Title	Intratumoral injection of dextran-CpG oligonucleotide conjugates for enhanced immune-stimulation for cancer therapy
Keywords (up to 5)	Cancer vaccine; CpG; Dextran; Polymer conjugate; Cell uptake
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Abstract	CpG oligonucleotide (CpG-ODN) is a Toll-like receptor (TLR) 9 agonist that activates antigen-presenting cells (APCs), which in turn activates innate immune cells required for effectively inducing antitumor immune response. Intratumoral injection of CpG-ODN has shown promise for tumor regression through focusing the immune stimulation in tumors and draining lymph nodes. The potential of intratumoral injection of CpG-ODN may be limited by its poor retention at the injection site. The objective of the current research is to test this hypothesis by conjugating CpG-ODN to a higher molecular-weight dextran polymer. The higher molecular weight delivery system will retard the diffusion of CpG from the injection site. CpG-1668 (5'-TCCATGACGTTCCTGATGCT-3') modified with a 3' amine (3' CpG-NH_2) is conjugated to the amino-dextran using bis-arylhydrazone-linking strategy. Conjugates were prepared using dextrans with molecular weights of 6 kDa, 20 kDa, 40 kDa, 70 kDa, and 110 kDa. For tracking purpose, the CpG-dextran was labelled with the fluorescent dye Dylight 633. Following dialysis against phosphate-buffered saline (PBS) at pH 7.4, the degree of CpG substitution was determined by the formation of bis-aryl hydrazine bond measured at 360 nm and the amount of dye conjugated was quantified by fluorescence spectroscopy (Ex = 638 nm and Em = 658 nm). The purity of the dye-labelled conjugate was assessed by size exclusion chromatography. The vaccine conjugates will be investigated for their *in vitro* uptake kinetics on various immune cell populations (i.e, dendritic cells, macrophages, T cells, and B cells) and their *in vivo* tumor retention. This work will determine whether this conjugation strategy improves efficacy either by improved uptake by APC and/or increased retention time at tumor site.
References	1. Zhang et al., *Bioconjugate Chem.* 2017, 28, 1993-2000 2. Kramer et al., *Mol. Ther.* 2016, 25, 62-70 3. Ma et al., *J. Immunother.* 2017, 40, 11–20