From syngeneic to humanized mouse models: addressing the needs for novel immunotherapies (efficacy evaluation and biomarker discovery)

P. Slos[1], F. Bichat[1], C. Mignard[1], O. Duchamp[1], F. Ghiringhelli[2,3], J.-F. Mirjolet[1]

[1]**Onco***design*, Dijon, [2]INSERM 866, Université de Bourgogne, Dijon, [3]Center Georges François Leclerc, Dijon (France)

For more information: contact@oncodesign.com

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

INTRODUCTION

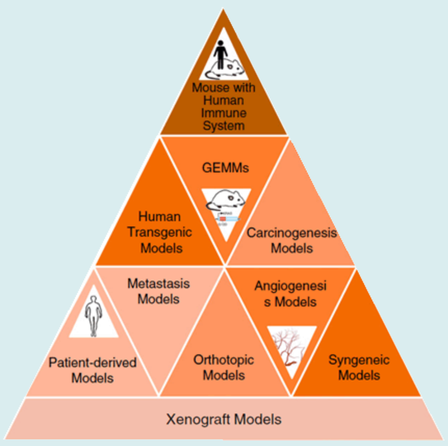
Treatments targeting immune cells such as immune checkpoint modulators, bispecific antibodies or adoptive T cell transfer have now demonstrated clinical efficacy and some of them are already approved.

However, preclinical development of these therapies requires models adapted for each target and each class of therapeutic. To address these needs, case studies using syngeneic mouse models as well as tumor bearing humanized mouse models will be presented.

Fit-for purpose use of mouse models to improve predictivity of cancer therapeutics evaluation

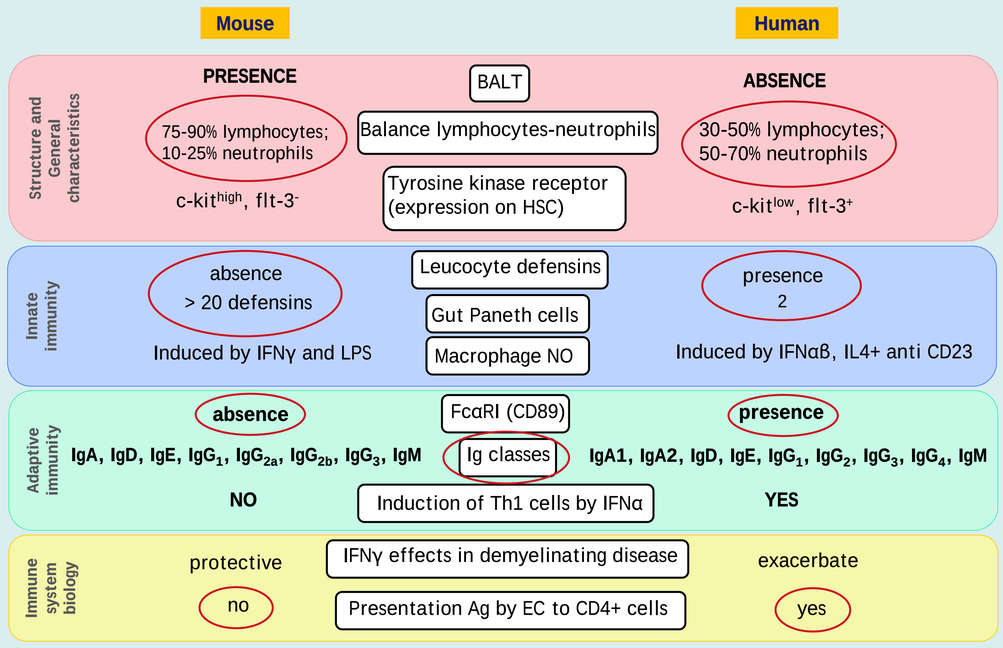
Suitable models for immuno-oncology?

