Layer-by-Layer Nanoparticles for Cytokine Delivery

Ivan S. Pires

Thesis supervisors: Paula T. Hammond and Darrell J. Irvine

In the past decade, cancer immunotherapy has been a promising therapeutic strategy for cancer treatment. However, immunotherapy has failed to improve responses in certain cancers such as ovarian cancer (OC). The action of cytokines in the tumor microenvironment (TME) is key to regulating immune responses, but dose-limiting toxicities limit the application of cytokines in cancer therapy. One promising approach to improve treatment with cytokines are nanoparticles (NPs) which, when modulated via layer-by-layer (LbL) assembly, can provide many of the desirable characteristics of cytokine-delivery vehicles including tumor cell targeting, subcellular localization, and improved pharmacokinetics.

In this thesis, we address some aspects of NPs that have limited their clinical utility including manufacturing, control over self-assembly, and mechanistic understanding of their interactions in biological environments The focus here was on using liposomal LbL-NPs coated with a bilayer of poly-L-arginine (PLR) and poly-L-glutamate (PLE). The coating of NPs with PLR/PLE enables targeting towards cancer cell surfaces which allows for extended extracellular presentation of cargos. This ability is used for targeted delivery of a potent immunostimulant – interleukin-12 (IL-12) to disseminated tumors in metastatic OC. Aspects on the manufacturing of other lipid-based nanocarriers such as discoidal assemblies and immune stimulating complexes (ISCOMs) are also explored.

We show that employing a bottom-up approach to produce lipid-based NPs from mixed micelles allows for greater control over NP self-assembly. With this procedure, we generated immune stimulating complexes (ISCOMs) co-loaded with monophosphoryl-lipid-A (MPLA) via a scalable approach for clinical-scale manufacturing of the adjuvant termed Saponin MPLA NanoParticles (SMNP). Moreover, we discover that this approach allows for precise control over liposome size from 50 nm to 1 μm with minimal polydispersity. Lastly, by exploiting the lipid headgroup charge repulsion, we find that multivalent charged lipids yield discoidal lipid nanoparticles through this approach. Unlike previous attempts to generate lipid-based discs, this new class of NPs termed charge-stabilized nanodiscs (CND) do not require disc-stabilizing agents such as proteins or polymers. CNDs are shown to be promising drug delivery vehicles, especially when coated with PLR/PLE via the LbL technique where they have greater tumor accumulation than LbL-coated liposomes.

On the use of LbL-NP for cytokine delivery via PLR/PLE coated NPs, we found that covalent conjugation of IL-12 to the liposomal core of LbL-NPs greatly improves targeting and retention of IL-12 in peritoneally-disseminated OC tumors, enabling immunological and therapeutic effects not observed with free cytokine treatment. Mechanistic investigations revealed that these LbL-NPs rapidly accumulated in tumor nodules upon intraperitoneal (i.p.) administration, wherein shedding of the LbL coating allowed for gradual release of IL-12-lipid conjugates via lipid extraction by

serum proteins present in interstitial fluid. Upon a single dose of IL-12 conjugated to LbL-NPs using an intraperitoneally disseminated OV2944 highly-metastatic (HM-1) mouse model, we observed a dramatic increase in T cell levels within the ascites and the tumor nodules dispersed within the i.p. space which was not observed with either free cytokine or unlayered IL-12-NPs. When evaluated for its effectiveness in this highly aggressive model, two doses could significantly enhance survival compared to even five times (5x) the amount of free cytokine. Remarkably, while the model was non-responsive to checkpoint inhibitor (CPI) therapy with anti-PD1 and anti-CTLA4, when combined with LbL-IL-12-NPs, we achieved complete responses with robust immune memory induction. The mice were able to rapidly clear rechallenges with fresh cancer cells in the i.p. space.

Towards the clinical translation of LbL-IL-12-NPs, we demonstrate that LbL assembly is readily performed via microfluidic mixing technology amenable for clinical-scale manufacturing. We also find that we can titrate the polymer amount used to omit time-consuming purification steps. We also find that the LbL film conformation is key to maintaining therapeutic efficacy as thicker films hinder IL-12 delivery. Lastly, we uncover that the binding target of PLE on the surface of cancer cells is SLC1A5, a glutamine amino acid transporter. Unlike PLD which can also interact with anionic amino acid transporters, PLE appears to be specific towards SLC1A5.