Intra-tumoral delivery of Interleukin-12 DNA via *in vivo* electroporation leads to regression of injected and non-injected tumors in Merkel cell carcinoma.

**Final results of a phase 2 study**

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COI disclosures

Adil Daud, MD: Stock (Oncosec), Consultant (Oncosec)

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Merkel cell carcinoma (MCC)

Aggressive disease with suboptimal therapeutic options
- 46% disease-associated mortality rate \{Lemos BD 2010\}
- High recurrence rate for patients with loco-regional disease despite surgery +/- radiation therapy (RT)
- High response rate with systemic chemotherapy (ORR ~ 50%), but responses seldom durable.

Strong rationale for immunotherapy
- Merkel cell polyoma virus (MCPyV) \{Feng H 2008\}
- Intra-tumoral (IT) CD8+ T-cell infiltration associated with better prognosis \{Paulson K 2011\}
- Multiple mechanisms of immune evasion
IL-12: A key mediator of IFNγ-driven T_H1-type pro-inflammatory response

Systemic rIL-12 administration is quite toxic
- Myelosuppression
- GI bleeding
- Death

Intra-tumoral delivery may improve its therapeutic index.
Intra-tumoral delivery of IL-12 plasmid DNA via *in vivo* electroporation (IT-pIL12-EP or *ImmunoPulse*™ IL-12)
Hypothesis: Local IT-pIL12-EP promotes tumor inflammation and a systemic anti-tumor immune response.
Phase II study of IT plL12-EP in MCC

• To the best of our knowledge, this study represents the first prospective clinical trial of immunotherapy in advanced MCC (First-patient first-visit: January 2012)

• Single-institution (University of Washington, Seattle)

• Key eligibility criterion: At least one injectable MCC lesion, defined as an easily-palpable superficial lesion (cutaneous, subcutaneous or lymph nodal metastasis), away from major nerves or blood vessels.
Study objectives and Trial design

**Primary Objective**
To demonstrate that IT-pIL12-EP leads to increased local expression of IL-12 in the tumor microenvironment

**Secondary Objectives**
- Safety and tolerability
- Efficacy – Local and systemic
- Immunologic changes and biomarkers

**Pre-treatment Biopsy**
Plasmid Dose = 0.5 mg/mL

**Post-treatment Biopsy (Day 22)**

**Cohort A** (Neo-adjuvant)
Definitive Surgery or Radiation → F/U

**Cohort B** (Metastatic)
Restaging at 6 weeks → Additional Treatment Cycles (Maximum 4 cycles total)

**Screening & Enrollment**
2 Weeks
## Study population: Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Cohort A (N = 3)</th>
<th>Cohort B (N = 12)</th>
<th>Overall (N = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median Age (years)</strong></td>
<td>59</td>
<td>69</td>
<td>66</td>
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<tr>
<td><strong>ECOG Performance Status</strong></td>
<td></td>
<td></td>
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<tr>
<td>0-1</td>
<td>3</td>
<td>11</td>
<td>14</td>
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<tr>
<td>2</td>
<td>0</td>
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<tr>
<td><strong>Stage at Enrollment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td><strong>Prior Therapies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>1</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Radiation</td>
<td>1</td>
<td>9</td>
<td>10</td>
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<tr>
<td>Systemic Therapy</td>
<td>0</td>
<td>6</td>
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<tr>
<td><strong>MCPyV Status</strong></td>
<td></td>
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<tr>
<td>(T-oncoprotein serology)</td>
<td></td>
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</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>
IT pIL12-EP was safe and well-tolerated

Mild, and mostly local AEs.
- EP-associated pain - Grade 1 and transient (lasting only a few seconds)
- Mild local inflammatory reaction

No treatment-related ≥Grade 3 AEs; no SAEs reported.

No treatment-discontinuation due to AEs.