- Two key components
  - ✓ Immune System
  - ✓ Tumor
- Immune system and tumor could be
  - ✓ from mouse : standard syngeneic models, GEMM
  - ✓ from human : standard xenografts or PDXs on humanized mouse



Adapted From Wartha et al. ***Pharmacology & Therapeutics, 2014***

Of mice and not men: differences between mouse and human immunology



Modified from Javier Mestas and Christopher C. W. Hughes, J Immunol 2004; 172:2731-2738

Syngeneic models

Summary table of major Immune Checkpoint Inhibitor (ICI) efficacy in syngeneic models

Model				CTLA-4		PD-1		PD-L1		
Name	Site	Type	Strain	n	T/C (median)	n	T/C (median)	n	T/C (median)	
4T1	OT	Breast	BALB/C	8	81	13	102	2	116	
A20	SC	BCL	BALB/C	1	25	2	34	0	NA	
B16-F10	SC	Melanoma	C57BL/6	3	100	5	80	2	121	
C38	SC	Colon	C57BL/6	2	6	1	11	0	NA	
CT-26	SC	Colon	BALB/C	8	22	16	72	7	68	
EMT6	SC	Breast	BALB/C	11	3	16	59	2	77	
EMT6	OT	Breast	BALB/C	0	NA	1	68	0	NA	
Hepa1-6	OT	Liver	C57BL/6	2	27	0	NA	0	NA	
LLC	SC	Lung	C57BL/6	2	121	2	97	1	88	
MBT2*	OT	Bladder	C3H	1	260	3	148	0	NA	* survival
MBT2	SC	Bladder	C3H	1	83	3	73	2	66	
RenCa*	OT	Kidney	BALB/C	1	100	1	100	0	NA	* survival
RenCa	SC	Kidney	BALB/C	0	NA	1	61	0	NA	

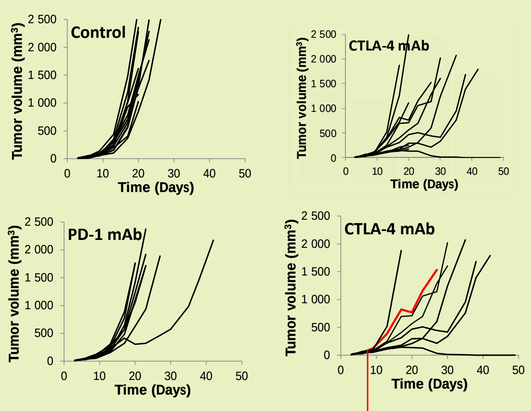
T/C < 42%
42% < T/C < 80 %
T/C > 80%

Other ICIs (such as antibodies against OX40, 4-1BB, GITR, etc.) were already tested. Please ask for details.

RESULTS

TV curve efficacy - CT-26 Colon Carcinoma Model

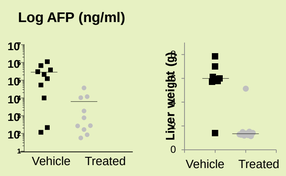
Combined Immune checkpoint inhibitors



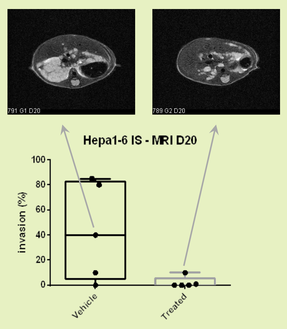
Mice were SC injected with CT-26 murine colon tumor cells at D0. Mice were randomized based on tumor volume at D9 and treated IP with mAb against CTLA-4 (clone 9H10) at 5 mg/kg/inj, PD-1 (clone RMP1-14) at 10 mg/kg/inj (TWx2) or combination of both. Mice were terminated at D20 and tumor analyzed by Flow Cytometry for T cells content.

