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

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Abstract # LB-063

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 become unresponsive to the presence of tumor antigens 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 Fas-L. T-cell regrowth following tumor immunobiardance. Our previous studies have demonstrated that murine anti-PD-1 and CTLA-4 therapy can effectively reactivate the antitumor response against multiple syngeneic tumor models. While these models proved instrumental for evaluating immune-specific checkpoint inhibitors (ICI), there is a need for further 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 antitumor 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 rMP1-14). 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 show underscores the value of the humanized PD-1 KI knock-in (KI) mouse model as a tool to evaluate human specific checkpoint-inhibiting therapeutics alone and in combination with other agents.

2 MATERIALS AND METHODS

Female C57BL/6-PD-1 KI mice from GenOway and control C57BL/6 were sixteen to sixteen weeks old at the start of the respective studies. MC38 tumors were initiated by subcutaneous implantation of 5 x 10^6 MC38 cells. Tumors were monitored as their volume approached the target size range of 50-500 mm³. GL261 tumors were initiated by intracranially implanting 1 x 10⁵ GL261 cells into the top of the skull of each test animal under anesthesia. Five days after implantation, animals were sorted into treatment groups. Statistical Significance 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 antitumor 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 rMP1-14). 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 show underscores the value of the humanized PD-1 KI knock-in (KI) mouse model as a tool to evaluate human specific checkpoint-inhibiting therapeutics alone and in combination with other agents.

3 RESULTS

Increased survival after pembrolizumab monotherapy in hPD-1-KI humanized mice.

4 SUMMARY AND CONCLUSIONS

- We evaluate 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 RMP1-14) monotherapy groups.
- The specificity of 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 development.