<table>
<thead>
<tr>
<th>Treatment-Related AEs</th>
<th>All Grades N (%)</th>
<th>≥ Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procedural Pain</td>
<td>15 (100%)</td>
<td>0</td>
</tr>
<tr>
<td>Treatment Site Reaction*</td>
<td>11 (73%)</td>
<td>0</td>
</tr>
<tr>
<td>Cellulitis</td>
<td>3 (20%)</td>
<td>0</td>
</tr>
<tr>
<td>Peripheral Edema</td>
<td>2 (13%)</td>
<td>0</td>
</tr>
<tr>
<td>Pain (other)</td>
<td>2 (13%)</td>
<td>0</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>1 (7%)</td>
<td>0</td>
</tr>
</tbody>
</table>

*includes local inflammation, discoloration, bruising, necrosis and erythema at the treatment site
IT pIL12-EP led to sustained local expression of IL-12 protein

11 out of 14 (79%) patients had increased IL-12 protein levels at day 22 in treated tumors.

Fold increase range: 1.7x to 3147x

Paired biopsy samples (Baseline and Day 22) of treated lesions were evaluated for IL-12 protein levels by ELISA.
IT pIL12-EP led to regression of treated as well as non-injected distant MCC tumors.

### Local Lesion Regression

| Proportion of treated lesions with major (>30%) regression | 12/27 (44%) |

### Distant Lesion* Regression

| Number of evaluable patients with a distant lesion | 10 |
| Proportion of patients with distant lesion regression | 3/10 (30%) |

*Defined as a non-injected MCC tumor, clearly distinct from treated lesions.
IT pIL12-EP led to objective clinical responses in metastatic MCC

Cohort B Patients | N = 12*
---|---
Complete Response (CR) | 0 (0%)
Partial Response (PR) | 3 (25%)
Stable Disease (SD) | 1 (8%)
Progressive Disease (PD) | 8 (52%)

NOTE: Modified-RECIST 1.1 was used.
- Up to 10 cutaneous target lesions were permitted (>2 lesions per organ)
- Combination of clinical and radiological measurements were allowed.

Cohort A (N = 3; all stage III B MCC)

- One patient had **pathologic CR** and remains free of recurrence at > 6 mos.
- Another patient has been **recurrence-free since 08/2012** (> 3 years).
- Third patient was recurrence-free for 9 mos before developing PD.
Patient 002 (Cohort B): Durable regression of treated and untreated tumors.

At 6 months  (confirmed Partial response; regression of distant non-injected lesions)

Prior therapies:
- Surgery
- RT
- Chemotherapy (PE)
- IFN- β
Patient 002: Enrichment of MCPyV-specific CD8+ T-cells in TILs of treated and distant tumors

IL12-2 TIL Pre and Post Treatment
Staining with B35/MCPyV "FPW" Tetramer

Negative Control (W876 REP)

3 Months Pre-Treatment (October 2011)

Immediately Pre-Treatment (January 2012)

2 Weeks Post-Treatment (February 2012)

8 Months Post-Treatment (September 2012)

10 Months Post-Treatment (November 2012)

Distant lesion

Comp-FITC-A :: CD8

Comp-APC-A :: CD8

Comp-PE-A :: Tetramer
PD1/PD-L1 mediated T-cell exhaustion could facilitate immune evasion despite increased TILs.

Despite clearly increased CD8+ T-cell infiltrate in treated tumors, this patient progressed in both local and distant tumors.
IT pIL12-EP mediated pro-inflammatory phenotype is characterized by NK-cell activation gene signature

- The majority of cases (70%) show increased expression of NK activation genes by Nanostring® evaluation
- NK cells are a key lymphocyte population involved in tumor surveillance and anti-tumor immunity
- IL-12 is a known activator of NK cells
Conclusions

- IT pIL12-EP leads to effective transfection of IL-12 plasmid DNA and sustained expression of IL-12 protein

- IT pIL12-EP is safe and well-tolerated in MCC patients
  - No major systemic toxicities; no ≥ Grade 3 AEs or SAEs

- IT pIL12-EP is associated with objective clinical responses in Merkel Cell Carcinoma

- IT-pIL12-EP results in a local pro-inflammatory response, which in turn promotes systemic anti-tumor response

- Combination studies with emerging systemic therapies (such as anti-PD-1/PDL-1) should be explored in advanced MCC.
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Questions?