Hepa1-6 Hepatocarcinoma Model

Efficacy data

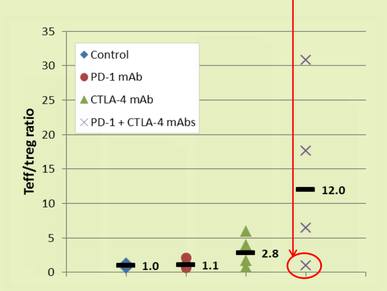


Axial MRI of the liver

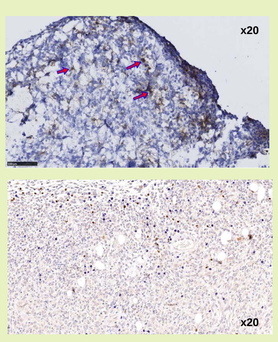


Circulating AFP level at D19 in mice bearing OT Hepa1-6 tumors. AFP was quantified by ELISA. Mice were IS injected with Hepa1-6 tumor cells at D0. Mice were randomized based on body weight at D5 and treated.

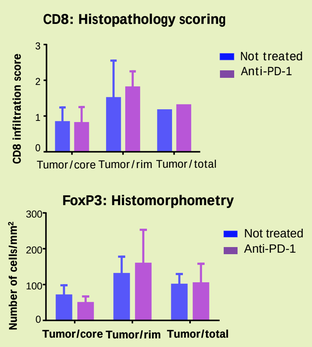
Immune infiltrates



Mice were SC injected with CT-26 murine colon tumor cells at D0. Mice were randomized based on tumor volume at D9 and treated IP with mAb against CTLA-4 (clone 9H10) at 5 mg/kg/inj, PD-1 (clone RMP1-14) at 10 mg/kg/inj (TWx2) or combination of both. Mice were terminated at D20 and tumor analyzed by Flow Cytometry for T cells content.

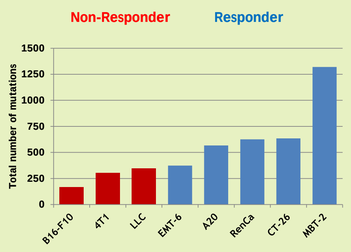


Representative images of the immuno histochemical staining of tumor tissue (EMT6).

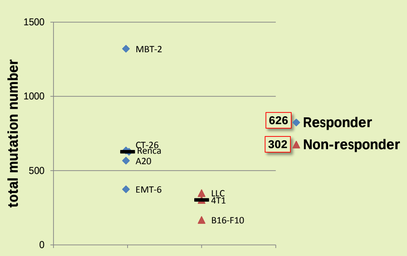


Anti PD-1 treatment did not modify CD8 and FoxP3 intratumoral immune cells distribution.

Whole exome sequencing



T/C (%) < 80 is used as cut-off criteria for responder and non-responder populations (SC models)



- **The genomic mutations were analyzed using whole exome sequencing,**
- **Responder have highest number of mutations in comparison to non responder cell lines**

Humanized mice models

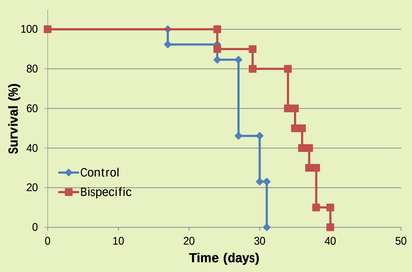
Summary table of tumor models tested in humanized conditions

Tumor model			PBMCs		T cells
Name	Histology	Injection	IP	SC	IV
786-O	Kidney clear cell adenocarcinoma	SC	3		
BT-474/matrigel	Her2+ Breast ductal carcinoma	SC	9	3	
Daudi	Burkitt's lymphoma, B cells	IV			x
FaDu	Head and Neck carcinoma	SC	1		
HCT116	Colorectal carcinoma	SC			x
Jeko-1	Mantle Cell Lymphoma, B cells	IV			x
KARPAS-299	Human T-cell non-Hodgkin lymphoma	IV	4		
LoVo	Colorectal adenocarcinoma	SC	2		
MCF-7	Breast adenocarcinoma	SC	2		
MOLM13	acute myeloid leukemia	IV			x
NCI-H929/matrigel	Plasma cell myeloma	SC	2		
NCI-N87	Gastric carcinoma (stomach)	SC		1	
NIH:OVCAR-3	Ovarian adenocarcinoma	SC	2		
Raji*	B cell lymphoma	IV	1		
Ramos	B cell lymphoma	IV	3		
RPMI 8226*	Multiple myeloma	SC	1		

