Novel in vivo preclinical humanized models for the evaluation of human specific immune checkpoint inhibitors.

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1 ABSTRACT

In the last few years, there has been an increasing demand for suitable pre-clinical mouse models for evaluating the efficacy of checkpoint inhibition-based cancer immunotherapies. During tumor progression, immune cells can become unresponsive and evade immune surveillance upon chronic activation and expression of the programmed cell death protein-1 (PD-1) the ligand PD-L1 on tumor cells or expression of the T lymphocyte associated antigen 4 (CTLA4) in T-cells cells resulting in tumor immune-tolerance. We have previously demonstrated that murine anti-PD-1, anti-PD-L1 and CTLA-4 blockade can effectively enhance immune normalization and 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 humanized PD-1 and CTLA-4 knock-in (KI) mouse models. The main advantage of these models is that human PD-1 or CTLA-4 proteins are expressed in the context of a fully functional immune system. To validate these models we evaluated the response to pembrolizumab or ipilimumab in a colorectal carcinoma and a glioblastoma preclinical tumor models. We observed significant tumor growth inhibition and growth delay in the MC38 tumor model with either monotherapy, but not when treated with the murine counterparts: anti-PD-1 (clone RPM1-14) or CTLA-4 (clone 9H10). 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. Furthermore, we found that pembrolizumab and ipilimumab therapy results in enhanced effector functions of $CD4^+$and $CD8^+$ T cells associated with increased expression of Granzyme B. In summary, the results shown here underscore the value of resourcing to humanized knock-in (KI) mouse models as tools to evaluate human specific immune-checkpoint based therapeutics alone and in combination with other agents.

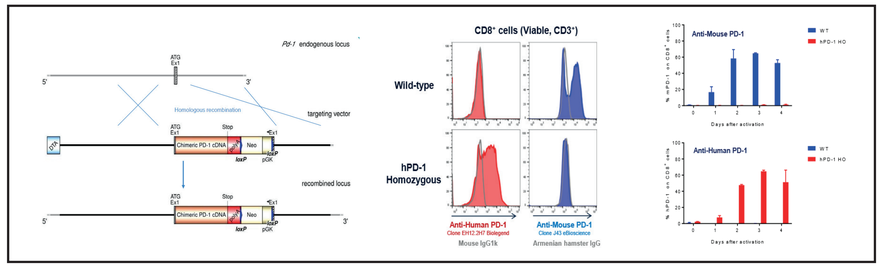
2 MATERIALS AND METHODS

Female C57BL/6-hPD1 KI, C57BL/6-hCTLA4 KI mice from **GenOway** and control C57BL/6 were eight weeks old at the start of the studies. **GenOway's** humanized PD1 or CTLA-4 knock-in mice were engineered by swapping the extracellular portion of the murine PD1 or CTLA-4 gene regions with the human counterpart. The intracellular domains were left intact to retain murine regulated endogenous signal transduction. MC38 tumors were initiated by subcutaneous implantation of 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 GL261 cells into the top of the skull of each test animal under anesthesia. Five days after implantation, animals were sorted into treatment groups. Flow cytometry analysis of MC38 treated groups were performed on Day 12. Spleen, blood and tumors were prepared into single cell suspensions and labeled with a panel of antibodies for cell identification. Panel antibodies included CD45, CD3, CD4, CD8, CD11b, CD25, Ly-6G, Ly-6C, FoxP3*, CD62L, CD44, KLRG1, GranzB*, TNF-α**, INFγ*, 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.).
*Internal marker **Ex Vivo Stimulation
Abbreviations:
Statistical Significance (Logrank test): ns = not significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, compared to Group 1 or group indicated

3 RESULTS

Generation of Human PD-1 Knock-In Mutant mice

Figure 1. Human PD-1 is detected on activated CD8 T cells from hPD1-KI humanized mice. Splenocytes isolated from WT and homozygous hPD-1 mice activated with αCD3/αCD28 (48h) corroborate the hPD-1 expression evaluated at two days post stimulation on viable CD3+CD8+T cells



Increased survival after pembrolizumab or ipilimumab monotherapy in Knock-in humanized mice.

