Evaluation of immuno-oncology related treatments using highly characterized syngeneic mouse tumor models

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INTRODUCTION

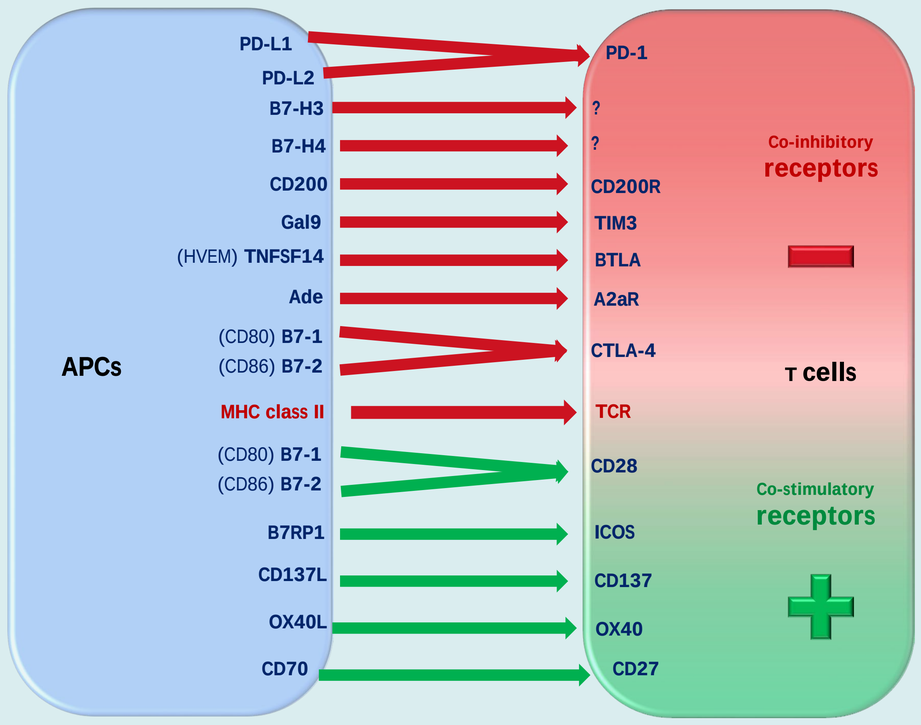
Immune-checkpoints: efficacy but still a lot of challenges

Monoclonal antibodies (mAbs) targeting co-inhibitory molecules such as PD-1, PD-L1 and CTLA-4 are now approved for treatment of melanoma, non-small cell lung carcinoma, renal cell carcinoma, Hodgkin's lymphoma and urothelial carcinoma.

A lot of compounds targeting new co-inhibitory or co-stimulatory molecules as well as combination strategies with anti-PD-1, anti-PD-L1 or anti-CTLA-4 targeting mAbs are under evaluation.

- **How to choose the best target, the best compound?**
- **Antibody format:**
  - IgG1, IgG2, IgG4,…
  - Humanized, fully human,…
- **Prognostic biomarker (genetic basis only?):**
  - Mutation loads (which cut off?),
  - MMR-D,
  - PD-L1,…
- **Early biomarker of response**
  - Imaging ?
- **Right way to analyse efficacy:**
  - RECIST, irRC, irRECIST
- **Durable response in absence of treatment (duration of treatment?)**

Are syngeneic mouse models relevant?



RESULTS

In-vivo efficacy

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

Model				CTLA-4		PD-1		PD-L1		OX40	
Name	Site	Type	Strain	n	T/C (median)	n	T/C (median)	n	T/C (median)	n	T/C (median)
4T1	OT	Breast	BALB/c	8	81	13	102	3	104	1	121
A20	SC	BCL	BALB/c	1	25	2	34	0	NA	0	NA
B16-F10	SC	Melanoma	C57Bl/6	3	100	5	80	2	121	0	NA
C38	SC	Colon	C57Bl/6	3	0	2	18	0	NA	0	NA
CT-26	SC	Colon	BALB/c	8	22	16	72	8	69	1	112
EMT6	SC	Breast	BALB/c	11	3	16	59	2	77	0	NA
EMT6	OT	Breast	BALB/c	0	NA	1	68	0	NA	0	NA
HEPA1-6	OT	Liver	C57Bl/6	2	27	0	NA	0	NA	0	NA
LLC	SC	Lung	C57Bl/6	2	121	2	97	1	88	1	108
MBT2*	OT	Bladder	C3H	1	260 **	3	148 **	0	NA	0	NA
MBT2	SC	Bladder	C3H	1	83	3	73	2	66	1	65
RenCa*	OT	Kidney	BALB/c	1	100	1	100	0	NA	0	NA
RenCa	SC	Kidney	BALB/c	0	NA	1	61	0	NA	1	65
PAN02	SC	Pancreas	C57Bl/6	1	5	0	NA	0	NA	0	NA

