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Pembrolizumab and Ipilimumab Reduce Human RKO Colon Carcinoma Tumor Growth in HLA Matched CD34-NSG Humanized Mice

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1 ABSTRACT

Over the past decade there has been an increasing demand for preclinical models useful for evaluating the efficacy of checkpoint inhibition-based cancer immunotherapies. The recently developed humanized mouse models composed of various types of human immune cells has the potential to recapitulate the human anti-tumor response modulated by checkpoint inhibitors. Tumor cells may develop multiple mechanisms to evade immune surveillance, including PD-L1 upregulation which suppresses T-cell effector responses towards the tumor. In the present study, we evaluated the efficacy of the checkpoint inhibitors pembrolizumab (anti-PD-1) and ipilimumab (anti-CTLA-4) in the human RKO colon carcinoma xenograft model in CD34-NSG humanized mice. These mice were engrafted with human hematopoietic stem cells (HSC) that matched the HLA haplotype of the RKO cells (HLA-A*01). Flow cytometry analysis confirmed high level of expression of PD-L1 in RKO colon carcinoma cells. The results from this study revealed that pembrolizumab and ipilimumab monotherapies significantly inhibited tumor growth. Surprisingly, combination therapy did not provide additive or synergistic effects and resulted in the same level of efficacy as the individual regimens. In order to understand the impact of these therapies on distinct immune cell populations, we analyzed the distribution of CD4 and CD8 T lymphocytes, Treg cells, NK cells and B cells in peripheral blood, spleen and tumor samples from tumor bearing humanized mice, via flow cytometry. We found that these checkpoint inhibitors can enhance effector functions of $CD4^+$ and $CD8^+$ T cells associated with increased expression of IFN-γ. A parallel study with these checkpoint inhibitors carried out with humanized mice engrafted with HSC of the same haplotype as the RKO cells but from a different donor resulted in identical anti-tumor efficacy. In summary, these studies validate the use of humanized mouse models to test the tumor response to immune-checkpoint based therapies as it shows significant anti-tumor growth as a result of pembrolizumab and ipilimumab monotherapies associated with activation of T lymphocytes.

2 MATERIALS AND METHODS

Female CD34+NSG™ mice (Jackson laboratory) (HLA-A*01), were 16 weeks old when implanted with $1x10^7$ RKO tumor cells in 50% Matrigel, subcutaneously in the right flank. Dosing began on Day 1 in mice with established RKO tumors (group mean 70 mm^3). The study endpoint was a tumor volume of 1500 mm^3 in the control group or 27 days, whichever came first. Treatment outcome was based on percent tumor growth inhibition (%TGI), defined as the percent difference between the median tumor volumes (MTVs) of treated and control mice. Percent TGI was calculated using the following formula: *%TGI = [1-(MTVdrug treated/MTVcontrol)] x 100.* Representative results of two independent studies are shown here. On Day 15, spleen, blood and tumors from satellite groups were processed and immune cell populations were analyzed by flow cytometry. All data were collected on a FACSCanto II (BD) and analyzed with FlowJo software (Tree Star, Inc.). Initial sequential gating on singlets (FSC-H vs. FSCA), leukocytes (SSC-A vs. FSC-A) and live cells was performed followed by identification of immune cells populations based upon the following signature markers: $hCD45^+$; CD4 ($hCD45^+CD3^+CD4^+$), CD8 ($hCD45^+CD3^+CD8^+$), Treg ($CD3^+$ $CD4+CD25^+$ $FoxP3^+$), Macrophages ($CD45^+HLA\text{-}DR^+CD68^+CD163^+$), gMDSC ($hCD45^+HLA\text{-}DR\text{-}CD33^+CD15^+$), mMDSC ($hCD45^+HLA\text{-}DR\text{-}CD33^+CD14^+$), and B cells ($hCD45^+CD19^+CD20^+$). T cell populations were further defined as naïve (N), effector (EFF), effector memory (EM) and central memory (CM), based upon CCR7 and CD45RO expression. Furthermore, intracellular staining was performed to detect the cytokine production in $CD4^+$ and $CD8^+$ cell populations (IFN-γ, IL-2, TNFα) after in vitro stimulation. Statistical Significance was determined using the Mann-Whitney U test.

