Validation of humanized PD-1 knock-in mice as an emerging model to evaluate human specific PD-1 therapeutics

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1 ABSTRACT

Over the past decade there has been an increasing demand in the use of syngeneic models for evaluating the efficacy of checkpoint inhibition-based cancer immunotherapies. During tumor development, immune cells can became unresponsive to the presence of tumor cells due to chronic activation and expression of the programmed cell death protein-1 (PD-1) or the T lymphocyte associated antigen 4 (CTLA4) in T-cells or the presence of FoxP3$^+$ Treg cells resulting in tumor immune-tolerance.

Our previous studies have demonstrated that murine anti-PD-1 and CTLA-4 therapy can effectively re-activate the antitumor response against multiple syngeneic tumor models. While these models proved instrumental for evaluating murine immune-checkpoint inhibitors (ICI), there is a clear need for additional mouse models to evaluate the efficacy of ICI specific for human targets. To address this need, we describe the development of a humanized PD-1 knock-in (KI) mouse model. This mouse model has the advantage of expressing the human PD-1 protein in the context of a fully functional immune system. We also show the response to clinically relevant immune checkpoint inhibitors in two preclinical tumor models. We evaluated the anti-tumor activity of pembrolizumab in the MC38 colorectal carcinoma and the GL261 glioblastoma models. We observed significant tumor growth inhibition and growth delay in the MC38 tumor model when treated with pembrolizumab monotherapy, but not when treated with the murine counterpart (anti-PD-1 clone RPM1-14). To extend our validation studies to other tumor models, we implanted GL261 glioblastoma orthotopically in the brain of PD-1 KI mice and achieved a significant increased life span in the group treated with pembrolizumab compared to both the control group and the group treated with murine anti-PD-1 antibody. The immune profile from control and treated these animals were also characterized in these studies by flow cytometry. In summary, the results shown here underscore the value of the humanized PD-1 knock-in (KI) mouse model as a tool to evaluate human specific immune-checkpoint based therapeutics alone and in combination with other agents.

2 MATERIALS AND METHODS

Female C57BL/6-hPD1 KI mice from GenOway and control C57BL/6 were eight to sixteen weeks old at the start of the respective studies. MC38 tumors were initiated by subcutaneous implantation of 5 x 10^5 MC38 cells. Tumors were monitored as their volumes approached the target start size range of 60-90 mm^3. GL261 tumors were initiated by intracranially implanting 1 x 10^5 GL261 cells into the top of the skull of each test animal under anesthesia. Five days after implantation, animals were sorted into treatment groups (Table 1). Flow cytometry analysis of blood, spleens and tumors were performed on Day 16. All tissues were prepared into single cell suspensions and labeled with a panel of antibodies for cell identification and evaluation of hPD-1 expression. Panel antibodies included: CD45, CD3, CD4, CD8, CD25, FoxP3*, hPD-1, LIVE/DEAD; sourced from Biolegend, BD biosciences, or Thermo Fisher. All data were collected on a Fortessa LSR (BD) and analyzed with FlowJo software version 10.0.7r2 (Tree Star, Inc.).

Abbreviations:

ILS = Increased life span, T/C*100% - 100%

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): ns = not significant, * = P < 0.05, ** = P < 0.01, *** = P < 0.001, compared to Group 1 or group indicated

n = number of animals in a group not dead from accidental or unknown causes, or euthanized for sampling

3 RESULTS

Increased survival after pembrolizumab monotherapy in hPD1-KI humanized mice.

Figure 1. GL261 murine glioma tumor cells were implanted orthotopically in hPD1-KI humanized mice. Dosing was initiated five days post engraftment. Survival was evaluated over a 60 days period.

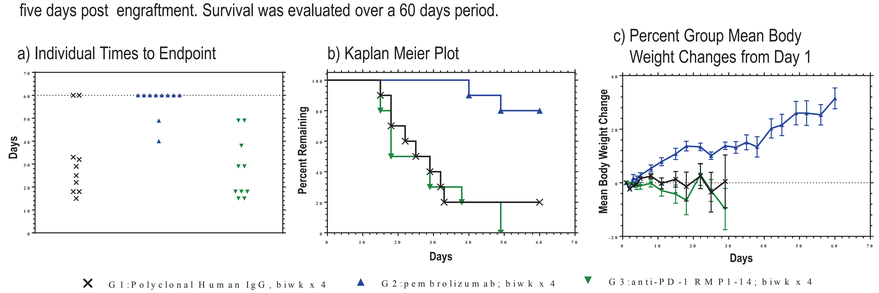

Table 1. Response summary in the GL261 glioma orthotopic model in hPD-1-KI humanized mice

		Treatment Regimen				Median		Statistical Significance		n Remaining
Group	n	Agent	mg/kg	Route	Schedule	TTE	ILS	vs G1	vs G2	Day 60
1	10	Polyclonal Human IgG	100[a]	ip	biwk x 4	27.0	---	---	**	2
2	10	pembrolizumab	100[a]	ip	biwk x 4	60.0	122	**	---	8
3	10	anti-PD-1 RMP1-14	2.5	ip	biwk x 4	23.5	-13	ns	***	0

a = µg/animal

Pembrolizumab demonstrate significant tumor growth delay in the MC38 colorectal cancer model In hPD1-KI mice humanized mice.

