THE FUTURE OF VACCINES

ASCO ANNUAL MEETING
JUNE 4, 2016
BAVARIAN NORDIC
INVESTOR & ANALYIST UPDATE & RECEPTION

Welcome and Introduction to Bavarian Nordic’s Cancer immunotherapy Programs

Paul Chaplin, Ph.D.
President & Chief Executive Officer, Bavarian Nordic

A Clinical Overview of PROSTVAC as Monotherapy and Combination Therapy

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Chief, Genitourinary Malignancies Branch, Head, Immunotherapy Section, Director, Medical Oncology Service, CCR Office of the Clinical Director, National Cancer Institute

Brachyury: A Novel Target with First in Man Data. Presentation of Clinical Data, Lessons Learned, and Potential Pathways Forward

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Associate Research Physician, Laboratory of Tumor Immunology and Biology, Director, Clinical Trials Group, National Cancer Institute
DEVELOPMENT OF BRACHYURY-TRICOM

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LABORATORY OF TUMOR IMMUNOLOGY AND BIOLOGY
CENTER FOR CANCER RESEARCH
NATIONAL CANCER INSTITUTE
Overview of Vaccine and Target

**MVA (modified vaccinia ankara)**
- non-replicating pox virus
- Safe (current smallpox vaccine)
- Can be given repeatedly

**Brachyury**
- T-box transcription factor
- Master driver of EMT
- Expression correlates with invasion, migration, and treatment resistance
- Poor prognostic factor
- Significant expression in Chordoma, rare mesodermal remnant tumor
- Also expressed in:
  - Colorectal (poor prognostic indicator)
  - Breast
    - ER+ tamoxifen adjuvant – poor prognostic indicator
    - Triple Negative – implicated as major driver
  - Lung cancer (Small cell and NSCLCa)
  - Hepatocellular Cancer (poor prognostic indicator)
  - Prostate Cancer (poor prognostic indicator)
  - Merkel Cell Carcinoma

**TRICOM**
- 3 human costimulatory molecules
- Not amenable to murine studies
Opportunities

Rare tumors:
- Chordoma
- Merkel Cell Carcinoma

“Immunogenic” tumors (respond to PD-1/L1 blockade)
- Lung
- Triple negative breast
- Hepatocellular
- Merkel Cell

“Cold” tumors (minimal responses to PD-1/L1 blockade)
- Colorectal
- ER+ breast cancer
- Prostate cancer
BRACHYURY OVER-EXPRESSION INDUCES EMT IN EPITHELIAL TUMOR CELLS

PANC-1-pcDNA
PANC-1-pBrachyury

Fernando...Palena. J Clin Invest. 2010; 120:533-44.
BRACHYURY CONTROLS METASTASIS IN LUNG CANCER MODEL

LUNG MICROMETASTASIS MODEL
- Nude mouse
- SC tumor implantation
- Day 0
- Day 15
- Tumor measurement
- Lung homogenates
- Subcutaneous tumor volume (mm³)
- H460-control.shRNA vs. H460-Brachyury.shRNA
- BRACHYURY DOES NOT AFFECT PRIMARY TUMOR
- Number of colonies per lung
- H460-control.shRNA vs. H460-Brachyury.shRNA

EXPERIMENTAL LUNG METASTASIS MODEL
- Nude mouse
- IV tumor implantation
- Day 0
- Day 45
- Evaluation of lungs for presence of tumor nodules
- Number of tumor nodules per lung
- H460-control.shRNA vs. H460-Brachyury.shRNA
- BRACHYURY CONTROLS TUMOR DISSEMINATION
- LUNG MACROMETASTASES
- p = 0.002

Fernando...Palena. J Clin Invest. 2010; 120:533-44.
Brachyury is absent in the majority of NORMAL TISSUES

