P16: Tumor Growth Inhibition Mediated by A Single Dose of Intratumoral TransCon™ TLR7/8 Agonist Associated with Activated Circulating T and B cells and Sustained Low Levels of Systemic Cytokines

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ABSTRACT

TLR agonists can elicit anti-tumor activity by activating innate immune cells and promoting a proinflammatory microenvironment.[1, 2, 3] Local delivery of TLR agonists has shown encouraging preclinical and clinical anti-tumor activity.[4, 5] However, intratumoral (IT) delivery of unmodified TLR agonists such as resiquimod, a TLR7/8 agonist, can lead to rapid diffusion from the tumor, resulting in acute systemic drug exposure and transient but high levels of peripheral proinflammatory cytokines, thus limiting anti-tumor benefit and increasing risk of cytokine-driven adverse effects.[6]

TransCon™ TLR7/8 Agonist was designed to elicit a sustained and local release of resiquimod following IT administration. In the syngeneic murine CT26 tumor model, a single IT injection of TransCon TLR7/8 Agonist monotherapy was sufficient to induce potent tumor growth inhibition. Following treatment, the induction of key cytokines and chemokines associated with innate immunity was determined. Proinflammatory cytokines (IL-1b, IL-6, and TNFα) were induced following IT TransCon TLR7/8 Agonist treatment, but peak levels were lower (difference ~10-fold or more) than those observed with an equimolar dose of free, soluble resiquimod. Circulating cytokine levels were sustained, but remained low, through Day 21. T_h1-associated IFNγ was induced with levels increased at Day 1 and maintained at Day 7. Additionally, sustained expression of myeloid-associated chemokines (MCP-1/CCL2, IP-10/CXCL10, and MIP-1α/CCL3) was detected in both tumor and periphery.

The increase in cytokines was consistent with an increase in circulating innate immune cells, such as NK and myeloid cells. Furthermore, evidence of adaptive immune cell activation was observed as indicated by expression of Ly6C, ICOS and Ki67, which were increased on peripheral $CD8^+$ T cells, $CD4^+$ T cells (Ki67, ICOS), and B cells (Ly6C), with evidence of sustained pharmacodynamic activity over the observation period of 21 days.

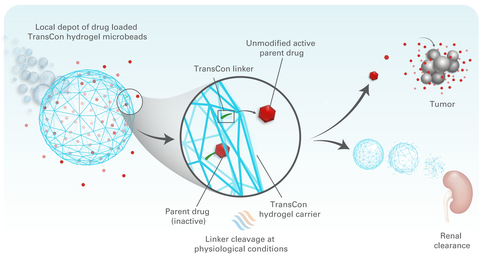
These data show that a single IT injection of TransCon TLR7/8 Agonist can elicit sustained expression of key cytokines and chemokines, promote innate immune cell mobilization, activate adaptive immune cells, and mediate robust anti-tumor activity. The peak levels of the cytokines remained relatively low through the observation period of 21 days, suggesting a low risk of systemic cytokine-associated adverse events.

The increase in activated B, T, and NK cells in blood was associated with induction of a potent anti-tumor response, further supporting TransCon TLR7/8 Agonist as a novel and potentially efficacious PRRA therapy.

METHODS

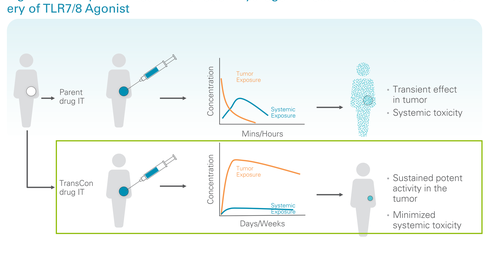
We generated TransCon TLR7/8 Agonist by conjugating resiquimod to a hydrogel carrier with a TransCon linker. TransCon TLR7/8 Agonist was assessed for anti-tumor efficacy, following a single intratumoral administration, using the murine syngeneic CT26 tumor model. Tumor volumes were estimated by using the formula: V = (L x W x W)/2, where V is tumor volume, L is tumor length and W is tumor width (both in mm). Tumors and plasma were harvested. Tumor lysates were assessed for protein concentration and diluted with PBS to a protein concentration of 5.5 mg protein/mL. 25 µL of concentration adjusted samples were then assessed for cytokine/chemokine levels via Luminex. Plasma cytokine/chemokine levels were determined via Luminex or MSD. Immunophenotyping of immune cell subsets from isolated PBMCs was performed by flow cytometry. Data analysis and visualization was performed using R.