Figure 2. MC38 murine colorectal cancer cells were implanted in hPD1-KI humanized or C57BL/6 mice. Dosing with pembrolizumab or murine anti-PD1 was initiated when tumors reached a 80-120 mm^3 tumor volume. Responders were followed 45 days.

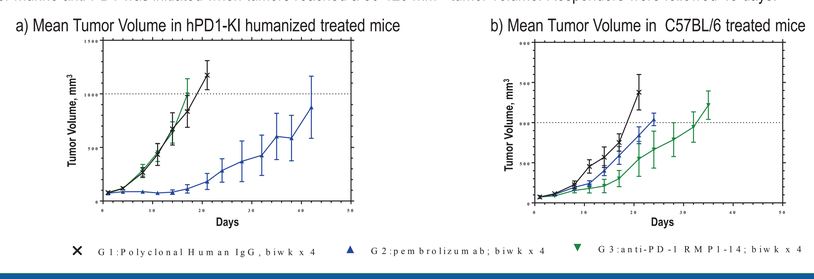

Table 2. Response summary in the MC38 colorectal carcinoma model in hPD-1-KI humanized mice

		Treatment Regimen				Median		Statistical Significance		MTV (n)	Regressions		
Group	n	Agent	µg/animal	Route	Schedule	TTE	%TGD	vs G1	vs G2	Day 45	PR	CR	TFS
1	7	Polyclonal Human IgG	100	ip	biwk x 4	18.7	---	---	**	---	0	0	0
2	7	pembrolizumab	100	ip	biwk x 4	40.2	115	**	---	1 (2)	0	2	2
3	7	anti-PD-1 RMP1-14	100	ip	biwk x 4	16.5	-12	ns	***	---	0	0	0

Table 3. Immuno-phenotype of tissues from hPD-1-KI and C57BL/6 mice bearing MC38 tumors

		PD-1-KI mice (N=3)			C57BL/6 mice (N=3)		
Tissue	Regimen	Human IgG	Pembrolizumab	anti-PD-1 RMP1-14	Human IgG	Pembrolizumab	anti-PD-1 RMP1-14
Blood	CD4$^+$ (% of CD45$^+$)	8.42 ± 1.1	7.35 ± 1.4	7.57 ± 0.5	7.36 ± 0.8	7.77 ± 0.6	10.4 ± 0.6
	hPD-1$^+$ (% CD4$^+$)	2.56 ± 0.3	0.72 ± 0.2	2 ± 0.1	---	---	---
	CD8$^+$ (% of CD45$^+$)	7.43 ± 1.1	5.99 ± 1.1	6.37 ± 0.4	6.42 ± 0.8	6.8 ± 0.2	9.15 ± 0.3
	hPD-1$^+$ (% of CD8$^+$)	0.81 ± 0.1	---	0.53 ± 0.1	---	---	---
Spleen	CD4$^+$ (% of CD45$^+$)	11.97 ± 0.8	12.57 ± 0.9	12.03 ± 1	10.57 ± 0.3	11.63 ± 0.5	13.13 ± 0.2
	hPD-1$^+$ (% of CD4$^+$)	8.61 ± 0.7	6.53 ± 1.6	5.86 ± 0.3	---	---	---
	CD8$^+$ (% of CD45$^+$)	9.44 ± 0.6	10.01 ± 0.5	9.7 ± 0.6	8.89 ± 0.4	9.7 ± 0.5	11.6 ± 0.6
	hPD-1$^+$ (% of CD8$^+$)	1.19 ± 0.2	0.29 ± 0	0.94 ± 0	---	---	---
Tumor	CD4$^+$ (% of CD45$^+$)	1.2 ± 0.7	1.26 ± 0.7	0.99 ± 0.2	1.6 ± 0.7	0.87 ± 0.3	2.12 ± 0.9
	hPD-1$^+$ (% of CD4$^+$)	48.45 ± 4.3	39.9	41.8	---	---	---
	CD8$^+$ (% of CD45$^+$)	3.79 ± 2.1	4.19 ± 1.7	3.1 ± 1.4	5.15 ± 3	4.6 ± 2	10.19 ± 4.7
	hPD-1$^+$ (% of CD8$^+$)	81.3 ± 1.4	83.3 ± 3	73.17 ± 5.7	---	---	---

4 SUMMARY and CONCLUSIONS

- We evaluated the efficacy of pembrolizumab in two syngeneic tumor models: MC38 colorectal cancer and GL261 glioma tumor models.
- We observed significant tumor growth delay (TGD) and survival following pembrolizumab monotherapy in the MC38 and GL261 tumor models, respectively, compared to the control and the murine anti-PD-1 (clone RPM1-14) monotherapy groups.
- The specificity to pembrolizumab anti-tumor response was validated in the MC38 model implanted in wild type C57BL/6 mice tumors (only the murine anti-PD1 produced a significant outcome.
- These results indicate that the response to clinically relevant immune checkpoint inhibitors directed to human targets can be evaluated in preclinical syngeneic tumor models with a fully functional immune system.
- The responses to a number of IO checkpoint inhibitors have been well characterized in most syngeneic tumor models, thus providing valuable tools for model decision making.