<table>
<thead>
<tr>
<th>Normal tissue</th>
<th>Brachyury positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis</td>
<td>3/3</td>
</tr>
<tr>
<td>Thyroid</td>
<td>4/6</td>
</tr>
<tr>
<td>Lung</td>
<td>0/5</td>
</tr>
<tr>
<td>Heart</td>
<td>0/3</td>
</tr>
<tr>
<td>Brain</td>
<td>0/3</td>
</tr>
<tr>
<td>Liver</td>
<td>0/3</td>
</tr>
<tr>
<td>Kidney</td>
<td>0/3</td>
</tr>
<tr>
<td>Spleen</td>
<td>0/3</td>
</tr>
<tr>
<td>Skeletal Muscle</td>
<td>0/3</td>
</tr>
<tr>
<td>Adrenal Gland</td>
<td>0/1</td>
</tr>
<tr>
<td>Skin</td>
<td>0/1</td>
</tr>
</tbody>
</table>

Brachyury is found in various types of CANCER

<table>
<thead>
<tr>
<th>Cancer types that are Brachyury positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
</tr>
<tr>
<td>NSCLC</td>
</tr>
<tr>
<td>SCLC</td>
</tr>
<tr>
<td>Breast</td>
</tr>
<tr>
<td>TNBC</td>
</tr>
<tr>
<td>Prostate</td>
</tr>
<tr>
<td>Colon</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>Gastric</td>
</tr>
<tr>
<td>Head and neck</td>
</tr>
<tr>
<td>Chordoma</td>
</tr>
<tr>
<td>Embryonal Carcinoma</td>
</tr>
</tbody>
</table>

Cancer types that are Brachyury positive:
- Lung
- NSCLC
- SCLC
- Breast
- TNBC
- Prostate
- Colon
- Liver
- Gastric
- Head and neck
- Chordoma
- Embryonal Carcinoma

BRACHYURY EXPRESSION IN LUNG TUMOR TISSUES CORRELATES WITH TUMOR STAGE

Brachyury mRNA expression relative to GAPDH

- Stage II, III, and IV represented by ■, △, and ×, respectively.

Fernando...Palena. J Clin Invest. 2010; 120:533-44.
BRACHYURY EXPRESSION IN BREAST TUMOR TISSUES CORRELATES WITH TUMOR GRADE

Brachyury mRNA expression relative to GAPDH

Grade 1: 0/4 (0.0%)
Grade 2: 4/36 (11.1%)
Grade 3: 13/57 (22.8%)
BRACHYURY AND TUMOR PROGNOSIS

Brachyury expression in PRIMARY TUMOR correlates with poor clinical outcome in lung, breast, triple negative breast ca., prostate, colon, oral squamous, GIST and hepatocellular carcinoma.

**Tamoxifen-treated Breast Ca.**

Palena et al, JNCI (2014)

**Lung Cancer**


**Hepatocellular Ca.**

**Expression of brachyury protein in primary and metastatic breast carcinoma lesions by immunohistochemistry utilizing a murine monoclonal anti-brachyury Ab**

<table>
<thead>
<tr>
<th>Pt</th>
<th>Tissue</th>
<th>Brachyury</th>
<th>% Positivity</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Breast primary tumor</td>
<td></td>
<td>30</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Met(^+) lymph node (a)</td>
<td></td>
<td>90</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Met(^+) lymph node (b)</td>
<td></td>
<td>90</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Non-met lymph node (c)</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>9</td>
<td>Breast primary tumor</td>
<td>Focal</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Met(^+) lymph node (a)</td>
<td></td>
<td>60</td>
<td>++</td>
</tr>
<tr>
<td>9</td>
<td>Met(^+) lymph node (b)</td>
<td></td>
<td>60</td>
<td>++</td>
</tr>
<tr>
<td>9</td>
<td>Non-met lymph node (c)</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>31</td>
<td>Pleura</td>
<td></td>
<td>90</td>
<td>+</td>
</tr>
<tr>
<td>32</td>
<td>Bone</td>
<td></td>
<td>90</td>
<td>++</td>
</tr>
<tr>
<td>33</td>
<td>Bone</td>
<td></td>
<td>90</td>
<td>+</td>
</tr>
<tr>
<td>34</td>
<td>Brain</td>
<td></td>
<td>70</td>
<td>++</td>
</tr>
</tbody>
</table>
Infection of human DCs in vitro with MVA-Brachyury-TRICOM