Figure 1: TransCon Localized Carrier for Sustained Intratumoral Drug Release



TransCon technology combines the benefits of conventional prodrug and sustained-release technologies and is broadly applicable to proteins, peptides, and small molecules. TransCon technology can be used for both sustained systemic and sustained localized delivery, including intratumoral administration. TransCon TLR7/8 Agonist consists of resiquimod transiently conjugated to an insoluble TransCon hydrogel microbead carrier. The hydrogel carrier allows for retention of the prodrug in the tumor microenvironment (TME) following IT administration and is designed to provide sustained local release of unmodified parent drug. Following drug release, the hydrogel carrier is degraded into small fragments that can be cleared renally.

Figure 2: Resiquimod-Loaded TransCon Hydrogel for Sustained Intratumoral Delivery of TLR7/8 Agonist



Current IT approaches to deliver PRRAs to the TME often do not allow local retention, thus rapid drug clearance and limited anti-tumor efficacy remains problematic. Furthermore, high systemic exposure of IT-delivered PRRAs can promote systemic treatment-related adverse events (eg, cytokine storm), leading to narrow therapeutic windows and necessitating frequent and often impractical dosing regimens. TransCon TLR7/8 Agonist was designed to provide weeks of drug exposure in the TME, stimulating a robust local anti-tumor immune response, with minimal systemic drug exposure or toxicity.

RESULTS

Figure 3: TransCon TLR7/8 Agonist Mediated More Potent Tumor Growth Inhibition and a Lower Concentration of Systemic Cytokines than a Comparable Dose of Resiquimod

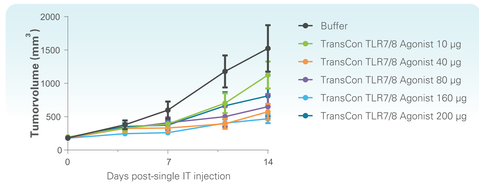
Female BALB/C mice were implanted with CT26 tumor cells. When tumors were grown to a mean tumor volume of ~80-105 mm^3, mice were randomized into treatment cohorts (day 0). The day following randomization, animals received either empty hydrogel (TransCon Vehicle), 20 µg leq. of resiquimod of TransCon TLR7/8 Agonist, or 20 µg of unconjugated resiquimod as a single intratumoral dose. A) Tumor volumes were calculated according to the formula: Tumor volume = (L x W) x 0.5 where L is the length of the tumor and W the width (both in mm). Values are represented as mean +/- SEM. B) Plasma samples were collected at various time points over a 24 hour period and assessed for cytokine/chemokine levels by Luminex. Log2 fold change in the abundance of each cytokine at different time points and treatment levels was calculated relative to TransCon Vehicle control one-hour post-single IT injection. The heatmap shows the up-regulation (red), down-regulation (blue), or no change (less than two-fold change, almond) status of each cytokine. C_{max} and T_{max} for each cytokine/chemokine are shown Table 1.

Table 1: Plasma Cytokine and Chemokine C_{max}/T_{max}

Mean C_{max} pg/mL [T_{max} h]				
Analyte	TransCon Vehicle	20 µg IT TransCon TLR7/8 Agonist	20 µg IT Resiquimod	Fold C_{max} difference (Resiquimod/ TransCon TLR7/8 Agonist)
TNF-α	4.7 [6]	27 [3]	831 [1]	31
IL-6	57.7 [24]	270 [24]	3619 [1]	13
IFN-γ	0.9 [LLOQ]	4.5 [10]	41 [6]	9.0
CCL2 / MCP-1	50 [24]	509[10]	4230 [3]	8.3

