Potential Antagonism Between PD-1 and CTLA-4 Antibody Treatment in the CT26.WT Murine Colon Cancer Model

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Abstract

Recently, the role of antibodies blocking the immune checkpoint inhibitors PD-1 and CTLA-4 have shown promise as anti-cancer therapies. Several antibodies that interfere with PD-1 and CTLA-4 have been approved for use in human melanoma, colon, and lung cancer patients. Since there is a continued interest to develop such anti-cancer strategies, Southern Research is developing small molecules to interfere with immune checkpoint inhibitors. Therefore, it is necessary to establish in vivo models for preclinical testing of the identified inhibitors. To this end, we are evaluating the efficacy of PD-1 and CTLA-4 in the CT26.WT murine colon tumor model in BALB/c mice. Additionally, we will characterize the immune response in the mice implanted with the CT26.WT tumor. In the current study we implanted 40 BALB/c mice with one hundred thousand CT26.WT cells from culture and placed the mice in four treatment groups with 10 animals per group (vehicle control, PD-1 alone, CTLA-4 alone, and PD-1 combined with CTLA-4). Mice were treated with PD-1 antibody on Days 3, 7, 10, and 14 at a dose of 100 micrograms and/or CTLA-4 antibody on Days 3, 8, 11, and 14 at a dose of 100 micrograms. Treatment was performed using Provantis®. Each mouse was injected SC in the right flank with one hundred thousand cells (1 x 10^6 cells) in 0.1 mL of PBS. A total of 40 female BALB/c mice were inoculated with tumor cells (Day 0). The 40 animals were placed in their respective treatment groups as indicated in figure legend above. Each animal was given an identification number by ear punch. Treatment began on Day 3 or 8 as indicated above. Mean tumor weights were plotted over time for all groups. Statistical analyses were performed using Provantis®, Version B (Instem Life Sciences Systems, Ltd.; Staffordshire, United Kingdom). A preliminary test (Levene’s test for homogeneity and Shapiro-Wilk’s test for normality) was performed on the tumor weight. 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).

Figure 2
Individual Response of SC-Implanted CT26.WT Murine Colon Tumors to Treatment with PD-1 Antibody or CTLA-4 Antibody Alone and in Combination

Vehicle

Anti-PD-1

Anti-CTLA-4

Combination

Summary

- The maximum tumor growth inhibition was 25.6% on Day 22 for PD-1 treatment, 91.5% on Day 36 for CTLA-4 treatment, and 53.3% on Day 32 for the combination treatment.
- There was one animal in the vehicle control group with no tumor growth. The PD-1, CTLA-4, and combination treated groups had 0, 7, and 3 animals, respectively, with no tumor growth.
- The CTLA-4 treatment was significantly different than the vehicle treated control group and the PD-1 treated group but not the combination group.

Conclusions

- The most effective treatment was CTLA-4 antibody alone.
- Although the difference between CTLA-4 alone and the combination treatment were not statistically significant there is a potential antagonistic interaction between the PD-1 and CTLA-4 combined treatments.