Immuno-oncology therapeutics technological progression toward a huge preclinical challenge of humanizing the immune component in mice

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How to choose the best animal models for your immunology program ?

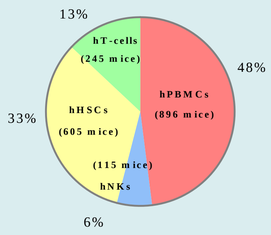
INTRODUCTION

Treatments targeting immune cells such as immune checkpoint modulators, bispecific antibodies or adoptive T-cell transfer have now demonstrated clinical efficacy and some of them are already approved.

However, preclinical development of these therapies requires models adapted for each target and each class of therapeutics. To address these needs, mouse models have been established to study the complex interactions of the immune system during cancer progression or other types of diseases.

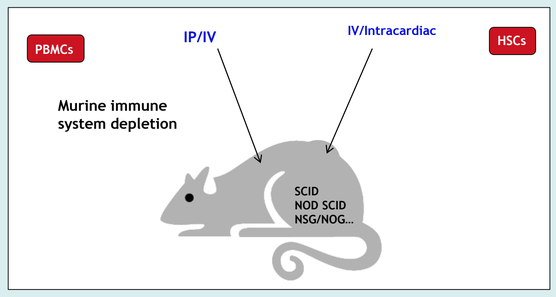
Human immune system should be reconstituted in immunodeficient mice using either human PBMCs or hematopoietic stem cells (HSCs). So called “Humanized” mice bearing human target tumor cells constitute relevant models for evaluation of immune-targeting cancer therapeutics.

Immune system reconstituted mice



A large panel of humanized mouse models to address specific immune cancer cell questions

Onco*design* has a large experience with immune system reconstituted mice xenografted with solid tumors (BT-474, FaDu, HCT116, LoVo, PDX…) as well as hematological tumors (Daudi, Karpas-299, Ramos…)



- **POC studies with hPBMCs reconstituted mice**
  - Injection of human PBMC in irradiated NOG mice
  - IV injection of B-cell lymphoma or SC injection of plasma cell myeloma
  - Tumor volume monitoring with caliper for SC tumor, mice termination when hind leg paralysis for IV tumor
  - Quantification of immune cell populations and tumor cells in blood bone marrow and spleen samples using flow cytometry analysis (IV tumor model)

- **POC studies with hHSCs reconstituted mice**
  - Injection of human HSC in BRGS mice
  - SC xenograft of lung and ovarian PDX tumor samples
  - Tumor volume monitoring with caliper
  - Mice termination for collection of blood, spleen, bone marrow and tumor
  - Quantification of immune cell populations using flow cytometry and immunohistochemistry analysis

RESULTS

Tumor models tested in humanized conditions

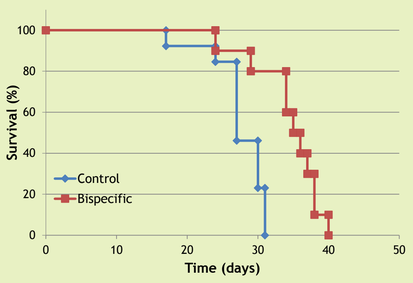
Name	Tumor model Histology	Injection	HSC -	NK IP	NK IV	PBMC IP	PBMC IV	PBMC SC	T cells IV
786-O	Kidney clear cell adenocarcinoma	SC				3			
BT-474/matrigel	Her2+ Breast ductal carcinoma	SC		5		9		3	
CaSki	HPV16+ epidermoid cervical cancer	SC							1
Daudi	Burkitt's lymphoma, B cells	IV			1				14
FaDu	Head and Neck carcinoma	SC				1			
HCT116	Colorectal carcinoma	SC							4
HL-60	Acute promyelocytic leukemia	SC				3			
HL-60*	Acute promyelocytic leukemia	IV				1			
IM-OVA-503	Ovary (PDX)	SC	1						
Jeko-1	Mantle Cell Lymphoma, B cells	IV							7
KARPAS-299	Human T-cell non-Hodgkin lymphoma	IV				4			
LoVo	Colorectal adenocarcinoma	SC	1			2			
LUN-NIC-001	NSCLC (PDX)	SC				2			
LUN-NIC-0084	NSCLC (PDX)	SC	1						
MCF-7	Breast adenocarcinoma	SC				2			
MDA-MB-231	Breast carcinoma (TNBC)	OT	2						
MKN74/matrigel	Stomach cancer metastasized to liver	SC		1					
MOLM13	Acute myeloid leukemia	IV							15
No tumor	NA	NA	2			17	3		6
NCI-H929/matrigel	Plasma cell myeloma	SC				2			
NCI-N87	Gastric carcinoma (stomach)	SC						1	
NIH:OVCAR-3	Ovarian adenocarcinoma	SC				2			
NIH:OVCAR-3	Ovarian adenocarcinoma	IP				1			
Raji*	B cell lymphoma	IV	1			1			1
Ramos	B cell lymphoma	IV	1			3			
RPMI 8226*	Multiple myeloma	SC				1			

