#294. Preclinical Assessment of Anti-Tumor Activity and Immune Response in Syngeneic Tumor Models

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Introduction and Background

- Preclinical immuno-oncology (I/O) needs identification and refinement of tumor models that recapitulate relevant biological dynamics.
- We tested several murine models for their response to checkpoint inhibitors like anti-CTLA-4, anti-PD-L1 and anti-PD-1 antibodies and found sensitive, moderately sensitive and insensitive models.
- Since the application of more sophisticated endpoints is critical to confidently assess drug sensitivities we also evaluated the immune profiles of these models following treatment.

Materials and Methods

- Female Balb/C mice (CT26, 4T1-Luc) or C57BL/6 mice (Pan02) were purchased from Envigo and were implanted SC in the high axilla (CT26, Pan02) or in the mammary fat pad (4T1-Luc).
- Mice were treated IP with In Vivo Plus antibodies from Bio X Cell (West Lebanon, NH) at 10 mg/kg two times/week for a total of four or five doses.
- In the 4T1-Luc model, localized radiation of 8 Gy at a rate of 1.50 Gy/min was delivered to the tumor area with an RS2000 Biological X-ray Irradiator (Rad Source Technologies, Alpharetta, GA).
- For flow cytometry, the tumors were processed into single-cell suspensions using the gentleMACS™ Dissociators (Miltenyi Biotec). Samples were acquired on an Attune™ NxT Flow Cytometer (Thermo Fisher Scientific) and data was analyzed using FlowJo software (Tree Star).

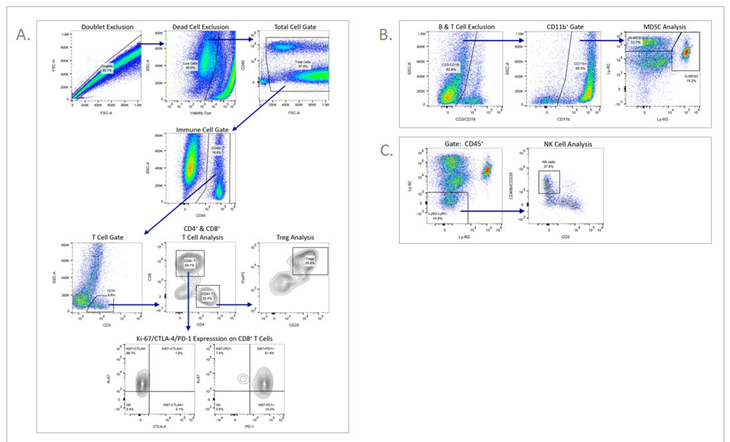

Figure 1. Gating strategies for flow cytometry. Similar gating strategies were used to analyze the 4T1 and Pan02 models. A) T cell analysis in CT26 tumors. Following exclusion of doublets and dead cells, total cells were analyzed for CD45+ immune cells. The CD3+ gate was then subdivided into CD4+ and CD8+ T cells. CD4+ T cells were further analyzed for the regulatory T cell subset (Tregs). Finally, CD8+ T cells were further analyzed for the Ki-67 proliferation marker and the CTLA-4/PD-1 exhaustion markers. B) MDSC analysis in CT26 tumors. B and T cells were first excluded from the CD45+ gate. CD11b+ cells were then further analyzed for M-MDSC and G-MDSC subsets. C) NK cell analysis in CT26 tumors. After exclusion of various myeloid subsets using Ly-6G and Ly-6C, NK cells were identified as CD3-CD49b+CD335+.

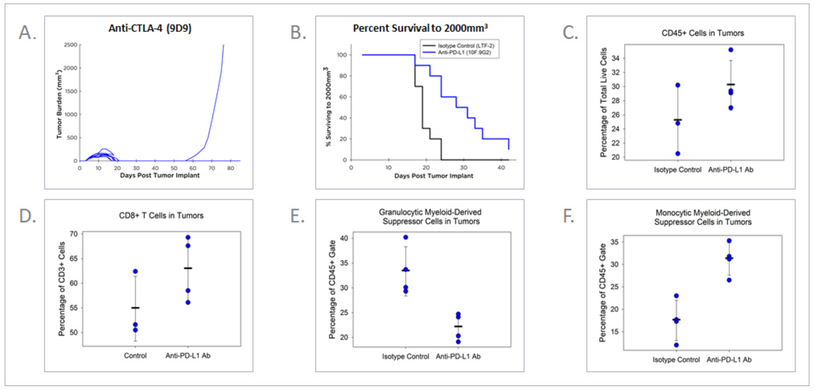

Figure 2. CT26: A model sensitive to checkpoint inhibitors (CPIs). Treatment effects of anti-CTLA-4 (A) or anti-PD-L1 (B) antibody in the CT26 mouse colon carcinoma model. Increased CD45+ cells (C) and CD8+ T cells (D) following treatment with anti-PD-L1 antibody. Treatment with anti-PD-L1 antibody shifts MDSC population from more granulocytic (E) to more monocytic (F).

