Background Novel therapy combinations utilizing PD-1 immune checkpoint inhibition have improved outcomes in patients with RCC; however, positive clinical responses are limited to select individuals. Various studies indicate resistance is mediated partly through STAT3 induced activity and a concomitant accumulation of tumor infiltrating myeloid derived suppressor cells and tumor promoting M2 macrophages capable of suppressing anti-tumor responses independent of PD-1 signaling. Indeed, in a separate study, we observed an increase in cytokines IL-6, IL-8 and persistent STAT3 activity within myeloid cells in RCC patients with resistance to anti-PD-1/CTLA-4 therapy. In this preclinical study, we investigated a novel treatment strategy combining anti-PD-1 therapy with our original CpG-STAT3ASO oligonucleotide strategy to knockdown STAT3 specifically within TLR9-positive myeloid cell populations associated with RCC tumors.

Methods 6-8 week female Balb/C mice were injected subcutaneously with 500,000 Renca cells in Matrigel. Mice were treated using CpG-STAT3ASO (IV, 5mg/kg) together with anti-PD-1 (IP, 200 ug), each of the two reagents alone, IgG control or only PBS. Immune alterations in tumors and tumor-draining lymph nodes were assessed via spectral cytometry. Statistical significance was determined using two-way ANOVA or Wilcoxon signed rank test with SEM.

Results CpG-STAT3ASO with anti-PD-1 significantly inhibited growth of syngeneic Renca tumors compared to either treatment alone or negative controls. To elucidate the immune-mediated mechanisms underpinning this efficacy, we undertook spectral cytometry analysis of tumor and tumor draining lymph nodes. In comparison to PBS, IgG and anti-PD-1 alone, combination CpG-STAT3ASO and anti-PD-1 treatment induced maturation and activation of intratumoral dendritic cells (DCs) and macrophages, including a decrease in immunosuppressive M2 macrophages, with a concomitant increase in anti-tumor M1 macrophages and an increase in intratumoral CD8+ T cells, decrease in CD4+ Tregs and increase CD8+ T cell to Treg ratios. In fact, anti-PD-1 alone treated cohorts was shown to have high percentages of alternatively activated (pro-tumor) M2 macrophages in comparison to combination treated cohorts where an increase in anti-tumor M1 macrophages was observed. This suggests anti-PD-1 alone efficacy may be limited by its failure to induce maturation/activation of myeloid cells.

Conclusions Our results indicate that combination of PD1 blockade with systemic administration of CpG-STAT3ASO significantly improves antitumor efficacy against the tested kidney cancer model in comparison to each treatment alone. The improved treatment efficacy is likely related to more potent activation and reprogramming of tumor-associated myeloid cells, particularly macrophages. Our results warrant further investigation into myeloid cell specific STAT3 silencing in combination with Anti-PD-1 in RCC.

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REFERENCES

Ethics Approval All protocols in this study were approved by the City of Hope Institutional Animal Care and Use Committee (IACUC protocol 10015)