REPLICATING RETROVIRUSES FOR MANIPULATION OF THE TUMOR IMMUNE ECOSYSTEM: PRECLINICAL AND CLINICAL OUTCOMES.

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Tocagen Inc.

PEGS 2018 IT Track Boston May 1 2018
Outline

• Phase 1 data from patients with recurrent high grade glioma
• Preclinical mechanism of action
• Clinical immune measurements & correlation with responses
• Level of transduction that leads to an anti-tumor response
• Phase 3 trial in recurrent high grade glioma (rHGG) enrolling
Toca 511 (vocimagene amiretrorepvec)
Retroviral replicating vector: encodes a prodrug activator enzyme, selectively infects tumor tissue, spreads without cell killing, prodrug administration leads to local chemotherapy

Tumor selectivity and replication in cancers cells is driven by:
- Defects in the innate immune system of cancer cells
- Virus may abortively infect some normal cells, but is rapidly eliminated by innate and acquired immunity
- Virus spreads through tumor without triggering immune system
- Virus only infects dividing cells

Optimized CD (cytosine deaminase)

5-FU has a very short half-life with direct cell killing localized to cancer microenvironment

RRV= Retroviral replicating vector
Pre-clinically, Toca 511 & 5-FC yields sustained high levels of 5-FU selectively in tumors

<table>
<thead>
<tr>
<th>Setting</th>
<th>Treatment</th>
<th>Tumor 5-FU (µg/g)</th>
<th>Plasma 5-FU (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat F98 glioma¹</td>
<td>Toca 511 &amp; 5-FC</td>
<td>69</td>
<td>0.4</td>
</tr>
<tr>
<td>Human CRC²</td>
<td>IV 5-FU</td>
<td>0.1-2.8</td>
<td>52</td>
</tr>
</tbody>
</table>

¹ Data on file

Reversed 5-FU from blood to tumor

- Toca 511 & 5-FC generates 100 fold IC 50 level of 5-FU in the tumor
- Toca 511 and 5-FC does not generate clinically relevant 5-FU in the blood
- 5-FU produced in tumor microenvironment has a very short half-life in blood
Toca 511 & Toca FC
Initial Development in Recurrent High Grade Glioma (rHGG)

Development in HGG: A Deadly and Aggressive Disease

Newly diagnosed GBM
- Surgery, radiation, Temodar SOC
- HGG includes glioblastoma (GBM) and anaplastic astrocytoma (AA)
- Limited treatment options

Recurrence by MRI

Recurrent GBM or AA
- Bevacizumab, lomustine, carmustine wafer
- NCCN guidelines recommend consideration of clinical trials
- ~ 8 months median survival

Hospice

≈ 8 months

Brain tumor by MRI

Surgery, radiation, Temodar

Approved drugs and or trials

≈ 8 months
Three phase 1 ascending dose trials* in recurrent high grade glioma setting evaluating delivery approaches (n=127)

**Resection**
Injection into cavity wall after removal of tumor

- NCT01470794 (n = 56)

**Intratumoral**
Direct injection into tumor

- NCT01156584 (n = 54)

**Intravenous**
Injection IV prior to resection and into cavity wall at resection

- NCT01985256 (n = 17)

*All three trials finished enrollment*
# Adverse Events Related to Toca 511 and Toca FC

Data Pooled Across Three Phase 1 Studies

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**Toca 511 associated with a very low % of treatment-related AEs, across all Grades**

