Lymphoid and myeloid biomarkers for clinical outcome of ipilimumab and Prostate GVAX treatment

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Tanja de Gruijl-disclosures

Research support from Cell Genesys Inc.

SITC 6 Nov 2011
Prostate cancer

- Most common malignancy in elderly men
- Second leading cause of cancer deaths in western countries
- 1 in 6 men affected

(www.cancer.org)
Prostate GVAX
Vaccine consisting of two AAV-GM-CSF transduced, irradiated prostate cancer cell lines (LNCaP, PC-3)

Ipilimumab (Yervoy)
*anti-human CTLA-4 Antibody
*high affinity and specificity
*fully human IgG1k antibody
*blocks the binding of CTLA-4 to B7
*does not mediate ADCC

Drake Nat Rev Immmnol 2010
Treatment and sampling scheme

GVAX every 2 weeks for a total of 13 i.d. doses

anti-CTLA4 mAb (Ipilimumab) every 4 weeks for a total of 6 infusions

<table>
<thead>
<tr>
<th>Dose level</th>
<th>patient #</th>
<th>anti-CTLA4 dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort 1</td>
<td>1-3</td>
<td>0.3 mg/kg</td>
</tr>
<tr>
<td>Cohort 2</td>
<td>4-6</td>
<td>1.0 mg/kg</td>
</tr>
<tr>
<td>Cohort 3</td>
<td>7-9</td>
<td>3.0 mg/kg</td>
</tr>
<tr>
<td>Cohort 4</td>
<td>10-12</td>
<td>5.0 mg/kg</td>
</tr>
<tr>
<td>Cohort 5</td>
<td>13-28</td>
<td>3.0 mg/kg</td>
</tr>
</tbody>
</table>
Clinical results

**Partial Response (PR);**
>50% PSA decline

**Stable Disease (SD);**
No PR or PD

**Progressive Disease (PD);**
>25% PSA increase

<table>
<thead>
<tr>
<th>Category:</th>
<th>Response</th>
<th>Number of patients</th>
<th>Duration of response (median and range in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA Partial Response (PR)</td>
<td>&gt;50% on-study PSA decline</td>
<td>5 / 28</td>
<td>305 (51-919)</td>
</tr>
<tr>
<td>PSA Stable Disease (SD)</td>
<td>No PR or SD</td>
<td>12 / 28</td>
<td>85 (82-190)</td>
</tr>
<tr>
<td>PSA Progressive Disease (PD)</td>
<td>&gt;25% on-study PSA increase</td>
<td>11 / 28</td>
<td>n.a.</td>
</tr>
</tbody>
</table>
Clinical results

• PSA declines were durable: 6 to 31 months
• Stable disease by bone scan was observed in 11 patients (>5 mns)
• Regressing bone and lymph node metastasis were observed in 2 patients

Toxicity: Auto-immune Breakthrough Events (irAE) in 9 patients
• 7 patients (5/5 PR!) showed hypophysitis with:
  - secondary adrenal insufficiencies
  - secondary hypothyroidism
• 1 PR patient developed a dose limiting grade 3 alveolitis (5 mg/kg Ipilimumab)
• 2 patients experienced low grade colitis; 1 patient grade 3 hepatitis
• irAE were successfully treated with standard hormone replacement therapy (endocrinopathies) or steroids.
Clinical results:

Treatment response correlated with survival

Actual survival was longer than Halabi-predicted survival
Immunomonitoring: principal question

Prostate Cancer as a learning model
Can we identify immune parameters that correlate with clinical activity and may be useful for clinical response prediction?

…or treatment resistance prediction? >>avoid autoimmune side effects

NB: Phase I study with non-randomized Phase II study: hypothesis generating.

Further validation of identified immune biomarkers in randomized trials with GVAX and/or ipilimumab required!
Immunomonitoring: principal question

Prostate Cancer as a learning model
Can we identify lymphoid and myeloid immune parameters that correlate with clinical activity and may be useful for clinical response prediction? …or treatment resistance prediction? >>avoid autoimmune side effects

1. **Serology**
   - tumor-specific antibodies
2. **Peripheral blood T\textsubscript{eff}/T\textsubscript{reg} cells**
   - frequency
   - activation status
   - effector/memory phenotype
3. **T cell Functionality**
   - TAA-specific reactivity
   - suppression assays
   - cytokine profiles
4. **Peripheral Blood DC (PBDC) and Myeloid Derived Suppressor Cells (MDSC)**
   - frequency
   - activation status
T cell activation: ICOS, FoxP3, CTLA-4, PD-1

→ Consistent upregulation of activation markers upon treatment: little association with survival
T cell activation: effector/memory phenotype

→ Increased Th differentiation on treatment: relation with survival
High Treg rates: associated with SD/PD and reduced survival

- Regulatory T cells (nTregs)

- PR
  - 50% increase in Tregs at w24
  - No increase in Tregs at w24

- SD/PD
  - *
  - **

- Percent survival
  - N=18; med. surv. 37.0 mths
  - N=6; med. surv. 20.5 mths

- Cut-off: 6.3%
  - p = 0.023
  - p = 0.089
Tumor-related elevated pre-treatment frequencies of CD4⁺CTLA4⁺ T cells have predictive value for survival on treatment.
**T cell activation profile**

