Targeted Layer-by-Layer Nanoparticles for Therapeutic Delivery to Antigen Presenting Cells and Ovarian Cancer

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Cancer is a devastating, complex disease driven by a multitude of biological and cellular factors. While there have been tremendous advancements in therapeutic strategies to target key vulnerabilities in cancer cells and the tumor microenvironment, the application of many promising agents has been hindered by safety concerns, poor pharmacokinetics, and low tumor accumulation. Nanomedicine seeks to address these limitations through the design of nanocarriers that encapsulate, protect, and target a range of therapeutic and diagnostic cargos to specific tissues and cell populations in order to improve their efficacy and safety. Achieving the necessary specificity of nanomedicine for the intended tissues and cells remains a challenge.

The overarching theme of this thesis work is to engineer the physiochemical properties of nanoparticles to improve the targeting of ovarian cancer cells as well as antigen presenting cells (APCs)—dendritic cells (DCs) and macrophages—in the tumor and lymph nodes to improve the delivery, safety and efficacy of various therapeutics. To accomplish this, we utilize Layer-by-Layer nanoparticles (LbL NPs) which are formed through the electrostatic adsorption of charged polymers onto a NP substrate. LbL NPs serve as a modular platform that enables independent optimization of the NP surface chemistry via choice of the outermost adsorbed polymer and the incorporation of cargo into the NP core or layers.

To improve the safety of a drug-combination targeting the intrinsic apoptosis pathway, we engineered a LbL NP platform encapsulating BCL-2/xL inhibitor ABT-263 and MCL-1 inhibitor S63845 to direct this combination to ovarian cancer via the choice of NP surface chemistry. To determine cell surface markers to target, a panel of ovarian cancer patient-derived xenograft (PDX) models were profiled; claudin-6 and folate receptor alpha (FRα) were identified as promising candidates. To highlight the modularity of the NP system, delivery of this combination was demonstrated using two strategies: anti-Claudin-6 antibodies conjugated onto the NP to target Claudin-6 and folate incorporated in the outer layer polymer to target FRα. In ovarian cancer PDX models, Claudin-6 and folate targeted NPs reduced toxicities and significantly extended survival.

Therapies directed to immune cells in tumors and lymphoid tissues are of interest to instruct anti-tumor immunity, but many exhibit toxicities upon systemic and off-target cell accumulation. A goal of this work was to identify NP physiochemical characteristics that facilitate specific delivery to APCs to modulate anti-cancer immune responses. The sulfated polysaccharide dextran sulfate was identified as a coating to enhance APC delivery while minimizing accumulation in off-target cells. Additionally, the effects of NP stiffness on cellular internalization were probed, and compliant liposomes were found to promote the internalization of LbL NPs in macrophages compared to stiffer liposomes.

These design parameters were applied to develop a targeted NP loaded with the toll-like receptor 3 agonist poly(I:C) for APC activation as a cancer immunotherapy. In a model of metastatic ovarian cancer, the LbL NP prolonged poly(I:C) retention in the peritoneal space, thereby reducing its systemic accumulation and associated toxicities compared to free drug. The NP formulation also enhanced activation of the target APC

population and ultimately slowed tumor growth and extended survival in combination with doxorubicin chemotherapy.

Motivated by the discovery that arginine is uniquely depleted in tumor-draining lymph nodes (tdLNs) and its depletion impairs the APC functions of DCs, a LbL NP encapsulating L-arginine and employing the DXS surface chemistry was developed to target this metabolite to DCs in tdLNs to restore their function. *In vitro,* LbL Arg NPs restored intracellular arginine levels and APC functions including the expression of costimulatory molecules and antigen presentation. In mouse models of breast cancer and melanoma, the addition of LbL Arg NPs improved the outcomes of multiple immunotherapies, including a cancer vaccine and immune checkpoint blockade, and improved the induction of T cell mediated immune memory.

Building upon the initial work of using the polysaccharide DXS to target APCs, rationally-designed anionic glycopolymers were developed to serve as the LbL surface chemistry to target carbohydrate binding receptors on APCs and cancer cells to direct NPs to these cells. The galactose-glycopolymer enhanced association with DCs over macrophages *in vitro* compared to DXS. Galactose, mannose, and lactose glycopolymer surface chemistries enhanced NP distribution in ovarian tumors compared the poly-L-aspartate control. Ongoing work is evaluating the cellular distribution of these NPs with cancer and immune cells in the tumor.

Overall, this thesis work demonstrates the applicability of engineering the surface chemistry of modular LbL NPs to improve the delivery to cancer and cancer-associated APCs, ultimately to improve the safety and therapeutic window of therapeutics to treat cancer.