Layer-by-Layer Nanoparticles for Cytokine Delivery to Treat Cancer

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Technical Summary

Immunotherapy is an attractive treatment for cancer because it utilizes the patient's own immune system to both recognize and fight the malignancy. Since the initial approval of checkpoint inhibition in 2011, immunotherapy has become an ever more present therapeutic strategy in the clinic and an increasingly large focal point in preclinical cancer research. Much of the success of immunotherapy in the clinic has focused on expanding indications of checkpoint inhibitors which "take the brakes off" the immune response to cancer. However, this strategy has seen limited success in many solid tumors, with only a small fraction of patients responding. Further research in the area suggests that a reason for this is that the immune response is lacking in other areas for many patients. For example, patients with poorly immune infiltrated or "cold" tumors respond very poorly to checkpoint inhibition as there is not enough of an immune presence within the tumor to effect the malignancy even with the "brakes off". An alternative strategy to utilize the immune system to fight the tumor in these and similar cases is to deliver a proinflammatory agent such as a cytokine to drive immune infiltration and activity within the tumor environment, or "hitting the gas" on the cancer immunity cycle. Unfortunately, many proinflammatory cytokines that have been translated to the clinic have shown high, schedule dependent toxicity at relevant doses, making translation infeasible. This is particularly true of interleukin-12 (IL-12). This is not unexpected as inflammatory cytokines are meant to act locally, being produced at high concentrations at the site of infection to elicit a robust immune response. Delivering cytokines systemically to reach this effective dose within the target site leads to high off-target activity and cytokine storm throughout the body.

One strategy to potentiate administration of therapies that are too toxic for systemic delivery is to use a nanoparticle delivery vehicle to concentrate the therapy within tumors and avoid off-target exposure. This strategy has been validated for many classes of therapeutics including chemotherapy, gene delivery, and even some immunotherapies. However, proinflammatory cytokines such as IL-12 pose unique design challenges for optimal delivery from a nanoparticle, including: 1) Cytokines are labile proteins and have been historically difficult to deliver using traditional encapsulation techniques; 2) NPs are often internalized by cells into endosomal compartments; however, IL-12 and other cytokines must engage external receptors to be effective and are rendered useless and often degraded upon internalization; 3) IL-12 is designed to be secreted and act locally in natural immune responses and is very toxic when allowed to circulate systemically, which necessitates a high degree of tumor association and display of IL-12 only within the tumor. In this thesis we utilize the layer-by-layer (LbL) nanoparticle technique to adjust the material properties of a nanoparticle delivery vehicle to meet these design criteria. We demonstrate extensive *in vitro* and *in vivo* characterization of the designed LbL nanoparticles; meeting each of the outlined design challenges. We show that our optimal designed LbL nanoparticles have a 90% encapsulation of IL-12 and maintain surface localization on cancer cells out to at least 24 hours. We also demonstrate that the designed nanoparticles show enhanced activity *in vitro* and *in vivo.* We demonstrate reduced toxicity and enhanced efficacy of systemic IL-12 therapy from optimized LbL nanoparticles not only compared to carrier-free IL-12 but also compared to a simpler nanoparticle design that does not incorporate targeting polymer layers.

Importantly, we demonstrate this effect in an orthotopic ovarian tumor model, a “cold” malignancy that has been particularly refractory to immunotherapies currently available in the clinic.

We also demonstrate in this thesis the method of action of IL-12 delivery from these particles and the opportunity to adjust that method of delivery in the future. The work herein lays a base for the delivery of not only cytokines, but many other therapeutic proteins that require delivery to an external receptor. Indeed, in the future, this work can be built upon to deliver not only other cytokines, but combinations of cytokines for more effective immunotherapy.