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REGIONAL DELIVERY OF A TLR9 AGONIST TO BOOST CHECKPOINT INHIBITOR RESPONSIVENESS IN LIVER METASTASES

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Background Class C TLR9 agonists, CpG oligodeoxynucleotides (ODNs) enhance responsiveness to anti-PD1 therapy in solid tumors through favorable modulation of the tumor microenvironment (TME) [1]. Recently, we reported that regional delivery of a TLR9 agonist eliminated myeloid derived suppressor cells (MDSC) and promoted pro-inflammatory/anti-tumorigenic M1 macrophage programming in the TME of liver metastases (LM) [2]. Further, we found enhanced TLR9 activation in LM following regional TLR9 agonist infusion compared to the systemic treatment. We hypothesize that regional delivery of a TLR9A into LM will enhance the responsiveness to systemically infused anti-PD1 therapy.

Methods In this study, we treated mice with established MC38-CEA-Luc LM with ODN-2395 (30µg/mouse) regionally with or without anti PD-1 antibody (250µg/mouse) intraperitoneally.

Results Control of LM growth (Figure 1) was significantly higher with combinatorial treatment as compared to anti-PD1 (p<0.01) or PBS treatments (p<0.05). To study the impact of TLR9 activation on human MDSC, we treated healthy donor PBMCs with ODN-2395 or SD101. We found that both reduced the hu-MDSC (CD11b+CD33+HLADR-) population in a dose-dependent manner with an increase in PD-L1 expression as determined by flow cytometry (FC) analysis (Figure 2). Moreover, by using Luminex, demonstrated that ODN-2395 and SD101 enhanced expression of IL 29, IFNα, and NFκB, along with downstream cytokines IL 6 and IL 10. To investigate the effect of SD101 in modulating the differentiation of huMDSC from huPBMC, we treated huPBMC with IL6+GM-CSF in the presence or absence of SD101. By FC analysis, we found that SD-101 blocked huMDSC development induced by IL6+GM-CSF, preferentially limited the more immunosuppressive monocytic MDSC subtype, and drove M1 macrophage polarization. Treatment of SD101 only once for 48hrs was sufficient to inhibit huMDSC differentiation for two weeks.

Abstract 607 Figure 1 Combinatorial treatment of CPI and ODN's reduces t
PV = portal vein; IP = intraperitoneal.

Eight to twelve weeks old male C57/BL6 mice were challenged intra-splenic with 0.5e6 MC38-CEA-Luc cells for a

week. Bioluminescence value was determined by IVIS on D0, and mice were randomized accordingly and treated with 30 µg/mouse ODN2395 via PV with or without 250 µg/mouse anti-PD1 antibody via IP on D0, D+3 and D+6. PBS served as the vehicle (Veh) control and administered via PV. Fold change of the tumor burden was calculated based on D0 baseline bioluminescence. Tumor progression was analyzed unpaired t test among groups. (*p <0.05).

Abstract 607 Figure 2 Human PBMC treated with ODN2395 and SD101 reduces
Ctrl = control; MDSC = ODN = oligodeoxynucleotide' PBMC = peripheral blood monocytes.

Human PBMC were isolated from the Leukoreduction system chamber. 1e6/ml PBMCs were treated with increasing concentrations (0.04–10 µM) SD101, ODN2395 along with ctrl ODN5328 (1µM) for 48 hours. Panels A and B: MDSC population and their corresponding PD-L1 expression were evaluated (n=12). Four donors with three replicates were used. Data represented as mean ± SEM.

Conclusions Both the in vitro and in vivo findings suggest that regional TLR9 stimulation in a model of LM improves responsiveness to systemic anti-PD-1 therapy through elimination of MDSC, and the effect on huMDSC was confirmed in vitro. Increased PDL-1 expression in response to TLR9 stimulation among MDSC may further enhance the anti-PD-1 effect. Therefore, combining regional infusions of a TLR9 agonist with systemic anti-PD-1 agents may be a promising approach for liver tumor treatment.

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<http://dx.doi.org/10.1136/jitc-2021-SITC2021.607>