P769: A Single Dose of Intratumoral TransCon™ TLR7/8 Agonist Monotherapy Promoted Sustained Activation of Antigen Presenting Cells Resulting in CD4+ and CD8+ T cell Activation and Tumor Growth Inhibition

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ABSTRACT

The use of pattern recognition receptor agonists (PRRAs) such as Toll-like receptor (TLR) agonists is an attractive approach for cancer immunotherapy. TLR agonism elicits anti-tumor activity by activating antigen presenting cells (APCs) to promote a proinflammatory microenvironment and anti-tumor immunity.[1] Local delivery of TLR agonists has shown encouraging preclinical and clinical anti-tumor benefit.[2,3] However, intratumoral (IT) delivery of naked PRRAs may lead to rapid effusion from the tumor microenvironment, potentially impacting their effectiveness in inducing local inflammation and may promote systemic cytokine release, increasing the risk of adverse effects.[4]

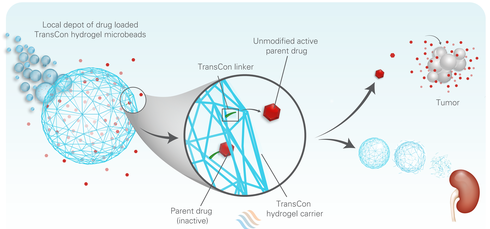
TransCon™ TLR7/8 Agonist was designed to address the current limitations of PRRA therapies and IT delivery through sustained and controlled release of resiquimod, a potent TLR7/8 agonist, following IT administration of a hydrogel depot. A single IT injection of TransCon TLR7/8 Agonist induced potent and sustained tumor growth inhibition in syngeneic mouse CT26 tumors. Following IT TransCon TLR7/8 Agonist treatment, acute and sustained upregulation of cell surface markers indicative of activation of APCs, such as CD54, CD69, and CD86, in the tumor was observed by flow cytometry. Additionally, TransCon TLR7/8 Agonist treatment was associated with an increase in the frequency of APCs with an activated phenotype in tumor–draining lymph nodes (LNs). Further, a concomitant potentiation in the frequency of activated CD4+ and CD8 T+ cells in tumor–draining LNs following IT TransCon TLR7/8 Agonist treatment was observed, as demonstrated by increased expression of Ki67, ICOS, or granzyme B.

These data support that a single IT dose of TransCon TLR7/8 Agonist can mediate robust anti-tumor activity as a monotherapy in the CT26 syngeneic mouse tumor model while promoting local activation of intratumoral APCs. Such activation may promote tumor antigen uptake and migration to tumor-associated lymphoid tissue, as evidenced by an increase in APCs with an activated phenotype in tumor–draining LNs following TransCon TLR7/8 Agonist treatment. Activated tumor antigen-bearing APCs can promote the priming and activation of tumor–specific T cells in the tumor-draining LNs.[5] Consistently, a dose-dependent increase in the frequency of T cells with an activated effector phenotype in tumor–draining LNs following administration of TransCon TLR7/8 Agonist was observed. These preclinical data further support TransCon TLR7/8 Agonist as a novel and potentially efficacious PRRA therapy. A clinical trial to evaluate safety and efficacy of TransCon TLR7/8 Agonist as monotherapy, and in combination with pembrolizumab, in cancer patients has been initiated (transcendIT-101; NCT04799054).

METHODS

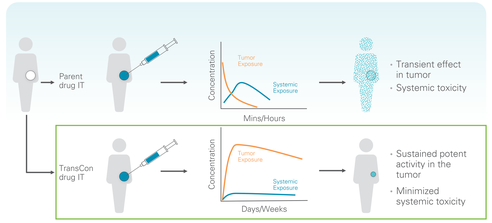
We generated TransCon TLR7/8 Agonist by conjugating resiquimod to a hydrogel carrier with a TransCon linker. TransCon TLR7/8 Agonist was assessed for anti-tumor efficacy using the murine syngeneic CT26 tumor model. Animals received either buffer control or 10, 40, 80, 160, or 200 µg (eq. of resiquimod) of TransCon TLR7/8 Agonist as a single intratumoral dose. 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. Immunophenotyping of immune cell subsets was performed by flow cytometry on single cell suspensions derived from tumors or tumor–draining inguinal lymph nodes harvested 1 or 7 days after dosing was initiated.

