Beneficial outcome of combination therapy with 4-1BB mAb targeting antibody

#293

M. RAMELET[1], J.-F. MIRJOLET[1], D. FRANCE[1], L. MORGAND[1], X. TIZON[1], L. ARNOULD[2], M. HILLAIRET de BOISFERON[1]
[1]Oncodesign, Dijon (France), [2]Centre Georges François Leclerc, Dijon (France)
For more information: contact@oncodesign.com

INTRODUCTION

Immune checkpoint: promising results and adverse effects

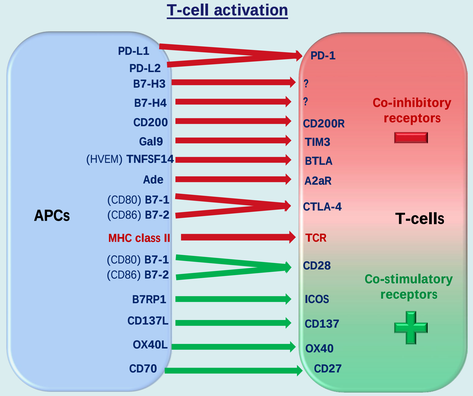
Immunotherapy based on mAbs targeting cancer cells is now developed as a valid approach to treat cancer. Suppressive mechanisms in immune responses normally play a critical role in maintaining immune homeostasis. However, these suppressive mechanisms are also considered as one of the main reasons for the failure of cancer immunotherapies because they induce peripheral tolerance of tumor-specific immune responses and allow tumor growth.

CD4+ CD25+ Foxp3+ regulatory T-cells have been revealed as the most important population of immune suppressors, and their depletion has been reported to enhance antitumor immune responses. CTLA-4 (CD152) was reported as a critical target for regulatory T-cell function [1] and thus blockade of CTLA-4 mediated signals has been suggested as a possible strategy to treat cancers. The first anti-CTLA-4 human monoclonal antibody (mAb), ipilimumab, was approved in 2011 by the FDA for use in metastatic melanoma. Success for ipilimumab was reported in a large phase III clinical trial involving patients with metastatic melanoma, who had undergone previous failed treatment [2].

Moreover, programmed death-1 (PD-1) mediated signals was also reported as a critical inhibitory mechanism regulating antitumor immune responses [3]. Nivolumab, a fully human mAb that blocks the programmed death-1 (PD-1) protein showed responses lasting over 1 year in previously treated metastatic melanoma patients. Combination therapy concurrently targeting PD-1, PD-L1 and CTLA-4 immune checkpoints leads to remarkable antitumor effects [4].

While these promising results have led to a great expectancy in treatments for cancer, these approaches are also show adverse toxicities associated with continuous treatment. To study these adverse side toxicities, we chose to characterize the murine orthotopic 4T1 mammary carcinoma model, known for his hypersensitivity reactions to monoclonal antibody (mAb) administration in this model. These effects were also studied with 4-1BB mAb combined to these immune checkpoint.

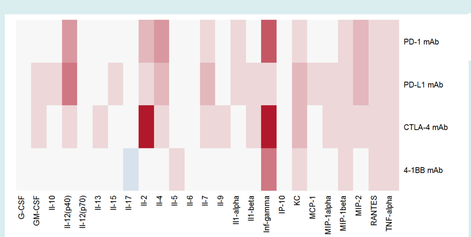
T-cell activation



In-vivo experiments

Mice (BALB/c, Charles River, FR) were orthotopically injected with 4T1 syngeneic breast tumor cell lines on D0. They were randomized on D18 (mean tumor volume of about 170 mm³) and then treated with anti-PD-1 (clone RMP1-14), anti-PD-L1 mAb (clone 10F.9G2 at 10 mg/kg (Q2Dx4), anti-CTLA-4 (clone 9H10), or with anti-4-1BB mAb (clone 3H3 at 10 mg/kg (Q3Dx3).

