ANTI-CTLA-4 AND ANTI-PD-1 IMMUNOTHERAPIES REPRESS TUMOR PROGRESSION THROUGH INDEPENDENT CELLULAR MECHANISMS

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Porsolt

TARGETING THE IMMUNE SYSTEM AGAINST CANCER

Recently, immunotherapies demonstrate robust efficacy gains and durable responses in a wide variety of cancers. Nevertheless many patients still present resistance to immunotherapies. Response to immunotherapy relies on dynamic interactions between tumor cells and the tumor micro environment through targeting immune cells using "checkpoints" molecules, such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) or programmed cell death protein 1 (PD-1). An improved comprehension of the basic mechanisms underlying tumor-immune system interactions may improve clinical management of cancer. Here, we report a preclinical strategy developed in Porsolt to test two FDA-approved drugs, e.g. anti-CTLA-4 and anti-PD-1. We demonstrate that the two antibodies can affect differently the tumor immune microenvironment even if they both display efficient anticancer activity.

MATERIAL AND METHODS

Anti-CTLA-4 and anti-PD-1 have been administrated at days 6, 9, and 12 post-cell inoculation in intraperitoneal (i.p.) at a respective dose of 100 µg and 200 µg per animal diluted in saline (vehicle). Mice were randomized at day 6. Tumor volume was calculated according to the following formula: $V = Length * Width^2 / 2$.

When applicable statistical differences between the groups were determined using by one-way or two-ways ANOVA with repeated measures followed by Tukey's or Dunnett's multiple comparisons test (* $p \leq 0.05$, ** $p \leq 0.01$, **** $p \leq 0.0001$). Data represent mean ± SD. n = 9-10 mice per group for the subcutaneous (s.c.) model, n = 5-8 for the intravenous (i.v.) metastasis model, and n = 3-6 for the FACS analysis.

RESULTS

ANTI–CTLA-4 AND –PD-1 REPRESS TUMOR DEVELOPMENT IN IMMUNOCOMPETENT MOUSE MODELS

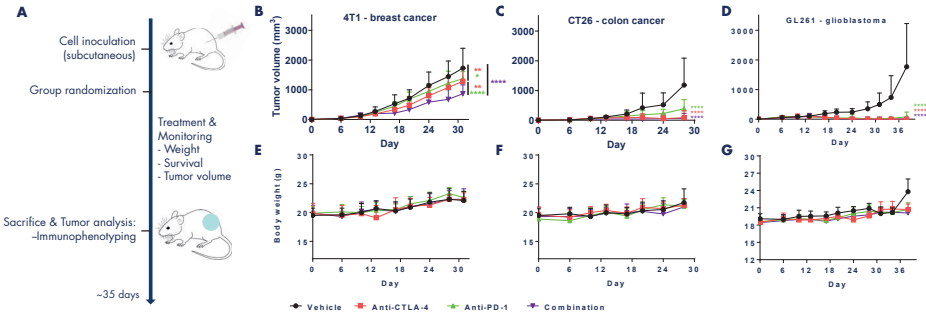


Figure 1: Effect of anti–CTLA-4 and –PD-1 immunotherapies in s.c. syngeneic tumor models

A. Description of the standard procedure for s.c. based syngeneic tumor grafting mouse models

B-G. Impact of anti–CTLA-4 and –PD-1 immunotherapies in monotherapy or in combination therapy on 4T1 (B, E), on CT26 (C, F), and GL261 (D, G) tumor growth and mice body weight.

