The OR as a Laboratory:
Investigating the Tissue Immune-Microenvironment in the Toca 511/Toca FC Clinical Trials

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Disclosure

• Indirect equity and Royalties: Infuseon Therapeutics, Inc.
• Honoraria:
  – Celgene
  – Tocagen
Gamma retrovirus Toca 511 selectively infects tumor, persists and spreads through tumor while delivering the CD gene.

Toca FC prodrug locally converts to 5-FU chemotherapeutic within the tumor, killing cancer cells and resulting in activation of antigen presenting cells and T cell priming.

5-FU also eliminates immunosuppressive myeloid cells (MDSCs and TAMs), activating antitumor immunity.
Locally, within tumor microenvironment, Toca 511 & Toca FC alter immune profile

- Increased T cell immune infiltrates in tumor (COLD → HOT)
- Immune effects are T cell-dependent (predominantly CD4) and correlate with immune-suppressive myeloid cells depletion

Adapted from Hiraoka et al., Neuro-Oncol. 2017.

Toca 511 & 5-FC Activate Immune System in the Tumor MicroEnvironment
Preclinical Mouse Glioma Model

Increase in immune activating lymphoid cells

Decrease in immune suppressive myeloid cells

T-regs not significantly impacted

Mitchell et al. Neuro-Oncology, 2017
Toca 511 & 5-FC Alter Immune Profile in the TME Over Time

*Observed in Intracranial, Subcutaneous, and Liver Metastases Models*

**T cells (CD4, CD8)**

**B cells**

**Immune-suppressive myeloid cells (MDSCs, TAMs, Monocytes)**

**T-reg not significantly impacted**

**Systemic immune cells spared effects of 5-FU**

**Tumor Burden**

**Placebo Control Baseline**

**Day 0**

**Day 3**

**Day 6**

**Day 9**

**Day 14**
Three Phase 1 Ascending Dose Trials in Recurrent HGG Setting Evaluating Delivery Approaches (n=127)

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

(n = 56)

**Intratumoral**
Direct injection into tumor

(n = 54)

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

(n = 17)
## Phase 1 Resection Study – Patients had Advanced Disease

<table>
<thead>
<tr>
<th>Population</th>
<th>All Patients N=56</th>
<th>Toca 5 Dose and Entry Criteria Subset N=23</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median Age (range)</strong></td>
<td>56 (24-75)</td>
<td>55 (24-70)</td>
</tr>
<tr>
<td><strong>n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>43 (77)</td>
<td>20 (87)</td>
</tr>
<tr>
<td><strong>Karnofsky Performance Score</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70-80</td>
<td>17 (30)</td>
<td>5 (22)</td>
</tr>
<tr>
<td>90-100</td>
<td>39 (70)</td>
<td>18 (78)</td>
</tr>
<tr>
<td><strong>Initial Tumor Histology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GBM</td>
<td>46 (82)</td>
<td>19 (83)</td>
</tr>
<tr>
<td>Anaplastic Astrocytoma</td>
<td>6 (11)</td>
<td>4 (17)</td>
</tr>
<tr>
<td>Other gliomas</td>
<td>4 (7)</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Number of Recurrences Including Current</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>28 (50)</td>
<td>19 (83)</td>
</tr>
<tr>
<td>2</td>
<td>13 (23)</td>
<td>4 (17)</td>
</tr>
<tr>
<td>≥ 3 or greater</td>
<td>15 (27)</td>
<td>N/A</td>
</tr>
</tbody>
</table>


Data cutoff date December 20th, 2017.
# Phase 1 Resection Study:
All 6 Responders Received Higher Toca 511 Dose and are Durable Complete Responders

