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Enhanced anti-tumor lymphocyte function and frequencies measured by multichromatic flow cytometry in human CTLA-4 knock-in mice in a colorectal carcinoma model after treatment with ipilimumab

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1 ABSTRACT

Targeting CTLA-4 has shown remarkable long-term benefits and thus remains a valuable approach for combating cancers of many types. A number of preclinical models have been developed over the years to evaluate the efficacy of immune checkpoint blockade in promoting antitumor immunity. In particular, knock-in (KI) humanized mouse models offer the possibility to study clinical grade immune checkpoint inhibitors (ICI) in the context of a fully functional immune system. Here we show the response to ipilimumab in a newly developed hCTLA-4 KI humanized mouse model. Our results demonstrate significant tumor growth inhibition as well as complete tumor regressions in the MC38 colorectal cancer model following treatment with ipilimumab. We have extended these studies by re-challenging the tumor-free surviving animals with tumors cells implanted opposite to the original tumor site. We established that all re-challenged hCTLA-4 KI mice remained tumor free suggesting potent T cell memory was maintained. Comprehensive multichromatic phenotyping and functional intracellular cytokine staining (ICS) using validated 18-color flow cytometry panels showed a significant increase in $CD8^+$ T cell frequencies when mice were treated with anti-hCTLA-4 but not with the mouse counterpart or the isotype control. Importantly, hCTLA-4 blockade reduced the regulatory T cell ($FoxP3^+$ Treg) frequency and increased leukocyte infiltration into the tumor in the hCTLA-4 treated mice but not in the other two treated groups. Furthermore, treatment of MC38 tumor-bearing mice with ipilimumab enhanced significantly the secretion of IFNγ and TNFα from $CD8^+$ and $CD4^+$ T cells. Altogether, the data presented here demonstrates that hCTLA-4 KI humanized mice are a robust model for evaluating the immune-modulatory effects and the activity of clinical grade ICI against tumors.

2 RESULTS

Increased survival after ipilimumab therapy in hCTLA-4-KI mice humanized mice.

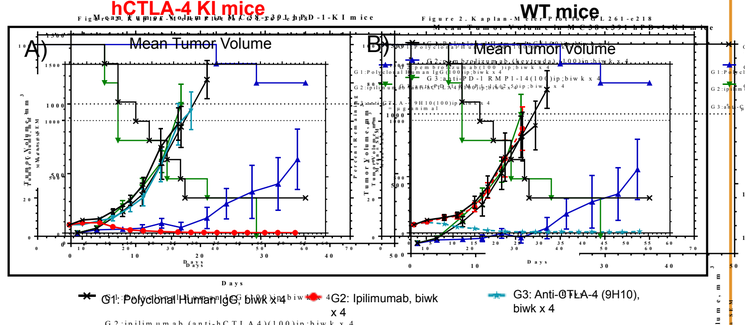


Figure 1. MC38 murine colorectal cancer cells were implanted in A) CTLA-4-KI humanized or B) WT-C57BL/6 mice. Dosing with ipilimumab or murine anti-CTLA-4 was initiated when tumors reached a 80 - 120 mm^3 tumor volume. Responders were followed 53 days.

Individual tumor volumes in CTLA-4-KI humanized and WT treated mice.

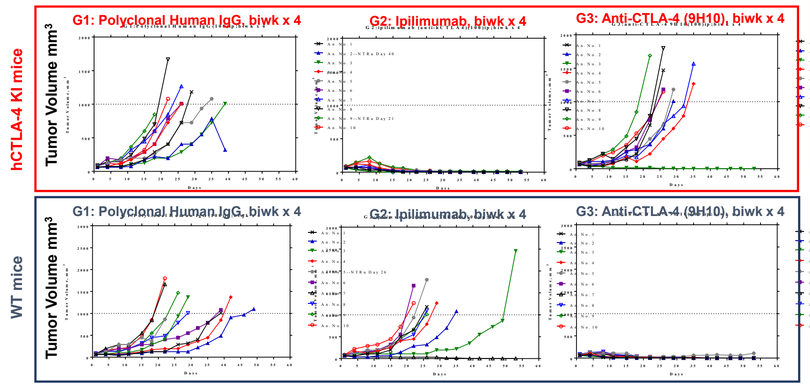
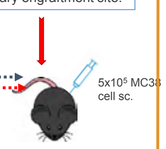


Figure 2. Individual MC38 tumor growth kinetics in CTLA-4-KI humanized and WT-C57BL/6 mice.

