumab

Characterization of cell line - derived xenografts (CDX)

PD-L1 expression by flow cytometry



CDX	Entity	PD-L1 expression	expression level	%
MCF-7	Breast	negative	-	
MDA-MB-231	Breast	positive	+++	85
ZR-75-1	Breast	negative	-	
Colo205	Colon	positive	++	53
HT-29	Colon	negative	-	
TC-71	Ewing's Sarcoma	positive	+	38
MeWo	Melanom	positive	+	2
UKF-NB-3	Neuroblastoma	positive	++	53
AsPC1	Pancreas	negative	-	
CWR-22R	Prostate	positive	+	35
PC-3	Prostate	negative	-	
ES-2	Ovcar	positive	+++	91
Ovcar3	Ovcar	positive	+	7

- differential expression of PD-L1 in CDX models
- gene expression data for CDX models of different entities are available on our website - www.epo-berlin.com

Characterization of patient - derived xenografts (PDX)

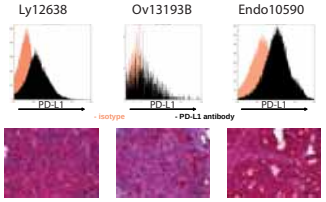
PD-L1 expression by immunohistochemistry and flow cytometry

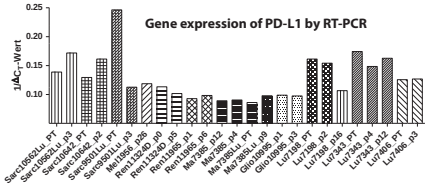
	PD-L1 (IH)	PD-L1 (FACS)
Ov6645	+	u.i.
Ov6668	+	u.i.
Ov6677	+	u.i.
Ov6719	-/+	u.i.
Ov10584	+	u.i.
Ov10716	+	u.i.
Ov11556	+	u.i.
Ov12963	+	u.i.
Ov13215	+	u.i.
Ov13329	+	-
Ov13370	+	u.i.
Ov13938	+	-
Ov13387	+	u.i.
Ov13439A	u.i.	u.i.
Ov13538	+	u.i.
Ov13577	+	u.i.
Ov13616	+	u.i.
Ov13726A	+	u.i.

	PD-L1 (IH)	PD-L1 (FACS)
Ly12212	+	+
Ly12318	+	u.i.
Ly12540	+	-/+
Ly12609	+	+
Ly12638	+	+
Ly12657	u.i.	-
Ly13005	+	+
Ly13136	u.i.	++
Ly13668	u.i.	+
Ly13693	+	u.i.
Ly13943	u.i.	u.i.
Ly13976	+	-
Ly13977	u.i.	++
Ly13847A	+	u.i.
Ly13847B	+	u.i.
Ly13847C	+	+
Ly13847E	+	u.i.

	PD-L1 (IH)	PD-L1 (FACS)
Endo6489	+	u.i.
Endo7502	+	-/+
Endo10561	+	u.i.
Endo10590	+	+
Endo10689	+	u.i.
Endo10987	+	u.i.
Endo11296	+	u.i.
Endo11339	+	u.i.
Endo12961	-/+	u.i.

u.i. - under investigation
\- - negative expression
-/+ - low expression
\+ - positive expression
++ - high expression

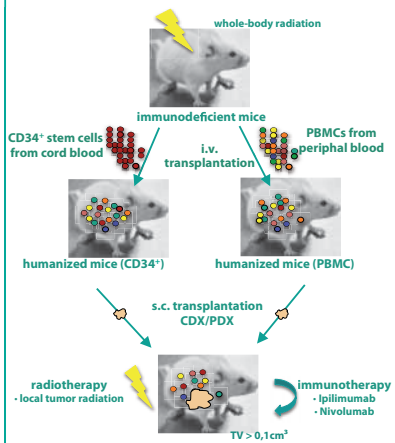




- differential expression of PD-L1 in PDX models of different entities
- PD-L1 expression correlated mostly between patient samples and passages of PDX
- in most cases, high expression of PD-L1 on RNA level could also detected on protein level in investigated PDX

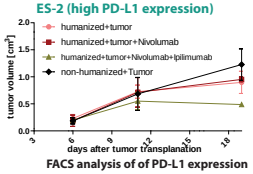
Cell line - and patient derived xenografts on humanized mice

Immuno-oncology model of humanized mice

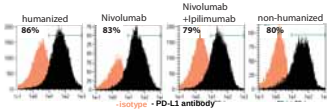


Tumor growth of CDX under immunotherapy treatment

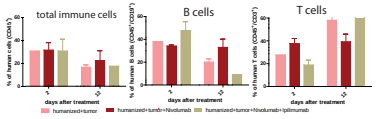
ES-2 (high PD-L1 expression)



FACS analysis of of PD-L1 expression



FACS analysis of immune cells in blood



- growth delay of PD-L1 positive CDX on humanized mice compared to non-humanized mice
- combined treatment of Nivolumab and Ipilimumab could decrease tumor growth of CDX with high PD-L1 expression

Tumor growth of PDX models with different PD-L1 expression

low PD-L1 expression

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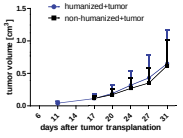
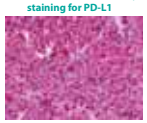
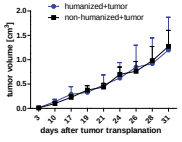


Immunohistochemistry staining for PD-L1

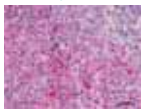


medium PD-L1 expression

Mel4988

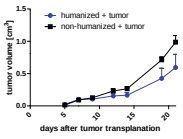


Immunohistochemistry staining for PD-L1



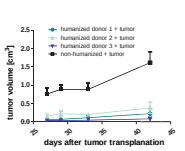
high PD-L1 expression

Mel1956

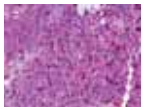


Immunohistochemistry staining for PD-L1

Ma7385



Immunohistochemistry staining for PD-L1

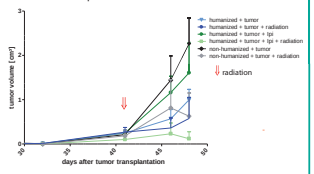


- growth of human PDX on humanized mice was confirmed
- engraftment delay seems to be dependent on PD-L1 expression of PDX (the higher PD-L1 expression, the higher growth delay)

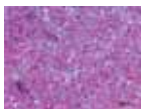
Tumor growth of PDX models under immunotherapy treatment in combination with radiation

Ly14651/A (low PD-L1 expression)

Ipilimumab + radiation



Immunohistochemistry staining for PD-L1



- Ly14651/A showed tumor growth delay compared to non-humanized mice
- radiation therapy showed a positive effect in combination with immunotherapy treatment

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