Local delivery of pattern recognition receptor agonists (PRRAs) to the tumor microenvironment (TME) stimulates innate immune sensors such as toll-like receptors (TLRs), which can enhance antigen uptake and presentation, induce proinflammatory immune cell recruitment, and reverse tumor-associated immunosuppression.1 Local delivery of PRRAs, such as TLR or STING agonists, has shown encouraging preclinical and clinical anti-tumor benefit.2-4 However, current approaches to intratumoral delivery of PRRA treatments suffer from the lack of local retention in the TME, thus limiting anti-tumor benefit, promoting systemic treatment-related adverse events (e.g., cytokine storms), and necessitating frequent and often impractical dosing regimens. Additionally, systemic toxicity associated with current PRRA treatments may limit combination therapies.1,2

We developed TransCon™ technology to improve the therapeutic index of TLR7/8 Agonist. A long-acting prodrug, designed to provide prolonged intratumoral retention of a therapeutic TLR7/8 Agonist, was transformed into a biologically active TLR7/8 Agonist by enzymatic activation following reconstitution in hydrogels. This process has been demonstrated in rodents demonstrating long-term reconstitution release over several weeks with minimal systemic exposure compared to an equivalent dose of unconjugated reconstituted resiquimod. Furthermore, in a syngeneic CT26 tumor model, a single intratumoral injection of TransCon TLR7/8 Agonist was well-tolerated, leading to significant and dose-dependent tumor growth inhibition, and only associated with significantly lower systemic proinflammatory cytokine induction when compared to an equivalent dose of unconjugated resiquimod. In a bilateral syngeneic tumor model, TransCon TLR7/8 Agonist treatment resulted in significant tumor growth inhibition in injected and non-injected tumor-bearing mice at the on-treatment and in combination with systemic IL-2. Complete regression of treated and untreated tumors was observed following combination treatment. TransCon TLR7/8 Agonist treatment was associated with an increase in frequency and activation of antigen-presenting cells and CD4+ T cells in tumor draining lymph nodes (BLM). Finally, tumor rechallenge with the colon cancer cell line CT26 demonstrated complete tumor growth inhibition in mice treated 2 months earlier with a single dose of TransCon TLR7/8 Agonist and IL-2.

These data provided strong evidence that a single dose of TransCon TLR7/8 Agonist can mediate long-term intratumoral release of resiquimod with minimal systemic exposure compared to unconjugated resiquimod. Moreover, TransCon TLR7/8 Agonist provided potent anti-tumor activity in a syngeneic murine tumor model and in a bilateral syngeneic tumor model treated with a single dose of TransCon TLR7/8 Agonist and IL-2. TransCon TLR7/8 Agonist was designed as a novel sustained-release PRRA therapy class and has the potential to overcome the shortcomings of existing PRRA treatments by providing a potent anti-tumoral response while reducing systemic drug exposure and related adverse events.

Our data showed that a single intratumoral dose of TransCon TLR7/8 Agonist:

- Provided drug exposure for weeks
- Avoided a high systemic Cmax, compared to equimolar dose of parent drug
- Demonstrated potent anti-tumor effects as a monotherapy
- Enhanced the anti-tumor effect of systemically administered 5 IU in injected and non-injected tumors, leading to several complete regressions
- Promoted anti-tumor memory when combined with IL-2 treatment through TransCon TLR7/8 Agonist-mediated expansion of activated anti-cancer T cells and potentiated CD8+ T-cell activation and memory.

**METHODS**

We generated TransCon TLR7/8 Agonist by first converting conjugated resiquimod to a hydrogel microcarrier with a TransCon linker. TransCon TLR7/8 Agonist was assessed for in vivo drug release in vivo using in vivo administration in rats or intratumoral administration in mice. Plasma drug levels were determined using UPLC-MS/MS. TransCon TLR7/8 Agonist was assessed for anti-tumor efficacy using the murine syngeneic CT26 tumor model in either monolateral or bilateral tumor models. TransCon TLR7/8 Agonist treatment was administered intratumorally with or without or without systemic IL-2 treatment started on the day of tumor xenograft. Tumor volumes were determined using the formula V = L x W² x 0.5, where V is tumor volume, L is a tumor length, and W is tumor width. Body weights were determined by scale measurement. Plasma cytokines were determined via Luminex. Immunohistochemistry of immune cell subsets was performed by fluorescence-activated cell sorting on single-cell suspensions derived from tumor tissue harvested by caliper measurement at 7 days after dosing with or without systemic IL-2 treatment. Tumor rechallenge experiments, mice that experienced complete regressions in both TransCon TLR7/8 Agonist-treated and non-injected tumors were re-inoculated with CT26 tumor cells and monitored for tumor growth. Tumor burden was determined using the formula V = L x W² x 0.5, where V is tumor volume, L is a tumor length, and W is tumor width. Body weights were determined by scale measurement.

**CONCLUSIONS**

TransCon TLR7/8 Agonist has the potential to:

- Induce potent anti-tumoral responses while reducing the risk of systemic adverse events
- Enable efficacy with dosing intervals of months
- Enhance local innate immune activation in the TME, thereby promoting anti-tumor immunity

TransCon technology for novel localized and systemic delivery have the potential to broadly impact the immune cycle and may offer new combination approaches in cancer therapy.

**REFERENCES:**


