P676: 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

Luis A. Zuniga[1], Torben Leßmann[2], Lars Holten-Andersen[3], Nicola Bisek[2], Joachim Zettler[2], Sebastian Stark[2], Frank Faltinger[2], Oliver Kracker[2], Samuel Weisbrod[2], Robin Müller[2], Tobias Voigt[2], Kornelia Bigott[2], Mohammad Tabrizifard[1], Vibeke Breinholt[3], Kennett Sprogøe[3], Juha Punnonen[1]

[1]Ascendis Pharma, Inc., Palo Alto, California, USA; [2]Ascendis Pharma GmbH, Heidelberg, Germany; [3]Ascendis Parma A/S, Copenhagen, Denmark

ABSTRACT

Local delivery of pattern recognition receptor agonists (PRRAs) to the tumor microenvironment (TME) stimulates innate immune sensors such as toll-like receptors (TLR), which can enhance antigen uptake and presentation, induce proinflammatory immune cell recruitment, and reverse tumor-associated immunosuppression.[1, 2] Local delivery of PRRAs, such as TLR or STING agonists, has shown encouraging preclinical and clinical anti-tumor benefit.[3-5] However, current approaches to intratumoral delivery of PRRAs suffer from the lack of local retention in the TME, thus limiting anti-tumor benefit, promoting systemic treatment-related adverse events (eg, cytokine storm), and necessitating frequent and often impractical dosing regimens. Additionally, systemic toxicity associated with current PRRA treatments may limit combination therapies.[2, 6]

We developed TransCon™ (transient conjugation) TLR7/8 Agonist, a long-acting prodrug of resiquimod, designed to provide prolonged intratumoral release of unmodified resiquimod by transiently conjugating resiquimod to hydrogel microbeads via a TransCon linker. A single intratumoral or subcutaneous injection of TransCon TLR7/8 Agonist in rodents demonstrated long-term resiquimod release over several weeks with minimal systemic exposure compared to an equimolar dose of unconjugated resiquimod. Furthermore, in a syngeneic CT26 tumor model, a single intratumoral injection of TransCon TLR7/8 Agonist was well tolerated, led to significant and dose-dependent tumor growth inhibition, and was associated with significantly lower systemic proinflammatory cytokine induction when compared to an equimolar 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 tumors as a monotherapy and in combination with systemic IL-2 treatment. 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 CD8+ T cells in tumor draining lymph nodes (DLN). 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.

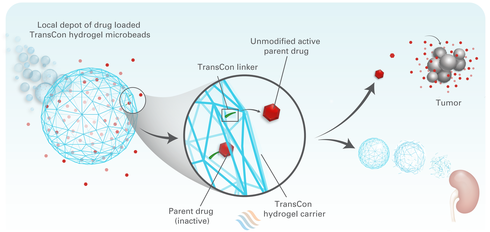
These data provided strong evidence that a single dose of TransCon TLR7/8 Agonist can mediate long-term intratumoral release of resiquimod with minimal systemic exposure compared to an equimolar dose of unconjugated resiquimod. Moreover, TransCon TLR7/8 Agonist provided potent anti-tumor effects as a monotherapy and in combination with cytokine therapy (eg, 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.

METHODS

We generated TransCon TLR7/8 Agonist by transiently conjugating resiquimod to a hydrogel microbead carrier with a TransCon linker. TransCon TLR7/8 Agonist was assessed for *in vivo* drug release via subcutaneous administration in rats or intratumoral administration in mice. Plasma drug levels were determined via UPLC-MS. TransCon TLR7/8 Agonist was assessed for anti-tumor efficacy using the murine syngeneic CT26 tumor model in either monolateral or bilateral tumor bearing mice. TransCon TLR7/8 Agonist treatment was administered intratumorally either with or without systemic IL-2 treatment started on the same day. Tumor volumes were estimated by using the formula: $V = (L \times W^2) \times 0.5$, where V is tumor volume, L is tumor length and W is tumor width. Body weights were determined by scale measurement. Plasma cytokines were determined via Luminex.