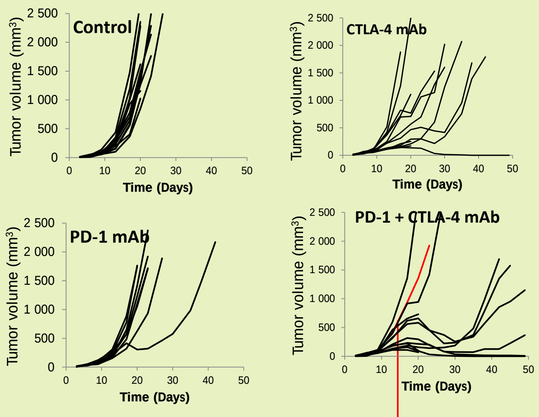
** for all models except*
*** survival*

Model				4-1BB		GITR	
Name	Site	Type	Strain	n	T/C (median)	n	T/C (median)
4T1	OT	Breast	BALB/c	1	65	0	NA
CT-26	SC	Colon	BALB/c	0	NA	3	17
HEPA1-6	OT	Liver	C57Bl/6	1	22	0	NA

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

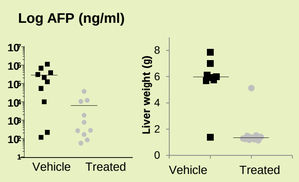
TV curve efficacy / CT-26 colon carcinoma model

Combined Immune checkpoint inhibitors



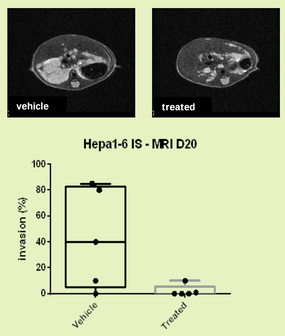
Hepa1-6 hepatocarcinoma model

Efficacy data

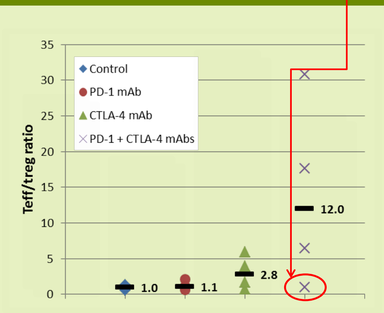


Circulating AFP level on D19 in mice bearing OT Hepa1-6 tumors. AFP was quantified by ELISA. Mice were IS injected with Hepa1-6 tumor cells on D0. Mice were randomized based on body weight on D5 and treated.

Axial MRI of the liver

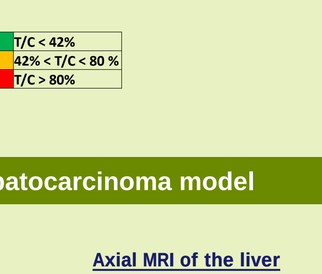


Immune infiltrates



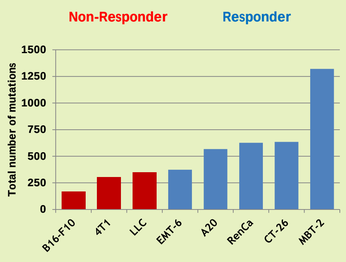
Mice were SC injected with CT-26 murine colon tumor cells on D0. Mice were randomized based on tumor volume on D9 and treated IP with mAb against CTLA-4 (clone 9H10) at 5 mg/kg/inj, PD-1 (clone RMP1-14) at 10 mg/kg/inj (TWx2) or combination of both. Mice were terminated on D20 and tumor analyzed by flow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

Representative images of the immune histochemical staining of tumor tissue (EMT6)

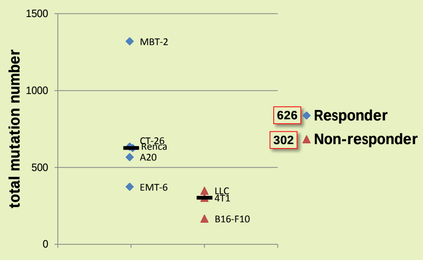


Anti PD-1 treatment did not modify CD8 and FoxP3 intratumoral immune cells distribution