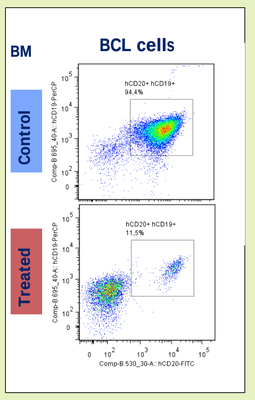
*** Do not grow properly in humanized condition**

PBMCs

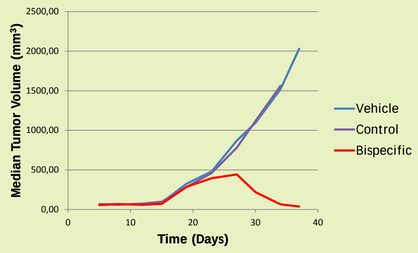
IV B cell lymphoma



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



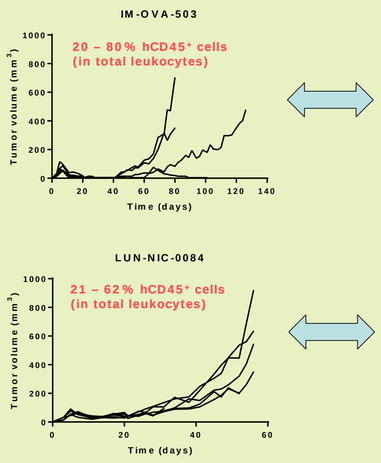
SC plasma cell myeloma



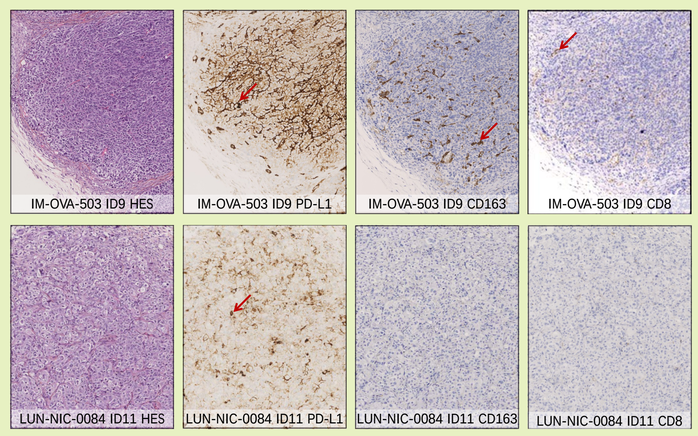
Mediam tumor volume growth of NOG mice bearing SC plasma cell myeloma and humanized with PBMCs.

HSCs

PDX tumor growth is not affected by humanization in BRGS mice



Spleen : non-tumor bearing mice vs tumor bearing mice



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

Conclusions and perspectives

- **10 well characterized syngeneic models are effective approach for immune oncology research and drug development,**
- **Cytometry, NGS and IHC technologies are available for drug efficacy monitoring and biomarker identifications,**
- **Humanization of immune system of mice with either hPBMCs of hHSCs permits 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.**

World Preclinical Congress (WPC) Annual Meeting / *Boston, MA. USA. June 14-17, 2016*

Oncodesign Biotechnology — synergie mtl — Une division d'Oncodesign — Inserm — CGFL Centre Georges François Leclerc — IMODI