Engineering kinetics of immunotherapies and vaccines

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The dynamic progression of immune responses to infections & tumors points to the possibility of an optimal temporal window for immune modulation as a key parameter that could influence protective outcomes. Altering kinetics in an attempt to orchestrate an immune response in resonance with the biological rhythm of innate and adaptive immunity could have significant returns with respect to improved efficacy and decreased toxicity; all without necessitating the approval of new agents. In this thesis, we explored two distinct strategies to engineer immunotherapy and vaccine kinetics. We show how these kinetics can significantly impact cellular and humoral immune responses, and carried out detailed investigations into the underlying mechanisms that govern these temporal effects.

Firstly, imidazoquinolines (IMDs), small molecule agonists of Toll like receptor (TLR)-7 and/or TLR-8, are of great interest as potential anti-cancer therapeutics due to their ability to activate innate immune cells. Nevertheless, safe and effective systemic administration of these compounds in the clinic is an unsolved challenge due to dose-limiting toxicities, poor bioavailability, and severe immune-related adverse events upon intravenous administration. While attempts to deliver them via nanoparticle technologies have improved the potency of IMDs, achieving these outcomes while minimizing acute systemic inflammation has proven difficult.

Here, we developed a bottlebrush prodrug (BPD) IMD library as a tool to provide a detailed understanding of how the kinetics of drug release impacts safety and tumor immune stimulation. Cylindrical BPDs featuring antibody-like dimensions (~10 nm), coaxial PEG chains, and TLR-7/8 agonists linked through cleavable linkers along their backbone were synthesized using ring-opening metathesis polymerization (ROMP). By tuning the cleavable linker molecular structure, IMD-BPD constructs were identified that allowed for potent stimulation of innate immune cells in tumors while avoiding systemic increases in inflammatory cytokines, reductions in white blood cell counts, or liver toxicity. These BPDs enabled significant reductions in tumor growth in syngeneic tumor models and improved responses to anti-PD-1 checkpoint blockade. Single-cell RNA-sequencing revealed that IMD-BPDs promote dendritic cell activation and reduce immunosuppressive macrophages in the tumor microenvironment, changes that free TLR7/8 agonists were unable to achieve.

Secondly, “extended prime” immunization regimens that prolong exposure of the immune system to the first dose of a vaccine have shown promise in enhancing humoral immune responses to a variety of subunit vaccines in preclinical models. We previously showed that escalating-dosing immunization (EDI), where a vaccine is dosed every other day in an increasing pattern over 2 weeks dramatically amplify humoral immune responses. Such a dosing regimen is impractical for prophylactic vaccines, but we hypothesized that simpler dosing regimens might replicate key elements of the immune response triggered by EDI. Thus, here we explored "reduced ED" immunization regimens, assessing the impact of reducing the number of injections and varying

dose levels and dosing intervals during EDI. Using a stabilized HIV Env trimer as a model antigen combined with a potent saponin adjuvant, we found that a two-shot extended-prime regimen consisting of 20% of a given vaccine dose administered on day 0 followed by a second shot with the remaining 80% of vaccine 7 days later resulted in increased total GC B cells, 10-fold increased antigen-specific GC B cells, and an order of magnitude higher serum antibody titers compared to bolus immunization. Computational modeling of the GC response suggested that this enhanced response is mediated by antigen delivered in the second dose being captured as immune complexes in follicles, an effect that can be amplified by prolonged antigen exposure in the second dose administration, predictions we verified experimentally. These results suggest that a two-shot priming approach can be used to substantially enhance responses to subunit vaccines.

These results pave the way to safer and more potent cancer immunotherapies and vaccines via engineered kinetic approaches to administering these compounds.

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