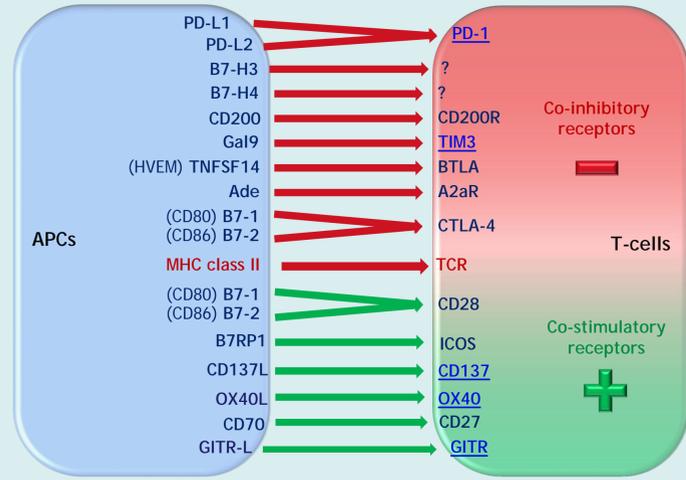


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INTRODUCTION

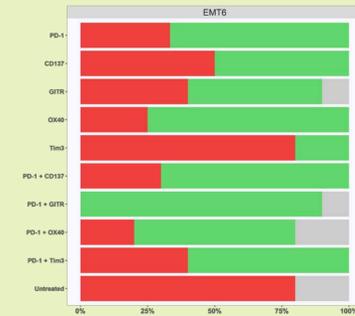
PD-1 targeting antibodies (nivolumab, pembrolizumab) are now approved in various tumor types either as second line treatment (locally advanced or metastatic urothelial carcinoma, advanced renal cell carcinoma, recurrent or metastatic head and neck squamous cell carcinoma, classical Hodgkin lymphoma) or even as first line therapy (metastatic melanoma, metastatic non-small cell lung cancer). Despite an increase in response rate as well as survival, there is still issue with resistance to PD-1 targeting therapies. Selection biomarkers as well as response biomarkers are still under intensive research. In addition, combination strategies are needed to increase response rate and overcome resistance.



## Efficiency of combinations - EMT6

Group	T/C (D22)	Median TV (mm <sup>3</sup> at D22)
PD-1 mAb	76	470
CD137 mAb	58	360
GITR mAb	61	375
OX40 agonist mAb	20	126
Tim3 mAb	85	524
PD-1 + Anti-CD137 mAbs	47	292
PD-1 + Anti-GITR mAbs	11	66
PD-1 + OX40 agonist mAbs	1	4
PD-1 + Anti-Tim3 mAbs	23	142
Untreated	100	617

Tumor growth inhibition (T/C %) defined as the ratio of the median tumor volumes of treated groups versus Vehicle group was calculated.



Proportion of responders (green) vs non-responders (red). Response was defined using relative tumor volume values (RTV: tumor volume at end of experiment divided by tumor volume at treatment start). Grey cells represent animals with no available efficacy endpoint at the end of the study.

RESULTS

RESULTS

## EMT6

### Responder vs. non responder



Cytokine quantification using multiplex assay in the blood of mice the day of termination (day 27). 8 cytokines are plotted (out of 25 measured). Color of dots represent response : responders (green) and non-responders (red).

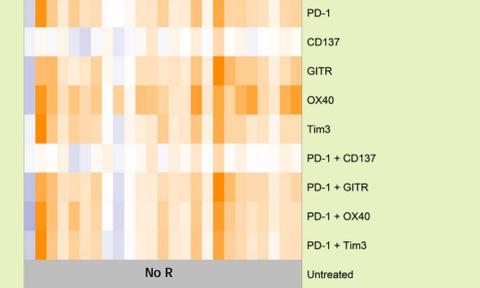
Cytokine concentration values have been normalized by group using the following formula:

$$\text{normalized value} = \log\left(\frac{\text{value}}{\text{mean(untreated group)}}\right)$$

### Non-Responders



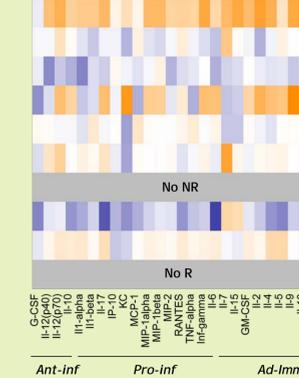
### Responders



Cytokine heatmaps. Normalized values were color-coded as follows:

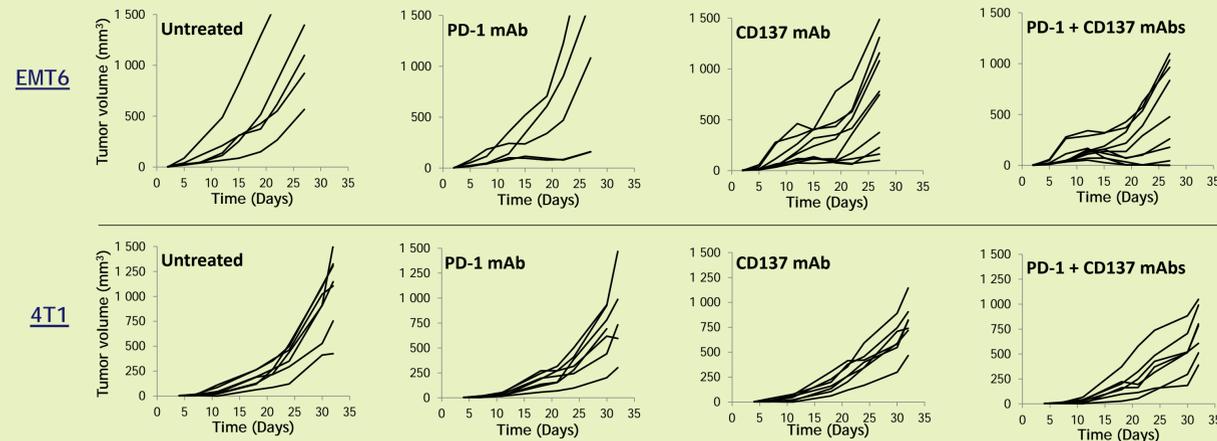
- Blue: under-expressed values
- Orange: over-expressed values
- White: no difference

### Non-responders - Responders

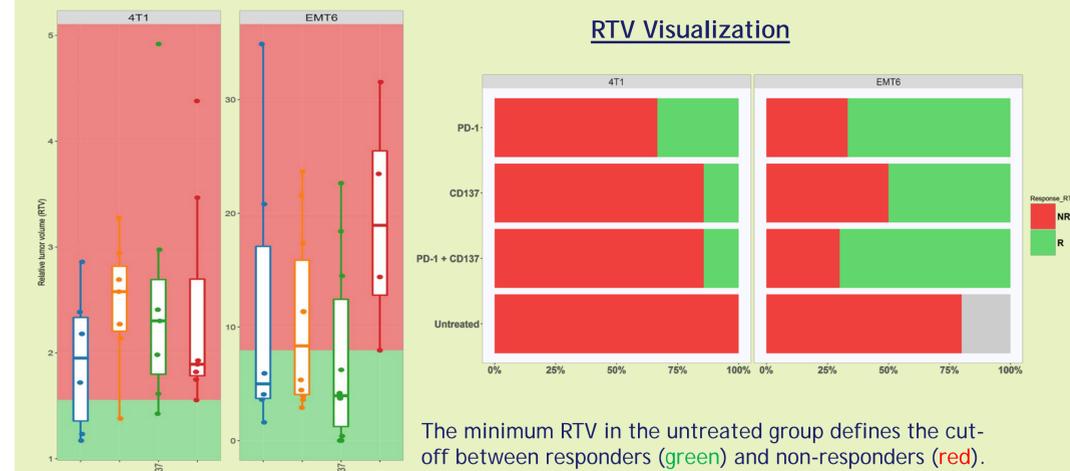


Variation of cytokine profile with treatment response. For each normalized cytokine value, the mean value of non-responders was subtracted from the mean value of responders.

- Blue: R < NR
- Orange: R > NR
- White: N = R

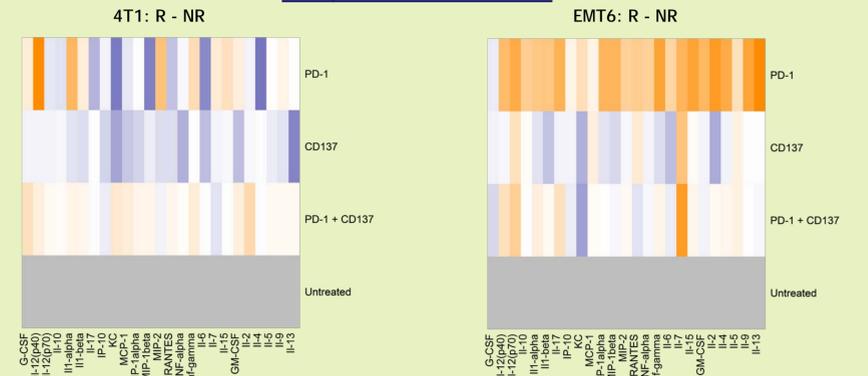


## EMT6 vs 4T1: CD137 mAb combined to PD-1 mAb



The minimum RTV in the untreated group defines the cut-off between responders (green) and non-responders (red).

### Comparison EMT6 & 4T1



## CONCLUSIONS

- EMT6 model
  - PD-1 mAb: global increase of the cytokine expression by the responders,
  - Cytokine profiles after CD137 mAb treatment are different compared to all other tested mAbs,
  - No obvious difference in cytokine profiles analyzed at the end of the study could be evidenced, between responders and non-responders.
- EMT6 model compared to 4T1 model
  - PD-1 mAb treatment induces profound changes in cytokine profiles,
  - Cytokine profiles differ between a sensitive model EMT6, and a more resistant one 4T1.
- Summary
  - Cytokines network is a complex system (that changes in a dynamic manner),
  - Cytokine profile analysis combined with heatmap visualization are valuable tools,
- Additional data are needed to identify robust cytokine-based biomarkers