

Characterization of a MC38 Mouse Syngeneic Tumor Model Expressing Human PD-L1 in the Transgenic C57BL/6J Mouse System Expressing Human PD-1 and PD-L1

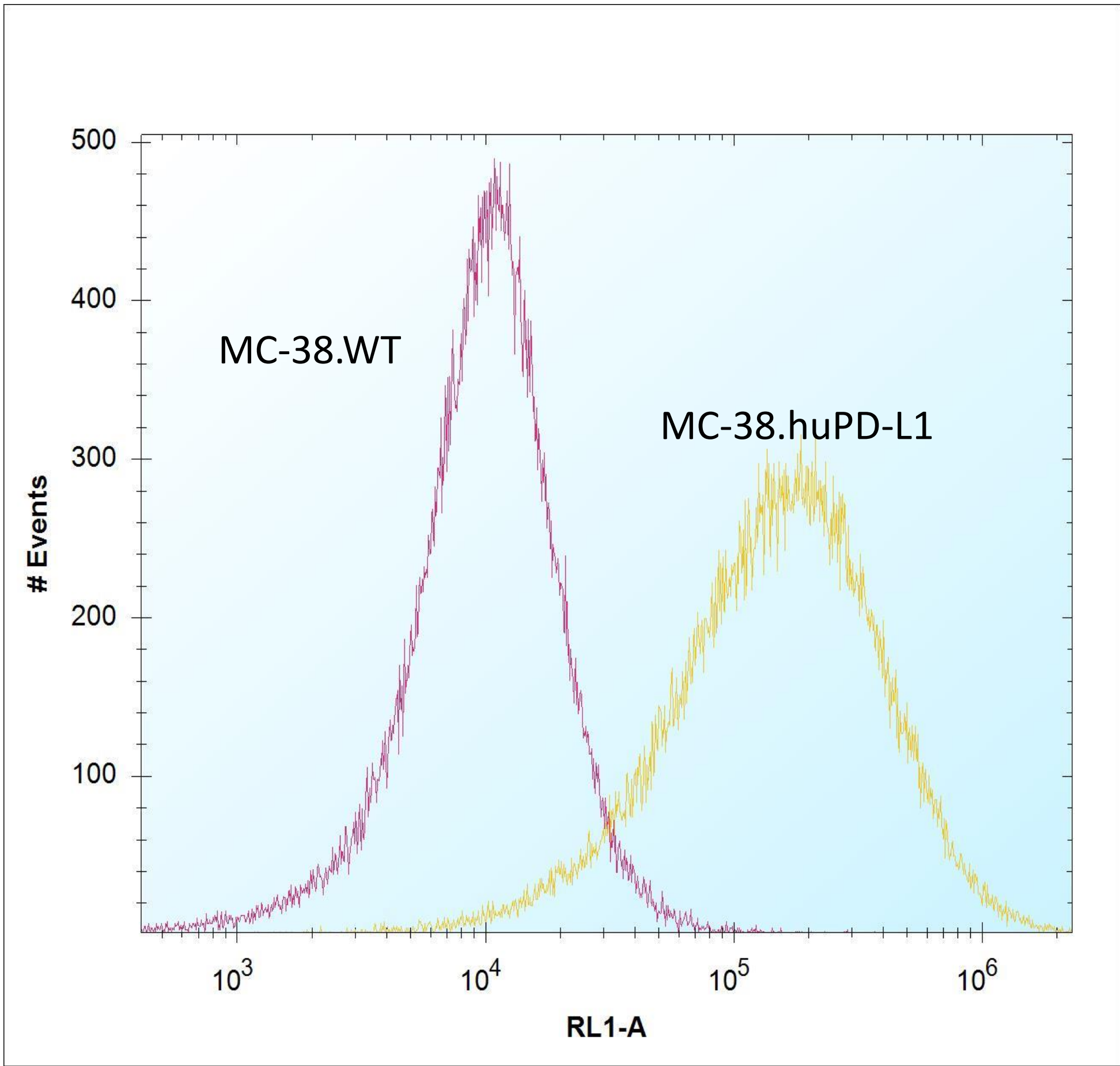
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Abstract #1504

