Combination Immune Checkpoint Inhibitors for the Treatment of Human Colon Carcinoma in hPBMC-NCG Humanized Mouse Model

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1 ABSTRACT

Over the past decade there have been great advances in the development of new preclinical models for the evaluation of the efficacy of checkpoint inhibition-based cancer immunotherapies. In particular, the availability of NSG, NCG and NOG superimmune-deficient mice lacking the expression of B-cells, T cells and NK cells, have proven instrumental in the development of humanized mouse models. These humanized models offer unique tools to assess the anti-tumor response to immune-checkpoint inhibitors in animals bearing a human immune environment. The work presented here shows that NCG mice successfully engraft with hPBMC and develop graft versus host disease (GvHD) five to six weeks post engraftment, providing a suitable model for pre-clinical immune-oncology studies. We investigated the response to the checkpoint inhibitors pembrolizumab (anti-PD-1) and ipilimumab (anti-CTLA-4) in the human RKO colon carcinoma xenograft model following the engraftment of hPBMC in NCG mice. Combination of pembrolizumab with ipilimumab therapy resulted in a significant inhibition of tumor growth. Analysis of the human immune cell subsets showed that the majority of the human CD45$^+$ cells were T-cells. A significant increase in the T-cell population, specifically CD8$^+$ T-cells was observed in the ipilimumab and pembrolizumab combination therapy group as compared to the control group treated with non-specific control IgG. In summary, this study supports the use of the huPBMC-NCG mouse model to test the tumor response to immune-checkpoint based therapies as it shows significant tumor growth inhibition associated with CD8$^+$ T-cell expansion. In addition, this study establishes an appropriate therapeutic window to evaluate cancer treatments before the onset of xenogeneic GvHD in this model

2 RESULTS

Human PBMC Donor Evaluation for Humanized Studies in NCG mice

- hPBMC from a single donor can be engrafted in a large cohort of animals.
- Body weight loss used as a read out of GvHD.
- Weekly flow cytometry analysis of hCD45 to evaluate hPBMC engraftment levels.

Table I: Body weight post hPBMC engraftment

Group	N on Day 66	Treatment Regimen: Agent	#cells/ animal	Route	Schedule	Mean BW Nadir	Number of Deaths
1	4/4	D204229	1 x 10^7	iv	Day 1	-3.8% Day 55	0
2	3/4	D204229	2 x 10^7	iv	Day 1	-3.3% Day 66	1 (Day28)
3	1/4	D270229	1 x 10^7	iv	Day 1	-6.5% Day 66	3 (Days 41, 48, 57)
4	0/4	D270229	2 x 10^7	iv	Day 1	---	4 (Days 38, 41, 55, 59)
5	4/4	D136302	1 x 10^7	iv	Day 1	-0.2% Day 55	0
6	4/4	D136302	2 x 10^7	iv	Day 1	-0.9% Day 55	0
7	4/4	D136302	3 x 10^7	iv	Day 1	-1.0% Day 55	0
8	4/4	D203727	1 x 10^7	iv	Day 1	-5.9% Day 66	0
9	0/4	D203727	2 x 10^7	iv	Day 1	---	4 (Days 36, 38, 43, 48)
10	0/4	D203727	3 x 10^7	iv	Day 1	---	4 (Days 36, 43, 55, 55)