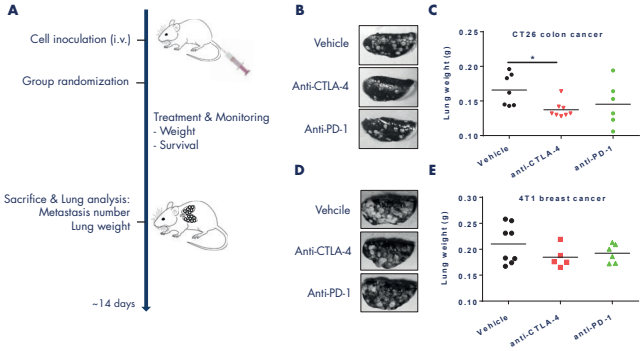
- Anti-CTLA-4 and anti-PD-1 reduce tumor progression in the three syngeneic mouse cancer models, e.g. 4T1triple negative breast cancer, CT26 colon cancer, and GL261 glioblastoma (Figure 2 B-D).
- Combination therapy demonstrates significant anti-cancer efficiency compared to monotherapies in the 4T1 model suggesting that cancer model with low sensibility to monotherapies might be overcame by combined therapy (Figure 2 B).
- Immunotherapies showed drastic anti-cancer effect in the GL261 model with tumor regression in the large majority of tumors (Figure 2 C).
- No obvious drug toxicity was observed during the experiment as revealed by the absence of body weight difference between groups (Figure 2 E-G).

CTLA-4 TARGETING STRATEGY REDUCES METASTASIS FORMATION IN OUR COLON CANCER MODEL

Figure 2: Effect of anti-CTLA-4 and –PD-1 immunotherapies in syngeneic i.v. based experimental metastasis models

A. Description of the standard procedure for the i.v. based syngeneic experimental metastasis model. B, C. Represented pictures (B) and quantification of lung weight (C) after necropsy in the CT26 mouse colon cancer model. D, E. Represented pictures (D) and quantification of lung weight (E) after necropsy in the 4T1mouse breast cancer model.

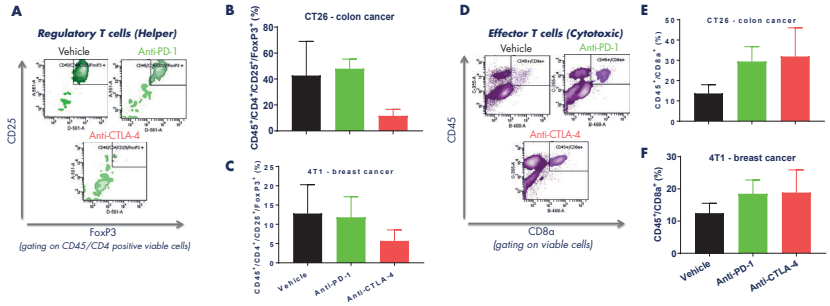
- Anti-CTLA-4, but not anti-PD-1, significantly reduces lung metastasis formation in CT26 colon cancer model (Figure 4 B, C).
- Immunotherapies do not significantly affect lung metastasis formation in 4T1 triple negative breast cancer model (Figure 4 D, E).



ANTI-CTLA-4 AND ANTI-PD-1 EXHIBIT INDEPENDENT CELLULAR MECHANISMS ON THE IMMUNE T CELL TUMOR MICROENVIRONMENT

Figure 3: Evaluation of tumor-infiltrating lymphocytes

A-E. Represented FACS multicolor analysis (A, C) and quantification of regulatory (B, C) and effector (E, F) T cells population after tissue processing of CT26 and 4T1 treated tumors.



- Anti-CTLA-4 reduces regulatory T cell (helper) population within the tumors but conversely anti-PD-1 does not (Figure 3 B, C).
- Anti-CTLA-4 and anti-PD-1 increase effector (cytotoxic) T cell population (Figure 3 E, F).
- Despite efficient anti-cancer activity (Figure 1), immuno-phenotyping analysis demonstrates independent cellular mechanisms between CTLA-4 and PD-1 blocking strategy. Targeting CTLA-4 leads to reduction of regulatory T cell population and recruitment of cytotoxic T cells while targeting PD-1 induces only recruitment of cytotoxic T cells (Figure 3).

CONCLUSION

The immunotherapy strategy aims to recruit anti-tumoral immune cells such as cytotoxic T cells in order to kill cancer cells and improve patient survival. Immune competent mouse models remain relevant tumor models useable to evaluate cancer tumor response to immunotherapy. Immunophenotyping analysis brings new insight on the effect of anti-CTLA-4 and anti-PD-1 immunotherapies. This new knowledge will participate to better understand the mechanisms of action of these new therapeutic strategies and may help to improve treatment efficiency or unravel tumor resistance.

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