EFFICACY OF PD-1 / PD-L1 PATHWAY DISRUPTORS IN SYNGENEIC MODELS

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 # B94

Abstract

Immune checkpoint modulators, such as antibodies targeting CTLA-4 or PD-1, are now being approved for treatment of patients with unresectable or metastatic melanoma and advanced squamous non-small cell lung cancer (NSCLC) who have progressed on or after platinum-based chemotherapy. Efficacy was also evidenced on other tumor types (renal cell carcinoma, bladder, Hodgkin lymphoma, colorectal carcinoma (CRC) …). However, there is still needs to identify predictive biomarkers of response in order to select patients who will benefit from treatments. PD-L1 expression was proposed to be a good candidate for NSCLC, even if PD-L1 expression is a difficult parameter due to its expression on both tumor cells and immune cells as well as technical challenges to use immunohistochemical detection. The dynamic of the immune system as well as the site and time where interactions between tumor cells and immune cells take place, increase the complexity of having a solid biomarker identified. In addition, for other pathologies like colorectal carcinoma, genomic biomarkers were evidenced. For example, CRC patients with mismatch repair (MMR) deficiencies have an objective response rate of 62% compared with 0% in patients with MMR-proficient tumors.
We propose here the use of syngeneic models to address mechanism of action and biomarker related questions for agent targeting PD-1 / PD-L1 axis. Syngeneic model systems remain one of the only options to analyse physiologically relevant interactions between tumor and immune cells. Up to now, eight models were characterized for response to either PD-1 and/or PD-L1 targeting antibodies. Among them, 4T1, LLC and Renca were identified as non-responders and B16-F10, CT-26, EMT-6, MBT-2 identified as partial responders. Most of the time, targeting PD-1 is more effective than targeting PD-L1, even if there is exception (e.g. B16-F10). Attempting to identify key parameters that could help us understand efficacy of PD-1 /PD-L1 axis disruptors, intratumoral immune infiltrate was analyzed in depth using flow cytometry: regulatory T cells (Treg), effector T cells (Teff), M-MDSCs, G-MDSCs, TAMs were phenotyped and quantified. In contrast to CTLA-4 targeting therapy, where Teff/Treg ratio was correlated to treatment efficacy, this is not the case for PD-1 or PD-L1 targeting therapies. It is now hypothesized that a more complex signature (e.g. detailed phenotype of CD8 positive T cells, tumor neoantigen expression…) will be needed to identify valuable biomarkers of response. Preliminary results using syngeneic models, both subcutaneously or orthotopically engrafted with tumors, will be presented.

Material and Methods

***In vivo* experiments**

Immunocompetent mice were obtained from Charles River (France). Animals were orthotopically (OT) or subcutaneously (SC) injected with syngeneic tumor cell lines on D0. The animals received repeated injections of antibodies directed against PD-1 and PDL-1. Isoflurane was used to anaesthetize the animals before cells injection 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, France). During the course of the experiment, animals were sacrificed 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° B21231011EA). 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

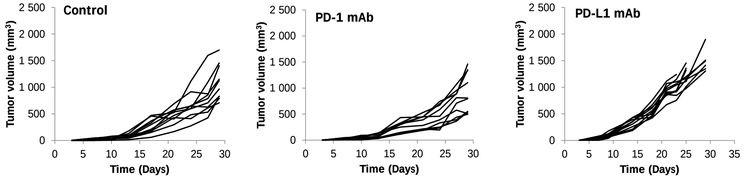
The tumor and tumor draining lymph nodes were collected for subsequent FACS analysis to study the immune response in mice. Cells suspensions were prepared from tissues either by mechanistic dissociation or by enzymatic digestion. The antigens associated antibodies used for FACS analyses are described in Table on right. The stained cells were analyzed with a LSR II flow cytometer (BD Biosciences) equipped with 3 excitation lasers at wavelengths 405, 488 and 633 nm.

Panels (up to 10 colors)	populations	markers
CD45, CD3, CD4, CD8, CD25, FoxP3	Teff	CD45+ CD3+ CD4- CD8+
	Treg	CD45+ CD3+ CD4+ CD8- (CD25+) FoxP3 +
CD45, CD3, CD8, TNFα, Perforin, Granzyme B	Teff	CD45+ CD3+ CD8+ (TNFα, Perforin, Granzyme B)
CD45, CD3, CD11b, Gr1, Ly6G, Ly6C, iNOS, Arg1	Total MDSCs	CD45+ CD3- CD11b+ Gr1+
	M-MDSCs	CD45+ CD3- CD11b+ Gr1+ Ly6G- Ly6C+ (iNOS, Arg1)
	G-MDSCs	CD45+ CD3- CD11b+ Gr1+ Ly6G+ Ly6C- (iNOS, Arg1)
CD45, CD3, CD11b, Gr1, CD68, CD80, CD206	Total TAMs	CD45+ CD3- CD11b+ Gr1-
	M1 TAM	CD45+ CD3- CD11b+ Gr1- CD68+ CD206-
	M2 TAM	CD45+ CD3- CD11b+ Gr1- CD68- CD206+

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.

