EVALUATION OF IMMUNO-ONCOLOGY RELATED TREATMENT IN SYNGENIC MOUSE MODELS

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 # 1325

Introduction

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]. BMS-936558, 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 and CTLA-4 immune checkpoints leads to remarkable antitumor effects [4].

A comprehensive panel of tools was constructed and validated aimed at evaluating the modulation of the immune system by new therapies. In immunocompetent mice, immune cells were studied for the detection of their cell markers using FACS phenotyping. We report on a panel of syngenic tumor models (4T1, A20, AB12, B16-F10, C1498, C26, C-51, CT26, EMT6, Hepa1-6, L1210, LLC, MBT-2, MPC-11, P388, Renca and TC-1) our capacity to correlate subpopulation of immune infiltrating cells and the therapeutic effects of critical antibodies directed against Cytotoxic T lymphocyte antigen-4 (CTLA-4), programmed death 1 membrane protein receptor (PD-1) and ligand (PDL-1).

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.

Material and Methods

In vivo experiments

Immunocompetent mice were obtained from Charles River (France). Animals were orthotopically or subcutaneously injected with syngenic tumor cell lines on D0. The animals received repeated injections of antibodies directed against CTLA-4, PD-1 and PDL-1. Isoflurane was used to anaesthetize the animals before cells injection, IV treatments and termination. All logistical parameters of the study (dosing, collection, measurements, raw data, lethality, behavior and results of autopsy…) were managed using Vivo Manager software (Biosystemes, Dijon). During the course of the experiment, animals were terminated under anesthesia when they displayed significant signs of physiological changes. Animal housing and experimental procedures were realized according to the French and European Regulations and NRC Guide for the Care and Use of Laboratory Animals. Animal facility is authorized by the French authorities (Agreement N° A21231011EA). All procedures using animals were submitted to the Animal Care and Use Committee of Oncodesign (Oncomet) agreed by French authorities (CNREEA agreement N° 91) [1, 2, 3].

Immune cell detection in mice tissues

The tumor and tumor draining lymph nodes were collected for subsequent FACS analysis to study the immune response in mice. Cells suspensions were prepared from tissues either by mechanistic dissociation or by enzymatic digestion. The antigens associated antibodies used for FACS analyses were CD45, CD3 and CD8 for T Cell lymphocytes, were CD45, CD11b, Ly6G, Ly6C, F4/80 for tumor-associated macrophages and were CD45, CD3, CD4, FoxP3 and CD25 for regulatory T Cell lymphocytes. The stained cells were analyzed with a CyFlow® space flow cytometer (LSR II, BD Biosciences) equipped with 3 excitation lasers at wavelengths 405, 488 and 633 nm.

1-Principe d'éthique de l'expérimentation animale, Directive n°2010/63 CEE du 22 septembre 2010, Décrêt n°2013-118 du 01 février 2013.
2-NRC Guide for the Care and Use of Laboratory Animals.
3-United Kingdom co-coordinating committee on cancer research guidelines for welfare of animals in experimental neoplasia, Br. J. Cancer 2010, 102: 1555-1577.