Cytokine et chemokine heatmap - Monotherapies



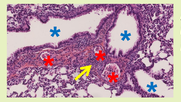
Luminex values (in pg/mL) were first normalized, by cytokine/chemokine, with respect to the untreated group (value-mean $_{untreated}$)/mean $_{untreated}$.

RESULTS

Survival increase by co-treatment with anti-4-1BB highlights anti-tumor activities of immune checkpoint antibodies

Lymphocyte infiltration lungs
Scoring in blind manner at x200 magnification

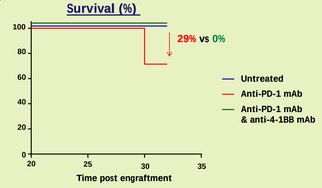
Untreated
Lymphocyte infiltration score: 0



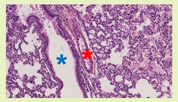
Anti-4-1BB mAb
Lymphocyte infiltration score: 1+

PD1

Survival (%)

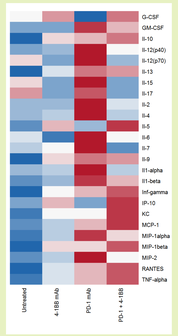


H&E stain of lungs

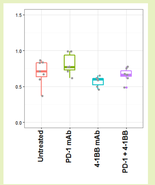


Anti-PD1 mAb
Lymphocyte infiltration score: 0

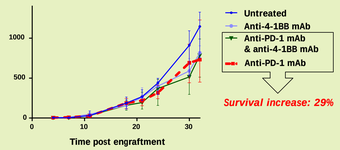
Cytokine & chemokine heatmap

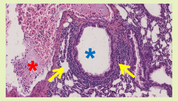


Spleen weights (g)



Median tumor volume ± IQR (mm³)

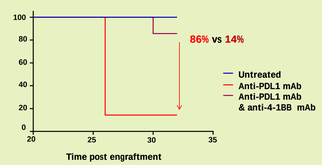


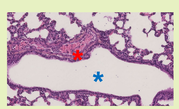


Anti-PD1 mAb & anti-4-1BB mAb
Lymphocyte infiltration score: 2+

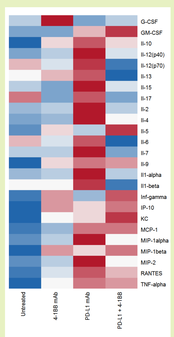
PD-L1

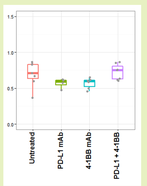
Survival (%)



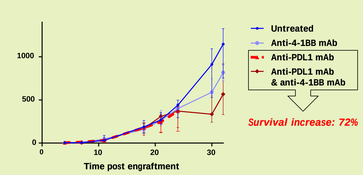


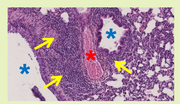
Anti-PD-L1 mAb
Lymphocyte infiltration score: 0





Median tumor volume ± IQR (mm³)

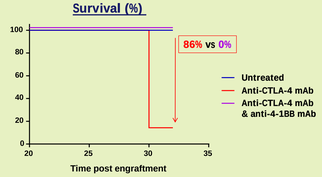


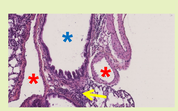


Anti-PD-L1 & anti-4-1BB mAb
Lymphocyte infiltration score: 3+

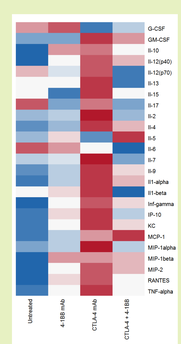
CTLA-4

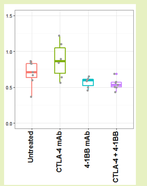
Survival (%)