Whole exome sequencing



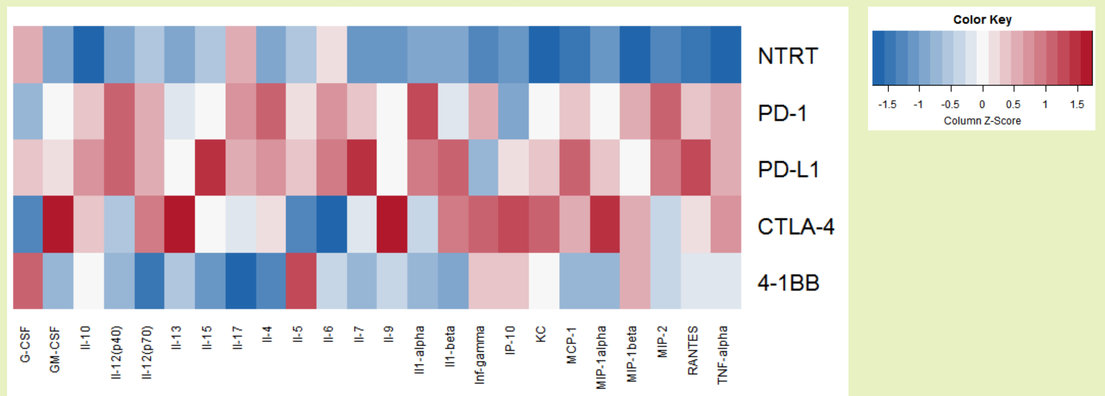
T/C (%) < 80 is used as cut-off criteria for responder and non-responder populations (SC models)



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

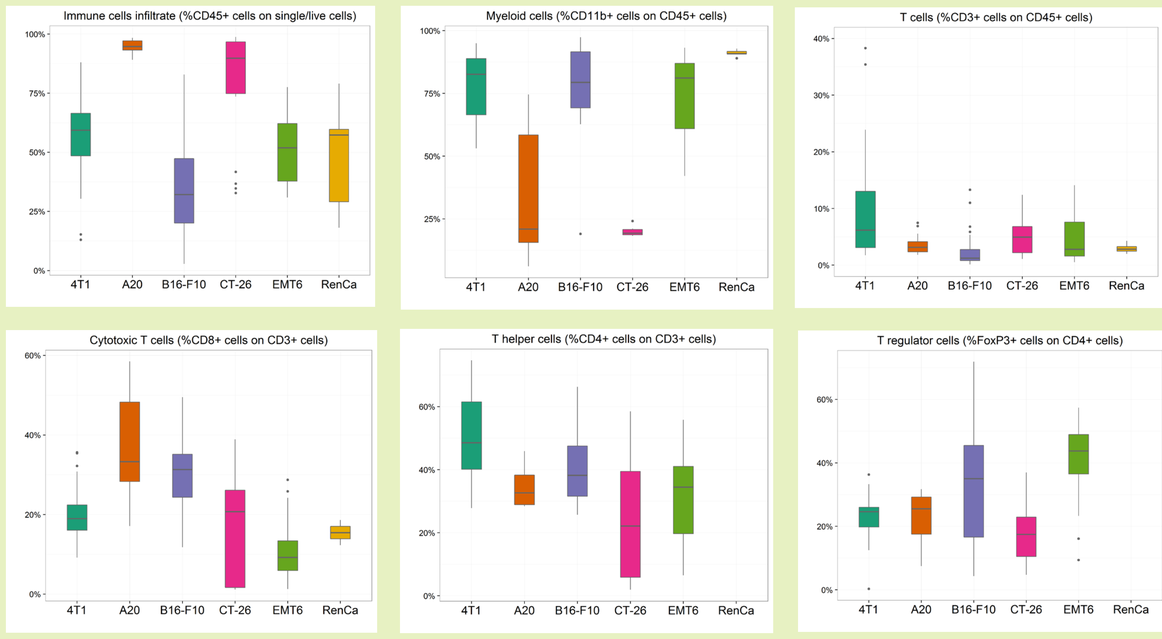
Cytokine release / 4T1 breast carcinoma model

Cytokine heatmap



Luminex values (in pg/mL) were first normalized, by cytokine/chemokine, with respect to the untreated group (value-mean untreated)/mean untreated. A z-score was then calculated from the normalized values, within each column of the heatmap.

Tumor infiltrates characterizations by flow cytometry



Boxplot representation of immune cell populations in tumors of untreated mice assessed by flow cytometry. Each tumor model represents the average of 1 to 5 independent experiments, with n=4 to 10 animals per experiment

Conclusions and perspectives

- **The use of a panel of well characterized syngeneic models is an effective approach for immuno-oncology research and drug development,**
- **Flow cytometry, NGS and IHC technologies are available for drug efficacy monitoring and biomarker identifications,**
- **In addition of this immune profile, other information such as sensitivity to chemotherapy, radiotherapy are also available,**
- **Moreover, a lot of *ex-vivo* / *in-vitro* assays such as ADCC, ADCP, MLR, CTL or ELISPOT are available or under development.**

World Preclinical Congress (WPC) Annual Meeting / *Lisbon, Portugal. November 14-16, 2016*

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