Efficacy of Three Checkpoint Inhibitors, Five Chemotherapeutic Agents, and the Experimental Drug Thiarabine Against MC38 Colon Cancer Expressing Human PD-L1 in Transgenic C57BL/6 Mice Expressing human PD-1 and PD-L1 Checkpoint Genes

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Poster 4087

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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. Combining these checkpoint inhibitors with standard chemotherapeutic agents is an important development for these inhibitors. Animal models that can use the human clinical antibodies and chemotherapeutic agents will be beneficial for evaluating new therapies.

A novel transgenic mouse model was developed that expresses the human PD-1 and PD-L1 genes in place of the murine genes. Additionally, a murine MC38 colon tumor cell line was modified to express human PD-L1. We initially evaluated the early treatment efficacy of human check point antibodies nivolumab, pembrolizumab, and atezolizumab in the transgenic animal model with modified MC38 colon cancer cells. Five hundred thousand transgenic MC38 cells were implanted in the C57BL/6 PD-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.

To extend the utility of the model we implanted mice and allowed the tumors to grow to 63 to 126 mg before treatment initiation (Day 10). Similar results were observed in established tumors as for early treatment with the PD-1 inhibitors nivolumab and pembrolizuman. In contrast there was little activity from the PD-L1 inhibitor atezolizumab in established tumors. To prepare for combination studies, we also tested cisplatin, irinotecan, gemcitabine, decitabine, rucaparib, and thiarabine near the MTD for each agent with various treatment schedules against the established tumors. The doses for cisplatin, irinotecan and decitabine resulted in excess animal deaths and require reduced doses for combination therapies. Gemcitabine, decitabine, and thiarabine (in house development drug) were too active with complete regressions observed; the doses for combination treatment will be reduced. Due to the effectiveness of the PD-1 checkpoint inhibitors the number of doses will be reduced from four to two treatments in combination studies; no change for atezolizumab.

We have shown that PD-1 inhibitors are effective in treating genetically modified MC38 colon tumors in transgenic mice in early treatment and in established tumors while the PD-L1 inhibitor was only effective in early treatment. We also have identified doses and schedules of five chemotherapeutic agents to use in future combination studies in this model.