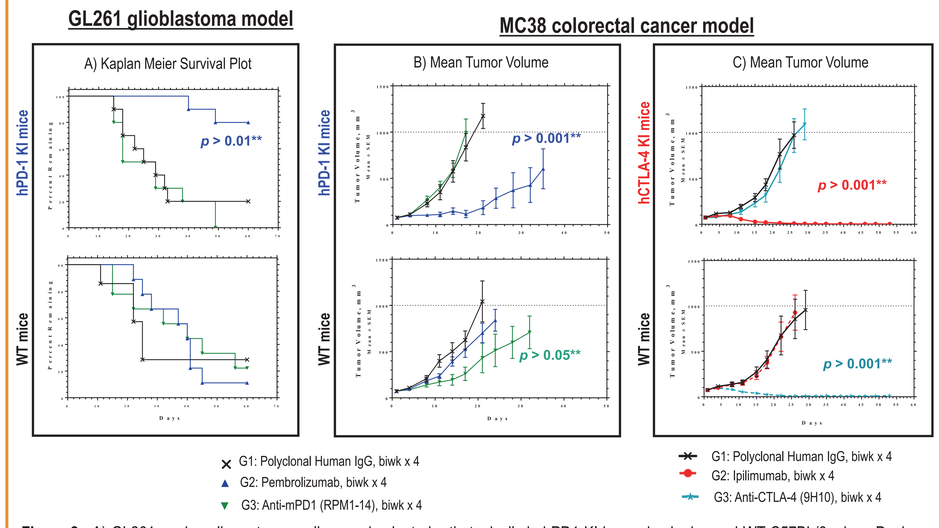


Figure 2 . A) GL261 murine glioma tumor cells were implanted orthotopically in hPD1-KI humanized mice and WT C57BL/6 mice. Dosing was initiated five days post engraftment. Survival was evaluated over a 60 days period. B) MC38 murine colorectal cancer cells were implanted in hPD1-KI and CTLA-4-KI humanized or WT C57BL/6 mice. Dosing with pembrolizumab or murine anti-PD1 and ipilimumab and murine anti-CTLA-4 was initiated when tumors reached a 80-120 mm^3 tumor volume. Responders were followed 45 days.

Immuno-phenotype of tissues from hPD-1-KI bearing MC38 tissues

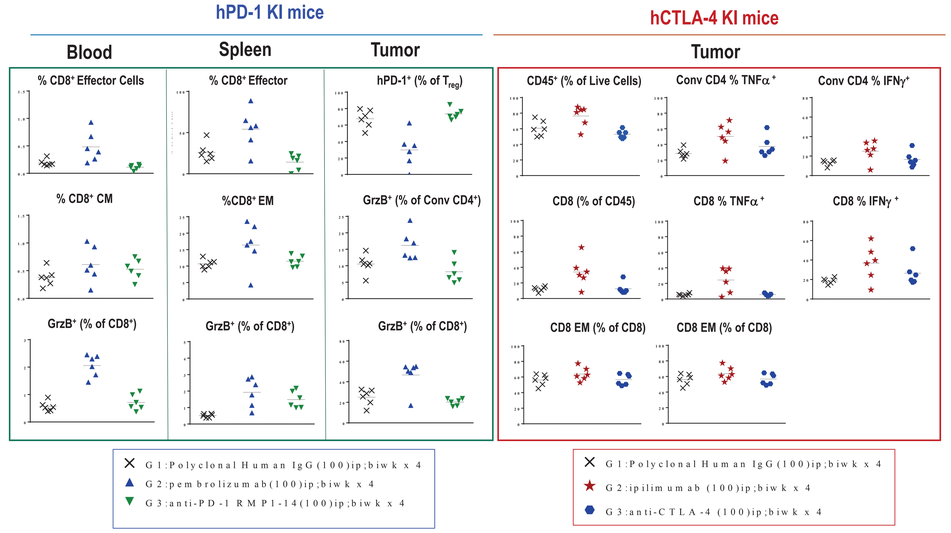


Figure 3 . A) MC38 tumors from hPD1-KI and CTLA-4-KI humanized treated (days 5,8,11) with pembrolizumab or murine anti-PD1 and ipilimumab or murine anti-CTLA-4 was initiated when tumors reached ~80 mm^3. Whole blood, spleens and tumors were collected on day 12 and immunophenotype via Flow Cytometry.

4 SUMMARY and CONCLUSIONS

- We observed significant tumor growth delay (TGD) and survival following pembrolizumab monotherapy in hPD1-KI humanized mice bearing MC38 and GL261 tumor, respectively, compared to the control and the murine anti-PD-1 (clone RPM1-14) monotherapy groups.
- 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. Assessment of durable responses in all surviving animals is been evaluated in a tumor re-challenge model.
- The established the specificity to pembrolizumab and ipilimumab anti-tumor responses by running comparative studies in the same tumor models implanted in wild type C57BL/6 mice. The results of the studies demonstrate that expression of human PD1 or CTLA-4 drive the efficacy of clinically relevant IO therapies directed to human targets can be evaluated in preclinical syngeneic tumor models with a fully functional immune system.
- The results of the immunophenotype of tissues from these humanized mice show enhanced T-cell function correlating with the activity observed in the groups treated with human-directed therapies