Figure 1. hCD45 engraftment kinetics

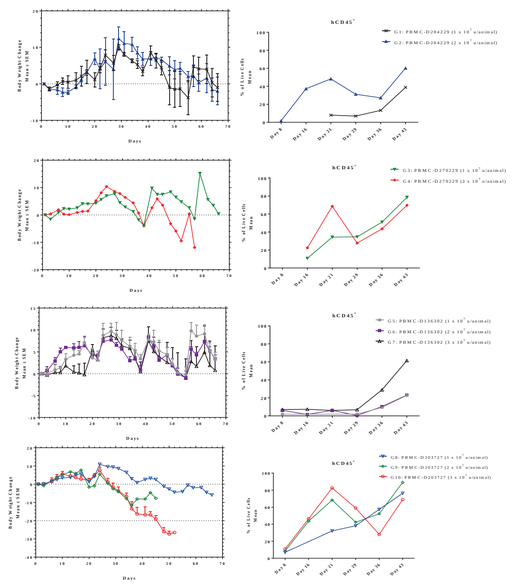


Figure 1. Female NCG mice (Charles River, NOD-Prkdc^{em26Cd52}Il2rg^{em26Cd22}/NjuCrl) were 8-10 weeks of age, when engrafted intravenously (i.v.) with human peripheral blood mononuclear cells (hPBMC) at 1, 2 or 3x10^7 cells/animal (Hemacare) on Day 1 of the study. Body weight measurements were taken twice weekly. Peripheral blood was collected in tubes containing K_2EDTA 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+, CD3+ (hCD45+CD3+). All hCD45+ cells were CD3+ T-cells (data not shown).

Ipilimumab (anti-hCTLA-4) and Pembrolizumab (anti-hPD-1) activity in the Human RKO Colorectal Carcinoma in NCG Mice Humanized with hPBMC.

Table II: Protocol Design

Group	n	Treatment Regimen: Agent	µg/animal	Route	Schedule
1	10	Polyclonal Human IgG	200	ip	biwk x 4
2	10	ipilimumab	100	ip	biwk x 4
3	10	pembrolizumab	100	ip	biwk x 4
4	10	ipilimumab pembrolizumab	100 100	ip ip	biwk x 4 biwk x 4