<table>
<thead>
<tr>
<th>Infection with</th>
<th>CD80</th>
<th>CD54</th>
<th>CD58</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MVA-WT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 MOI</td>
<td>15.6 (23)</td>
<td>86.6 (254)</td>
<td>89.0 (123)</td>
</tr>
<tr>
<td>10 MOI</td>
<td>17.3 (21)</td>
<td>81.2 (188)</td>
<td>82.9 (91)</td>
</tr>
<tr>
<td><strong>MVA-Brachyury-TRICOM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 MOI</td>
<td>83.3 (490)</td>
<td>94.9 (847)</td>
<td>95.4 (394)</td>
</tr>
<tr>
<td>10 MOI</td>
<td>81.4 (587)</td>
<td>91.8 (792)</td>
<td>93.9 (381)</td>
</tr>
</tbody>
</table>

Mean Flourescence Instensity (MFI) in parentheses

**Western blot**

MW | Uninfected | MVA-WT | MVA-Brachyury-TRICOM
---|------------|--------|----------------------
[Image of Western blot showing Brachyury and GAPDH bands for MW, Uninfected, MVA-WT, and MVA-Brachyury-TRICOM]
Brachyury-specific CD8+ T cell stimulation by MVA-Brachyury-TRICOM infected DCs compared with MVA-TRICOM infected DCs
### Patients Who Have Generated T-Cell Responses to Brachyury Post-Vaccination

<table>
<thead>
<tr>
<th>Pt</th>
<th>Vaccine</th>
<th>Metastatic Tumor Type</th>
<th>PSA/CEA</th>
<th>ELISPOT Brachyury</th>
<th>HIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PSA-TRICOM + αCTLA-4</td>
<td>Prostate Ca.</td>
<td>Pre: &lt;1/200,000&lt;br&gt;Post: 1/150,000</td>
<td>&lt;1/200,000&lt;br&gt;1/46,000</td>
<td>&lt;1/200,000&lt;br&gt;&lt;1/200,000</td>
</tr>
<tr>
<td>2</td>
<td>PSA-TRICOM + αCTLA-4</td>
<td>Prostate Ca.</td>
<td>Pre: &lt;1/200,000&lt;br&gt;Post: 1/40,000</td>
<td>&lt;1/200,000&lt;br&gt;1/41,000</td>
<td>&lt;1/200,000&lt;br&gt;&lt;1/200,000</td>
</tr>
<tr>
<td>3</td>
<td>Yeast-CEA</td>
<td>Medullary Thyroid Ca.</td>
<td>Pre: &lt;1/200,000&lt;br&gt;Post: 1/9,677</td>
<td>&lt;1/200,000&lt;br&gt;1/12,766</td>
<td>&lt;1/200,000&lt;br&gt;&lt;1/200,000</td>
</tr>
<tr>
<td>4</td>
<td>Yeast-CEA</td>
<td>Colorectal Ca.</td>
<td>Pre: &lt;1/200,000&lt;br&gt;Post: 1/200,000</td>
<td>&lt;1/200,000&lt;br&gt;1/60,000</td>
<td>&lt;1/200,000&lt;br&gt;&lt;1/200,000</td>
</tr>
</tbody>
</table>
**MVA-BRACHYURY-TRICOM**

**PHASE I**

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>Dose and Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 n = 3 to 6</td>
<td>1 site of injection at (2 \times 10^8) IU given every 28 days for 3 doses. If no evidence of disease progression, 3 more monthly doses may be given.</td>
</tr>
<tr>
<td>2 n = 3 to 6</td>
<td>2 sites of injection at (2 \times 10^8) IU given every 28 days for 3 doses. If no evidence of disease progression, 3 more monthly doses may be given.</td>
</tr>
<tr>
<td>3 n = 3 to 6</td>
<td>4 sites of injection at (2 \times 10^8) IU given every 28 days for 3 doses. If no evidence of disease progression, 3 more monthly doses may be given.</td>
</tr>
</tbody>
</table>

- **MVA (modified vaccinia ankara)**
  - non-replicating pox virus
  - Safe (current smallpox vaccine)
  - Can be given repeatedly

- **Brachyury**
  - T-box transcription factor
  - Master driver of EMT
  - Expression correlates with invasion, migration, and treatment resistance
  - Poor prognostic factor
  - Significant expression in Chordoma, rare mesodermal remnant tumor

