RNA NANOPARTICLE VACCINES OVERCOME THE IMMUNOSUPPRESSIVE TUMOR MICROENVIRONMENT OF METASTATIC OSTEOSARCOMA

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. We tested this approach to circumvent the lack of specific targets, overcome antigenic heterogeneity, and 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 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 RNA-NP as a 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+ lymphocytes 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, 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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