Small molecule drugs and biological macromolecules are currently being investigated as therapies for various types of disorders such as cancer and infectious diseases. Generally, these agents are systemically administered. However, many immunomodulatory agents are active primarily on specific immune cells such as dendritic cells (DCs). This ‘targeted delivery’ of drugs is thus important. The purpose of this study was to evaluate the efficacy of the novel delivery platform (DPX) for targeted delivery of immunomodulatory agents to the lymph node. As the lymph node is the primary site for priming and activation of immune cells, this approach may improve delivery over systemic administration resulting in increased drug efficacy, the use of less drug, and reduced off-target toxicity.

To evaluate the efficacy of the DPX platform for lymph node-targeted drug delivery, we used the CT26 mice model. DPX formulation of small molecule drugs allowed delivery of the target of lymph-node-specific delivery by visual assessments and by flow cytometry. To test impacts on tumor directed immune responses, the HPV16 E7 mRNA vector (pE7-1) was used. Novel formulations tested in conjunction with DPX/FP (a C3 tumor-specific antigen HPV16 E7-V645-57 conjugated to a FP helper epitope) DPX-mediated delivery of low doses of cyclophosphamide (CPA) were delivered subcutaneously as a single injection. Using DPX to deliver CPA resulted in a significant increase in the number of lymph node cells after a single administration, showing equivalent therapeutic benefit was achieved with significantly less active ingredient.

We also evaluated DPX delivery of the checkpoint inhibitor anti-CTLA-4. We found that administration of DPX was equivalent to intraperitoneal injection in terms of overall survival of tumor-bearing mice and in the proportion of CD3+ T cells bound by anti-CD3 (anti-CD3). This, coupled with fewer administrations of antibody. This demonstrates maintenance of biological functionality of macromolecules in the DPX platform.

In conclusion, DPX holds promise in terms of lymph node-targeted delivery of functional agents. This delivery is facilitated by a sustained uptake of the drug from the one injection and can offer reduced dosing and lower toxicity as compared to systemic delivery. Many approaches to targeted therapies have failed to provide a therapy that maximizes their promise through systemic delivery, with a challenge in achieving selectivity for the target cell type. Here, we show the DPX drug delivery platform has the potential to overcome these challenges and thereby increase the efficacy of immune and cancer therapies.

**ABSTRACT**

**Distribution**

DPX-formulation of small molecule dyes demonstrated targeted delivery compared to systemic aqueous delivery

**Functionality**

DPX-formulation with functional biological molecules

**Stability**

No degradation in DPX-formulation

**Targeted Delivery**

DPX-drug delivery achieved equivalent LN-specific effects with significantly less active agent

**CONCLUSIONS**

• Targeted delivery of small molecules and immunomodulatory agents to lymph node is demonstrated using DPX platform technology. DPX studies demonstrated that DPX can deliver small molecules to lymph nodes with minimal to no systemic exposure.
• Low dose CPA has been shown to act as a bivalent immunomodulatory agent when delivered systemically over a week. As expected, using DPX to deliver CPA resulted in a significant reduction in the number of lymph node cells with significantly less active agent.
• Meanwhile, with DPX/anti-CTLA-4, developed ADA against anti-CTLA-4. While ADA can hinder therapeutic efficacy, their generation supports that large biological macromolecule structure is maintained in DPX. Importantly, in this study, the formation of ADAs did not impede the effect of anti-CTLA-4 delivered in DPX in restricting tumour growth nor binding to circulating T cells.