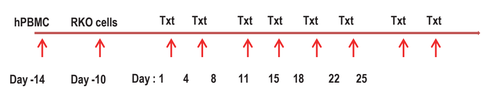


Figure 2a: Group Mean Tumor Volume

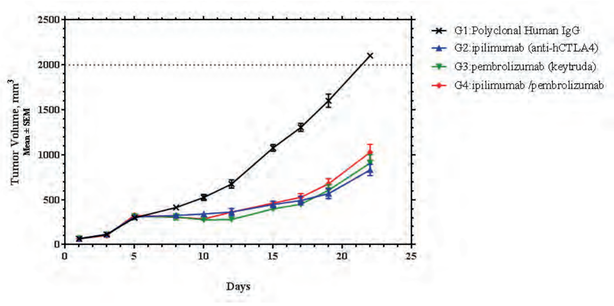


Figure 2b: Individual Animal Tumor Volume

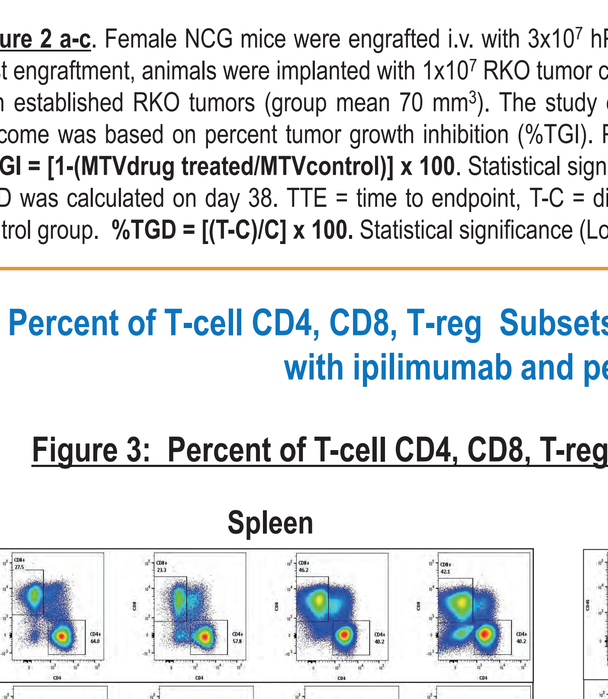


Figure 2c: Group body weight changes

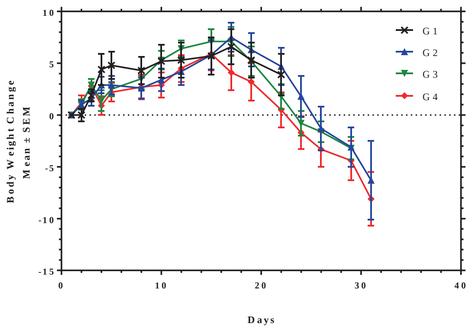


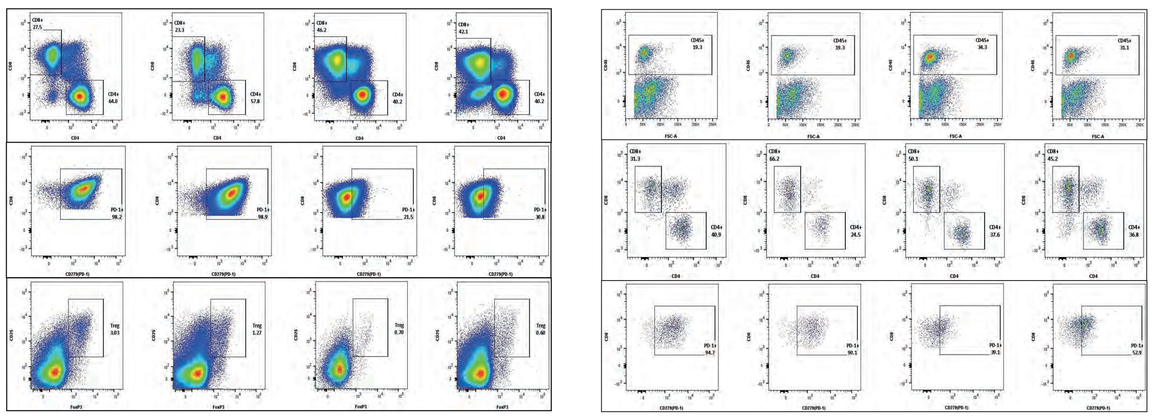
Table III: Response summary. TGI and TGD analysis

		Treatment Regimen	MTV (n)		Statistical Significance	Median		Statistical Significance	Mean BW	
Group	n	Agent	Day 22	%TGI	vs G1	TTE	%TGD	vs G1	Nadir	NTR
1	10	Polyclonal Human IgG	2112 (10)	---	---	21.5	---	---	---	0
2	10	ipilimumab	817 (10)	61	***	29.9	39	***	-6.3% Day 31	0
3	9	pembrolizumab	936 (10)	56	***	28.7	33	***	-3.2% Day 29	1
4	10	ipilimumab pembrolizumab	1060 (10)	50	***	29.8	39	***	-8.1% Day 31	0

Figure 2 a-c. Female NCG mice were engrafted i.v. with 3x10^7 hPBMC cells/animal (Hemacare donor D136302). A week post engraftment, animals were implanted with 1x10^7 RKO tumor cells in 50% Matrigel, sc. Dosing began on Day 1 in mice with established RKO tumors (group mean 70 mm^3). The study endpoint was a tumor volume of 2000 mm^3. Treatment outcome was based on percent tumor growth inhibition (%TGI). Percent TGI was calculated using the following formula: **%TGI = [1-(MTVdrug treated/MTVcontrol)] x 100**. Statistical significance was determined using the Mann-Whitney *U* test. TGD was calculated on day 38. TTE = time to endpoint, T-C = difference between median TTE (Days) of treated versus control group. **%TGD = [(T-C)/C] x 100.** Statistical significance (Logrank test). Non-treatment related (NTR) deaths.

Percent of T-cell CD4, CD8, T-reg Subsets in Humanized NCG Mice treated with ipilimumab and pembrolizumab

Figure 3: Percent of T-cell CD4, CD8, T-reg, and PD-1 in spleen and tumors



On Day 12 (24 hrs. post dose), spleen and tumors from satellite groups were processed for single cells suspensions and immune cell populations were analyzed by flow cytometry. 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$^+$, CD279), CD8 (hCD45$^+$CD3$^+$CD8$^+$, CD279), T-reg (CD3$^+$ CD4+CD25$^+$ FoxP3$^+$).

3 SUMMARY AND CONCLUSIONS

I. Multiple hPBMC donors were evaluated for engraftment in NCG mice which showed various levels of engraftment which depended on the donor and the number of cells engrafted.
II. By day 22 all of the hCD45+ cells were CD3+ distributed between CD4 and CD8 subsets (data not shown).
III. There therapeutic window in the hPBMC-NGG model is donor dependent (~40 days).
IV. The RKO colorectal carcinoma model is responsive to ipilimumab and pembrolizumab monotherapies. Combination therapy does not produce additive or synergistic effects.
V. FACS analysis revealed that CD8 T cells subsets appear to play a role in tumor rejection.

4 ACKNOWLEDGEMENTS

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