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**IMPACT OF SD-101, A TOLL-LIKE RECEPTOR 9 CLASS C (TLR9C), AGONIST ON MYELOID DERIVED SUPPRESSOR CELLS**Chandra Ghosh\*, Steven Katz, Chandra Ghosh. *Trisalus Life Sciences, Westminster, CO, USA*

**Background** Specific immunosuppressive pathways, including unique programming of myeloid derived suppressor cells (MDSC), may limit the success of immunotherapy in the liver. SD-101, a TLR9C agonist delivered via intravascular Pressure Enabled Drug Delivery™ (PEDD™), is currently under study in combination with systemic checkpoint inhibition across multiple intrahepatic tumor indications (NCT04935229, NCT05220722). There are multiple classes of TLR9 agonists, with differential effects on plasmacytoid DC (pDC) IFN $\alpha$  production, B and NK cell activation, and other immune cell populations. TLR9C agonists have relatively broad immunologic effects, but the impact on MDSC is less clear. We investigated the impact on myeloid cells by various TLR agonists (TLR4, TLR7, TLR9B and TLR9C) to expand our understanding of their differential effects on MDSC and potential to immunomodulate liver tumor microenvironments (TME)

**Methods** Peripheral Blood Mononuclear Cells (PBMCs) obtained from healthy donors were cultured in IL6+GMCSF (20ng/ml) to induce MDSCs and treated with various TLR agonists. Flow cytometry was performed to evaluate MDSCs (CD33<sup>+</sup>CD11b<sup>+</sup>HLADR<sup>-</sup>), subtypes of MDSCs (monocytic/granulocytic CD14<sup>+</sup>/CD15<sup>+</sup>MDSCs), M1 macrophages (CD14<sup>+</sup>CD86<sup>+</sup>), and monocytic dendritic cells (CD14<sup>+</sup>CD11C<sup>+</sup>CD123<sup>-</sup>). Nanostring analysis (n=3) was performed on total RNA isolated from day 2 samples, verified by qRT-PCR. Bone marrow (BM) murine MDSCs (CD11b<sup>+</sup>GR-1<sup>+</sup>) were treated for 72h with GMCSF and effects on MDSC were evaluated by flow cytometry.

**Results** SD-101, a TLR9C agonist, significantly inhibited MDSC expansion compared to vehicle (Veh), TLR9B, TLR4 and TLR7 agonists on day 2 (p<0.01; n=11). Similar results were obtained with murine BM derived MDSCs (p<0.001; n=5). SD101 significantly reduced the human M/G MDSC ratio as compared to Veh, TLR4, TLR7 and TLR9B agonists (p<0.05; n=11). In addition, SD101 shifted DC towards a myeloid program compared to Veh, TLR4 and TLR7 agonists (p<0.05; n=6). Nanostring gene expression analysis revealed that SD101 induced higher adaptive immunity, innate immunity, immunometabolism scores compared with Veh and TLR9B agonist (p<0.05; n=3). There was greater induction of TLR, Th1, NF $\kappa$ B and lymphocyte activation signatures in SD101 as compared to Veh and TLR9B agonist (p<0.05; n=3). SD101 induced more PD-L1, IFN $\gamma$  and IP10 gene expression compared to Veh and TLR9B (p<0.05; n=6), in addition to B and NK cell expansion compared to Veh (p<0.05; n=6).

**Conclusions** SD101 inhibited MDSC expansion and enhanced polarization towards M1 macrophages. The favorable impact on MDSC, in addition to broad immune activating effects, suggests that TLR9C agonists, if delivered effectively, have the potential to enable better performance of other immunotherapy agents within hostile liver TMEs.

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