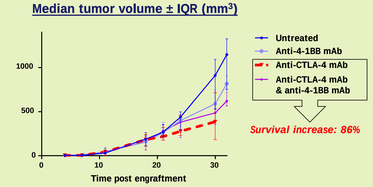


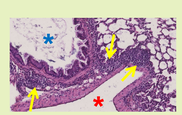
Anti-CTLA-4 mAb
Lymphocyte infiltration score: 1+





Median tumor volume ± IQR (mm³)





Anti-CTLA-4 & anti-4-1BB mAb
Lymphocyte infiltration score: 3+

Protected side effect (toxicity/survival)

Repeated IP injections of anti-PD-1, anti-PD-L1 and anti-CTLA-4 monoclonal antibody alone led to mortality of 29%, 86% and 86% of tumor bearing mice, respectively, within 2 weeks of the initiation of treatment.

No death was observed when mice were treated with repeated IP injections of anti 4-1BB (clone 3H3) agonist antibody. From different experiments, no death was observed when tumor bearing mice were treated with irrelevant IgG controls of the corresponding origin. When anti 4-1BB antibody was combined to either anti-PD-1, anti-PD-L1 or anti-CTLA-4 monoclonal antibody, a significant increase in survival was observed with no death observed in case of 4-1BB targeting antibody combined to either anti-PD-1 or anti-PD-L1 monoclonal antibodies and only 14% of death when anti-4-1BB antibody was combined to anti-CTLA-4 antibody.

Antitumor efficacy

Due to lethality in mice bearing orthotopic 4T1 tumor, the use of anti-PD-1, anti-PD-L1 or anti-CTLA-4 as single therapy is compromised and don't permit to observe anti-tumor activity of these therapies. In contrast, when used in combination with 4-1BB antibody, the increase of the mice survival observed permit to highlight an antitumor activity of anti-PD-1, anti-PD-L1 or anti-CTLA-4 antibodies. This antitumor activity was significant for mice treated with anti-PD-L1 antibody combined with 4-1BB targeting antibody (optimal T/C value of 37% on D30). In group receiving 4-1BB targeting antibody alone, a moderate antitumor efficacy was observed with an optimal T/C value of 65% on D30.

Immune response

Interestingly, Hematoxylin and Eosin (H&E) stain representative of lung shows a slight lymphocyte infiltration score for mice treated with anti-PD-1, anti-PD-L1, anti-CTLA-4 or anti-4-1BB antibodies whereas the scoring was increase for all mice treated in combination.

On D26, a cytokine release was evidenced in blood of mice treated with anti-PD-1, anti-PD-L1 or anti-CTLA-4. This release was decreased for mice treated with these immune checkpoint inhibitors in combination with 4-1BB.

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 row of the heatmap.

Splenomegaly was observed in mice receiving anti-PD-1 or anti-CTLA-4 monoclonal antibody alone, whereas splenomegaly was decreased when both monoclonal antibodies were administrated in combination with anti-4-1BB mAb.

Conclusions and perspectives

- Combinations of anti-4-1BB mAb with immune checkpoint inhibitors prevent adverse side effects of ICIs alone in the murine 4T1 mammary carcinoma model. This increased survival then allowed to observe antitumor activity of checkpoint inhibitor antibodies when combined to anti-4-1BB antibody,
- Fatal hypersensitivity reactions in murine 4T1 mammary tumor bearing mice treated with anti-PD-1, anti-PD-L1 or anti-CTLA-4 seem to be in correlation with a specific cytokine storm
- Highly characterized models (exome sequencing, immune infiltrate, response to standard of care therapies : radiotherapy, chemotherapy, immunotherapy,...) as well as high level technologies (*ex-vivo* assays, flow cytometry, imaging, hCS,...) are valuable tools to identify potent combination therapies.

References
1. WING K. *et al.*, Science, 322: 271-275, 2008
2. HODI F.S. *et al.*, N Engl J Med., 363:711-723, 2010
3. DONG H. *et al.*, J. Mol. Med., 81: 281-287, 2003
4. DAS R. *et al.*, The Journal of Immunology 194(3):950-959, 2014.