Abstract

The human checkpoint genes PD-1 and PD-L1 have been the target of extensive research for the treatment of human malignancies. Checkpoint inhibitor therapy has shown great promise in the clinic. Animal models that can use the human clinical antibodies will be beneficial for evaluating new therapies. A novel transgenic mouse model was developed that expresses the human PD-1 and PD-L1 in place of the murine genes. Additionally, a murine MC38 colon tumor cell line was modified to express human PD-L1. We evaluated the efficacy of human checkpoint antibodies nivolumab, pembrolizumab, and atezolizumab in the transgenic animal model with modified MC38 colon cancer cells. Five hundred thousand transgenic MC38 cells were implanted subcutaneously (SC) in the C57Bl/6 hPD-1/­PD-L1 knock in mice and allowed to grow for three days. The tumor-implanted mice were treated with nivolumab and pembrolizumab at 100 µg per animal and atezolizumab at 1 mg per animal on Days 3, 7, 10, and 14. Treatment with nivolumab and pembrolizumab caused tumor regression by Day 17. Growth inhibition was 84%, 58%, and 94% on Day 17 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. We have shown that human clinical anti-PD-1 and anti-PD-L1 antibodies are effective in treating genetically modified MC38 colon tumors in our transgenic mice. Further research will involve combining each of the checkpoint antibodies with various chemotherapeutic agents.

Figure 1

Mean Response of SC-Implanted MC38/hPD-L1 Murine Colon Tumors in C57Bl/6 hPD-1/PD-L1 Mice Treated with Nivolumab, Pembrolizumab, or Atezolizumab

Each mouse was injected SC in the right flank with five hundred thousand MC38/hPD-L1 colon tumor cells (5 x 10^6 cells) in 0.1 mL of complete media. A total of 18 male and 13 female C57Bl/6 hPD-1/PD-L1 transgenic mice were inoculated with tumor cells (Day 0). The 31 animals were placed in their respective treatment groups as indicated in figure legend above.

Treatment began on Day 3 as indicated above. Mean tumor weights were plotted over time for all groups. Statistical analyses were performed using Provantis®, Version 8 (Instem Life Sciences Systems, Ltd.; Staffordshire, United Kingdom). A preliminary test (Levene’s test for homogeneity and Shapiro-Wilk test for normality) was performed. If the preliminary test was not significant a One-way analysis of variance and Dunnett’s test was performed. If the preliminary test was significant a Kruskal-Wallis and Wilcoxon Mann-Whitney U was performed. The level of significance was p<0.05 (p<0.01 for preliminary tests) and is shown in the graph by the symbol *.

Figure 2

Individual Response of SC-Implanted MC38/hPD-L1 Murine Colon Tumors in C57Bl/6 hPD-1/PD-L1 Mice Treated with Nivolumab, Pembrolizumab, or Atezolizumab

Summary

- The maximum tumor growth inhibition was 90.4%, 70.2%, and 95.3% on Day 24 for Nivolumab, Atezolizumab, and Pembrolizumab treatment, respectively.
- The Nivolumab, Atezolizumab, and Pembrolizumab treated groups had 4, 0, and 5 animals, respectively, with complete tumor regressions (CR) and 3, 0, and 4 animals tumor free (TF) on Day 77.
- All antibody treatments were significantly different than the vehicle treated control group on Days 14-28.

Conclusions

- We have shown that MC38/hPD-L1 cells in transgenic C57Bl/6 hPD-1/PD-L1 mice respond to treatment with the human clinical agents Opdivo™, Tecentriq™, and Keytruda™.
- 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.