Repetitive DNA Vaccination Elicits PAP Antigen-Specific T-Cell Immune Responses in Patients with Castration-Resistant Prostate Cancer

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Disclosures

Dendreon Corporation – consultant

Intellectual property – WARF
Outline

• Background
  • Prostate cancer immunotherapy
  • Previous experience
    DNA vaccine encoding prostatic acid phosphatase (PAP)

• Pilot Clinical Trial
  • Evaluation in patients with non-metastatic prostate cancer
  • Immune monitoring to answer questions of vaccine schedule
Prostate Cancer

• Most commonly diagnosed cancer in the United States
• Second leading cause of cancer-related death in men
• Approximately 1/3 of patients have recurrent disease after “definitive” local therapy
• 240,890 projected new cases in 2011
• 33,720 projected deaths in 2011
Immunotherapy for Prostate Cancer

- Sipuleucel-T approved by FDA in 2010 – first approved anti-tumor vaccine in U.S.
- Approved on the basis of improved overall survival
- Time to progression endpoint in previous trial not met
- Similar findings with PSA-TRICOM vaccine approach

Questions Regarding Immunotherapy for Prostate Cancer

Why was there no clear association between TTP and OS? Or T-cell immune response and OS or TTP?

[Graph showing the percentage of patients without disease progression over time to disease progression, comparing positive and negative PAP immune responses.]

- Positive PAP Immune Response
- Negative PAP Immune Response

n=20
n=11
p<0.03

• Other preclinical studies and anti-tumor vaccine trials have suggested that anti-tumor vaccines might “take time to work” and/or be most effective in the minimal-residual-disease setting.

• Is it possible that the advanced stage of prostate cancer that has been most evaluated is not optimal to detect time-to-progression and immunological readouts?
Prostate Cancer

Organ Confined

- Locally Advanced

Rising PSA Hormone Naïve (D0)

Metastatic Disease (D2)

Rising PSA Castrate-Resistant (D0.5)

Metastatic CR Asymptomatic (D3)

Metastatic CR Symptomatic

PSA = prostate-specific antigen
CR = castrate-resistant
Prostate Cancer

Timeline

PSA doubling time highly associated with time to progression in stages of disease with only rising PSA
Prostatic Acid Phosphatase – Vaccine Target Antigen

• Expression essentially restricted to prostate tissue in humans

• Permits evaluation of serum PSA as an independent assessment of response in human trials

• Previous experience targeting this antigen in rodent models and human clinical trials:
  – Vaccinia, pulsed dendritic cell (Fong, Stanford)
  – Antigen-presenting cell vaccine (Dendreon corp.)
Antigen-Specific DNA Vaccines

Advantages:
• Simpler, less costly, manufacturing and storage
• Non-autologous
• Not MHC-restricted
• No foreign viral antigens
  – Safety
  – No need for heterologous immunization approach
• Validated in non-human (companion dog) trials

Disadvantage:
• Less immunologically potent
Phase I Trial – DNA Vaccine Encoding PAP Study Design

Patients with stage D0 prostate cancer

Tetanus
DNA Vaccine + 200 µg GM-CSF

Month: -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
* * Leukaphereses
* * * * * * * * * * * *
Timepoints for which PBMC available for immune monitoring

Dose Escalation Schedule

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>pTVG-HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 µg</td>
</tr>
<tr>
<td>2</td>
<td>500 µg</td>
</tr>
<tr>
<td>3</td>
<td>1500 µg</td>
</tr>
</tbody>
</table>

McNeel, JCO ’09 27:4047
Prostate Cancer – Stage D0 Trial
Lessons Learned

- PAP-specific T-cell immune responses elicited
  - CD8+ T cells – IFN\(\gamma\)-secreting
  - CD4+ and CD8+ T-cell proliferation
  - HLA-A2-restricted cytolytic activity
  - Immune responses elicited irrespective of dose
- No PAP-specific antibody responses elicited
- No significant adverse events
Prostate Cancer – Stage D0 Trial
Lessons Learned (cont)

Immune responses detectable after immunization appeared to require several vaccinations
Prostate Cancer – Stage D0 Trial
Lessons Learned (cont)

Immune responses were “boostable”
Detection of PAP-specific IFN\(\gamma\) responses at least twice in 1 year of follow up (*) associated with favorable change in PSA doubling time \((P=0.001)\)
Hypotheses

- Multiple immunizations may be necessary to elicit responses in some individuals
- Development of long-term, durable memory immune responses may be associated with long-term stable disease
- Periodic booster immunizations may be necessary to maintain Th1-type response
Objectives / Endpoints

Primary Clinical Endpoint:
To determine the safety of multiple serial immunizations in a castrate-resistant, non-metastatic population

Primary Immunological Endpoints:
• Determine whether long-term, memory PAP-specific T cells can be elicited
• Determine an optimal schedule of immunization to maintain effector/memory T-cell response

Secondary Endpoints:
• Determine if immunization associated with prolonged PSA doubling time
• 1-year metastasis-free survival
Trial Schema

- PAP DNA vaccine 100 µg + 200 µg rhGM-CSF (adjuvant) intradermally
- Tetanus immunization given prior
- Patients remain on study until:
  - Radiographic progression
  - Toxicity
  - Personal choice to discontinue
  - 2 years or maximum of 24 immunizations

