Background Patients with relapsed and metastatic osteosarcoma have a 5 year overall survival <25%. Our group tested our novel multilamellar RNA lipid nanoparticle aggregate vaccine (RNA-LPA) as an approach to reprogram the immunosuppressive tumor microenvironment (TME) present in osteosarcoma, where immunotherapy has not yet been effective.

Methods Total-tumor mRNA was amplified from tumor cell lines in mice or tumor biopsy in canines before complexation in lipid nanocarriers/cationic lipids, generating RNA-LPA for systemic administration. Preclinical murine models were generated using K7M2, KHOS or 143B osteosarcoma cells in either C57Bl/6, BALB/c or BALB/c SCID mice inoculated by tail vein injection to mimic minimal residual metastatic disease from pulmonary osteosarcoma. We treated client-owned canine patients (pet-dogs) with osteosarcoma through a comparative phase I trial with the UF College of Veterinary Medicine, Gainesville, FL, USA; Lerner Research Institute, Cleveland, OH, USA; Children’s National Medical Center, Bethesda, MD, USA; University of Connecticut, Hartford, CT, USA; Duke University, Durham, NC, USA; UF College of Veterinary Medicine, Gainesville, FL, USA.

Results Tumor-specific RNA-LPAs elicited anti-tumor efficacy in the murine K7M2 model with long term survival (7/8 mice), with some survival benefit even with irrelevant pp65 RNA-LPAs (p<0.05); these findings correlated with an increase in intratumoral central memory T cells. Both tumor-specific and irrelevant RNA-LPAs reprogrammed the innate immune microenvironment (decreased tumor associated macrophages and myeloid derived suppressor cells, p<0.01), but tumor-specific RNA-LPAs additionally resulted in activation of adaptive immunity (dendritic cells and T-cells). Five pet-dogs with osteosarcoma were safely treated with total-tumor RNA-LPAs, which were immunologically active, demonstrating changes in complete blood counts and serum cytokines within 6 hours of vaccine administration. One pet dog received 4 total RNA-LPAs every 2 weeks with initial objective radiographic resolution of pulmonary metastases, though this subject relapsed with new metastases. Osteosarcoma tumors from mice, canines, and human patients were used to successfully establish 3D tumoroid tissue culture for at least 32 days ex vivo.

Conclusions Both tumor-specific and nonspecific ‘off the shelf’ RNA-LPA vaccines, redirected immunosuppressive myeloid cells, a hallmark of the osteosarcoma TME. This agent, which is FDA-IND approved (BB-19304, Sayour) and in human clinical trials for patients with brain tumors (NCT04573140) will enter clinical trials for patients with osteosarcoma in the coming year (NCT04837547, not yet recruiting). Our 3D tumoroid system offers the opportunity to study the immune-modulating effect of RNA-LPAs and may be a meaningful correlate to both canine and human subjects enrolled on clinical trials.