Enhanced Efficacy and Combinability of Low Dose Toca 511 and 5-FC with Metronomic Chemotherapy in Preclinical Models

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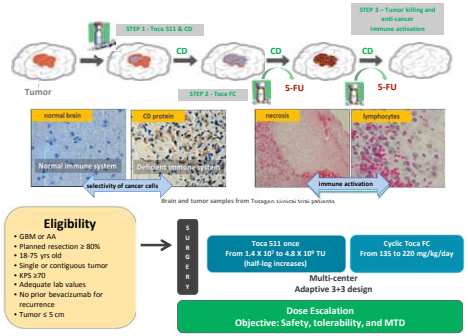
Abstract

Toca 511 (vocimagene amiretrorepvec) is an investigational, conditionally lytic, retroviral replicating vector that selectively infects cancer cells due to specificity for dividing cells combined with the immune-suppressed tumor microenvironment[1]. Toca 511 spreads and stably delivers optimized yeast cytosine deaminase (CD) that converts subsequent Toca FC (an investigational, extended-release version of 5-fluorocytosine [5-FC]) into 5-fluorouracil (5-FU). 5-FU kills infected dividing cancer cells and, in preclinical models, local immunosuppressive myeloid cells leading to therapeutically active anti-tumor immunity. A similarly derived antitumor response may occur in cancer patients, as local injection of recurrent high grade gliomas with Toca 511 followed by treatment with Toca FC has been associated with prolonged survival and durable complete responses (median duration of follow-up for response: 37.4+ months); responses were delayed in onset, consistent with an immunological mechanism. Not all patients responded and, clinically, only portions of the tumors were infected. These data led to a phase III trial (NCT02414165). To model submaximal infections, and look for clinically-compatible synergistic treatments, we implemented a novel preclinical model. We used this model system to test the immuno-stimulatory and antiangiogenic properties of cyclophosphamide (CTX) following a metronomic/low dose regimen and its previously reported ability to cross the blood-brain-barrier, in a combination therapy.

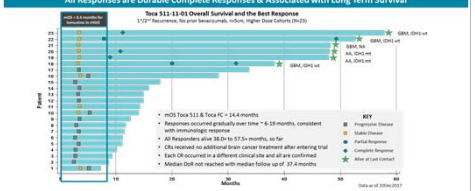
Introduction

The combination of Toca 511 and Toca FC (extended-release 5-FC) has undergone clinical investigation in Phase I trials with Toca 511 delivered intratumorally or via intravenous infusion (NCT01156584), into the wall of the resection cavity (NCT01470794), or via intravenous infusion followed by injection into the wall of the resection cavity (NCT01985256) in patients with recurrent high-grade glioma (HGG). A phase Ib study in patients with solid tumors or lymphoma (Toca 6) (NCT02576665) and a randomized Phase III trial in recurrent glioblastoma and anaplastic astrocytoma (Toca 5) (NCT02414165) are completed (Toca 6) or underway (Toca 5). Multiyear durable and complete responses by independent radiology review have been reported[2,3].



Phase I ascending dose trial of safety and tolerability of Toca 511 & Toca FC in rHGG
Toca 511 administered into the resection cavity

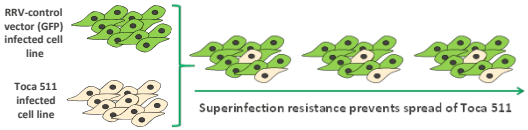
Long-Term Survival in Higher Dose Cohort



- Preclinical work with Toca 511 and 5-FC has shown that this combination can promote T cell priming, which is at least partially due to depletion of immunosuppressive myeloid cells[4]. Treg cells were not shown to be affected by Toca 511 and 5-FC and therefore combination with a compound that targets this cell population may provide added benefit, as previously shown with anti-CTLA-4.
- Cyclophosphamide (CTX) is an alkylating agent indicated in the treatment of malignant diseases and used as a conditioning regimen for cell transplantation and mobilization. In addition to its anti-tumor activity, CTX is endowed with immuno-modulatory and anti-angiogenic properties[5], especially when administered following a metronomic dosage. In particular, CTX has been shown to deplete Treg cells[6,7].

Methods and Results

Utilizing superinfection resistance to block the spread of Toca 511 is an effective research tool to explore the potential of combination therapies



- Mouse glioma cell line Tu-2449SQ has been adapted to grow subcutaneously.
- Tu-2449SQ cells were first 100% infected *in vitro* with Toca 511 or a sister vector that expresses GFP (Green Fluorescent Protein) instead of CD.
- In order to control Toca 511 spread, these cells were then admixed at various percentages as Toca 511 does not readily infect cells already infected with the GFP virus (superinfection resistance).
- Naïve B6C3F1 mice were implanted in the right flank with the admixed Tu-2449SQ with either 2% or 10% Toca 511-infected cell mix.
- Cycles of 500 mg/kg 5-FC and 20 mg/kg metronomic CTX were started when tumors reached >50mm³ volume (Figure 1).