- **TRICOM**
  - 3 human costimulatory molecules
  - Not amenable to murine studies

*An additional modality for sequential therapy*
Key Eligibility Criteria

Standard Phase I eligibility except for:

Inclusion:
- Measurable or non-measurable disease (must be evaluable)
- ECOG 0-1
- Prior immune therapy is allowed

Expansion Inclusion allowed ongoing treatment for patients with stable disease on:
- Hormonal therapy in ER+ breast ca and prostate cancer,
- Her2-targeted therapy in HER2+ breast cancer
- Erlotinib in EGFR mutation+ lung adenocarcinoma
- Capecitibine (oral 5-FU precursor drug) and bevacizumab in mCRC

Exclusion:
- HIV, hepatitis
- Active autoimmune disease
- Systemic steroid use (except physiologic replacement doses, local (topical, nasal, inhaled) or to treat or prevent allergic reactions (contrast)
Endpoints

Primary

- Safety and tolerability

Secondary

- CD8 and CD4 immunologic response ELISPOT and proliferation in response to Brachyury
- Clinical benefit
- General immune activation: immune cell subsets in PBMCs

Correlative Studies

- Correlation of clinical findings with general immune activation
- Correlation of clinical findings with CD8 and CD4 immunologic response
- Correlation of clinical findings with brachyury expression and EMT markers
## Baseline Demographics

### All Patients (n = 38)

<table>
<thead>
<tr>
<th>Gender</th>
<th># (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>18 (47%)</td>
</tr>
<tr>
<td>Female</td>
<td>20 (53%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age - Median (range)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>60 (35-86)</td>
<td></td>
</tr>
</tbody>
</table>

### Primary Disease

<table>
<thead>
<tr>
<th>Primary Disease</th>
<th># (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chordoma</td>
<td>13 (34.2%)</td>
</tr>
<tr>
<td>Colorectal</td>
<td>9 (23.7%)</td>
</tr>
<tr>
<td>Breast (ER+)</td>
<td>5 (13.2%)</td>
</tr>
<tr>
<td>NSCLCa (EGFR mutated)</td>
<td>4 (10.5%)</td>
</tr>
<tr>
<td>Prostate (D0)</td>
<td>3 (7.9%)</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>2 (5.3%)</td>
</tr>
<tr>
<td>Ovarian</td>
<td>1 (2.6%)</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>1 (2.6%)</td>
</tr>
</tbody>
</table>

### Maintenance Therapy Expansion

<table>
<thead>
<tr>
<th>Disease</th>
<th># (% of tumor type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal (Capecitibine +/- Bevacizumab)</td>
<td>4 (44.4%)</td>
</tr>
<tr>
<td>NSCLCa (EGFR mutated – erlotinib)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Breast (ER+ - hormonal therapy)</td>
<td>5 (100%)</td>
</tr>
</tbody>
</table>
## ADVERSE EVENTS

<table>
<thead>
<tr>
<th></th>
<th>Grade 1</th>
<th></th>
<th>Grade 2</th>
<th></th>
<th>Grade 3</th>
<th></th>
<th>Total All Grades</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Patients</td>
<td>Events</td>
<td>Patients</td>
<td>Events</td>
<td>Patients</td>
<td>Events</td>
<td>Patients</td>
</tr>
<tr>
<td>Creatinine Increased</td>
<td>1 (1.0%)</td>
<td>1 (2.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (1.0%)</td>
<td>1 (2.7%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>3 (2.9%)</td>
<td>2 (5.4%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (1.0%)</td>
<td>1 (2.7%)</td>
<td>4 (3.9)</td>
<td>3 (8.1%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1 (1.0%)</td>
<td>1 (2.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (1.0%)</td>
<td>1 (1.0%)</td>
</tr>
<tr>
<td>Fever</td>
<td>9 (8.8%)</td>
<td>7 (18.9%)</td>
<td>1 (1.0%)</td>
<td>1 (2.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>10 (9.8%)</td>
<td>7 (18.9%)</td>
</tr>
<tr>
<td>Flu like symptoms</td>
<td>20 (19.6%)</td>
<td>13 (35.1%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>20 (19.6%)</td>
<td>13 (35.1%)</td>
</tr>
<tr>
<td>Headache</td>
<td>1 (1.0%)</td>
<td>1 (2.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (1.0%)</td>
<td>1 (2.7%)</td>
</tr>
<tr>
<td>Injection site reaction</td>
<td>25 (24.5%)</td>
<td>18 (48.6%)</td>
<td>25 (24.5%)</td>
<td>18 (48.6%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>50 (49.0%)</td>
<td>28 (75.7%)</td>
</tr>
<tr>
<td>Pruritus</td>
<td>1 (1.0%)</td>
<td>1 (2.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (1.0%)</td>
<td>1 (2.7%)</td>
</tr>
<tr>
<td>Rash acneiform</td>
<td>1 (1.0%)</td>
<td>1 (2.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (1.0%)</td>
<td>1 (2.7%)</td>
</tr>
<tr>
<td>Pain in extremity</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>2 (2.0%)</td>
<td>1 (2.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>2 (2.0%)</td>
<td>1 (2.7%)</td>
</tr>
</tbody>
</table>

