

**COMBINATORIAL HSCS AND ANTI-PD-1 THERAPY IN  
MICROSATELLITE STABLE COLORECTAL CANCER**

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**Background** Colon cancer (CRC) is the second leading cause of cancer-related deaths in the US. CRC incidence is on the rise and there is an alarming increase in young onset CRC cases. Immune checkpoint inhibitors (ICIs) have yielded promising anti-tumor results in microsatellite instable high (MSI-high) patients, which represent only 15% of tumors. The remaining 85% are denoted as microsatellite stable (MSS) and are unresponsive to ICI. Using a murine glioma model, our group has previously found the combination of anti-PD-1 and a transfer of hematopoietic stem cells (HSCs) can sensitize mice that are resistant to anti-PD-1 alone. We evaluated survival after treatment with this combinatorial platform 3 or 5 days post-implantation in subcutaneous CRC-bearing mice and also phenotyped the splenic compartment of mice at endpoint. **Methods**  $1 \times 10^6$  MSS CRC cells, CT26, were subcutaneously injected into the right flank of BALB/cJ mice. 3 or 5 days later, HSCs were isolated from naïve BALB/cJ mice and injected through the tail vein into CT26-bearing mice and were also given 10 mg/kg anti-PD-1. Mice were given 3 additional doses of anti-PD-1 for a total of doses either every 3 or 5 days. Mice were sacrificed when tumors reached 1.5 cm at its widest point and spleens were excised and stained for flow cytometry.

**Results** When mice were treated with HSC/anti-PD-1 3 days post-tumor implantation, we observed a statistically significant increase in survival in mice that received combinatorial HSCs and anti-PD-1 relative to no treatment control mice ( $p=0.0034$ , Mantel-Cox long-rank test) as well as mice that received HSCs alone ( $p=0.0462$ , Mantel-Cox log-rank test). In the same 3 day cohort, no differences in the frequency of T cell populations were observed. However, we found mice that received this combination therapy had a significant increase in the frequency of splenic CD11c+ MHC II+ dendritic cells (DCs) relative to no treatment control mice ( $p=0.0364$ , Mann-Whitney t test). When mice were treated with HSC/anti-PD-1 5 days post-tumor implantation, we found a statistically significant increase in survival of mice treated with combinatorial HSCs and anti-PD-1 compared to no treatment control mice ( $p=0.0024$ , Mantel-Cox log-rank test) and relative to mice that received HSC monotherapy ( $p=0.0462$ , Mantel-Cox log-rank test).

**Conclusions** These results suggest combinatorial HSCs and anti-PD-1 represents a promising therapeutic axis in a murine model of MSS CRC. In addition, the increase in splenic DCs suggests the mechanism behind this anti-tumor response may be expansion of DCs within the periphery.

**Ethics Approval** All animal work approved through University of Florida IACUC # 201910777

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