<table>
<thead>
<tr>
<th>Treatment-Related Adverse Events</th>
<th>Toca 511 n = 127</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 1/2 n (%)</td>
</tr>
<tr>
<td>Any treatment-related event</td>
<td>32 (25.2)</td>
</tr>
<tr>
<td>Treatment-related event in ≥ 3 patients</td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>14 (11.0)</td>
</tr>
<tr>
<td>Headache</td>
<td>6 (4.7)</td>
</tr>
<tr>
<td>Convulsion</td>
<td>6 (4.7)</td>
</tr>
<tr>
<td>Confusional state</td>
<td>5 (3.9)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>5 (3.9)</td>
</tr>
<tr>
<td>Nausea</td>
<td>4 (3.1)</td>
</tr>
<tr>
<td>Hemiparesis</td>
<td>3 (2.4)</td>
</tr>
<tr>
<td>Vasogenic cerebral edema</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Any treatment-related SAE</td>
<td>1 (0.8)</td>
</tr>
</tbody>
</table>

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**Toca FC has very limited Grade ≥ 3 treatment-related toxicities**

<table>
<thead>
<tr>
<th>Treatment-Related Adverse Events</th>
<th>Toca FC n = 122</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 1/2 n (%)</td>
</tr>
<tr>
<td>Any treatment-related event</td>
<td>50 (41.0)</td>
</tr>
<tr>
<td>Treatment-related event in ≥ 3 patients</td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>27 (22.1)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>16 (13.1)</td>
</tr>
<tr>
<td>Nausea</td>
<td>12 (9.8)</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>6 (4.9)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>4 (3.3)</td>
</tr>
<tr>
<td>Rash</td>
<td>3 (2.5)</td>
</tr>
<tr>
<td>Any treatment-related SAE</td>
<td>0</td>
</tr>
<tr>
<td>AE Leading to Discontinuation</td>
<td>1 (0.8)</td>
</tr>
</tbody>
</table>

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*No deaths considered related to Toca 511; ^No deaths considered related to Toca FC.
Data cutoff 17 Apr 2017; excludes data from continuation study.

Adapted from Cloughesy et al. AACR-NCI-EORTC Conference, 2017.
Overall survival comparable in both surgical and non-surgical settings

<table>
<thead>
<tr>
<th>Method</th>
<th>Group</th>
<th>mOS, months (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resection</td>
<td>Tg 511-11-01</td>
<td>14.4 (11.3, 28.1)</td>
</tr>
<tr>
<td></td>
<td>(n = 23)</td>
<td></td>
</tr>
<tr>
<td>Intratumoral</td>
<td>Tg 511-08-01</td>
<td>13.8 (7.1, 22.2)</td>
</tr>
<tr>
<td></td>
<td>(n = 24)†</td>
<td></td>
</tr>
<tr>
<td>Intravenous</td>
<td>Tg 511-13-01</td>
<td>13.6 (5.8, 19.7)</td>
</tr>
<tr>
<td></td>
<td>(n = 17)</td>
<td></td>
</tr>
</tbody>
</table>

*Patients at 1\textsuperscript{st}/2\textsuperscript{nd} recurrence with no prior Avastin, tumor ≤ 5 cm, in high-dose cohorts.
†Patients receiving Toca 511 via biopsy needle.

Preliminary, unaudited data – cutoff Aug 2017 for Tg511-11-01, May 2016 for Tg511-08-01, Apr 2017 for Tg511-13-01
Phase 1 Experience in 127 Patients

**RESECTION**
Injection into cavity wall after tumor de-bulking
- n=56

**INTRATUMORAL**
Direct injection into the brain tumor
- n=54

**INTRAVENTOUS**
Injection IV prior to resection and into cavity wall at resection
- n=17

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**Resection Study – Data Highlights**

- **Increased survival** versus historic benchmarks
- 6 CRs – all remain alive up to 57.5 months and counting
- Favorable safety profile
- Phase 1 patients at Toca 5 dose and entry criteria
  - 43.5% clinical benefit rate
  - mOS of 14.4 months
  - 26.1% survival at 3 years

*Efficacy evaluable patients (n=53), which includes 10 patients who received combination therapy that included Toca 511 & Toca FC.
^ n=23 with data cutoff date August 15th, 2017.*
A positive association of durable response with overall survival
Best response & survival post progression

All Responses are Durable Complete Responses & Associated with Long Term Survival