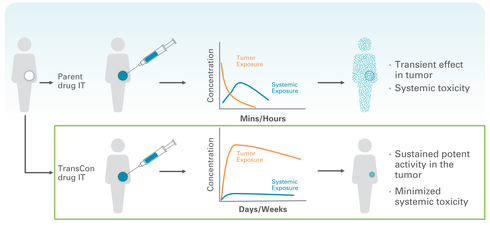
Immunophenotyping of immune cell subsets was performed by fluorescence-activated cell cytometry on single-cell suspensions derived from tumor draining lymph nodes harvested 7 days after dosing was initiated. For tumor rechallenge experiments, mice that experienced complete regressions in both TransCon TLR7/8 Agonist–injected and non-injected tumors were re-inoculated with CT26 tumor cells and monitored for tumor growth. Naïve mice were used as a tumor growth control.

Figure 1: Insoluble TransCon Carrier Technology for Sustained Localized Intratumoral Delivery



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 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.

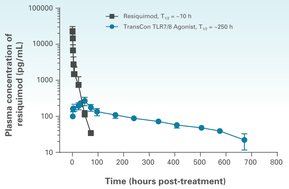
Figure 2: TLR7/8 Agonist-Loaded TransCon Hydrogel for Sustained Intratumoral Drug Delivery



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.

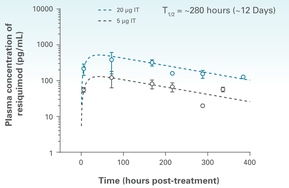
RESULTS

Figure 3: TransCon TLR7/8 Agonist Resulted in ~25-fold Longer Effective Half-life and ~100-fold Lower Systemic C_{max} of Resiquimod Compared to Equimolar Unconjugated TLR7/8 Agonist



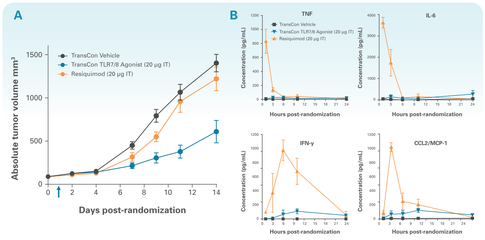
Male Wistar rats (n=3 per group) received a single subcutaneous injection of either unconjugated resiquimod (25 µg) or TransCon TLR7/8 Agonist (25 µg eq. of resiquimod). Blood samples were taken and used for plasma generation over the course of 28 days. The resiquimod concentration in the plasma samples was quantified by LC-MS/MS. Values are represented as mean +/- SD.

Figure 4: TransCon TLR7/8 Agonist Allowed for Sustained, Dose-Dependent Release of Resiquimod Following Intratumoral Administration in Mice



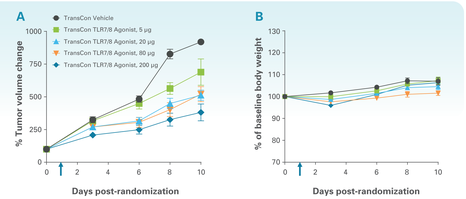
Female BALB/C mice were implanted with CT26 tumor cells. When tumors were grown to a mean tumor volume of ~115 mm³, mice were randomized into treatment cohorts (Day 0). The day following randomization, animals received either 5 or 20 µg (eq. of resiquimod) of TransCon TLR7/8 Agonist as a single intratumoral dose. Blood samples were taken and used for plasma generation over the course of the study. The concentration of resiquimod in the plasma samples was quantified by LC-MS/MS. Values are represented as mean +/- SD.

Figure 5: A Single Dose of TransCon TLR7/8 Agonist Mediated Potent Tumor Growth Inhibition With Minimal Systemic Cytokine Release When Compared to an Equimolar Dose of Resiquimod



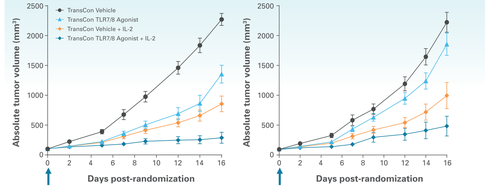
Female BALB/C mice were implanted with CT26 tumor cells. When tumors were grown to a mean tumor volume of ~80 mm³, mice were randomized into treatment cohorts (Day 0). The day following randomization, animals received either empty hydrogel (TransCon Vehicle), 20 µg (eq. of resiquimod) of TransCon TLR7/8 Agonist, or 20 µg of unconjugated resiquimod as a single intratumoral dose (arrow). A) Tumor volumes were calculated according to the formula: Tumor volume = (L × W²) × 0.5 where L is the length of the tumor and W the width (both in mm). B) Plasma samples were collected at various time points and assessed for cytokine levels by Luminex. Values are represented as mean +/- SEM.

