Circulating regulatory T-cell function and overall survival in metastatic castration-resistant prostate cancer (mCRPC) patients treated with poxviral-based vaccine

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BACKGROUND

Increased levels of regulatory T cells (Tregs) have been reported in both the tumor microenvironment and in the peripheral blood of patients with several types of malignancies…

A low number of tumor-infiltrating FOXP3-positive cells during primary systemic chemotherapy correlates with favorable anti-tumor response in patients with breast cancer.

The prevalence of FOXP3+ regulatory T-cells in peripheral blood of patients with NSCLC.

Incidence and prognostic impact of FoxP3+ regulatory T cells in human gliomas.

Intratumoural FOXP3-positive regulatory T cells are associated with adverse prognosis in radically resected gastric cancer.

Prognostic impact of tumor infiltrating FOXP3 positive regulatory T cells in diffuse large B-cell lymphoma at diagnosis.

Correlation of NK T-like CD3+CD56+ cells and CD4+CD25+(hi) regulatory T cells with VEGF and TNFalpha in ascites from advanced ovarian cancer: Association with platinum resistance and prognosis in patients receiving first-line, platinum-based chemotherapy.

Tumor-infiltrating Foxp3-CD4+CD25+ T cells predict poor survival in renal cell carcinoma.

Increased frequency of regulatory T cells in peripheral blood and tumour infiltrating lymphocytes in colorectal cancer patients.

...AND are generally related to poor clinical outcome
Enhanced functionality of CD4+CD25(high)FoxP3+ regulatory T cells in the peripheral blood of patients with prostate cancer.
Yokokawa J, Cereda V, Remondo C, Gulley JL, Arlen PM, Schom J, Tsang KY.

Frequency of CD4⁺CD25<sup>high</sup>FoxP3⁺ Tregs

Function of CD4⁺CD25<sup>high</sup>FoxP3⁺ Tregs
Peripheral Tregs and overall survival in metastatic castration-resistant prostate cancer (mCRPC) patients treated with a poxviral-based vaccine (PSA-TRICOM)
A vaccine formulation consisting of recombinant vaccinia (rV) or fowlpox (rF) virus, encoding:

<table>
<thead>
<tr>
<th>Costimulatory Molecule</th>
<th>Ligand on T cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>B7-1 (CD80)</td>
<td>CD28/CTLA-4</td>
</tr>
<tr>
<td>ICAM-1 (CD54)</td>
<td>LFA-1</td>
</tr>
<tr>
<td>LFA-3 (CD58)</td>
<td>CD2</td>
</tr>
</tbody>
</table>

TRICOM = B7-1/ICAM-1/LFA-3

PSA/TRICOM = PSA/B7-1/ICAM-1/LFA-3 (PROSTVAC)

All vaccines contain: rV- as a prime vaccine
avipox (fowlpox, rF-) as multiple booster vaccines
Randomized Multicenter Placebo-controlled Vaccine Therapy Trial in Castrate-resistant Metastatic Prostate Cancer Patients

Patients (n = 125)
- Metastatic prostate cancer (CT or bone scan +)
- Gleason score ≤ 7; no visceral disease
- Chemotherapy naïve

Vaccine: rV, rF-PSA-TRICOM (PROSTVAC) + GM-CSF

Control arm: empty vector

Randomization: 2:1 (double blind)

P.I.: P. Kantoff, Dana-Farber Cancer Center

Analyses: W. Godfrey, BNIT
B. Blumenstein, statistician
Randomized Multicenter Placebo-controlled Vaccine Therapy Trial in Castrate-resistant Metastatic Prostate Cancer Patients

Observations:

A. Time to Progression: no difference in arms

B. Median survival at 4 years
   Placebo: 16.6 months
   Vaccine: 25.1 months (p=0.006)

C. 40% reduction in death rate in vaccine arm

Phase III Trial Planned
PSA-TRICOM - PHASE I/II CLINICAL TRIAL: STUDY DESIGN

- Androgen refractory
- Metastatic disease

Patients treated → Vaccinia PSA-TRICOM Priming Dose → Monthly Fowlpox PSA-TRICOM Boost