Toca 511-11-01 Overall Survival and the Best Response
1st/2nd Recurrence, No prior bevacizumab, <=5cm, Higher Dose Cohorts (N=23)

- Responses occurred gradually over time ~ 6-19 months, consistent with immunologic response
- All Responders alive 38.0+ to 57.5+ months, so far
- All CRs confirmed
- CRs received no additional brain cancer treatment after entering trial
- Each CR occurred in a different clinical site
- Median duration of response not reached

mOS Toca 511 & Toca FC* = 14.4 months

GBM, IDH1 wt
GBM, IDH1 wt
GBM, IDH1 wt
GBM, NA
AA, IDH1 mt
AA, IDH1 mt
GBM, IDH1 wt

Cloughesy MD, AAN, April 24th, 2018
Data cutoff date Dec. 20th, 2017
Complete Response in a Patient with Progressive GBM, IDH1 wt

<table>
<thead>
<tr>
<th>Date</th>
<th>Event Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/13/14</td>
<td>Tumor progression</td>
</tr>
<tr>
<td>10/2/14</td>
<td>Screening scan</td>
</tr>
<tr>
<td>10/10/14</td>
<td>Resection of enhancing tumor then Toca 511 injection</td>
</tr>
<tr>
<td>10/10/14</td>
<td>1 day post-op</td>
</tr>
<tr>
<td>11/12/14</td>
<td>Baseline scan for response assessment</td>
</tr>
<tr>
<td>1/7/15</td>
<td>Stable disease</td>
</tr>
<tr>
<td>6/2/16</td>
<td>Partial response</td>
</tr>
<tr>
<td>5/11/17</td>
<td>Complete response</td>
</tr>
<tr>
<td>5/11/17</td>
<td>Toca FC cycles (1 week per 6 weeks)</td>
</tr>
</tbody>
</table>

Alive and with ongoing complete response > 36 months

Toca FC cycles (1 week per 6 weeks)

Cloughesy MD, AAN, April 24th, 2018

PR and CR by independent Radiology Review, Macdonald criteria.
Toca 511 & 5-FC
Activates a Durable Anti-Cancer Immune Response

Multiple cycles of tumor-associated antigen (TAA) release during FC treatment cycles, coupled with simultaneous depletion of immune suppressive cells in the tumor microenvironment, leads to immune activation, effective TAA presentation, and lymphocytic infiltration, resulting in gradual killing and shrinkage of the tumor, sometimes completely, over a long period of time.

Pre-Clinical Evidence of Durable Immune Activation

CONTROL

IMMUNE DEFICIENT

IMMUNE COMPETENT

“Cured” Mice Reject Re-Challenge of Same Tumor in Flank

T Cells From “Cured” Mice Increase Survival in Adoptive Transfer Model


Images modified for illustrative purposes.
Toca 511 and 5-FC efficacy in a subcutaneous glioma model
efficacy based on tumor selective, high dose, 5-FU

Mitchell et al. NeuroOnc 2017
Toca 511 and 5-FC alters the tumor microenvironment
immunosuppressive myeloid cell depletion followed by T cell infiltration

Mitchell et al. NeuroOnc 2017
Toca 511 and 5-FC alter immune profile in the tumor microenvironment true in intracranial, subcutaneous, and liver metastases models

Systemic immune cells spared effects of 5-FU

T cells (CD4, CD8)

B cells

T-regs not significantly impacted

Immune-suppressive myeloid cells (MDSCs, TAMs, Monocytes)
Clinical Immune Measurements & Correlation with Clinical Responses
High DNA Mutation Frequency Not Associated with Response or Survival

Left barplot shows the total number of high confidence mutations called by MUSE from exome sequencing data of patient tumors before treatment with Toca 511 and Toca FC; OS in months is shown in the Right-side barplot.
Longitudinal Patient Cytokine Measurements in Patient Sera