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.

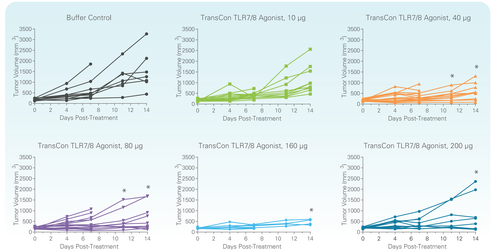
Figure 2: Resiquimod-Loaded TransCon Hydrogel for Sustained Intratumoral Delivery of TLR7/8 Agonist



Current IT approaches to deliver PRRAs to the TME often do not allow local retention, thus rapid drug clearance and limited anti-tumor efficacy remain 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.

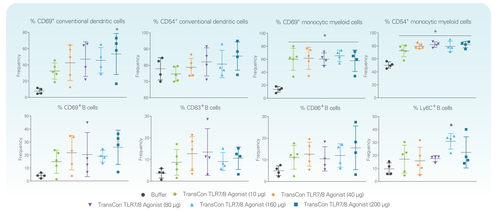
RESULTS

Figure 3: A Single Monotherapeutic Dose of TransCon TLR7/8 Agonist Mediated Potent and Sustained Tumor Growth Inhibition



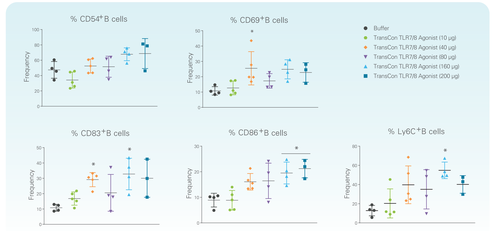
Female BALB/C mice were subcutaneously implanted with CT26 tumor cells. When tumors were grown to a mean tumor volume of ~185 mm³, mice were randomized into treatment cohorts (Day 0). On the same day, animals received either control vehicle buffer or 10, 40, 80, 160, or 200 µg (eq. of resiquimod) of 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 individual tumor volumes over time. *p<0.05 vs Buffer treated animals. Significance was determined by Two-way ANOVA followed by multiple comparisons using Tukey's Honest Significant Differences post-hoc test.

Figure 4: Intratumoral Administration of TransCon TLR7/8 Agonist Induced Early Markers of Activation on Antigen Presenting Cells in Treated Tumors



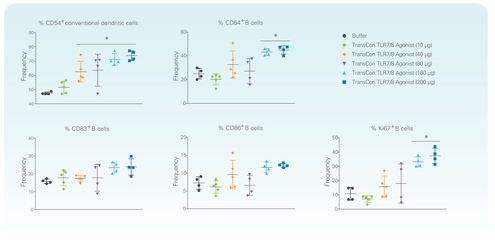
BALB/c mice were established with CT26 tumors as described in Figure 3 and the mice were sacrificed one day following treatment initiation, and tumors were harvested. Tumor infiltrating lymphocytes were isolated and assessed for markers of immune-cell subsets and activation via flow cytometry. Values are represented as mean +/- SEM. *p<0.05 vs Buffer treated animals. Significance was determined by Ordinary One-way ANOVA followed by multiple comparisons using Tukey's Honest Significant Differences post-hoc test.

Figure 5: Intratumoral Administration of TransCon TLR7/8 Agonist Promoted Sustained Activation of Tumor-Associated B cells



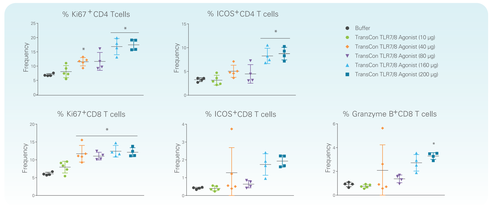
Mice from the experiment described in Figure 3 were sacrificed on Day 7 following treatment initiation, and tumors were harvested. Tumor infiltrating lymphocytes were isolated and assessed for markers of immune-cell subsets and activation via flow cytometry. Values are represented as mean +/- SEM. *p<0.05 vs Buffer treated animals. Significance was determined by Ordinary One-way ANOVA followed by multiple comparisons using Tukey's Honest Significant Differences post-hoc test.