### Dose Level 1
- n = 3 patients enrolled, 0 DLT

### Dose Level 2
- n = 17 patients enrolled, 0 DLT

### Dose Level 3
- n = 18 patients enrolled, 0 DLT

---

**Dose level 1** – 3 patients enrolled, 0 DLT
**Dose level 2** – 17 patients enrolled, 0 DLT
**Dose level 3** – 18 patient enrolled 0 DLT

---

Dose Level 2 n = 17 patients 49 doses

<table>
<thead>
<tr>
<th></th>
<th>Events</th>
<th>Patients</th>
<th>Events</th>
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<th>Events</th>
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<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine increased</td>
<td>1 (2.0%)</td>
<td>1 (5.9%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (2.0%)</td>
<td>1 (5.9%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1 (2.0%)</td>
<td>1 (5.9%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (2.0%)</td>
<td>1 (5.9%)</td>
</tr>
<tr>
<td>Fever</td>
<td>3 (6.1%)</td>
<td>2 (11.8%)</td>
<td>1 (2.0%)</td>
<td>1 (5.9%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>4 (8.2%)</td>
<td>2 (11.8%)</td>
</tr>
<tr>
<td>Flu like symptoms</td>
<td>3 (6.1%)</td>
<td>3 (17.6%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>3 (6.1%)</td>
<td>3 (17.6%)</td>
</tr>
<tr>
<td>Headache</td>
<td>1 (2.0%)</td>
<td>1 (5.9%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (2.0%)</td>
<td>1 (5.9%)</td>
</tr>
<tr>
<td>Injection site reaction</td>
<td>10 (20.4%)</td>
<td>6 (35.3%)</td>
<td>11 (22.4%)</td>
<td>8 (47.1%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>21 (42.9%)</td>
<td>13 (76.5%)</td>
</tr>
<tr>
<td>Pruritus</td>
<td>1 (2.0%)</td>
<td>1 (5.9%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (2.0%)</td>
<td>1 (5.9%)</td>
</tr>
<tr>
<td>Pain in extremity</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>2 (4.1%)</td>
<td>1 (5.9%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>2 (4.1%)</td>
<td>1 (5.9%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (2.0%)</td>
<td>1 (5.9%)</td>
<td>1 (2.0%)</td>
<td>1 (5.9%)</td>
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Dose Level 3 n = 17 patients 44 doses

<table>
<thead>
<tr>
<th></th>
<th>Events</th>
<th>Patients</th>
<th>Events</th>
<th>Patients</th>
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<th>Patients</th>
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<tbody>
<tr>
<td>Diarrhea</td>
<td>1 (22.7%)</td>
<td>1 (5.9%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (22.7%)</td>
<td>1 (5.9%)</td>
</tr>
<tr>
<td>Fever</td>
<td>6 (13.6%)</td>
<td>5 (29.4%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>6 (13.6%)</td>
<td>5 (29.4%)</td>
</tr>
<tr>
<td>Flu like symptoms</td>
<td>17 (38.6%)</td>
<td>10 (58.8%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>17 (38.6%)</td>
<td>10 (58.8%)</td>
</tr>
<tr>
<td>Injection site reaction</td>
<td>13 (29.5%)</td>
<td>10 (58.8%)</td>
<td>13 (29.5%)</td>
<td>9 (52.9%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>26 (59.1%)</td>
<td>13 (76.5%)</td>
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### Carcinoma – By Cycle

