SIPULEUCEL-T: IMMUNOTHERAPY FOR PROSTATE CANCER

Nadeem Sheikh, PhD
Dendreon Corporation,
Seattle, USA
Sipuleucel-T is approved by the U.S. FDA for the treatment of asymptomatic or minimally symptomatic metastatic castrate resistant prostate cancer. It is not approved in the EU.

Challenges for immunotherapy of cancer

Sipuleucel-T manufacturing and product attributes

IMPACT immune responses and correlation with overall survival

The vaccinology of sipuleucel-T
  - Immunological attributes of sipuleucel-T
  - Immune responses elicited by sipuleucel-T
CHALLENGES FOR IMMUNOTHERAPY OF CANCER
There are now 10 hallmarks of cancer pathogenesis: Sipuleucel-T targets one hallmark.

Key Events in the History of Cancer Immunotherapy

1890
First cancer vaccine developed (Coley)

1960s
Adjuvants (e.g. BCG) shown to eradicate some tumors

1953
Coley’s work first published

1985
Adoptive immunotherapy for patients with cancer

1986
IFN-α approved as cancer immunotherapy

1991
First tumor-associated antigen cloned (MAGE-1)

1992
IL-2 approved as cancer immunotherapy

2002
Lymphopenia/Reconstitution enhances adoptive TIL therapy

2010
Sipuleucel-T approved by FDA as the first autologous cellular immunotherapy

2011
Ipilimumab approved for Treatment of melanoma

BCG = Bacille Calmette-Guerin
IFN = interferon
IL = interleukin
TIL = tumor infiltrating lymphocyte
“Cancer treatment vaccines are designed to treat cancers that have already developed. They are intended to delay or stop cancer cell growth; to cause tumor shrinkage; to prevent cancer from coming back; or to eliminate cancer cells that have not been killed by other forms of treatment.”

- NCI (2011)
SIPULEUCEL-T MANUFACTURING, PRODUCT ATTRIBUTES AND CORRELATION WITH SURVIVAL
Dendreon Current Clinical Trials and Prostate Cancer Disease Stages

Sipuleucel-T: An Autologous Cell Immunotherapy That Stimulates the Immune System to Target Tumor Cells

PBMCs (including resting APC)

PAP-GM-CSF antigen combines with resting APC

APC takes up the PAP-GM-CSF

PAP-GM-CSF is processed and presented on the surface of the APC

PAP-GM-CSF–loaded APCs are now the active component of sipuleucel-T

Inactive T cell

Active T cell

Sipuleucel-T activates T cells in the body
Patient cells are incubated with PA2024, a fusion protein of prostatic acid phosphatase (PAP) and GM-CSF.
Cellular Composition Profiles of Pre-Cultured Cells for Early- and Late-Stage Disease

<table>
<thead>
<tr>
<th>Early Stage</th>
<th>Late Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neoadjuvant</td>
<td>Minimally Symptomatic/Asymptomatic mCRPC</td>
</tr>
<tr>
<td>NeoACT (n=41)</td>
<td>IMPACT (n=330)</td>
</tr>
<tr>
<td></td>
<td>OpenACT (n=98)</td>
</tr>
</tbody>
</table>

Week 0

Week 2

Week 4

- CD3⁺ (T cells)
- CD54⁺ (APC)
- CD19⁺ (B cells)
- CD56⁺ (NK cells)
CD54 Upregulation During *Ex Vivo* Generation of Sipuleucel-T

- **Day 0**: Apheresis → Buoyant Density Separations
- **Day 2**: PA2024 Culture → Wash → Resuspend Cells in Lactated Ringer’s Solution

Large Cell CD54 Determination by FLOW → Product Potency = Fold CD54 Upregulation → Large Cell CD54 Determination by FLOW
APCs Are Activated Ex Vivo During Sipuleucel-T Treatment

Day 0 (Pre-Culture Cells)

Day 2 (Post-Culture Cells)