The human checkpoint targets PD-1 and PDL-1 continue to demonstrate great promise in the clinic. With much of the current focus turning toward combination regimens, an animal model using the human clinical antibodies will be beneficial for evaluating new combination strategies. Here we continue characterization of a C57BL/6 transgenic mouse model expressing both the human PD-1 and PD-L1 combined with a modified murine MC38 colon tumor cell line expressing human PD-L1. PD-1 expression of the transgenic mice were verified in ex vivo stimulated splenocytes by flow cytometry analysis. Expression of PD-L1 on the genetically modified MC38 cells was also demonstrated by flow cytometry. For in vivo efficacy evaluation, tumor-implanted mice were treated with the clinical agents nivolumab and pembrolizumab at 100 µg and atezolizumab at 1 mg on Days 3, 7, 10, and 14 post implant. Treatment with nivolumab and pembrolizumab initiated tumor regression by Day 17. Complete tumor regressions were seen at Day 28 in nivolumab, 62.5% complete regression, and pembrolizumab, 71.4% complete regression. Growth inhibition was 83.9%, 69.7%, and 95.0% on Day 28 for nivolumab, atezolizumab, and pembrolizumab, respectively, compared to the control animals. There was no significant body weight loss and no signs of toxicity in any of the treated animals. To compare the human anti-PD-1 and PD-L1 clinical agent specificity, the non-transgenic parent C57BL/6 mouse strain was implanted with the unmodified MC38 colon tumor cells. Treatment with pembrolizumab and atezolizumab was conducted as with the transgenic animals. Through 28 days no growth inhibition, tumor size 105.9% of control, was seen in the pembrolizumab treated group, demonstrating lack of cross reactivity of the human therapeutic in the standard mouse model. Atezolizumab did demonstrate a 56.9% growth inhibition compared to controls and is consistent with the known cross reactivity of atezolizumab between human and mouse. We have shown a genetically modified MC38 colon tumor expressing human PD-L1 in transgenic mice expressing both human PD-1 and PD-L1 to be a suitable model for checkpoint inhibitor evaluation of the human form of the particular checkpoint therapeutic. Further research will involve combining each of the checkpoint antibodies with various chemotherapeutic agents.