<table>
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<tr>
<th>Cancer</th>
<th>Dose Level</th>
<th>CD4</th>
<th>CD8</th>
<th>CD4</th>
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<td></td>
<td>Late</td>
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### Chordoma – By Cycle

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<thead>
<tr>
<th>Cancer</th>
<th>Dose Level</th>
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<td>CD8</td>
<td>CD4</td>
<td>CD8</td>
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<tr>
<td></td>
<td>Cycle III</td>
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<td>Late</td>
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</tr>
</tbody>
</table>

>250 | >2,000 | >500 | >5,000 | >1,000
Conclusions to Date with MVA-Brachyury-TRICOM

1. Safety profile established and very good
2. Brachury-specific immune responses observed in early sample analysis
3. Other correlative studies pending (paired biopsy analysis, circulating tumor cell, 123-subset analysis)
Brachyury-TRICOM studies in Chordoma
## Agent, Source, Isotype Table

<table>
<thead>
<tr>
<th>Agent</th>
<th>Source</th>
<th>Isotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMS-936559</td>
<td>MDX-1105</td>
<td>Bristol-Myers Squibb</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fully human IgG4</td>
</tr>
<tr>
<td>MEDI4736</td>
<td>Durvalumab</td>
<td>Medimmune/AstraZeneca</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fc-modified IgG1</td>
</tr>
<tr>
<td>MPDL3280A</td>
<td>Atezolizumab</td>
<td>Genetech/Roche</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fc-modified IgG1</td>
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<tr>
<td>MSB0010718C</td>
<td>avelumab</td>
<td>EMD Serono</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fully human IgG1</td>
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</tbody>
</table>

**Anti PD-L1 antibody induces tumor death via check point blockade**

*Diagram showing the interaction between T cells and tumor cells, including the roles of PD-1 and B7-H1 in the context of PD-L1 blockade.*

---

MARIO SZNOl, AND LIEPING CHEN
Induces tumor death via ADCC (Antigen-dependent cell-mediated cytotoxicity)

From Dr. David

Wayne et al/ Nature Reviews Immunology Apr.2003
PD-L1 Expression in Chordoma

The therapeutic potential of anti-PD-L1 antibody for chordoma is unknown
### Chordoma Cell Lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Derivation</th>
<th>MHC-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>JHC7</td>
<td>61-year old Female Primary chordoma</td>
<td>HLA A24(+)</td>
</tr>
<tr>
<td>UM-Chor1</td>
<td>66-year old Male Primary clival chordoma</td>
<td>HLA A2(+)</td>
</tr>
<tr>
<td>UCH2</td>
<td>72-year old Female Recurrent sacral chordoma</td>
<td>HLA A2(-)/A24(-)</td>
</tr>
<tr>
<td>MUG-Chor1</td>
<td>57-year old Female Sacral chordoma</td>
<td>HLA A11(+)</td>
</tr>
</tbody>
</table>

JHC7 and UM-Chor1 were obtained from the Chordoma Foundation
U-CH2 and MUG-Chor1 were obtained from ATCC
What is the baseline expression of MHC-I in chordoma and can it be modulated with IFN-γ treatment?

Expression of MHC-I was significantly upregulated by IFN-γ in 4/4 cell lines.28
What is the baseline expression of PD-L1 in chordoma and can it be modulated with IFN-γ treatment?

Expression of PD-L1 was significantly upregulated by IFN-γ in 4/4 cell lines.

Tumor cells
+ IFN-γ 50 ng/ml
24 hours
Flow cytometry
Can the ADCC capability of anti-PD-L1 mAb (Avelumab) be exploited for chordoma?