<table>
<thead>
<tr>
<th>Response Category</th>
<th>All Efficacy Evaluable Patients N=53&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Higher Doses and Toca 5 Entry Criteria Subset N=23</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Durable response rate (CR or PR ≥ 24 weeks), n (%)</strong></td>
<td>6 (11.3); All CRs</td>
<td>5 (21.7); All CRs</td>
</tr>
<tr>
<td><strong>Median duration of durable response</strong></td>
<td>Not reached (median follow-up 36.5 mos)</td>
<td>Not reached (median follow-up 37.4 mos)</td>
</tr>
<tr>
<td>Stable disease, n (%)</td>
<td>12 (22.6)</td>
<td>5 (21.7)</td>
</tr>
<tr>
<td>Progressive disease, n (%)</td>
<td>35 (66.0)</td>
<td>13 (56.6)</td>
</tr>
<tr>
<td>Clinical Benefit Rate, n (%) (CR, PR, and SD ≥ 8 wks)</td>
<td>16 (30.2)</td>
<td>10 (43.5)</td>
</tr>
</tbody>
</table>

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2 Of 56 safety evaluable patients, 53 patients who received Toca 511 & Toca FC are efficacy evaluable and of these 2 were not evaluable for response.
Includes 4 IDH wildtype and 2 IDH mutant patients. All 6 responders were in the higher dose cohorts and include 1 patient who received the Toca regimen in combination with bevacizumab.
3 Higher doses (cohorts 4-7a) and meet Toca 5 entry criteria of 1<sup>st</sup> and 2<sup>nd</sup> recurrence, no prior bevacizumab in rAA or rGBM, tumor not > 5cm.

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response duration compares favorably:
- Bev ~4.2 mos
- Lomustine ~6.2 mos

Wick, JCO 2010; Avastin PI
Phase 1 Resection Study: Long-Term Survival in Higher Dose Cohort

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)

- mOS Toca 511 & Toca FC = 14.4 months
- Responses occurred gradually over time ~ 6-19 months, consistent with immunologic response
- All Responders alive 3.2+ years to 4.8+ years, so far
- CRs received no additional brain cancer treatment after entering trial
- Each CR occurred in a different clinical site and all are confirmed
- Median DoR not reached with median follow up of 37.4 months

KEY
- Progressive Disease
- Stable Disease
- Partial Response
- Complete Response
- Alive at Last Contact

Cloughesy et al. JSGCT, 2018; Data cut off 20 Dec 2017
### Adverse Events Related to Toca 511 and Toca FC

Data Pooled Across Three Phase 1 Studies

#### Low Incidence of Grade 3/4 Treatment-Related AEs

<table>
<thead>
<tr>
<th>Treatment-Related AEs</th>
<th>Toca 511 &amp; Toca FC</th>
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<tr>
<td></td>
<td>N=127</td>
<td>Grade 1/2</td>
<td>Grade 3/4</td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Any treatment-related event</td>
<td>62 (48.8)</td>
<td>11 (8.7)</td>
<td></td>
</tr>
<tr>
<td>Treatment-related event ≥5% patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>37 (29.1)</td>
<td>1 (&lt;1)</td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>17 (13.3)</td>
<td>1 (&lt;1)</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>14 (11.0)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>8 (6.2)</td>
<td>1 (&lt;1)</td>
<td></td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>7 (5.5)</td>
<td>0</td>
<td></td>
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#### Low Incidence of Treatment-Related SAEs

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<td></td>
</tr>
<tr>
<td>Any treatment-related event</td>
<td>1 (&lt;1)</td>
<td>8 (6.3)</td>
<td></td>
</tr>
<tr>
<td>Treatment-related event ≥1% patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vasogenic cerebral oedema$^1$</td>
<td>0</td>
<td>2 (1.6)</td>
<td></td>
</tr>
</tbody>
</table>

$^1$ Both SAEs were Grade 3; no Grade 4 SAE

Are there Immune Correlates of Enhanced Anti-Tumor Immunity in Ph1 Responders?