Week 0  Week 2  Week 4

Week 0  Week 2  Week 4

IMPACT (D9902B)
APC activation prime-boost profile is consistently observed

![Graph showing APC activation profile across different stages and conditions](Image)
Correlation between overall survival and product parameters

<table>
<thead>
<tr>
<th>Cell Product Parameters</th>
<th>Unadjusted $P$ Value</th>
<th>$P$ Value Adjusted for PSA and LDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative CD54 Upregulation</td>
<td>0.002</td>
<td>0.041</td>
</tr>
<tr>
<td>Cumulative CD54+ Cells ($x 10^9$)</td>
<td>0.016</td>
<td>0.005</td>
</tr>
<tr>
<td>Cumulative Total Nucleated Cell Counts ($x10^9$)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

IMPACT IMMUNE RESPONSES AND CORRELATION WITH OVERALL SURVIVAL
IMPACT: Long-Lasting and Robust Antibody Responses

- Antibody response maintained up to 6 months post-treatment
- PA2024 responses significantly greater magnitude compared to PAP

IMPACT: Antibody Response Is Predominantly IgM and Seroconverts to IgG

IMPACT: Long-Lasting T-Cell Responses to PA2024 and PAP

- Proliferative response maintained up to 6 months post-treatment
- PA2024 responses significantly greater magnitude compared to PAP

IMPACT: Treatment Generates Antigen-Specific Memory T-Cell Responses

- ELISpot response maintained up to 6 months post-treatment
- PA2024 responses greater magnitude compared to PAP

Sipuleucel-T Induces Peripheral Immune Responses in the Majority of Treated Patients

IMPACT: OS Correlates With Antigen-Specific Immune Response

THE VACCINOLOGY OF SIPULEUECEL-T

The Immunological attributes of sipuleucel-T
Sipuleucel-T induces cytokine production during manufacture of doses at week 0, week 2 and week 4.
Sipuleucel-T induces cytokine production during manufacture of doses at week 0, week 2 and week 4.
A Generalized Pattern of Enhanced Cytokine Levels Is Observed at Weeks 2 and 4
Cytokine Accumulation in Culture at Week 2 Correlates With Enhanced CD54 Upregulation

Week 2 Cytokine Production: Unguided Clustering of Individual Patients

- **n=34**

Group 1
- CD54 high
- CD54 low

Group 2
- CD54 high
- CD54 low

Group 3
- CD54 high
- CD54 low

**Cytokines Present:**
- IL-6
- IL-1β
- TNF-α
- MCP-1
- IFN-γ
- IL-8
- IP-10
- MIP-1β
- IL-2
- TARC
- MCP-4
- Eotaxin
- IL-10
- IL-13
- IL-5
- IL-4
- IL-12
Kaplan-Meier Plots: Stratification by Cytokine Secretion

- Group 1: n=8 (2 events)
- Group 2: n=12 (6 events)
- Group 3: n=14 (2 events)

10 events/deaths in 34 patients
Sipuleucel-T/T-Cell Ex Vivo Interaction

Week2 PROACT Product
T cell activation gating strategy

- **Naïve (CD45RA^{hi})**
- **Transitional (CD45RA^{int})**
- **Activated (CD45RA^{low})**

La partie de l'image avec l'ID de relation r1d8 n'a pas été trouvé dans le fichier.
Early activation markers are increased on product after the first infusion.

**ProACT (P07-2) trial data**
Early activation markers are increased on product after the first infusion

ProACT (P07-2) trial data
The percent of T-regulatory cells are increased after culture but remain low in vivo.