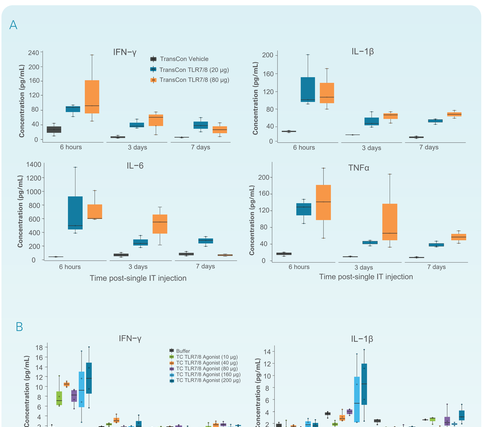
RESULTS

Figure 4: TransCon TLR7/8 Agonist Mediated Potent Tumor Growth Inhibition



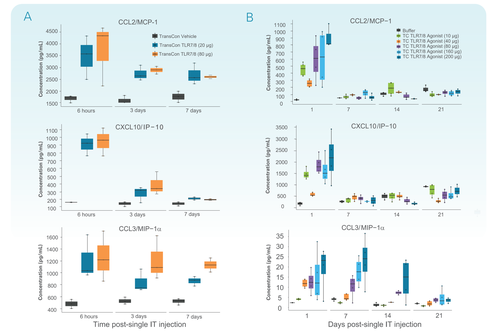
Female BALB/C mice were subcutaneously implanted with CT26 tumor cells. When tumors were grown to a mean tumor volume of ~185 mm^3, mice were randomized into treatment cohorts (Day 0). On the same day, animals received either control vehicle buffer or 10, 40, 80, 160, or 200 µg leq. of resiquimod of TransCon TLR7/8 Agonist as a single intratumoral dose. Tumor volumes were calculated according to the formula: Tumor volume = (L x W) x 0.5 where L is the length of the tumor and W the width (both in mm). Values are represented as mean +/- SEM (n =5-20 animals per time point).

Figure 5: TransCon TLR7/8 Agonist Promoted Sustained Expression of Myeloid- and T_h1-associated Cytokines in the Tumor with Low but Detectable Cytokine Levels in Plasma



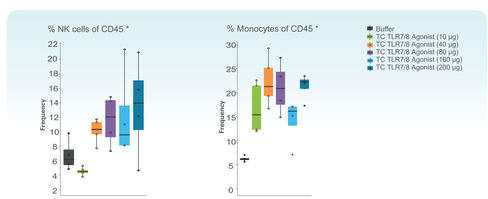
A) Female BALB/C mice were implanted with CT26 tumor cells. When tumors were grown to a mean tumor volume of ~115 mm^3, mice were randomized into treatment cohorts (day 0). The day following randomization, animals received either empty hydrogel (TransCon Vehicle), or 20 or 80 µg leq. of resiquimodl of TransCon TLR7/8 Agonist as a single intratumoral dose. Mice were sacrificed at various times following treatment initiation and tumors were collected and assessed for cytokine levels by Luminex. Data is from 2-3 animals per time point. B) Female BALB/C mice implanted with CT26 tumors were treated with TransCon TLR7/8 Agonist as described in Figure 4. The mice were sacrificed at various times following treatment initiation, and plasma was collected and assessed for cytokine levels by MSD. Data is from 3-5 animals per time point. Box plots in A) and B) represent the minimum, 25th percentile, median, 75th percentile, and maximum values.

Figure 6: TransCon TLR7/8 Agonist Promoted Sustained Expression of Inflammatory Chemokines in the Tumor with Low, but Detectable Chemokine Levels in Plasma