- Preclinical data showing immunological mechanism of action

- In the Phase 1 study, initial responses from 6-19 months after initial treatment, consistent with immunologic effect

- Observation of immune correlates in the Phase 1 responders
  - Tumor samples before Toca 511 administration
  - Serum samples over time of treatment

- Neoantigen load*, similar to mutation frequency, is not associated with response
  - Tumor samples before Toca 511 administration

*Typically high for checkpoint inhibitors to be efficacious
Observation of Immune Correlates from Tumor and Blood Samples in the Phase 1 Responders

Objective Response: CR (n = 5); PD or SD (n = 39)
Wilcoxon rank sum p-values are displayed above the boxplot

Data cutoff date: Aug2017
TOCA 5 Phase 2/3 Study Design for Recurrent High Grade Gliomas

Largest randomized study conducted in this setting (recurrent HGG)

Eligibility
- GBM or AA
- 1st and 2nd recurrence
- Tumor ≤ 5cm

Stratification by:
- IDH1 mutation status
- KPS (70-80 vs. 90-100)
- Region

Primary Endpoint
- Overall Survival (OS)

Secondary Endpoints
- Durable response rate (DRR: CR or PR ≥ 24 weeks)
- Durable clinical benefit rate (DCBR: CR or PR ≥ 24 weeks or SD ≥ 18 months)
- Duration of durable response (DDR)
- Overall survival at 12 months (OS12)

SOC/Control arm:*
- lomustine, or
- temozolomide, or
- bevacizumab

1:1 randomization
* Administered at time of surgery
** Begins 6 weeks post-surgery

Conducted in US, Canada, Israel, S. Korea
ClinicalTrials.gov Identifier: NCT02414165

Toca5 Presentation: 11:50 AM (Plenary) on Friday
No significant differences were observed between the two arms at baseline.

Baseline Immune Balance Between Toca Arm and SOC Arm
Baseline Immune Differences Observed Between GBM and AA Patients

More robust immune profile for AA patients at baseline

#All significant

Tumor#

PBMCs

AA

GBM

Macrophages M0

B cells naive

T cells CD4 memory/resting

T cells follicular helper

Monocytes

Dendritic cells activated

NK cells resting

T cells CD8

T cells CD8 naive

Monocytes non classical

AA (n = 31)

GBM (n = 265)

Wilcoxon, p = 0.058

Wilcoxon, p = 0.31

Wilcoxon, p = 0.0069

Wilcoxon, p = 0.045

*
Baseline Immune Differences Observed Between IDH1wt and IDH1mt

Greater potential to generate anti-tumor responses for patients with IDH1mt at baseline

Tumor#

#All significant

PBMCs

*All significant
Baseline Immune Differences Observed Between 1\textsuperscript{st} and 2\textsuperscript{nd} Recurrence

More Robust immune profile for patients with 2\textsuperscript{nd} recurrence at baseline

#All significant

PBMCs

1\textsuperscript{st} recurrence 2\textsuperscript{nd} recurrence

Tumor#

Dendritic cells--activated
NK cells--resting
T cells CD4 naive
T cells CD4 memory--resting
T cells regulatory
Baseline Immune Profile Summary

- No significant arm imbalances from baseline tumor infiltrating lymphocyte or peripheral blood mononuclear cell profiles
- AA patient tumors and baseline blood appear to have a significantly more robust immune profile compared to GBM patients
- Compared to IDH1 wt patients, IDH1 mutant tumors and baseline blood also appear to have greater potential to generate anti-tumor responses based on significantly increased infiltrating lymphocytes and higher levels of CD8+ T cells
- Similar to the previous two subgroups, second recurrence patient tumors and baseline blood have better immune profile to potentially generate anti-tumor responses compared to patients at first recurrence
- Overall, there are distinct significant difference in baseline tumor infiltrating lymphocyte or peripheral blood mononuclear cell profiles across the subgroups analyzed
Conclusion

• Toca 511 & 5-FC activates durable T-cell mediated immune responses pre-clinically
  • CD4 and CD8 T cell dependent, with local myeloid suppressor cell depletion

• Three phase 1 trials
  • Treatment was well tolerated – limited Grade ≥ 3 drug-related toxicities (127 patients)

• In the Phase 1 resection trial
  • Preliminary association of response to a favorable biomarker profile (tumor and blood) is consistent with an immunotherapeutic mechanism of the regimen