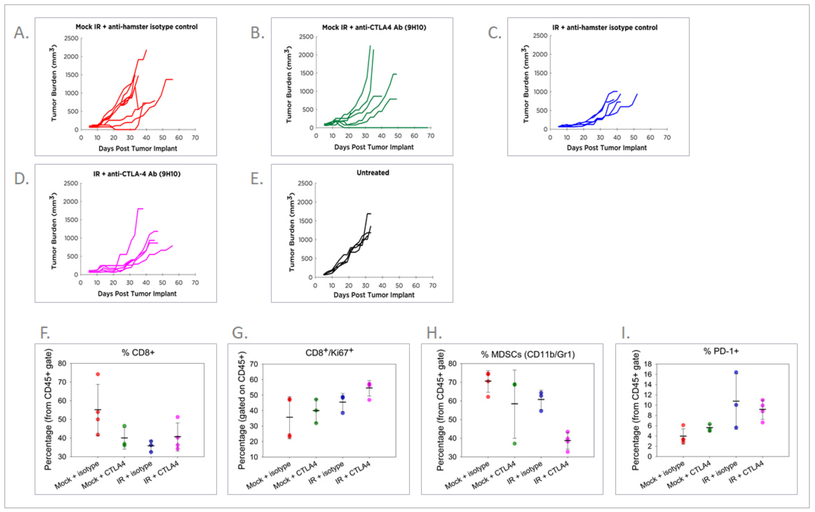

Figure 3. 4T1-Luc: A model moderately sensitive to CPIs. Treatment effects of an anti-CTLA-4 antibody, localized radiation (IR) or the combination in the 4T1-luc mouse mammary carcinoma model. Individual growth over time following treatment with isotype control (A), anti-CTLA-4 antibody (B), radiation (C) or the combination (D). Combination treatment triggers both pro- and anti-tumor signaling pathways thus providing a possible explanation for the marginal anti-tumor responses we observed in this model. Use of precise focal radiation could provide improvements in either single agent IR or IR combined with checkpoint inhibitors.

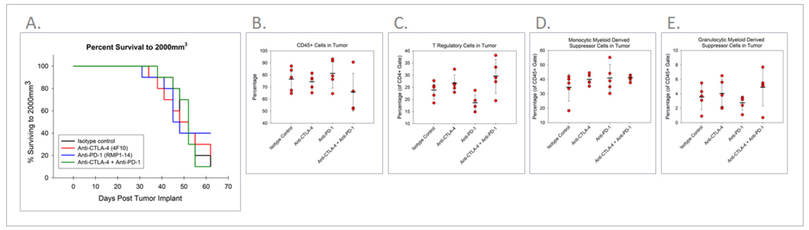

Figure 4. Pan02: A model insensitive to CPIs. Treatment with anti-CTLA-4 or anti-PD-1 antibodies as single agents or in combination displayed no anti-tumor activity (A). Treatment with CPIs did not substantially modulate the immune profile of the tumors (B – E) providing some possible rationale for the lack of efficacy observed.

Table 1. Comparison of Immune Profiles

Model/Treatment		Endpoint						
		CD45⁺ (%Total Cells)	CD4⁺ T cells (%CD3⁺)	CD8⁺ T cells (%CD3⁺)	Tregs (%CD4⁺ T cells)	NK (%CD45⁺)	G-MDSC (%CD11b)	M-MDSC (%CD11b)
CT26	Control	25.2 ± 4.9	25.6 ± 5.1	54.8 ± 6.6	47.7 ± 3.1	9.05 ± 3.0	33.3 ± 5.0	17.5 ± 4.5
	CTLA-4	ND	ND	ND	ND	ND	ND	ND
	PD-1	ND	ND	ND	ND	ND	ND	ND
	PD-L1	30.2 ± 3.5	19.7 ± 4.1	62.9 ± 6.6	47.9 ± 11.5	9.3 ± 1.4	22.1 ± 2.8	31.2 ± 3.6
4T1	Control	59.4 ± 8.1	63.6 ± 16.5	19.5 ± 16.6	27.7 ± 10.6	0.5 ± 0.3	54.0 ± 8.6	6.7 ± 2.7
	CTLA-4	75.0 ± 7.0	41.2 ± 24.3	15.5 ± 7.8	10.7 ± 9.6	1.4 ± 1.3	39.9 ± 10.3	13.6 ± 5.4
	PD-1	ND	ND	ND	ND	ND	ND	ND
	PD-L1	ND	ND	ND	ND	ND	ND	ND
Pan02	Control	80.4 ± 8.6	19.4 ± 5.7	46.0 ± 9.7	23.7 ± 3.6	1.1 ± 0.1	3.5 ± 1.7	34.3 ± 9.5
	CTLA-4	77.9 ± 8.2	15.4 ± 1.2	46.9 ± 11.1	26.5 ± 3.6	1.0 ± 0.3	4.0 ± 2.0	39.7 ± 3.8
	PD-1	84.3 ± 8.2	19.6 ± 3.9	47.9 ± 3.6	18.5 ± 3.4	1.1 ± 0.1	2.7 ± 1.0	40.7 ± 9.6
	PD-L1	ND	ND	ND	ND	ND	ND	ND

Results and Conclusions

- The CT26 model is sensitive to immune CPIs with 100% of the mice showing anti-tumor response following treatment with anti-CTLA-4 antibody and 40% demonstrating response following treatment with anti-PD-L1 antibody.
- Treatment of CT26 tumor-bearing mice with anti-PD-L1 results in an increase of CD45+ lymphocytes and modifies the composition of the myeloid derived suppressor cell population.
- Treatment of 4T1-Luc mice with radiation and anti-CTLA-4 antibody triggers both pro- and anti-tumor signaling pathways thus providing a possible explanation for the marginal anti-tumor responses we observed in this model.
- Pan02 is non-immunogenic, similar to human pancreatic cancers. No treatments had anti-tumor effects. The treatments did not alter the immune phenotype of this model. Pan02 may be useful to test CPIs in combination with other I/O agents, targeted agents, chemotherapies or radiation.

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