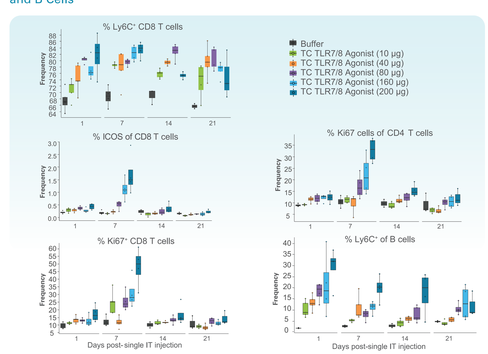
A) Female BALB/C mice were implanted with CT26 tumor cells. When tumors were grown to a mean tumor volume of ~115 mm^3, mice were randomized into treatment cohorts (day 0). The day following randomization, animals received either empty hydrogel (TransCon Vehicle), or 20 or 80 µg leq. of resiquimodl of TransCon TLR7/8 Agonist as a single intratumoral dose. Mice were sacrificed at various times following treatment initiation and tumors were collected and assessed for chemokine levels by Luminex. Data is from 2-3 animals per time point. B) Female BALB/C mice implanted with CT26 tumors were treated with TransCon TLR7/8 Agonist as described in Figure 4. The mice were sacrificed at various times following treatment initiation, and plasma was collected and assessed for cytokine levels by MSD. Data is from 3-5 animals per time point. Box plots in A) and B) represent the minimum, 25th percentile, median, 75th percentile, and maximum values.

Figure 7: TransCon TLR7/8 Agonist Treatment Associated With An Increase in Peripheral Myeloid and Natural Killer Cells



Female BALB/C mice implanted with CT26 tumors were treated with TransCon TLR7/8 Agonist as described in Figure 4. The mice were sacrificed one day following treatment initiation and PBMCs were isolated and assessed for markers of immune-cell subsets via flow cytometry. Box plots represent the minimum, 25th percentile, median, 75th percentile, and maximum values. Data is from 3-5 animals.

Figure 8: TransCon TLR7/8 Agonist Promoted Sustained Activation of Peripheral T and B Cells



Female BALB/C mice implanted with CT26 tumors were treated with TransCon TLR7/8 Agonist as described in Figure 4. The mice were sacrificed on Days 1, 7, 14, and 21 following treatment initiation and PBMCs were isolated and assessed for markers of immune-cell subsets via flow cytometry. Box plots represent the minimum, 25th percentile, median, 75th percentile, and maximum values. Data is from 3-5 animals per time point.

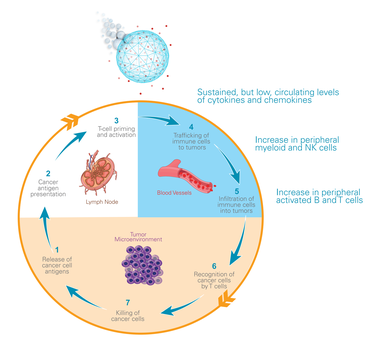
SUMMARY

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

- Mediated robust anti-tumor activity that associated with sustained expression of key cytokines and chemokines in the tumor.
- Induced peak plasma cytokine levels were substantially lower than those induced by an equimolar dose of unconjugated resiquimod.
- Promoted inflammatory cytokine/chemokine protein expression that was sustained in tumor and blood but remained low in circulation through the observation period of 21 days.
- Induced expression of several inflammatory chemokines known to attract T cells and myeloid cells (MCP-1/CCL2, IP-10/CXCL10, MIP-1α/CCL3) that were elevated in both tumor and circulation.
- Potentiated the frequency of peripheral NK and activated B and T cells in blood. Evidence for sustained immune cell activation was observed through the observation period of 21 days.

CONCLUSIONS

- TransCon TLR7/8 Agonist Mediated robust anti-tumor activity which associated with sustained activation of both innate and adaptive immune mechanisms
  - Sustained intratumoral cytokine and chemokine expression has the potential to enhance tumor infiltrating lymphocytes and potentiate anti-tumor immune responses
  - Elevated levels of peripheral NK and activated B and T cells in blood may contribute to systemic anti-tumor responses
- The current data further support TransCon TLR7/8 Agonist as a novel and potentially efficacious PRRA therapy with low risk of systemic adverse events
  - Consistent with the low systemic cytokine levels observed in this study, a GLP toxicity study in cynomolgus monkeys showed no adverse observations related to systemic resiquimod exposure from TransCon TLR7/8 Agonist (data not shown)
- A clinical trial to evaluate the safety and efficacy of TransCon TLR7/8 Agonist, in monotherapy and in combination with a checkpoint inhibitor, in cancer patients is currently underway (transcendIT-101; NCT04799054)



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