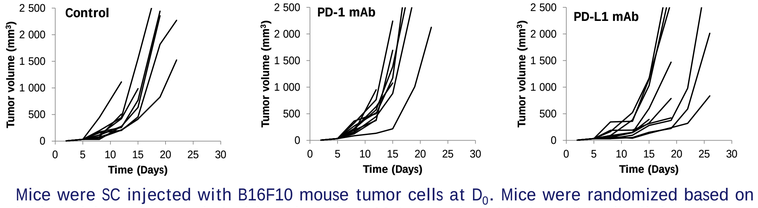
Results

4T1 Breast Carcinoma Model



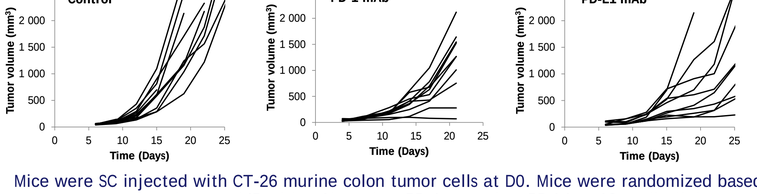
Mice were OT injected with 4T1 murine breast tumor cells at D0. Mice were randomized based on tumor volume and treated IP with mAb against PD-1 or against PD-L1 at 10 mg/kg/inj.

B16-F10 Malignant Melanoma Model



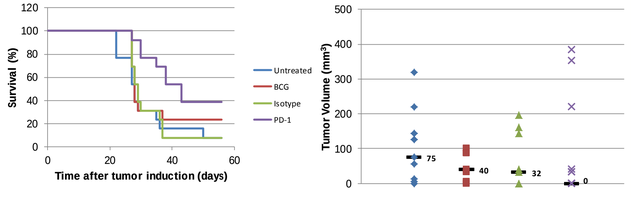
Mice were SC injected with B16F10 mouse tumor cells at D_0. Mice were randomized based on body weight and treated IP with mAb against PD-1 or against PD-L1 at 10 mg/kg/inj.

CT-26 Colon Carcinoma Model



Mice were SC injected with CT-26 murine colon tumor cells at D0. Mice were randomized based on tumor volume and treated IP with mAb against PD-1 or against PD-L1 at 10 mg/kg/inj.

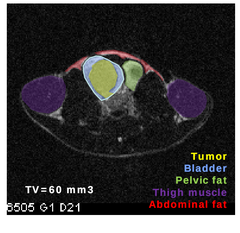
MBT-2 Bladder Carcinoma Model



Mice were OT injected with MBT-2 mouse tumor cells at D0. Mice were randomized based on body weight and treated with repeated intravesical instillations of BCG at 1.35 mg/kg/inj, with IP injection of isotype mAb or mAb against PD-1 at 10 mg/kg/inj. Tumor volume was determined by MRI.

MRI image

Representative MRI axial image of the pelvic region of a mouse OT injected with MBT-2 mouse tumor cells



Mouse immune checkpoint *in vivo* efficacy

summary		PD-1		PD-L1	
name	site	n	T/C (median)	n	T/C (median)
4T1	OT	11	97	1	104
A20	SC	1	37	0	NA
B16-F10	SC	3	78	1	33
C38	SC	0	NA	0	NA
CT26	SC	10	69	6	67
EMT6	SC	12	66	2	63
EMT6	OT	1	68	0	NA
HEPA1-6	OT	0	NA	0	NA
LLC	SC	1	110	1	88
MBT2*	OT	2	149**	0	NA
MBT2	SC	1	73	1	74
Renca*	OT	1	100**	0	NA

* Survival
** ILS

Conclusions and perspectives

- Agents targeting PD-1/PD-L1 axis, alone or in combination with other molecules, may be evaluated using these syngeneic mouse models.
- Biomarkers of response to immune checkpoint modulators are evaluated by flow cytometry as well as immunohistochemistry analyses.
- Newly available RNA sequencing data will help to understand how genomics information could be used as biomarker of response for these new therapies.