**Potential biomarkers:**

predictive vs prognostic; no relation according to Halabi-predicted survival

### On-treatment predictive

<table>
<thead>
<tr>
<th>Immune parameter</th>
<th>Median Survival between groups</th>
<th>P-value</th>
<th>Median Halabi Predicted Survival</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-naïve CD4+ cells</td>
<td>41.0 vs. 20.0</td>
<td>0.036</td>
<td>16.5 vs. 21.4</td>
<td>0.086</td>
</tr>
<tr>
<td>CD8+ICOS+</td>
<td>21.0 vs. 57.0</td>
<td>0.043</td>
<td>16.7 vs. 19.3</td>
<td>0.622</td>
</tr>
<tr>
<td>CD4+CD25intFoxP3+</td>
<td>41.0 vs. 21.0</td>
<td>0.030</td>
<td>19.6 vs. 15.0</td>
<td>0.401</td>
</tr>
<tr>
<td>CD4+CD25hiFoxP3+ Tregs</td>
<td>37.0 vs. 21.0</td>
<td>0.045</td>
<td>15.0 vs 19.6</td>
<td>0.201</td>
</tr>
</tbody>
</table>

### Pre-treatment predictive

<table>
<thead>
<tr>
<th>Immune parameter</th>
<th>Median Survival between groups</th>
<th>P-value</th>
<th>Median Halabi Predicted Survival</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-naïve CD8+ cells</td>
<td>n.r. vs. 20.5</td>
<td>0.028</td>
<td>21.6 vs. 17.3</td>
<td>0.222</td>
</tr>
<tr>
<td>Non-naïve CD4+ cells</td>
<td>19.0 vs. 41.0</td>
<td>0.02</td>
<td>21.4 vs. 16.7</td>
<td>0.021</td>
</tr>
<tr>
<td>CD4+PD-1+</td>
<td>41.0 vs. 18.0</td>
<td>0.014</td>
<td>20.5 vs. 15.9</td>
<td>0.194</td>
</tr>
<tr>
<td>CD4+CTLA-4+ (conv. T cells)</td>
<td>52.0 vs. 20.5</td>
<td>0.011</td>
<td>19.0 vs. 15.9</td>
<td>0.097</td>
</tr>
<tr>
<td>CD4+CD25hiFoxP3+ Tregs</td>
<td>20.0 vs. 36.0</td>
<td>0.087</td>
<td>20.9 vs. 19.0</td>
<td>0.230</td>
</tr>
</tbody>
</table>

n.r.= not reached
T cell activation profile

Unsupervised cluster analysis:

CD4+CDTLA4+ as dominant predictor of survival on treatment

Min. Max. Stat. cut-off

004 011 013
002 008 007
014 016 019
003 015 020
022 002 008
023 017 009
026 007 009
018 014 017
006 006 006
013 011 013
015 010 015
025 020 025
010 021 021
027 022 027
019 023 023
028 021 028

Treg increase
Non-naïve CD4+ T cell increase
CD4+CD25intFoxP3+ increase
Treg pre
Non-naïve CD4+ T cell pre
Non-naïve CD8+ T cell pre
CD4+CTLA-4+ pre
CD4+PD-1+ pre

Patient codes

N=14; med.surv. 46.5 months
N=9; med.surv. 21 months

p=0.036
Myeloid subsets: also targets for GVAX and ipilimumab?

Oosterhoff Immunother 2011

Liu CII 2009

Suzuki Cell Transplant 2010
PBDC: subset activation

- **cDC1**: BDCA1/CD1c
  - w0, w4, w8, w12, w16, w20, w24, fu

- **cDC2**: BDCA3/CD141
  - w0, w4, w8, w12, w16, w20, w24, fu

- **cDC3**: CD14\textsuperscript{dim}/MDC8
  - w0, w4, w8, w12, w16, w20, w24, fu

- **pDC**: BDCA2/CD123
  - w0, w4, w8, w12, w16, w20, w24, fu
PBDC: cDC1 and cDC3 activation

Increased activation of cDC1 and cDC3 (also known as 6-sulfo LacNAc+ or SLAN-DC) is related to survival.
MDSC: monocytoid

High pre-treatment levels of mMDSC are associated with poor survival

Filipazzi et al. JCO 2007
MDSC: granulocytic

Granulocytic MDSC (CD11b⁺CD14⁻CD15⁺)

On-treatment increases in grMDSC are associated with poor survival

Zea et al. Cancer Res 2005
A predictive T cell and myeloid marker profile

Unsupervised cluster analysis:

High DC activation and Th CTLA4 and PD-1 expression and low suppressive MDSC and Treg levels together predict survival on GVAX+ipilimumab treatment
Conclusions

→ Potential immune biomarkers for patient selection prior to treatment:
→ mMDSC, Tregs, effector/memory and CD4+PD-1+/CD4+CTLA4+ T cell rates

Next: validation
• Treatment specific? (GVAX, ipilimumab monotherapies; other therapies?)
• Disease stage specific? (Early versus advanced prostate cancer?)
• Disease specific? (Melanoma vs prostate cancer)
Anita Stam & Saskia Santegoets

Fighting the Blues...

...with wine...

...and awards
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Petra Scholten  
Martine Reijm  
Mary von Blomberg  
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Tanja de Gruijl

Medical Oncology Clinic  
Helen Gall  
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Winald Gerritsen

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Natalie Sacks  
Kristen Hege

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