*** Do not grow properly in humanized conditions**

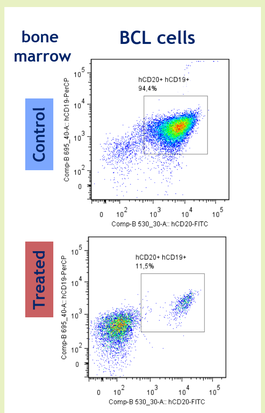
Summary table of tumor models used in humanized conditions. Mice are reconstituted with either human HSC, PBMC, NK or T cells for evaluation of cell therapies or therapeutic antibodies.
Each number indicate independant studies performed

hPBMCs reconstituted mice

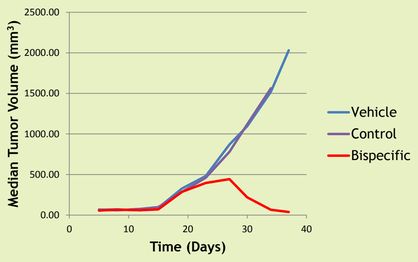
IV B cell lymphoma



Survival curves of NOG mice bearing IV disseminated B cell lymphoma and humanized with PBMCs

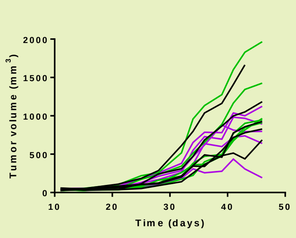


SC plasma cell myeloma



Mediam tumor volume growth of NOG mice bearing SC plasma cell myeloma and humanized with PBMCs

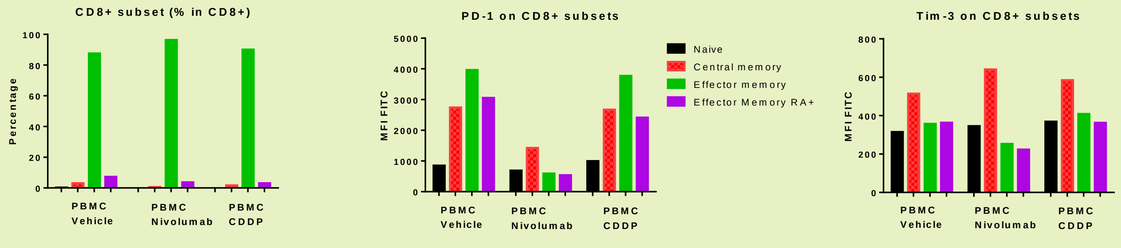
SC PDX epidermoïd lung carcinoma lung (PD-L1)



Tumor volume curves of NOG mice bearing SC PDX lung tumor and humanized with PBMCs

Nivolumab (3 mL/kg - Q5Dx4) and CDDP (3 mL/kg - Q7Dx3) monotherapies did not induce any significant antitumor activty against PDX lung tumor in PBMC-engrafted mice. Nevertheless, effector T-cell response was evidenced in mouse blood.

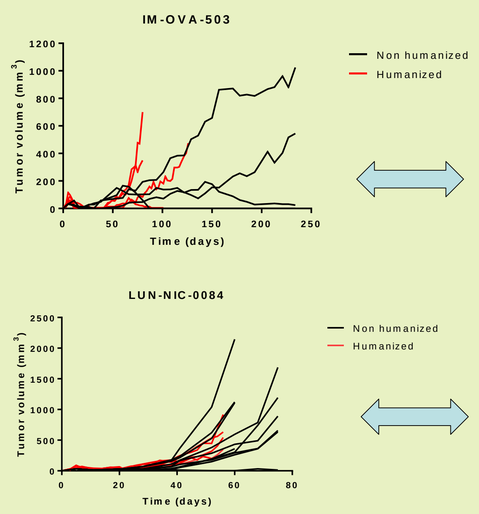
On going immunohistochemistry analyses of tumor samples should confirm that Nivolumab and CDDP treatments lead to recruitment of effector T-cells.