Primary Endpoint
Immune Response

Secondary Endpoint
Clinical response

Evaluation Predicted Survival (HN)
Evaluation Immune Response

Re-staging Scans Q 3 months

Evaluation Immune Response
Evaluation Overall Survival

D1 D29 D57 D85 D113 D141 D169

V F F F F F F
### Halabi Nomogram to Predict Survival

<table>
<thead>
<tr>
<th>Points</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visceral Disease</td>
<td>Yes</td>
<td>No</td>
<td>8-10</td>
<td>2-7</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gleason Score</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>20</td>
<td>70</td>
<td>300</td>
<td>5000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Performance Status</td>
<td>17</td>
<td>15</td>
<td>13</td>
<td>11</td>
<td>9</td>
<td>8</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline PSA</td>
<td>6</td>
<td>8</td>
<td>20</td>
<td>100</td>
<td>200</td>
<td>400</td>
<td>1000</td>
<td>2000</td>
<td>4000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH</td>
<td>10</td>
<td>20</td>
<td>70</td>
<td>150</td>
<td>500</td>
<td>2500</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>12-Month Survival Probability</td>
<td>0.9</td>
<td>0.8</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
<td>0.2</td>
<td>0.1</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>24-Month Survival Probability</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
<td>0.2</td>
<td>0.1</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Points</td>
<td>Median Survival Time (months)</td>
<td>72</td>
<td>48</td>
<td>36</td>
<td>30</td>
<td>24</td>
<td>18</td>
<td>12</td>
<td>8</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>
# OVERALL SURVIVAL IN PSA-TRICOM CLINICAL TRIAL

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Median PS (mos)</th>
<th>Actual Median OS (mos)</th>
<th>Difference in Survival (mos)</th>
<th>Patients with OS longer than PS</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>17.4</td>
<td>26.6</td>
<td>9.2</td>
<td>22/32 p=0.05*</td>
</tr>
<tr>
<td>&lt;18 PS</td>
<td>12.3</td>
<td>14.6</td>
<td>2.3</td>
<td>10/17 p=0.63*</td>
</tr>
<tr>
<td>≥18 PS</td>
<td>20.9</td>
<td>Not reached</td>
<td>23.7+</td>
<td>12/15 p=0.035*</td>
</tr>
</tbody>
</table>

*two-tailed p-value is listed.

<table>
<thead>
<tr>
<th>Pts with Predicted Survival &lt;18 months</th>
<th>Pts with Predicted Survival ≥18 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 17</td>
<td>n = 15</td>
</tr>
</tbody>
</table>

*Immunologic and Prognostic Factors Associated with Overall Survival Employing a Poxviral-based PSA Vaccine in Metastatic Castrate-resistant Prostate Cancer*

T-REG (CD4⁺CD25⁺CD127⁻FoxP3⁺) FUNCTION CORRELATES WITH OVERALL SURVIVAL

Treg Frequency

Shorter Survivors

Longer Survivors

% CD4⁺CD25⁺CD127⁻FoxP3⁺ in CD4⁺

PRE  POST  PRE  POST  PRE  POST

p = 0.1

p = 0.745

p = 0.107

Treg Function

Increased Suppression in 75% pts

Decreased Suppression in 80% pts

% suppression

PRE  POST

PRE  POST

PRE  POST
POSSIBLE ROLE OF CTLA-4 EXPRESSION ON T-REGS

Zheng Y, Manzotti CN, Burke F, Dussably L, Qureshi O, Walker LS, Sansom DM.

Acquisition of suppressive function by activated human CD4+ CD25- T cells is associated with the expression of CTLA-4 not FoxP3.


**CTLA-4 control over Foxp3+ regulatory T cell function.**


**Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor.**


CD4⁺ CD25⁽⁺⁺⁾ FoxP3⁽⁺⁾ CD45RA⁻ (CTLA-4⁽⁺⁾ DR⁽⁺⁾) activated Tregs (aTregs) Suppressive +++
CORRELATION BETWEEN CTLA-4 EXPRESSION ON T-REGS AND T-REG SUPPRESSIVE FUNCTION

INCREASED SUPPRESSION

DECREASED SUPPRESSION

87.5%

67%
CORRELATION BETWEEN CTLA-4 EXPRESSION ON T-REGS AND PATIENT SURVIVAL

SHORTER SURVIVORS

LONGER SURVIVORS

% CTLA-4+ Tregs
(CD4+CD25+CD127-FoxP3+)

PRE
POST

*p = 0.019

% CTLA-4+ Tregs
(CD4+CD25+CD127-FoxP3+)

PRE
POST

p = 0.127
CORRELATION BETWEEN PATIENT SURVIVAL AND 
EFFECCTOR/CTLA4⁺ EXPRESSING T-REG RATIO

\[ *p = 0.0006 \]

Ratio Effectors (CD4⁺CD25⁺)/CTLA4⁺ Tregs
(Fold Increase Post vs Pre)

Shorter Survivors
Longer Survivors
NO DIFFERENCE IN THE % OF EFFECTOR CELLS IN SHORTER AND LONGER SURVIVORS
CONCLUSIONS

1. No difference in Treg numbers pre- and post-vaccination

2. Significant correlations between:
   - Treg suppressive function and overall survival
   - Frequency of CTLA-4 expressing Tregs and Treg suppressive function
   - Patient survival and the ratio between effectors and CTLA4 expressing Tregs

These data suggest that the clinical benefit of vaccine immunotherapy with PSA-TRICOM can be due in part to a decreased Treg suppressive activity post vaccination

Further studies are ongoing to confirm and extend this observation
ACKNOWLEDGEMENTS

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