Figure 6: Intratumoral TransCon TLR7/8 Agonist Potentiated Activation of Antigen Presenting Cells in Tumor–Draining Lymph Nodes



Mice from the experiment described in Figure 3 were sacrificed on Day 7 following treatment initiation, and tumor–draining lymph nodes were harvested. Lymphocytes were isolated and assessed for markers of immune-cell subsets and activation via flow cytometry. Values are represented as mean +/- SEM. *p<0.05 vs Buffer treated animals. Significance was determined by Ordinary One-way ANOVA followed by multiple comparisons using Tukey's Honest Significant Differences post-hoc test.

Figure 7: Intratumoral TransCon TLR7/8 Agonist Promoted Activation of T cells in Tumor–Draining Lymph Nodes



Mice from the experiment described in Figure 3 were sacrificed on Day 7 following treatment initiation, and tumor draining lymph nodes were harvested. Lymphocytes were isolated and assessed for markers of immune-cell subsets and activation via flow cytometry. Values are represented as mean +/- SEM. *p<0.05 vs Buffer treated animals. Significance was determined by Ordinary One-way ANOVA followed by multiple comparisons using Tukey's Honest Significant Differences post-hoc test.

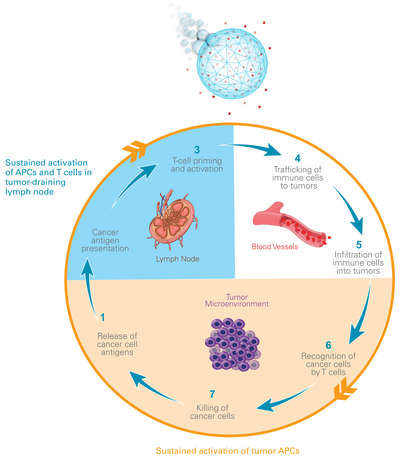
SUMMARY

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

- Resulted in potent and sustained anti-tumor effects as a monotherapy
- Promoted early and sustained induction of activation markers on APCs in treated tumors
- Led to an increased frequency of antigen presenting cells, such as dendritic cells and B cells, with an activated phenotype in tumor draining lymph nodes
- Potentiated the frequency of T cells with an activated effector phenotype in tumor–draining lymph nodes
  - Increased frequencies of Ki67+ and ICOS+ CD4+ T cells
  - Increased frequencies of Ki67+ and Granzyme B+ CD8+ T cells

CONCLUSIONS

- TransCon TLR7/8 Agonist is a novel and potentially efficacious PRRA therapy
- A single IT dose of TransCon TLR7/8 Agonist:
  - Mediated robust anti-tumor activity as a monotherapy while promoting local activation of intratumoral APCs
  - Has potential to stimulate tumor antigen uptake by APCs, APC migration to tumor–associated lymphoid tissue, and tumor antigen presentation to adaptive immune cell subsets
  - May potentiate cytotoxic immune cell activation to promote adaptive, cytotoxic anti-tumor activity
- TransCon technologies have the potential to broadly impact the immunity cycle and may offer new combination approaches in cancer therapy
- 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)



REFERENCES
1) Hamid O, Ismail R, Puzanov I. Intratumoral Immunotherapy-Update 2019. Oncologist. 2020 Mar;25(3):e423-e438.
2) Rook AH, et al. Topical resiquimod can induce disease regression and enhance T-cell effector functions in cutaneous T cell lymphoma. Blood 2015;126:1452-1461.
3) Clark CM, Furnes M, Mackay-Wiggan JM. Basal cell carcinoma: an evidence-based treatment update. Am J Clin Dermatol 2014;15:197-216.
4) Zuniga LA, et al. Intratumoral Delivery of TransCon TLR7/8 Agonist Provides Potent Anti-tumor Activity as a Monotherapy and in Combination With IL-2 While Minimizing Systemic Cytokine Induction. J Immunother Cancer. 2019;7:283.
5) van Pul KM,et al. Immunotherapy Goes Local: The Central Role of Lymph Nodes in Driving Tumor Infiltration and Efficacy. Front Immunol. 2021;12:643291.

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