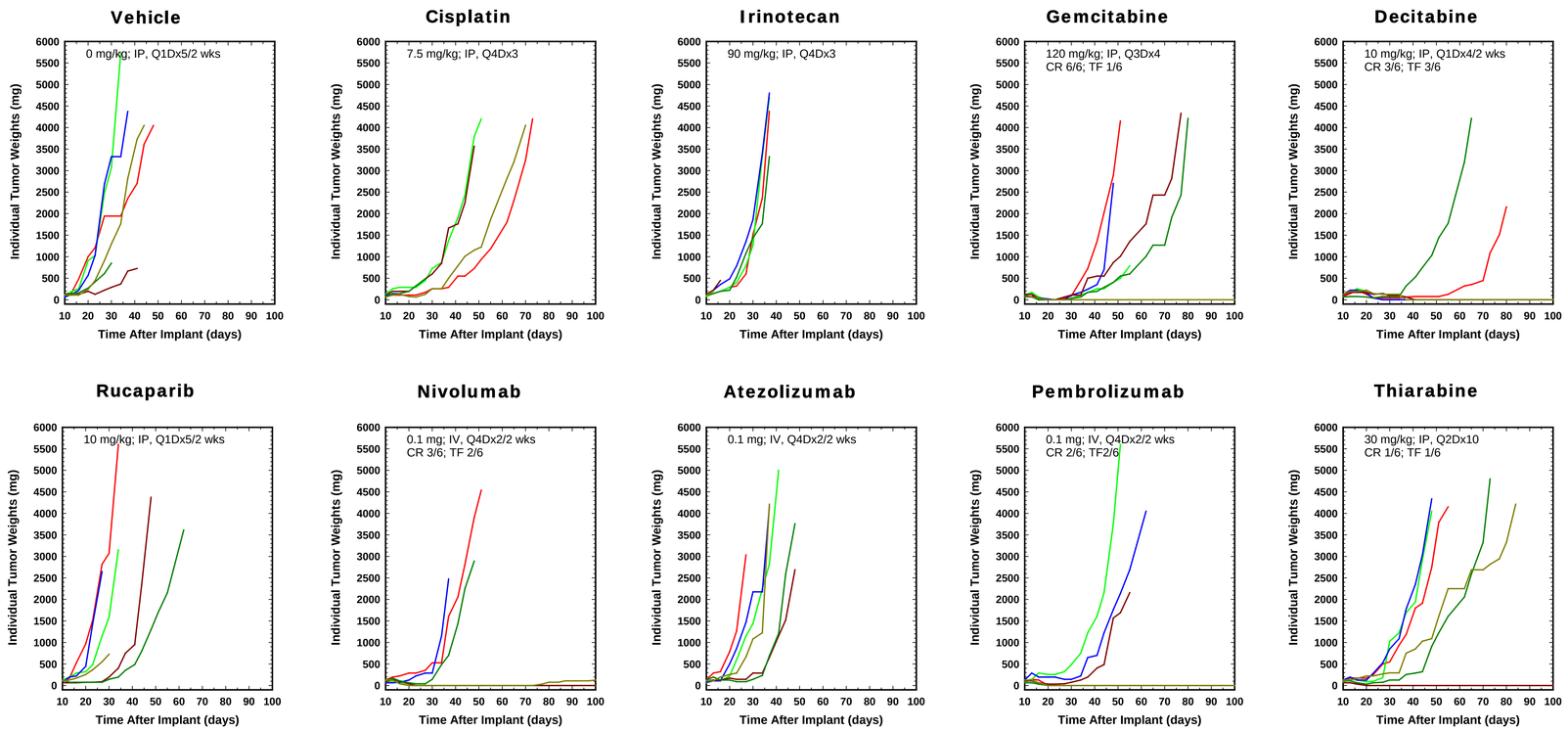
Materials and Methods

Each mouse was injected SC in the right flank with five hundred thousand MC38/hPD-L1 colon tumor cells (5 x 10^5 cells) in 0.1 mL of complete media. A total of 54 male and 65 female C57Bl/6 hPD-1/PD-L1 transgenic mice were inoculated with tumor cells (Day 0). On Day 10, 60 animals (30 male and 30 female) with tumors from 63 to 125 mg were placed in their respective treatment groups. Some animals were switched among groups to assure the mean tumor weights were as close as possible. Treatment began on Day 10 for all compounds. The doses and schedules are in the individual graphs.. Individual 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-Wilks 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 table by the symbol * for the maximum % tumor difference.

Figure 1

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



Data Summary Table

Treatment	Maximum % Body Weight Loss (Day)	Deaths (Day)	Maximum % Tumor Difference (Day)
Control	1.5 (13)	0	--
Cisplatin	16.8 (22)	1 (18)	78.8 (34)
Irinotecan	3.6 (11)	2 (16, 18)	39.6 (20)
Gemcitabine	4.4 (15)	0	100* (23)
Decitabine	9.5 (23)	1 (21)	97.4* (34)
Rucaparib	1.5 (12)	0	36.3 (30)
Nivolumab	2.2 (11)	0	92.3* (27)
Atezolizumab	0	0	53.0 (34)
Pembrolizumab	1.2 (12)	1(22)	93.2* (27)
Thiarabine	2.3 (16)	0	82.5 (27)

*** p < 0.05**

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™. The response is greatest in Opdivo™ and Keytruda™; Keytruda™ was used in combination studies (ongoing). Tecentriq™ was also used in combination studies (ongoing).
- Due to toxicity the doses of Cisplatin, Irinotecan, and Decitabine (not used) were reduced 20-50% for ongoing combination studies
- Due to high activity the doses of Gemcitabine, Decitabine (not used), and Thiarabine were reduced 50-75% for ongoing combination studies
- 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 alone and in combination with standard chemotherapeutic agents.