Background Myeloid-derived suppressor cells (MDSCs) are pathologically activated monocytes and neutrophils that promote an immunosuppressive milieu by inhibiting T-cell activation and recruitment, leading to resistance to immune checkpoint therapies and poor clinical outcomes. C/EBPβ is a critical transcription factor that regulates the immunosuppressive state of MDSCs and is activated by prostaglandin E2 (PGE2) and interleukin 10 (IL10). Both PGE2 and IL10 are involved in MDSC development, accumulation and functional stability.

Methods We have developed a proprietary engineered exosome loaded with antisense oligonucleotides (ASO) targeting C/EBPβ (exoASO-C/EBPβ™), that selectively delivers ASOs to MDSCs, thereby inhibiting C/EBPβ expression, promoting the immune-modulation of MDSCs to a pro-inflammatory phenotype, and demonstrating single agent anti-tumor activity in checkpoint refractory tumor models.

Results Human MDSCs were generated and characterized in vitro using a combination of putative cell surface markers: CD11b, CD33, HLA-DR, CD14 and CD15. Exosome surface glycoproteins enabled preferential delivery of ASO to MDSCs, as demonstrated by a 3-fold improvement in delivery with exoASO vs free ASO. Treatment of MDSCs with exoASO-C/EBPβ resulted in a dose-dependent target gene knockdown, and a cytokine secretion profile consistent with a proinflammatory phenotype. Biodistribution in tumor-bearing mice of systemically administered exoASO demonstrated up-to 11-fold improvement in selective ASO delivery as compared to free ASO, in MDSCs and other myeloid cells in the tumor, blood, and bone marrow. Administration of exoASO-C/EBPβ as a single agent demonstrated significant anti-tumor activity and survival benefits in multiple MDSC rich, checkpoint refractory tumor models: E0771-breast cancer (30% complete responses (CRs)), MB49-bladder cancer (70% CRs) and 4T1-breast cancer (44% Tumor growth inhibition (TGI)). Combination treatment with anti-PD1 further increased the anti-tumor efficacy, while the complete responders were resistant to tumor re-challenge, demonstrating immunological memory associated with exoASO-C/EBPβ treatment. In a lung metastasis model of B16F10 with disseminated tumors in the abdominal cavity, IV administration of exoASO-C/EBPβ significantly attenuated tumor growth (92% TGI with monotherapy, 98% TGI with a combination with anti-PD1), as observed with ex-vivo IVIS imaging. Additionally, exoASO-C/EBPβ treatment resulted in a significant infiltration of cytotoxic CD8 T cells in the lung and tumor. Finally, a myeloid cell specific C/EBPβ/PGE2/IL10 gene signature was generated to identify cancer indications where exoASO-C/EBPβ therapy may have the most therapeutic significance.

Conclusions exoASO-C/EBPβ is a novel therapeutic that selectively targets and attenuates a critical transcription factor in immunosuppressive myeloid derived suppressor cells, resulting in their immune-modulation and potent anti-tumor activity across multiple MDSC rich tumor models.