**Immunological activation in responding patients with recent HGG after treatment with Toca 511 & Toca FC: Results from a phase 1 trial**

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**Introduction and Background**

Toca 511 (cytostatin cistransmetabolized) is an investigational retroviral replicating vector (RRV) that encodes the transgene cytosine deaminase (CD) gene. Toca FC is administered into the resection cavity at the time of surgery. Toca 511 is delivered by multiple routes and oscillates effects and effects on temporal tumor control. CD gene expression in vivo: CD gene delivery: CD-mediated transformation to the tumor cell, where the enzyme drops the bacterial antitumor drug 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU). Toca FC selectively destroys cancer cells within the body, while leaving healthy cells unaffected.

**Clinical Results**

Response Category

<table>
<thead>
<tr>
<th>All Patients</th>
<th>Phase 2-eligible subgroup</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete response</td>
<td>(11.3%)</td>
</tr>
<tr>
<td>Partial response</td>
<td>0</td>
</tr>
<tr>
<td>Stable disease</td>
<td>10 (40.9)</td>
</tr>
<tr>
<td>Progression</td>
<td>7 (28.8)</td>
</tr>
<tr>
<td>Clinical benefit rate (CR+PR+SD ≥ 6 weeks)</td>
<td>16 (60.0)</td>
</tr>
<tr>
<td>Durable response rate (PR or CR ≥ 24 weeks)</td>
<td>6 (11.3)</td>
</tr>
<tr>
<td>Median Duration of Response (months)</td>
<td>35.3 (0.2 - 44.9)</td>
</tr>
</tbody>
</table>

**Molecular Characterization of patient tumors: low tumor DNA mutation burden**

Summary of RNA and DNA sequencing results from patient tumors (n=31), categorized by phase II/III trial eligibility criteria. The total number of high-confidence somatic mutations called by MiSeq from exome sequencing data. The event file left RNA sequencing analysis revealed high-confidence somatic mutations. Estimates of somatic mutations per Mb were estimated by SomaticSeq (a subset of the TCGA data analysis) and the number of mutations observed. The number of mutations was determined by clinical site pathologist (grade IV = grey, grade III = brown, grade II = orange, grade I = black). The number of mutations was determined by clinical site pathologist (grade IV = grey, grade III = brown, grade II = orange, grade I = black). The number of mutations was determined by clinical site pathologist (grade IV = grey, grade III = brown, grade II = orange, grade I = black). The number of mutations was determined by clinical site pathologist (grade IV = grey, grade III = brown, grade II = orange, grade I = black).

**Conclusions**

- **PH1 dosing study** of Toca 511 & Toca FC in HGGs showed:
  - 6 complete responses (IDH1 and IDH2) are ongoing with a median duration of response >35.1 months
  - Durable response (objective response > 24 weeks) rate may be a valuable end point for immunotherapists
  - Responders do not show a higher DNA mutational burden in tumors compared to tumors from patients that progressed
  - Of the two patients at first recurrence with IDH1 tumor mutations, both responded, suggesting an enrichment of durable complete responses in IDH1 mutant patients at first or second recurrence treated with Toca 511 & Toca FC
  - In tumors collected before the start of treatment, responders have significantly higher TITL fractions compared to non-responders who did not respond
  - PCA of multiplex inflammatory cytokine ELISAs from longitudinal patient blood shows at least 2 panels of cytokines which expression is associated with response to therapy

**Inflammatory cytokine changes in blood are associated with patient outcomes identified by multivariate analysis**

Cytokines were measured by multiple ELISAs. Log-transformed values for 30 cytokines were subjected to principal components analysis (PCA) and PCA scores were used in multivariate statistical models to identify putative associations with patient outcomes, including best clinical response (logistic regression models) and survival (Cox proportional hazards models).

**Increased Clonality and T Cell Infiltrating Lymphocyte (TITL) Fracion in Tumor Pre-Toca 511 treatment**

Brain and tumor samples from Tocagen clinical trial patients

Toca 511- optimized RRV expressing CD, a prodrug activator gene

Toca FC - investigational extended-released oral formulation of 5-FC

- 5-FU crosses blood-brain barrier and is therefore effective for fungal infections of the brain
- CD converts 5-FU to 5-fluorouracil within infected cells
- GBM cell lines and MOGO are sensitive to 5-FU
- 5-FU inhibits thymidylate synthase, perturbs RNA synthesis, and affect glycosylation of proteins and lipids
- 5-FU mediated killing triggers antitumor immunity from within tumor with systemic benefit
- Toca 511 & SJC activates a durable T cell mediated immune response

**Response Rates**

- **Molecular Characterization of patient tumors:** low tumor DNA mutation burden
- 35 patients were evaluable for efficacy: 32 with fresh frozen tumor samples and 3 with patient blood. 32 patients had clear histological tumor sections, which were evaluable for mutation analysis. 8 patients had CD3+ lymphocytes from the tumor core.
- Clinical benefit rate (CR+PR+SD ≥ 6 weeks) was 60.0% for all patients and 43.5% for phase 2-eligible patients. Durable response rate (PR or CR ≥ 24 weeks) was 11.3% for all patients and 21.7% for phase 2-eligible patients. Median response duration was 35.3 months (0.2 - 44.9 months) for all patients and 35.2 months (14.4 - 44.9 months) for phase 2-eligible patients.

**Immune Monitoring:** Sustained PD-1+ T cells in Patient Samples Who Have Not progressed

- **Regulatory genes**
  - Regulatory gene expression was not significantly altered by Toca 511 & Toca FC treatment
  - Toca 511 & Toca FC treatment did not significantly alter expression of regulatory genes

**Stability**

- Log-transformed values for 30 cytokines were subjected to principal components analysis (PCA) and PCA scores were used in multivariate statistical models to identify putative associations with patient outcomes, including best clinical response (logistic regression models) and survival (Cox proportional hazards models).

**Response Rates**

- More than 20 cytokines that are known to be associated with tumor response were measured with multiplex ELISA. These cytokines were clustered using PCA and then associated with clinical endpoints (survival, response) using multivariate linear regression models.

**Conclusions**

- Responders have significantly higher TITL fractions than non-responders
- Responders also have a trend towards higher TITL clonality than non-responders (not significant)
- This suggests that TITL clonality may provide additional predictive information
- ROC methods to determine spatial orientation of infiltrating T cells in progress

**Data for Aims:**

- **Aims:**
  - To assess the safety and tolerability of Toca 511 & Toca FC in patients with recurrent HGG
  - To assess the immunology of responding patients

**Table:**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Baseline</th>
<th>Toca 511 &amp; Toca FC</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>CD3+ T cells</td>
<td>5.2%</td>
<td>6.3%</td>
<td>0.31</td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>29%</td>
<td>30%</td>
<td>0.62</td>
</tr>
<tr>
<td>CD8+ T cells</td>
<td>18%</td>
<td>19%</td>
<td>0.74</td>
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</tbody>
</table>

**Figure:**

- **Figure 1:** Flow cytometry analysis of CD3+ T cell populations before and after Toca 511 & Toca FC treatment.

**Graphs:**

- **Graphs A and B:** Comparison of CD3+ T cell populations before and after Toca 511 & Toca FC treatment.

**Research supported by:**

- **Research supported by:**
  - Tocagen Inc.
  - National Institutes of Health, National Cancer Institute, National Institute of Neurological Disorders and Stroke, National Institute of Biomedical Imaging and Bioengineering, and National Institute of Allergy and Infectious Diseases.