1390 RNA NANOPARTICLE VACCINES OVERCOME THE IMMUNOSUPPRESSIVE TUMOR MICROENVIRONMENT OF METASTATIC OSTEOSARCOMA

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Background We previously developed a complex of tumor-derived mRNA (whole tumor transcriptome) with a liposomal nanoparticle to allow systemic delivery of tumor-specific antigens to antigen presenting cells for induction of antigen specific T cell immunity.[1-3] We tested this approach to circumvent the lack of specific targets, overcome antigenic heterogeneity, and reprogram the immunosuppressive tumor microenvironment (TME)[4] present in osteosarcoma, where immunotherapy has not yet been effective.

Methods Total-tumor mRNA was amplified from tumor cell lines or tumor biopsy tissue before complexation in lipid nanocarriers/cationic lipids, generating an RNA-nanoparticle (RNA-NP) for systemic administration. We also developed non-specific RNA-NPs to measure the anti-tumor effect of an "off-the-shelf" immunomodulator. 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 launched a comparative oncology clinical trial for client-owned canine patients (pet-dogs) with osteosarcoma through collaboration with the UF College of Veterinary Medicine (UF IACUC#202111376, PI: Milner).

Results In mice inoculated with pulmonary osteosarcoma, total-tumor RNA-NPs elicit significant anti-tumor efficacy in the K7M2 model with long term survivor benefits (7/8 treated mice). These total-tumor RNA-NPs reprogram the TME with significantly less tumor associated macrophages and myeloid-derived suppressor cells ($p<0.01$). RNA-NPs localize to the perivascular region of the TME, transfect CD45+ myeloid cells, and induce upregulation of genes such as BATF3 known to be related to myeloid reprogramming into activated dendritic cells. Furthermore, monotherapy with non-specific pp65 RNA-NPs generated significant anti-tumor effect in SCID mice ($p<0.05$). Long-term survivor outcomes from tumor loaded mRNA-NPs correlates with an increase in intratumoral central memory T cells (not observed in animals vaccinated with GFP RNA-NPs). In our first three canine subjects with osteosarcoma, total-tumor RNA-NPs were safe and immunologically active with changes in peripheral blood markers of dendritic cell and T cell activation within 6 hours of vaccine administration.

Conclusions RNA-NPs redirect immunosuppressive myeloid cells which are a hallmark of the osteosarcoma TME[4], resulting in an immune activated state and leading to increased intratumoral central memory T cells. These vaccines bypass MHC restriction and can be made readily available for all patients/canines providing a renewable antigen resource that can be used to continuously vaccinate patients. This agent, which is FDA-IND approved (BB-19304, Sayour) and in human clinical trials for patients with brain tumors (NCT04573140), may be a promising potential novel therapy for patients with recurrent pulmonary metastatic osteosarcoma.

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