Tumor cells + IFN-γ 50 ng/ml 24 hours
4h NK assay +/- avelumab (2 ug/ml)

Purified normal human NK cells
50:1 E:T ratio

Similar observations with UM-Chor1, U-CH2 and MUG-Chor1
IFN-γ treated cells showed enhanced sensitivity to ADCC via avelumab
Model of indirect enhancement of ADCC by antigen-specific T-cells

- these studies used exogenous IFN-γ
- model system to recapitulate IFN-γ release from antigen-specific T-cells (from vaccine) into microenvironment and interrogate PD-L1 ADCC

1. VACCINE
   - tumor antigen specific T-cell recognition of tumor
2. Induce IFN-γ
3. PD-L1 upregulation
4. Increase binding of anti-PD-L1 Ab
5. Enhance NK mediated killing of tumor

Enhance NK mediated killing of tumor
Model of a patient receiving a brachyury vaccine

UM-Chor1 cells + Naïve CD8+ T cell
Brachyury specific CD8+ T cell line (Tp2A)

IFN-γ concentration (pg/ml)

<table>
<thead>
<tr>
<th></th>
<th>IFN-γ concentration (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>&lt; 3.76</td>
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<tr>
<td>Brachyury specific CD8+ T cell line (Tp2A)</td>
<td>1334.14</td>
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<tr>
<td>Normal donor naïve CD8+ T cells</td>
<td>9.58</td>
</tr>
</tbody>
</table>

PD-L1 expression

<table>
<thead>
<tr>
<th></th>
<th>PD-L1 expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td></td>
</tr>
<tr>
<td>% Positive</td>
<td>MFI</td>
</tr>
<tr>
<td>Brachyury specific CD8+ T cell line (Tp2A)</td>
<td>86</td>
</tr>
<tr>
<td>Normal donor naïve CD8+ T cells</td>
<td>42.7</td>
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<tr>
<td>% Positive</td>
<td>MFI</td>
</tr>
<tr>
<td>60.3</td>
<td>12.9</td>
</tr>
<tr>
<td>47.9</td>
<td>9.33</td>
</tr>
</tbody>
</table>

24hr ELISA (IFN-γ)
Flow cytometry (PD-L1)
ADCC assay
Model of a patient receiving a brachyury vaccine

Tumor cells + Naïve CD8+ T cell 24hr  ELISA (IFN-γ)
Naïve CD8+ T cell Flow cytometry (PD-L1)
Brachyury specific CD8+ T cell ADCC assay

PD-L1 expression

<table>
<thead>
<tr>
<th>control</th>
<th>IFN-γ</th>
<th>Brachyury specific CD8+ T cell line (Tp2A)</th>
<th>Normal donor naïve CD8+ T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Positive</td>
<td>MFI</td>
<td>% Positive</td>
<td>MFI</td>
</tr>
<tr>
<td>60.3</td>
<td>12.9</td>
<td>97</td>
<td>89.4</td>
</tr>
</tbody>
</table>

Brachyury specific T cells induced PD-L1 expression of chordoma

Chordoma cells increased the sensitivity to ADCC by avelumab

Potential of combination use of brachyury vaccine and avelumab
A randomized phase II study of Erlotinib with or without MVA-Brachyury-TRICOM vaccine in subjects with metastatic lung adenocarcinoma with activating EGFR mutations

Christopher Heery, MD
LTIB, CCR, NCI, NIH
EMT induces resistance to immune-mediated lysis

Objective: can reduction of EMT via EGFR blockade improve tumor susceptibility to immune attack?
ERLOTINIB TREATMENT DECREASES EXPRESSION OF BRACHYURY AND FIBRONECTIN

NSCLC cell line: H460 – high Brachyury, mesenchymal

Cells were treated with erlotinib (replenished daily)

↓

Protein extracts at 72hr for WB

Brachyury

Fibronectin

↓

Matrigel® coated membrane = ECM

↓

ECM invading cells on the underside of the membrane are counted under X100

Duration of the assay = 17hr

DMSO 0.1 1

erlotinib (µM)

Relative expression

0.0

0.5

1.0

DMSO 0.1 1

erlotinib (µM)

0

40

80

120

Number of cells

DMSO 0.1 1

Erlotinib (µM)
ERLOTINIB IMPROVES IMMUNE-MEDIATED LYSIS OF LUNG CARCINOMA CELLS

**Erlotinib sensitive lung cancer cell lines**

Cells were pre-treated with erlotinib 0.1uM for 72hr

- Cells were labeled with $^{111}$In
- In vitro lysis assay with NK (CD56+) cells
  - Effector to target ratio 25:1