Figure 1

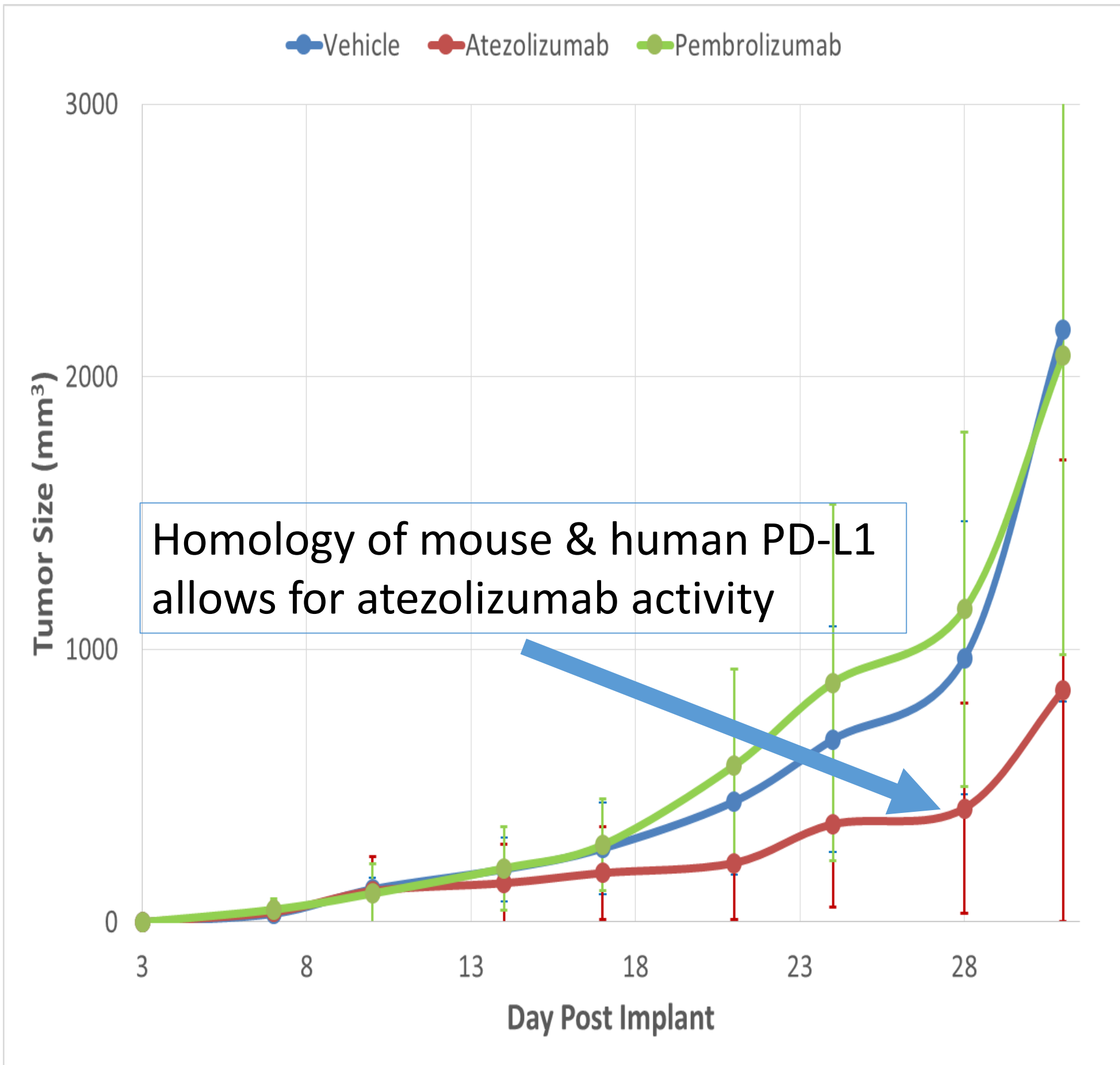
Human PD-L1 Expression in MC-38.WT versus MC-38.huPD-L1 transfected cells



Wild-type MC-38 mouse colon tumor cells and MC-38.huPD-L1 cells were stained with anti-human APC anti-human CD274 (B7-H1, PD-L1), Biolegend (Cat 124311) and analyzed for expression of human PD-L1.