**Gated on** CD3⁺CD4⁺CD8⁻CD25hiCD127⁻FoxP3⁺ **cells**

*ProACT (P07-2) trial data*
Mature, and memory B cells are activated during treatment

Gated on IgD+CD20+ cells  Gated on IgD-CD20+ cells

Percent CD27+ B cells

Percent CD86+ B cells

ProACT (P07-2) trial data
Significant Changes in Gene Expression Are Observed Upon Culture With PA2024

- ~800 genes upregulated (similar number downregulated) post-culture relative to pre-culture (fold change >3, corrected $P$ value <0.05)
- <10 genes change in the control arm above thresholds ($N = 5$)
Key Cytokines and Cell-Surface Markers Show Increased Expression Post-Culture

Cytokines

- IL-1β
- TNF-α
- IFN-γ
- IL-2
- IL-4
- IL-5
- IL-10
- IL-17

Surface Activation Markers

- CD54
- CD80
- CD25
- CD137

Week 0  Week 2  Week 4

Log₂ (Fold Change of Post-/Pre-Culture Ratio)

±3-fold change
No change
mRNA Changes Are Consistent With Phenotypic Measurements

4-1BB (CD137) on Activated CD4+ T Cells

Frequency of Cells (%)

log$_2$(Fold Change)

Week 0 2 4

BDS65 Final Product
Functional Interpretation of Gene Expression Changes by Pathway Enrichment

- Genes altered post-culture can be mapped to biological pathways based on their known functions (literature)
  - Provides a summarized functional interpretation of genes altered post-culture

- Method: Are known (literature-curated) pathways represented in the associated gene list?
  - **Pathway enrichment significance**: Inferred based on the overlap of a gene-list with genes in a known pathway
  - **Pathway ‘activation’ or ‘repression’**: Inferred based on (a) directionality of expression change post-culture and (b) a gene’s known influence (positive or negative) on a specific pathway.
Signatures of Leukocyte Activation, Differentiation, and Migration Observed Post-Culture

T Cell

- T cell migration
- T cell activation
- Th cell polarization

APC

- migration of DCs
- activation of monocytes
- migration of monocytes
- activation of macrophages
- differentiation of macrophages
- chemotaxis of macrophages
- stimulation of B-cells
- NK cell migration
- Apoptosis

Direction of Activation* – log (p Value)

Week 0
Week 2
Week 4

* Activation vs Repression

Dendreon: Proprietary and Confidential
Key Signaling Pathway Signatures Are Altered Post-Culture

- IL-10 pathway signaling is repressed (even though IL-10 expression level is not altered)
- Transcriptional pathways: STAT1 (regulates IFN-induced genes), STAT4 (regulates Th1 development from CD4+ cells)
THE VACCINOLOGY OF SIPULEUCEL-T

Immune responses elicited by sipuleucel-T
Memory T-Cell Subsets

- Naïve
- Central Memory
- Early
- Intermediate
- Late/T_{EMRA}

Effector function

Apoptosis Resistance
## Cell-Surface Markers for the Characterization of Memory T-Cell Subsets

<table>
<thead>
<tr>
<th>CD8+ T cells</th>
<th>CD4+ T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Naïve</strong></td>
<td><strong>Central Memory</strong></td>
</tr>
<tr>
<td>CD45RA+</td>
<td>CD45RA+</td>
</tr>
<tr>
<td>CD27+</td>
<td>CD27&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD28+</td>
<td>CD28&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCR7&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CCR7&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Effector Memory</strong></td>
<td></td>
</tr>
<tr>
<td>CD45RA~</td>
<td>CD45RA~</td>
</tr>
<tr>
<td>CD27&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD27&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD28&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD28&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCR7&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CCR7&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD45RA~</td>
<td>CD45RA~</td>
</tr>
<tr>
<td>CD27&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD27&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD28&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD28&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCR7~</td>
<td>CCR7~</td>
</tr>
<tr>
<td>CD45RA~</td>
<td>CD45RA~</td>
</tr>
<tr>
<td>CD27&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD27&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD28&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD28&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCR7&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CCR7&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD45RA~</td>
<td>CD45RA~</td>
</tr>
<tr>
<td>CD27&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD27&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>CD28&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD28&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCR7~</td>
<td>CCR7~</td>
</tr>
</tbody>
</table>

- **Early** memory T cells are characterized by intermediate expression of CD45RA and CCR7 with high CD27 and CD28 expression.
- **Intermediate** memory T cells show late expression of CD45RA and CCR7 with low CD27 and CD28 expression.
- **Late** memory T cells are characterized by further downregulation of CD45RA and CCR7, with low CD27 and CD28 expression.