![Graph showing percent lysis for HCC4006, H1650, H441 with DMSO and 0.1uM erlotinib]  

---

**Lung cancer cell line H522 (erlotinib resistant)**

Cells were pre-treated with erlotinib for 72hr

- In vitro lysis assay  
  - Effector to target ratio 50:1

**Effector cells: Brachyury-specific CD8+ T cells**

![Graph showing percent specific lysis for H522 with DMSO, 0.1uM, 1uM erlotinib]
Simultaneous erlotinib enhances T-cell immune-mediated lysis

Control CTL assay using antigen specific T cells against MUC1 or Brachyury
Erlotinib added to the assay (overnight, 16 h)

![Graph showing % specific lysis for HCC827, PC9, and HCC4006 cells with and without erlotinib](image)

Simultaneous erlotinib enhances T cell-mediated lysis
Proposed Phase II Clinical Trial

PHASE II

Randomized Phase II Trial of Erlotinib Alone or in Combination with MVA-Brachyury-TRICOM Vaccine in Patients with Previously Untreated Metastatic Adenocarcinoma of the Lung with Erlotinib-Sensitive EGFR Mutation Present

Metastatic Lung Adenocarcinoma with activating EGFR mutation*

Randomize

Erlotinib alone
n = 31

Erlotinib plus Vaccine
n = 31

*Activating mutations include exon 19 deletions or L858R (~15% patients)

Primary endpoint: PFS
- Erlotinib alone arm, estimated at 11 month median PFS
- Goal on combination arm = 20 months PFS
- n = 31pts per arm

Secondary endpoints: OS, immune endpoints

Trial PI: Dr. Chris Heery
Benefits of Trial Design

• Ample time for immune response to occur

• Erlotinib induces objective response
  – PR 60-80% of these patients (Zhou Lancet 2011 and Rosell Lancet 2012)
  – Cells killed by erlotinib may boost immunologic effects – antigen spreading, improved
tumor microenvironment

• For cells not killed by erlotinib, cells are made more amenable to T cell
killing
Randomized Phase 2 Study of Vaccine in Stage II Colon Cancer and High Risk Features Determined by “Immunoscore”
Galon Science 2006

(A) Disease-Free Survival vs. Survival (months) for UICC-TNM classifications.

(B) Disease-Free Survival vs. Survival (months) for CD3_{CT}^{Hi}CD3_{IM}^{Hi} and CD3_{CT}^{Lo}CD3_{IM}^{Lo}.

(C) Disease-Free Survival vs. Survival (months) for CD45RO_{CT}^{Hi}CD45RO_{IM}^{Hi} and CD45RO_{CT}^{Lo}CD45RO_{IM}^{Lo}.
CV-301 or Brachyury-TRICOM
Adjuvant “Immunoscore” Trial

Population:
Adjuvant setting Colorectal cancer
Patients with high risk (50% recurrence at 2 years) by Immunoscore

Design:
Randomized phase II → Standard of care +/- vaccine (PANVAC)

Endpoint: recurrence free survival

Goal: Convert RFS at 2 years from 50% to 75%

Estimated n = 40 per arm
Combined regions analysis

\[ \frac{93}{318} = 29\% \]
Stage I, II, III Patients

=249 → 30/249 = 12%
Patients with colon cancer post resection stage II or III

- **Good / intermediate score**
  - Standard of care management

- **Poor score**
  - Randomize
  - **Immunotherapy plus standard of care**
  - **Standard of care alone**

Endpoints:
- Primary: PFS
- Secondary: OS, peripheral immune responses
Figure 5 Proposed taxonomy of colorectal cancer, reflecting significant biological differences in the gene expression-based molecular subtypes. CIMP, CpG island methylator phenotype; MSI, microsatellite instability; SCNA, somatic copy number alterations.
Acknowledgements

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Renee Donahue, PhD
Sofia Gameiro Ph.D.
Italia Grenga M.D
Lauren Lepone Ph.D.

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Branch Chief
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Sheri McMahon
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Chrisa Thomas
Andrea Burmeister, PA-C
Amy Hankin, PA-C
Diana Martin, RN
Ana Couvillon, NP
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