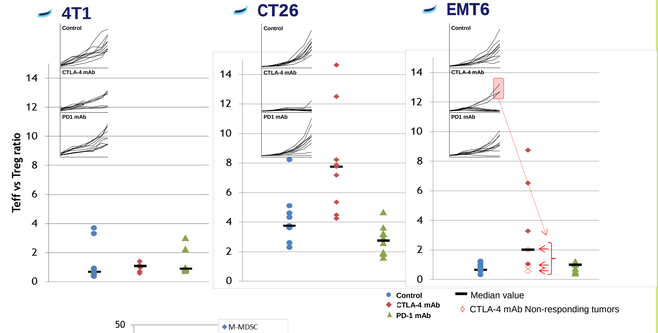
Results

Mouse immune checkpoint tumor target expression

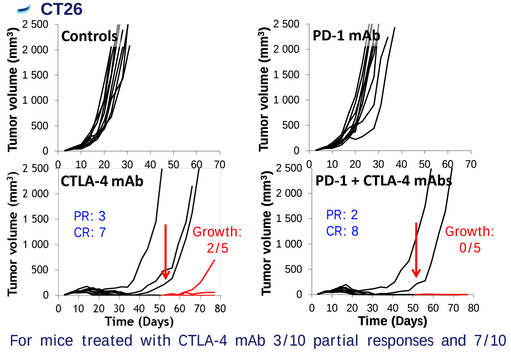
Cell line	Checkpoint Inhibitor related target				Cell surface antigen
	PD-L1	PD-L2	CD200	Galectin-9	Her-2
4T1					
A20					
AB12					
B16-F10					
C1498					
C26					
C51					
CT26					
EMT6					
Hepa1-6					
L1210					
LLC					
MBT-2					
MPC-11					
P388					
Renca					
TC-1					

Heat map of target expression levels shown as a percentile of the overall distribution of expression in the tumor panel (50% corresponds to median expression in the panel): 100% 70% 50% 30% 0%

Immune checkpoint effector cells (FoxP3+ CD4+ T) in tumor

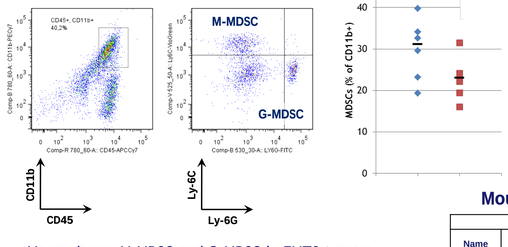


Combined immune checkpoint inhibitors.



For mice treated with CTLA-4 mAb 3/10 partial responses and 7/10 complete responses were observed. For mice treated in combination 2/10 partial responses and 8/10 complete responses were observed. The red arrow represents the tumor cells rechallenge subcutaneously grafted on D51 in 5 mice with a complete response (CR). A tumor growth was observed in 2 mice treated with CTLA-4 mAb, whereas no tumor growth was recorded for mice treated in combination.

EMT6 model characterization



Macrophages M-MDSC and G-MDSC in EMT6 tumors.

Tumors were analyzed by FACS for Lymphocyte Teffector *vs* Tregulator infiltrating immune cells *ratio*. In CT26 and EMT6 models, a *ratio* increase was observed for responding mice treated with CTLA-4 mAb. No *ratio* change was observed for mice treated with PD-1 mAb.
In 4T1 model, no *ratio* change was observed for mice treated with PD-1 or CTLA-4 mAb.

Marked antitumor activity
Moderate antitumor activity
Marginal antitumor activity

Mouse immune checkpoint *In vivo* efficacy

Model				Checkpoint Inhibitors			
Name	Type	Site	Strain mouse	CTLA-4	PD-1	PD-L1	Combo
A20	BCL	SC / IV	Balb/C	On-going			
4T1	Breast	OT	Balb/C			On-going	
B16-F10	Melanoma	SC	C57Bl/6		On-going	On-going	CTLA-4 / PD-L1
C1498	AML	SC / IV	C57Bl/6			On-going	
C38	Colon	SC	C57Bl/6	On-going			
CT26	Colon	SC	Balb/C				CTLA-4 / PD-1
EMT6	Breast	SC	Balb/C				
Hepa1-6	Liver	OT	C57Bl/6				
LLC	Lung	SC	C57Bl/6				
MBT-2	Bladder	OT	C3H	On-going	On-going		

Conclusions and perspectives

- Novel therapeutic strategies are being developed that aim to implicate the immune system in the initiation, development and progression of tumors, by resetting or redirecting the immune effectors against tumor cells.
- Pending new generation of humanized mouse models, the growing interest in immunology as a cancer therapy shows the limitation of conventional xenograft models in immunodeficient animals. A more effective approach is the use of syngeneic mouse models. Various examples of immunological readouts using syngeneic models are described here.
- We report on a panel of syngenic tumor models our capacity to correlate subpopulation of immune infiltrating cells and the therapeutic effects of new antibodies generation directed against CTLA-4, PD-1 and PDL-1 antigens.