Arm A: * (Radiographic studies) * * * * * * * * * * * * * * * * * (continuing until disease progression) (immunizations)

Leukapheresis

Arm B: * (Radiographic studies) * * * * * * * * * * * * * * * * * (continuing until disease progression)

(frequency of booster immunizations determined by immune monitoring)
Study Population – Entry Criteria

- Stage D0.5 prostate cancer, defined as:
  - Castrate-resistant
  - Rising serum PSA
  - No evidence of metastases by CT or bone scan
- All (minimum of 4) serum PSA values available over a 3-6 month period, last value > 2 ng/mL, all from same clinical laboratory – for pretx PSA DT
- ECOG PS < 2
- Normal hematological, renal, liver function
- Not on immunosuppressive therapy
Trial Conduct

Accrual: 14 patients as of October 2011, of whom
11 have completed 1 year

8 have come off study
  2 for PD (6, 15 months)
  1 for choice – rising PSA (9 months)
  1 for grade 3 allergic reaction (15 months)
  4 completed study
    3 received 24 immunizations
    1 on study for 2 years

9 of 11 have been / were on study > 1 year
6 remain on study
## Demographics

Age, median: 73.5 years (range 47-86)

Prior treatment:
- Prostatectomy: 7 (50%)
- Radiation therapy:
  - Primary treatment: 3 (21%)
  - Salvage treatment: 5 (36%)

Gleason Grade
- <7: 3 (21%)
- 7: 6 (43%)
- 8: 1 (7%)
- 9: 4 (29%)

Pre-treatment
- PSA, median: 5.35 ng/mL (range 2.3 – 54.4)
- PSA doubling time, median: 2.8 months (range 1.36 – 5.48)
## Adverse Events

<table>
<thead>
<tr>
<th>Category</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic / Hypersensitivity</td>
<td>2</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Dermatologic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection site reactions</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rash / desquamation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory / metabolic</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Elevated creatinine</td>
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Immune Analysis

Real-Time Immune monitoring:
• PAP-specific CD4+ and CD8+ T-cell proliferation (dye dilution to determine precursor frequency)
• PAP-specific IFN\(\gamma\) release (ELISpot)
• PAP-specific granzyme B release (ELISpot)

“Response” defined as statistically significant compared with media-only control, and at least 3x baseline value. Baseline cryopreserved sample evaluated with each timepoint.

Other measures:
• Memory phenotype of antigen-specific proliferating cells
• Cytokine expression of proliferating cells
• Tetramer analysis of HLA-A2+ individuals
• PAP-specific antibody (IgG) responses
Example Real-Time Immune Analysis

Immune Responder

CD4+ T-cell Proliferation

CD8+ T-cell Proliferation

IFNγ ELISPOT

Granzyme B ELISPOT
Baseline Immune Analysis
Reproducibility over Time

CD8 Proliferation

IFN\textsubscript{\gamma} ELISPOT

Responding Cells per Million CD8

SFU per million PBMC
PSA Monitoring with Immunization
Fixed Schedule

Days since beginning immunization
Serum PSA (ng/ml)

-250 -50 150 350 550 750

Days since beginning immunization
Serum PSA (ng/ml)

-250 -50 150 350 550 750

Days since beginning immunization
Serum PSA (ng/ml)

-250 -50 150 350 550 750

Days since beginning immunization
Serum PSA (ng/ml)

-250 -50 150 350 550 750
PSA Monitoring with Immunization
Variable Schedule with Monitoring

Days since beginning immunization

Serum PSA (ng/ml)
Changes in PSA Doubling Time Associated with Long-Term Th1-Type Immunity

![Graph showing PSA Doubling Time](image)

- **IFN-γ** vs Pre-treatment and Post-treatment: $P=0.02$
- **GZB** vs Pre-treatment and Post-treatment: $P=0.12$
- **CD4** vs Pre-treatment and Post-treatment: $P=0.35$

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**PSA Doubling Time (months)**

- Pre-treatment
- Post-treatment

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*Note: The image contains a graph showing the changes in PSA doubling time associated with long-term Th1-type immunity. The graph indicates the statistical significance of the differences between pre-treatment and post-treatment, with the following p-values:

- IFN-γ: $P=0.02$
- GZB: $P=0.12$
- CD4: $P=0.35$*
Summary and Preliminary Trial
Conclusions

- Multiple repetitive immunizations appears safe
- Long natural disease history appropriate for evaluating long-term effects of anti-tumor vaccines
- Different patterns of immune “response”
- To date, identification of optimal schedule challenging due to delayed immune responses
- IFN$_\gamma$-secreting responses identified at multiple times after immunization most associated with favorable changes in PSA doubling time
Unanswered Questions and Future Directions

• Does vaccination (and/or change in PSA doubling time occurring after vaccination) affect time to disease progression?

• Does establishment of long-term immune response confer benefit in terms of time to disease progression?

• What differences exist in some patients pre-treatment that make them not “immunizable”?
Ongoing Randomized Phase II Trial

Primary Objective:

To evaluate the 2-year metastasis-free survival of patients with non-castrate, non-metastatic prostate cancer (clinical stage D0) treated with a DNA vaccine encoding PAP, with GM-CSF as an adjuvant, versus GM-CSF only.

Patients with PSA doubling time < 12 months

2-center trial: UWCCC and UCSF (Larry Fong, PI)
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