Efficacy of PD-1/PD-L1 pathway disruptors in syngeneic models

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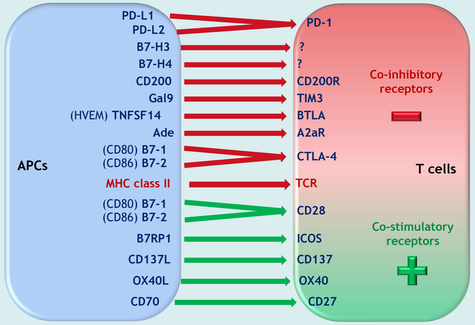
INTRODUCTION

Immune-checkpoints: efficacy but still a lot of challenges

- 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
- Right way to analyse efficacy:
  - RECIST, irRC, irRECIST
- Durable response in absence of treatment (duration of treatment?)

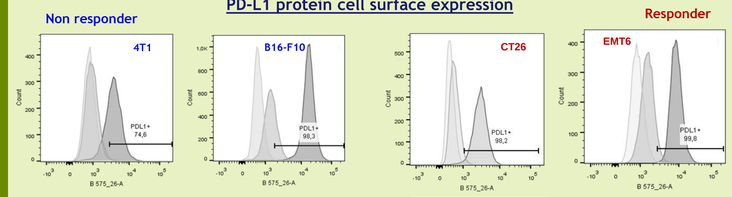
Are syngeneic mouse models relevant?



RESULTS

In-vitro modulation of PD-L1 and IRF expression after IFNɣ exposure

PD-L1 protein cell surface expression

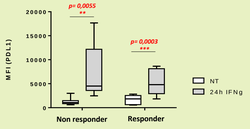


PD-L1 expression (using RT-qPCR, WB and flow cytometry) **was analyzed on cell lines** before and after IFNɣ.

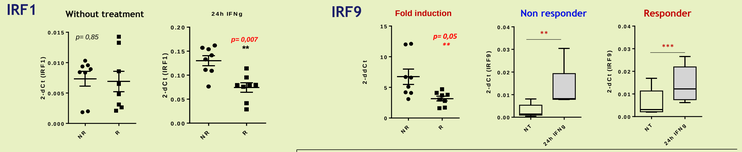
At baseline, **no significant difference between responders and non responders whatever the analysis** (mRNA, total PD-L1 protein expression or cell surface PD-L1 expression) **was observed.**

After incubation with IFNɣ, the level of PD-L1 (mRNA protein cell surface expression) **was highly increased for non responders** as compared to responders.

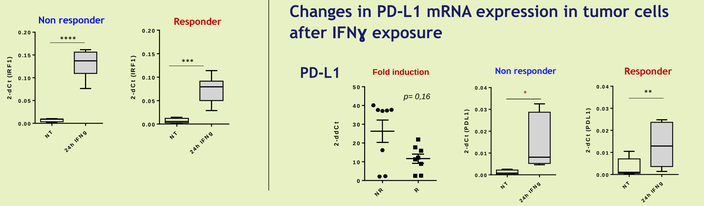
IFNɣ increases PD-L1 expression



Changes in IRF mRNA expression in tumor cells after IFNɣ exposure



Changes in PD-L1 mRNA expression in tumor cells after IFNɣ exposure



- **Expression of IRF1, IRF3, IRF7 and IRF9 mRNA was analyzed before and after IFNɣ exposure,**
- At baseline or after IFNɣ stimulation, **no significant difference for IRF3, and IRF7 mRNA expression was observed between responders and non-responders** (data not shown),
- In contrast, **IRF1 and IRF9 mRNA are less expressed at baseline in responders** as compared to non-responders,
- **After IFNɣ stimulation, a highest increase in IRF1, IRF9 and PD-L1 expression was observed in non-responders** compared to responders population.

In-vivo Efficacy

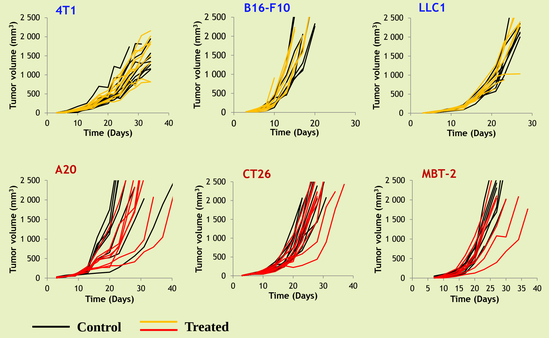
Model				PD-1		PD-L1	
Name	Site	Type	Strain	n	T/C (median)	n	T/C (median)
4T1	OT	Breast	Balb/C	13	102	2	116
A20	SC	BCL	Balb/C	1	37	0	NA
B16-F10	SC	Melanoma	C57Bl/6	5	80	2	121
C38	SC	Colon	C57Bl/6	1	11	0	NA
CT26	SC	Colon	Balb/C	15	70	7	68
EMT6	SC	Breast	Balb/C	15	63	2	77
EMT6	OT	Breast	Balb/C	1	68	0	NA
HEPA1-6	OT	Liver	C57Bl/6	0	NA	0	NA
LLC1	SC	Lung	C57Bl/6	2	97	1	88
MBT2*	OT	Bladder	C3H	2	149	0	NA
MBT2	SC	Bladder	C3H	2	79	2	66
Renca*	OT	Kidney	Balb/C	1	100	0	NA

* survival

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

10 mouse models were tested for response to PD-1/PD-L1 targeting antibodies:

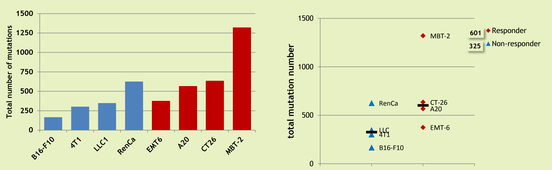
- 4T1, B16-F10, LLC1 and Renca models were characterized as non-responder,
- A20, C38, CT26, EMT6, MBT-2 showed sensitivity to PD-1/PD-L1 inhibitors,
- Efficacy study on HEPA1-6 OT model is on-going.



Mice were SC injected with murine tumor cells at D0.

Mice were randomized based on tumor volume (50-100 mm³) and treated IP with mAb against PD-1 (clone RMP1-14) at 10 mg/kg/inj (TWx2).

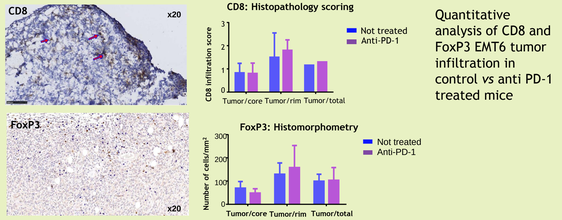
Number of gene mutations and sensitivity to ICIs



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.

Immune infiltrate



Quantitative analysis of CD8 and FoxP3 EMT6 tumor infiltration in control *vs* anti PD-1 treated mice

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.

Conclusions and perspectives

- **PD-L1 expression and genomic variation could be used for predicting tumor response to anti PD-L1/PD-1 therapy after IFNɣ exposure,**
- **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,**
- **New humanized mouse models are under development to circumvent limitations of syngeneic models.**

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