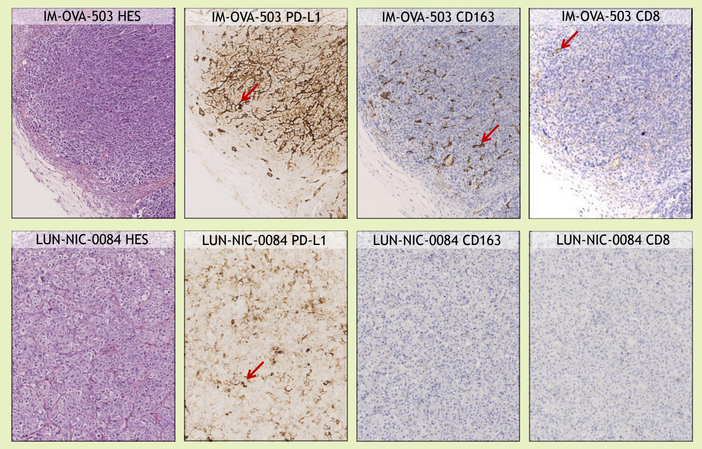


Characterization of CD8 T cells in mouse blood collected during the treatment period using flow cytometry (cell surface markers: CD3, CD8, CD45RA, CCR7, PD-1 & Tim-3)

hHSCs reconstituted mice

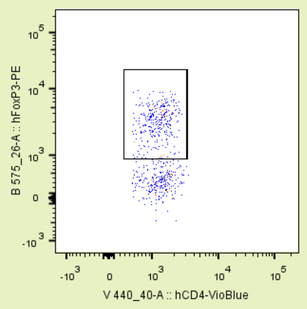
PDX tumor growth is not affected by humanization in BRGS mice

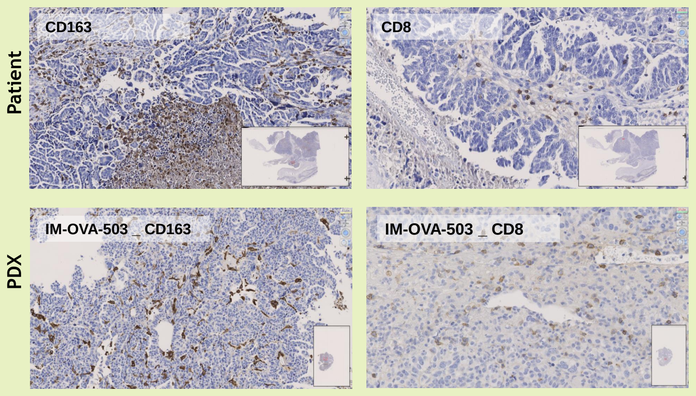




IHC analysis of SC PDX tumors growing in HSCs humanized BRGS mice. PD-L1, CD163 (macrophages) and CD8 (T cells) labeling are shown

Treg identified within the IM-OVA-503 tumor





Comparison between CD163 and CD8 staining for the patient tumor and the corresponding PDX model IM-OVA-503

- **Tumor Lymphoid cells & Myeloid cells (Flow cytometry analysis)**
  - T-cells: ~55%
  - B cells: ~4%
  - NK cells: ~6%
  - Monocytes: ~3%
  - Other myeloid cells: ~5%

Conclusions and perspectives

- **Humanization of immune system of mice with either hPBMCs of hHSCs did not change the growth of human tumors SC or IV xenografted,**
- **Humanized mouse models enable the study of immunological processes and the evaluation of immunomodulating agents in complement to our syngeneic mouse model platform,**
- **Efficacy of bispecific compounds as well as adoptive cell therapy was demonstrated in humanized models by both recruitment of T-cells and decreased tumor development,**
- **Lung and ovarian PDX developed in HSCs reconstituted BRGS mice induced an increase in Tregs and a change in T/B ratio in blood and spleen samples,**
- **The humanization of PDX tumor bearing mice through hHSCs shows tumor immune cell infiltrates in PDX tumor, those conditions validate the use of such model to investigate new immuno-oncology drugs,**
- **The presence of immune infiltrates within PDX tumor was found with a high degree of similarities with that of the patient tumor infiltration,**
- **HSCs reconstituted models still need further improvement, mainly regarding mouse host novel strains such as NSG-SGM3 and NOG-EXL are under evaluation.**

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