Combination of Pathogen-mimicking Polymer Particles and an Injectable, Synthetic Immune-Priming Center (sIPC) Significantly Enhances Cellular and Protective Immunity in Murine Models of Cancer

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Introduction: Most cancer immunotherapy approaches till date have been unsuccessful in generating strong antitumor immunity primarily due to weak T helper type 1 (Th1) and Cytotoxic T Lymphocyte (CTL) response against self tumor antigens. Here we report a pathogen-mimicking polymer particle formulation and an injectable, in-situ crosslinking synthetic immune-priming center (sIPC) to enhance these responses by effectively controlling several key immunological events such as: a) recruitment of a large number of antigen presenting cells (APCs) at the site of immunization, b) efficient delivery of antigens and multiple immune-stimulatory molecules to these APCs, c) strong activation of APCs for antigen presentation and d) effective immunomodulation to generate strong Th1 and CTL responses.

Methods: PLGA micro- and nano particles were prepared by oil/water or water/oil/water emulsions and then were covalently modified with polyethylenimine to generate cationic particles. The cationic PLGA particles were then surface loaded with pDNA/Ovalbumin antigen and/or with multiple toll-like receptor ligands including IL10 siRNA, CpG, poly (I:C), and poly(U). In-situ forming dextran hydrogels were synthesized using a Michael addition reaction. Murine bone marrow dendritic cells (BMDCs) were used for activation studies. *In vivo* immunization studies were conducted by injecting (IM/SC) various formulations of the microparticles with/without hydrogel. Immunotherapeutic efficacy was tested in two different murine tumor models: B lymphoma in Balb/c mice for pDNA antigen and Melanoma-OVA in C57BJ/6 mice for OVA protein antigen.

Results: Both micro and nanoparticles were successfully surface loaded with different levels (50-98%) of both single/multiple nucleic acids and antigen on the same particle (Fig.1) and were able to activate BMDCs strongly. Preliminary in vivo immunization studies with PLGA-MP in hydrogel system showed promising results in A20 B lymphoma model in a prophylactic immunization setting. 90% mice of PLGA microparticles co-surface loaded with pDNA, CpG and Poly IC group were tumor free as compared to 40% of naked pDNA injected mice following 16 days of lethal A20 tumor challenge. PLGA MP co surface loaded with pDNA and IL10-siRNA on one particle and CpG on separate particle did relatively well with 60% mice tumor free at 16 days post challenge. In melanoma-OVA tumor model, OVA and CpG dual surface loaded PLGA microparticles showed very promising results in both the therapeutic (Fig. 2) and prophylactic immunization settings with a significantly longer survival for OVA and CpG dual loaded particle as compared to PBS, Incomplete Freund's Adjuvant (IFA)-OVA or only OVA loaded microparticle groups.

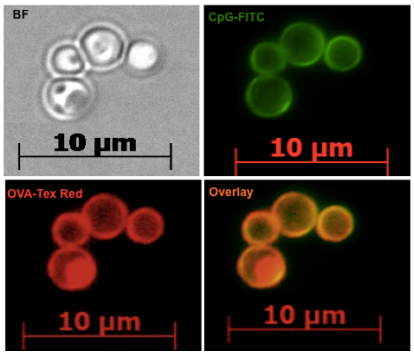

Fig. 1 CpG and OVA dual surface loaded PLGA MP

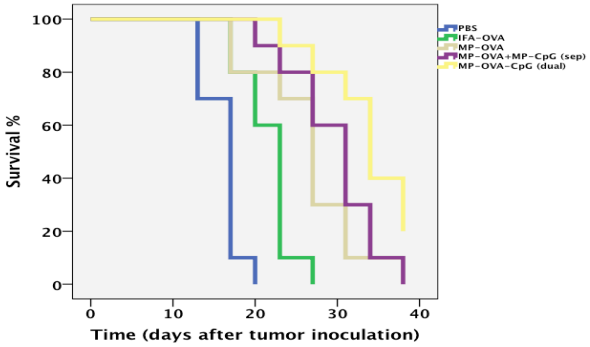

Fig. 2 Survival of therapeutically immunized melanoma-OVA tumor cell treated (sc injected) mice

Conclusions: Polymer based, pathogen-mimicking micro- and nanoparticles capable of delivering pDNA/protein antigens along with multiple TLR ligands on the surface of the same particles were developed. These particles can load tumor antigens and adjuvants both separately as well as simultaneously on a single particle with high loading efficiencies. This surface loaded particulate system is easy to formulate and the doses of antigens and various nucleic acid based TLR ligands per particle can be controlled. The particulate formulations were able to immunologically protect mice from lethal A20 lymphoma and Melanoma-OVA tumor challenge in prophylactic and therapeutic settings. Thus, a polymer based multifunctional versatile particulate delivery system has been developed for cancer immunotherapy. A comparative immunotherapeutic efficacy of polymer micro vs nanoparticles study is in progress.

References: Kasturi et al., J Control Release. 2006; 113: 261-270, Singh et al., Biomat. 2009; 30:5187-5200. Singh et al., J Control Release, 2011;155:184-192.