• Goals
  – Demonstrate immune signaling in responders vs non-responders
  – Find shorter-term blood marker of response than survival
    • to facilitate drug development and “adaptive” trial design

• Methods
  – Serum from 25 patients sent for multiplex ELISA
    • Up to 6 samples from different time points for each patient (6 responders, 19 SD or PD)
    • Total of 87 serum samples were assessed
    • Each serum sample had 40 different inflammatory cytokines measured
Cytokines putatively associated with patient outcomes
Identified by multivariate analysis

Survival

E-Selectin Maximum:  
- Above Median
- Below Median

Crude p-value = 0.34
Adjusted p-value = 0.45

MIP1 Beta Maximum:  
- Above Median
- Below Median

Crude p-value = 0.16
Adjusted p-value = 0.013

(Wald test p-values +/- age and gender adjustment displayed)
Survival-associated cytokine principal components (scores)
Identified by multivariate analysis

(p-values from Wald test, adjustment for Age and Gender)
Specific pre-existing immune cell subtypes in tumor may predict response to therapy

RNAseq analyses of resected tumors
What % of tumor infection is enough to elicit anti-tumor activity?
Three phase 1 ascending dose trials* in recurrent high grade glioma setting evaluating delivery approaches (n=127)

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IV Administration of Toca 511 Results in Expression of Cytosine Deaminase Protein in Tumor

- DAPI
- Cytosine Deaminase
- gag
- Merge

DAPI: Nuclear stain
Cytosine Deaminase: Target protein encoded by Toca 511
gag: 1 of 3 major proteins encoded by the retroviral genome
Merge: gag protein localizes with cytosine deaminase expression after IV delivery of Toca 511
Exploiting resistance mechanisms to generate translational models that represent lower infection rates in responding patients

- By limiting Toca 511 infection to a “known” percentage of tumor cells we can investigate:
  - Minimum effective transduction level necessary for efficacy
  - Minimum effective transduction level necessary to induce alterations in tumor immune infiltrate
  - A transduction level that lacks full efficacy so that successful combinations can be more readily evaluated
Dose-dependent response with Toca 511
100% tumor cell transduction is not necessary for efficacy, 3% is enough to see some benefit

Viral transduction over 15% did not provide additional benefit in this model
Conclusions

- Phase 1 trials showed overall survival comparable in both surgical and non-surgical settings
  - In the resection study
    - 6/23 patients in the Phase 3 eligible cohort survived over 36 months (longest is 57.5 months)
    - 6 had highly durable CR that have not relapsed (median duration of response = 35.7 mos)
  - Treatment was well tolerated – limited Grade ≥ 3 drug-related toxicities in three phase 1 studies (127 patients)
  - In the intravenous study: vector deposition in tumor
    - Same observation in liver metastases with solid tumors
- Toca 511 & 5-FC activates durable T-cell mediated immune responses pre-clinically
  - CD4 and CD8 T cell dependent, with local myeloid cell depletion
- Immunological correlates to responses in tumor and blood in clinical trials
- Evidence for anti-tumor efficacy at 2-3% transduction in several animal models
- Phase 3 trial in rHGG enrolling
Primary Endpoint: Overall Survival
Secondary Endpoints Include Durable Response Rate

Eligibility
- GBM or AA
- First and 2nd recurrence
- Tumor ≤ 5cm

Surgery And Randomization N=380

Toca 511* → Toca FC**

Chemotherapy**
(lomustine or temozolomide) or bevacizumab

Stratify by IDH1 mutation status KPS (70-80 vs. 90-100) and region

* Administered at time of surgery
** Begins 6 weeks post-surgery

Biomarker monitoring includes blood (lymphocytes, immune activation markers, cytokines) and tumor (TILS and immune-suppressive myeloid cells)

ClinicalTrials.gov Identifier: NCT02414165
Thanks to all the patients, their families and caregivers who have supported this work and to the Clinical Investigators for the Phase1 trials.

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