Figure 6: A Single Dose of TransCon TLR7/8 Agonist Promoted Dose-Dependent Tumor Growth Inhibition and Was Well Tolerated



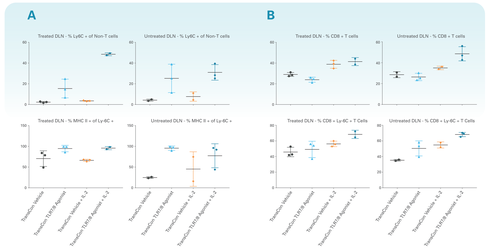
Female BALB/C mice were implanted with CT26 tumor cells. When tumors were grown to a mean tumor volume of ~115 mm³, mice were randomized into treatment cohorts (Day 0). The day following randomization, animals received either empty hydrogel (TransCon Vehicle) or 5, 20, 80, or 200 µg (eq. of resiquimod) of TransCon TLR7/8 Agonist as a single intratumoral dose (arrow). A) Tumor volumes were calculated as described in Figure 5. Data are represented as the average of the percentage change in tumor size from baseline (D0). B) On the same day as tumor measurements, mice were weighed for absolute body weight (g). Values are represented as mean +/- SEM.

Figure 7: A Single Dose of TransCon TLR7/8 Agonist Enhanced Anti-tumor Effects of IL-2 in Injected and Non-injected Tumors



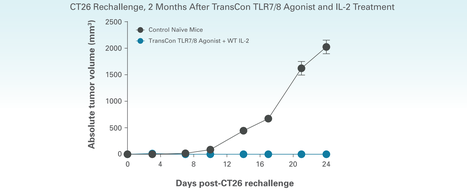
Female BALB/C mice were implanted with CT26 tumor cells into the left and right flanks. When tumors were grown to a mean tumor volume of ~100 mm³, mice were randomized into treatment cohorts (Day 0). On the day of randomization, animals received either empty hydrogel (TransCon Vehicle) or 216 µg (eq. of resiquimod) of TransCon TLR7/8 Agonist as a single intratumoral dose (red arrow). Some cohorts were further treated with 20 µg human IL-2 dosed twice daily on Days 0-4 and once daily on days 8-12. Tumor volumes were calculated as described in Figure 5. In this experiment, 3 out of 7 mice treated with TransCon TLR7/8 Agonist + IL-2 experienced complete regressions in injected and non-injected tumors. Values are represented as mean +/- SEM.

Figure 8: Intratumoral TransCon TLR7/8 Agonist Potentiated Antigen-Presenting Cell Frequency and T-Cell Activation in Tumor Draining Lymph Nodes as Monotherapy and in Combination With IL-2



Mice from the experiment described in Figure 7 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 via flow cytometry. A) Increase in antigen presenting cell content in tumor DLN and upregulation of MHCII with TransCon TLR7/8 Agonist treatment. B) Increase in CD8+ T cell content in tumor DLN and upregulation of Ly-6C with TransCon TLR7/8 Agonist treatment. Values are represented as mean +/- SEM.

Figure 9: A Single Dose of TransCon TLR7/8 Agonist With IL-2 Treatment Induced Immunological Memory and Prevented Tumor Growth Upon Rechallenge



Mice from the experiment described in Figure 7 that were treated with TransCon TLR7/8 Agonist and IL-2, and that experienced complete regressions in both treated and untreated tumors (n=3), were rechallenged with CT26 tumor cells and observed for tumor growth. Naïve mice were used as controls for tumor growth. Tumor volumes were calculated as described in Figure 5. Values are represented as mean +/- SEM.

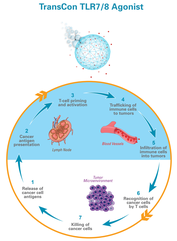
SUMMARY

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

- Provided drug exposure for weeks
- Avoided a high systemic C_{max} compared to equimolar dose of parent drug
- Demonstrated potent anti-tumor effects as a monotherapy
- Enhanced the anti-tumor effects of systemically administered IL-2 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–associated expansion of activated antigen presenting cells and potentiated CD8+ T-cell activation and memory

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 cell activation in the TME, thereby promoting anti-tumor immunity
- TransCon technologies for sustained localized and systemic delivery have the potential to broadly impact the immunity cycle and may offer new combination approaches in cancer therapy



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