Response Summary Table

Group	Strain	Treatment Regimen Agent	Median TTE	%TGD	Statistical Significance vs G1	vs G2	MTV (n) Day 53	Regressions CR	TFS
1	hCTLA-KI	Polyclonal higG	25.4	---	---	***	---	0	0
2	hCTLA-KI	ipilimumab	53.0	109	***	---	1 (10)	10	9
3	hCTLA-KI	anti-mCTLA-4 9H10	26.5	4	ns	***	0 (1)	1	1
1	C57BL/6	Polyclonal higG	28.9	---	---	ns	---	0	0
2	C57BL/6	ipilimumab	25.8	-11	ns	---	4 (1)	1	1
3	C57BL/6	anti-mCTLA-4 9H10	53.0	83	***	***	4 (10)	9	9

Tumor free survivors (TSF) and naïve mice were re-inoculated with MC38 tumor cells in the opposite flank from the primary engraftment site.



Durable anti-tumor response following MC38 tumor re-challenge .

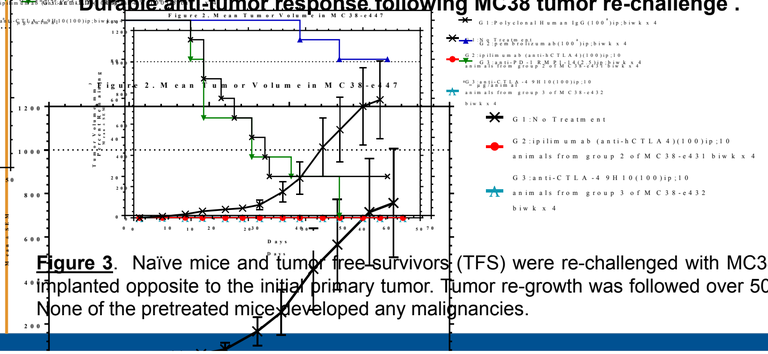


Figure 3. Naïve mice and tumor free-survivors (TFS) were re-challenged with MC38 cells implanted opposite to the initial primary tumor. Tumor re-growth was followed over 50 days. None of the pretreated mice developed any malignancies.

Immuno-phenotype of MC38 treated Tumors

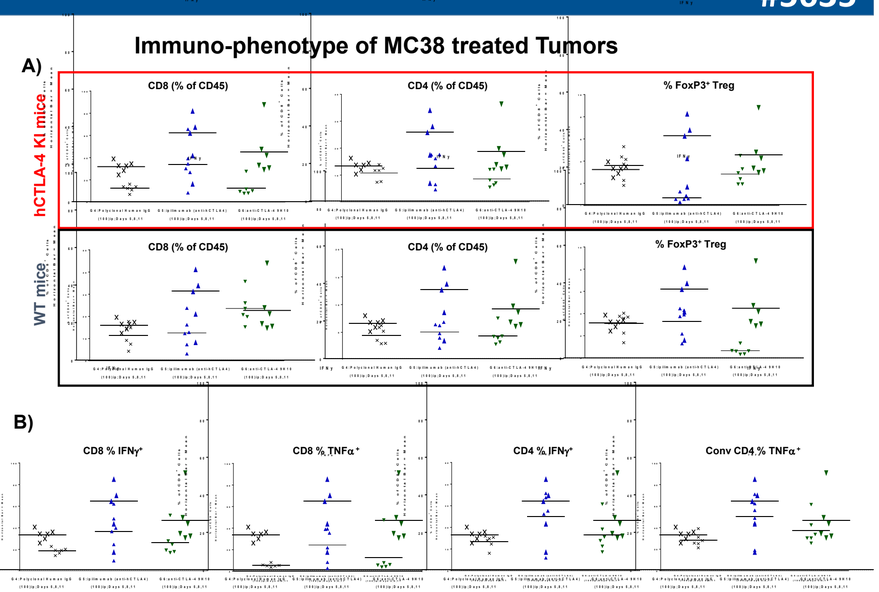


Figure 4. MC38 tumors from CTLA-4-KI humanized treated with ipilimumab or murine anti-CTLA-4 (days 5,8,11) were harvested 24 post last dose for immunophenotyping via Flow Cytometry. A) Percentage of T regs, CD4 and CD8 T cells present in the tumor tissue of CTLA-4-KI or WT mice. B) T cell ex-vivo stimulation shows increase levels of INFγ and TNFα in both CD4 and CD8 T cells of CTLA-4-KI humanized treated mice

4 SUMMARY and CONCLUSIONS

- We observed significant tumor growth delay (TGD) and survival following ipilimumab monotherapy in CTLA-4-KI humanized mice bearing MC38 tumors.
- Complete regressions (10/10) were observed in the CTLA-4-KI humanized receiving ipilimumab monotherapy. WT-C57BL/6 mice responded similarly to the murine CTLA-4 (9H10) therapy. All TF survivors showed durable responses in a tumor re-challenge model.
- These results demonstrate that hCTLA-4 KI humanized mice are a robust model for evaluating the immune-modulatory effects and the activity of clinical grade ICI against tumors.