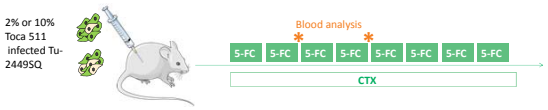


Figure 1. Toca 511 admixed Tu-2449SQ and 5-FC, CTX dosing schedule in Tu-2449SQ/B6C3F1 subcutaneous tumor model

Enhanced tumor burden control when CTX is combined with Toca 511 and 5-FC to treat subcutaneous Tu-2449SQ tumors

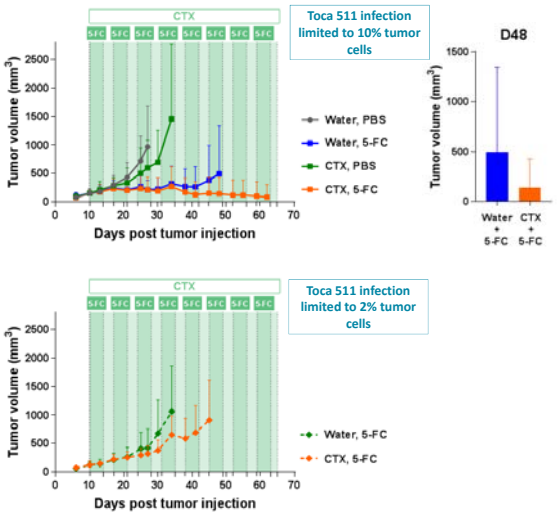


Figure 2. Tumor growth kinetics of mice implanted with subcutaneous glioma tumor cells, pre-transduced with Toca 511 (10% or 2%) and treated with 5-FC following cycles of 5 days on-2 days off and metronomic CTX continuously provided in the drinking water at 20 mg/kg.

- In order to limit the efficacy of Toca 511 and 5-FC, transduction superinfection resistance mechanisms were utilized to limit Toca 511 infection to only 2 or 10% of Tu-2449SQ tumor cells (see graphic above).
- When Toca 511 infection was limited to 10% of subcutaneous Tu-2449SQ tumor cells, treatment with 5-FC alone showed robust tumor control but when combined with CTX, this effect was maintained over a longer period of time, preventing relapse.
- Interestingly, in a submaximal infection setting with only 2% pre-transduced tumor cells, the addition of CTX improved Toca 511 and 5-FC efficacy.

Results

Superior CD8⁺/Treg ratio in the peripheral blood of mice treated with CTX + 5-FC

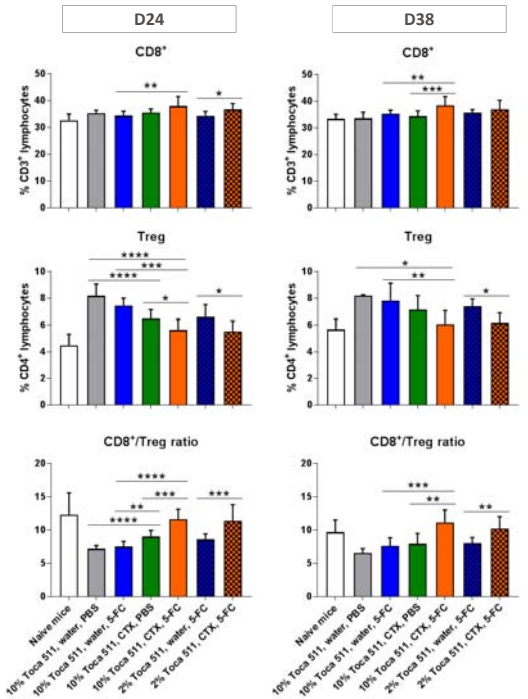


Figure 3. Analysis of CD8⁺ T and Treg cells in the peripheral blood by flow cytometry.

- When CTX is combined with 5-FC, after 2 (D24) and 4 (D38) cycles of treatment in the peripheral blood of 2% or 10% pre-transduced tumor-bearing mice, CD8⁺ T cells are increased significantly while Treg cells are decreased significantly, compared to 5-FC alone (Mann–Whitney test, *, P≤0.05, **, P≤ 0.01, ***, P≤ 0.001, ****, P≤ 0.0001).
- The resulting CD8⁺ / Treg ratio at D24 and D38 is statistically superior to 5-FC alone and is associated with enhanced tumor burden control.

Conclusion

- These data demonstrate that Toca 511 and 5-FC therapy can be combined with metronomic chemotherapeutics that modulate the immune system, like cyclophosphamide, to enhance efficacy of submaximal Toca 511 and 5-FC treatment in preclinical models.
- Superior CD8/Treg ratio observed in this combination therapy is hypothesized to compliment the known immunotherapeutic mechanism of action of Toca 511 & Toca FC therapy[8,4,9].
- Data from this study may inform future clinical development of Toca 511 & Toca FC.

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