*Note: CD45RA<sup>int/~</sup>, CD27<sup>~/low</sup>, and CCR7<sup>low/~</sup> denote intermediate or low levels of expression.*
An Increased Frequency of Central Memory T Cells Is Observed On CD4\(^+\) T Cells At Earlier Clinical Settings

**CD4\(^+\) T\(_{\text{CM}}\) cells**

- Percent of CD45RA\(^{\text{Neg}}\)CD4\(^+\) cells that express CD27\(^+\) CD28\(^+\) CCR7\(^+\)
- Frequency of Cells (%)

**CD8\(^+\) T\(_{\text{CM}}\) cells**

- Percent of CD45RA\(^{\text{Neg}}\)CD8\(^+\) cells that express CD27\(^+\) CD28\(^+\) CCR7\(^+\)
- Frequency of Cells (%)

**NeoACT (Early)**

- Sample Size:
  - Pre-culture: Wk0 30, Wk2 29, Wk4 29
  - Post-culture: Wk0 30, Wk2 29, Wk4 29

**ProACT (Late)**

- Sample Size:
  - Pre-culture: Wk0 20, Wk2 24, Wk4 23
  - Post-culture: Wk0 20, Wk2 24, Wk4 23
Sipuleucel-T generates antigen-specific responses during treatment

**IFNγ ELISPOT**

- **NeoACT (P07-1)**
- **ProACT (P07-2)**

**Proliferation**

- **NeoACT (P07-1)**
- **ProACT (P07-2)**

**Stimulation Index**

- **NeoACT (P07-1)**
- **ProACT (P07-2)**
Sipuleucel-T generates antigen-specific responses during treatment

**IgM**

- WK0: Initial titers
- WK2: Two weeks post-treatment
- WK4: Four weeks post-treatment
- WK6: Six weeks post-treatment

**IgG**

- WK0: Initial titers
- WK2: Two weeks post-treatment
- WK4: Four weeks post-treatment
- WK6: Six weeks post-treatment

**Antibody titer**

- PA2024
- PAP
Long-Term Memory Immune Responses Are Evident Prior to Retreatment, and are Boosted

- P10-1 patients were formerly treated with sipuleucel-T 8-10 years prior on P-11
Long-Term Memory Immune Responses Are Evident Prior to Retreatment, and are Boosted

- P10-1 patients were formerly treated with sipuleucel-T 8-10 years prior on P-11
Antigen-Specific Proliferative Responses Are Diminished

- P10-1 patients were formerly treated with sipuleucel-T 8-10 years prior on P-11
Summary

- Immune response profile is similar to classical vaccine-mediated prime-boost
- Sipuleucel-T generates antigen-specific immune responses
- Antigen-specific response are generated after the first infusion of sipuleucel-T
- Immune responses have an activated memory phenotype
- Immune responses are boosted upon subsequent infusions of sipuleucel-T
Acknowledgements

- **Patients and physicians from Dendreon’s clinical trials**
  - D9902B, P07-1, P07-2, P11, P10-1, P10-2 & P11-3
- Mark Frohlich
- James Trager
- **Clinical Immunology**
  - Corazon dela Rosa
  - Dwayne Campogan
  - Heather Haynes
  - Nikole Perdue
  - Amanda Sanders
  - Tuyen Vu
  - Jessica Wei
- **Clinical Operations**
  - Debbi Humble
  - Kara Moss
  - Tara Allen
- **Biometrics**
  - James Whitmore
  - Todd Devries
  - Frances Stewart
- **Systems Biology**
  - Debraj Guhathakurta
  - Harini Kandadi