4 SUMMARY and CONCLUSIONS

- Pembrolizumab and ipilimumab monotherapies significantly inhibited tumor growth in the human RKO colon carcinoma xenograft in the $CD34^+$ NSG humanized mouse model.
- Combination therapy with these checkpoint inhibitors does not enhance the efficacy demonstrated by either monotherapy.
- Moderate toxicity was observed with combination therapy
- FACS analysis revealed that activated effector T cells appear to play a role in tumor rejection
- mMDSC, gMDSC and macrophages are poorly represented in this model (data not shown).
- Lack of professional APC may restrict the overall tumor specific T cell response

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3 RESULTS

Table I and Figure 1

Group	N	Treatment Regimen: Agent	µg/animal	Route	Schedule	MTV (n): Day 27	%TGI	Statistical Significance	Mean BW Nadir
1	8	Polyclonal Human IgG	100	ip	biwk x 3	1800 (8)	---	---	---
2	8	ipilimumab	100	ip	biwk x 3	663 (8)	63	$p < 0.001$	---
3	8	pembrolizumab	100	ip	biwk x 3	936 (7)	48	$p < 0.001$	---

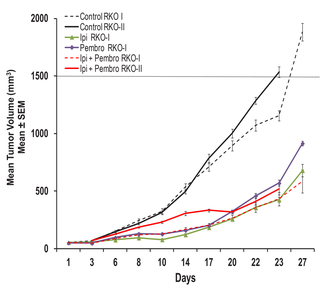

Table I and Figure 1: Treatment table and mean tumor growth in the RKO-I study following treatment with ipililumab and pembroluzimab monotherapies. Statistical Significance was determined using the Mann-Whitney U test.

Table II and Figure 2

Group	N	Treatment Regimen: Agent	µg/animal	Route	Schedule	MTV (n): Day 24	%TGI	Statistical Significance	Mean BW Nadir
1	16	Polyclonal Human IgG	200	ip	biwk x 3	1470 (10)	---	---	-3.0% Day 13
2	16	ipilimumab pembrolizumab	100 100	ip ip	biwk x 3	498 (10)	66	$p < 0.001$	-8.7% Day 24

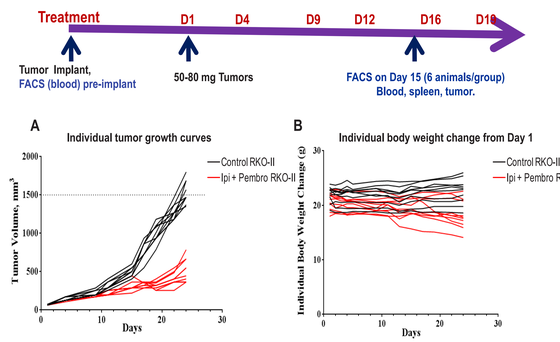

Table II and Figure 2: Treatment table and mean tumor growth in the RKO-II study following treatment with ipililumab and pembroluzimab combination therapy. A) Individual tumor growth curves. B) Individual body weight change from Day 1. Mean BW Nadir = lowest group mean body weight, as % change from Day 1.

Figure 3: Flow analysis of blood samples on Day 15

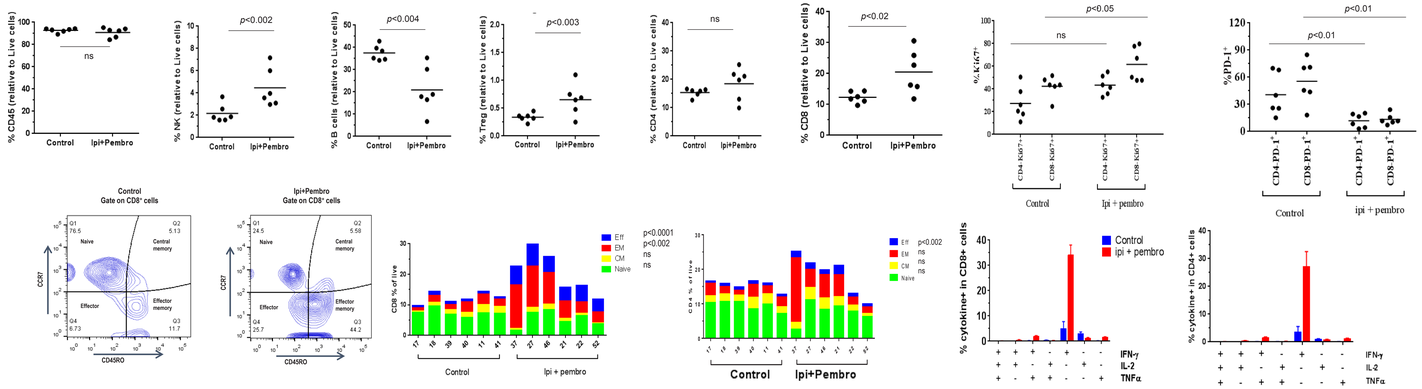

Figure 4: Flow analysis of spleen samples on Day 15