Figure 2

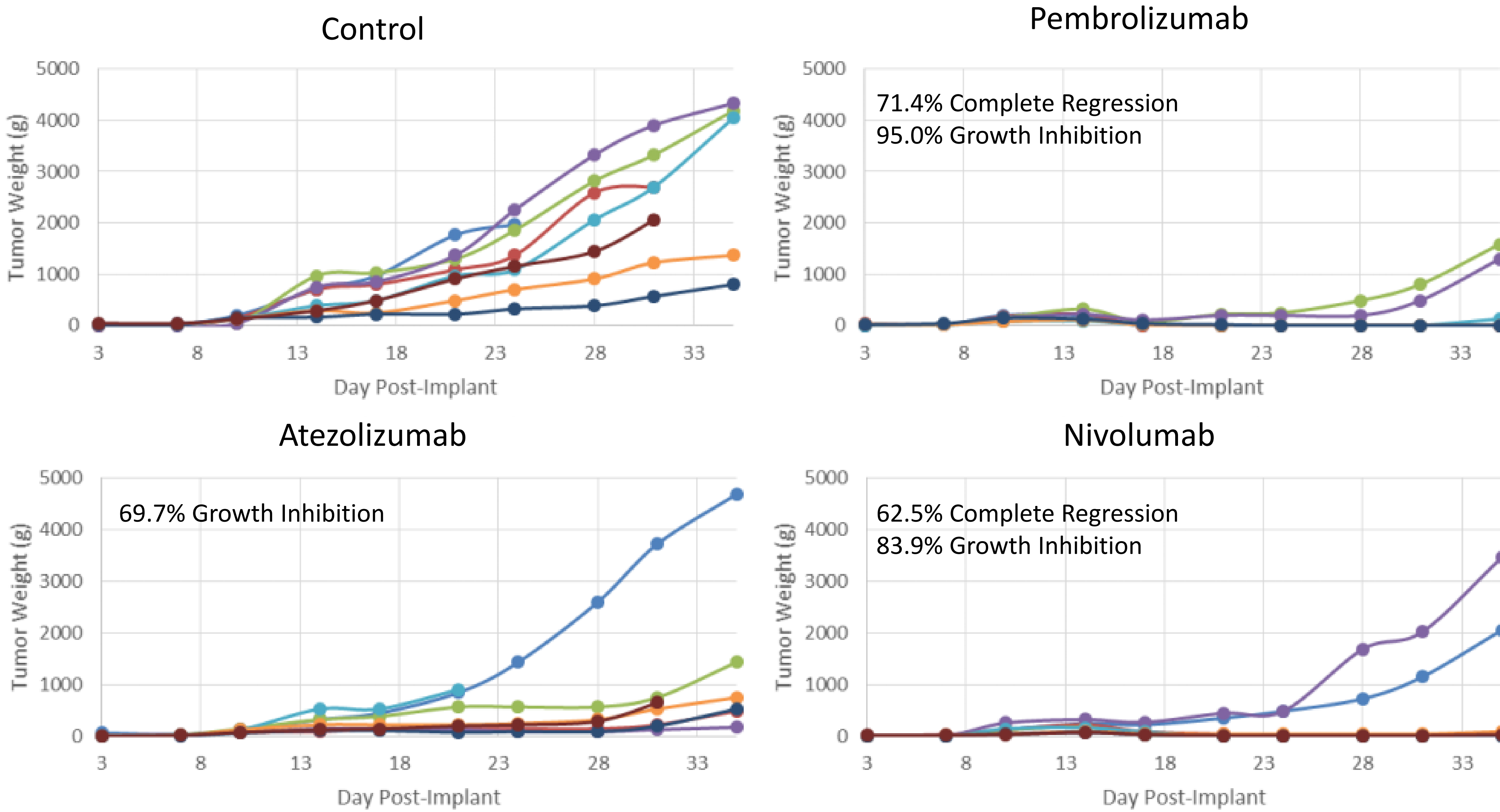
Atezolizumab and Pembrolizumab Efficacy in Wild-Type C57BL/6:MC-38 Model System



Wild-type C57BL/6 mice were implanted with wild-type MC-38 mouse colon tumor cells and dosed with clinical agents atezolizumab (1 mg) or pembrolizumab (100 µg) on Days 3, 7, 10, & 14. Tumor measurements collected twice weekly.

Figure 3

Atezolizumab, Pembrolizumab, & Nivolumab Efficacy in huPD-1/L1 Transgenic C57BL6J Mouse model with MC-38 Tumor Expressing huPD-L1



Transgenic huPD-1/L1 C57BL/6J mice implanted with MC-38.huPD-L1 cells and dosed with clinical agents atezolizumab (1 mg), pembrolizumab (100 µg), or nivolumab (100 µg), on Days 3, 7, 10, & 14. Tumor measurements collected twice weekly. Individual animal data presented.

Summary

- Genetically modified MC-38 cells stably express human PD-L1.
- Homology of mouse and human PD-L1 allows for activity of atezolizumab in the wild-type syngeneic MC-38 model.
- Lack of mouse/human homology for PD-1 requires use of transgenic model system to evaluate human therapeutics.
- Nivolumab, atezolizumab, and pembrolizumab demonstrated growth inhibition of MC-38.huPD-L1 tumor in the huPD-1/L1 transgenic C57BL/6J mouse model.
- Nivolumab and pembrolizumab demonstrated complete tumor regression of MC-38.huPD-L1 tumor in the huPD-1/L1 transgenic C57BL/6J mouse model.

Conclusions

- We have shown that MC38.huPD-L1 cells in transgenic C57BL/6J huPD-1/PD-L1 mice respond to treatment with the human clinical agents atezolizumab, pembrolizumab, and nivolumab.
- Importantly, the genetically modified MC38 tumor and transgenic mouse model expressing human checkpoint genes allows direct evaluation of human checkpoint inhibitor therapies without testing the murine analogs.
- The wild-type C57BL/6 with wild-type MC-38 tumor may be a suitable model for anti-PD-L1 therapeutics, but not for PD-1.