• In the Phase 3 Toca 5 trial
  • Immune balance between the two arms at baseline
  • Baseline immune differences observed between GBM and AA, IDH1wt and IDH1mt, and 1st and 2nd recurrence
NRG-BN006*: Trial of Toca 511 & Toca FC in ndGBM
Toca 511 & Toca FC + Standard of Care vs. Standard of Care Alone in Newly Diagnosed GBM (ndGBM)

Surgery

1:1 Randomization

Stratification Factors
- Age
- KPS

Toca 511
Chemoradiation → maintenance TMZ & Toca FC
(No Optune® allowed)

SOC
Chemoradiation → maintenance TMZ
(Optune® allowed)

*NRG Oncology is the study sponsor

Poster Presentation (RBTT-11) at 5-7pm; Nov. 23
NRG-BN006*: Trial of Toca 511 & Toca FC in ndGBM
Toca 511 & Toca FC + Standard of Care vs. Standard of Care Alone in Newly Diagnosed GBM (ndGBM)

• Target accrual:
  – Phase II: 312 patients
  – Phase III: 900 patients, including phase II

• Primary endpoints:
  – Phase II: PFS, by central review
  – Phase III: OS

• Safety analyses to occur at 15 and 30 patients on experimental arm
Now, About Delivery...

• The Phase 1 Toca 511 trials investigated multiple questions, including method of delivery of the vector
• The intratumoral delivery studies started with use of a biopsy needle for both biopsy and injection
• A separate cohort was infused with the vector with use of a CED optimized cannula (MRI Interventions SmartFlow)
### Subjects at Risk:

<table>
<thead>
<tr>
<th>Method</th>
<th>Subjects at Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Needle</td>
<td>21, 18, 12, 8, 4, 2, 2, 1, 1, 0</td>
</tr>
<tr>
<td>RTMG</td>
<td>18, 10, 6, 1, 0, 0, 0, 0, 0</td>
</tr>
</tbody>
</table>

### Biopsy Needle vs. CED/RTMG:

- **Log-rank p-value:** 0.0249
- **Hazard Ratio (95% CI):** 0.44 (0.21-0.92)

### Biopsy Needle

- Median OS: **13.8 months** (95% CI 7.1, 22.2)

### CED/RTMG

- Median OS: **7.9 months** (95% CI 4.4, 13.0)

CED/RTMG = Convection Enhanced Real Time MRI Guided Drug Delivery
Delivery of Toca511 via SmartFlow Cannula

Target (IV Gd-DTPA)

Post CED (Infused Gd-DTPA)
Delivery of Toca511 via SmartFlow Cannula

Target (IV Gd-DTPA)

Post CED (Infused Gd-DTPA)
Real Time Visualization of Biopsy Needle Administration of Toca 511 in MRI Suite

Subject 160
iMRI
- Tumor
- Vector
Toca5 Study - Delivery

- For the post-resection studies, a Touhy needle was used (and will be for next study).
Toca5 Study - Delivery

- A subset of patients underwent infusion of Toca511 + Gd-DTPA
Could More Robust Delivery Help?
Summary

• Studies to date indicate that Toca511 is altering both the tumor and systemic immune profile in favor of an anti-tumor profile
• As might be expected, lower grade, IDHmut and patients who have recovered from RT/TMZ seem to have the strongest immunological response
• Delivery of Toca511 may not be optimized and there may be room for further stimulation of an immune response with delivery that ensures vector coverage of more of the tumor-infiltrated brain
• We are not likely to make progress without incorporating correlative biological analysis into our clinical trials
Acknowledgements

• Phase 1 and Toca5 co-investigators
  – Tim Cloughesy
  – Manish Aghi

• BN006 co-Chairs and the NRG Oncology Clinical Trials Team

• Tocagen
  – Dan Pertschuk, Harry Gruber, Doug Jolly, Derek Ostertag, Joan Robbins, Tiffany Montellano, Mohamed Ladha