**Aim**

Find evidence of treatment response, immune activation and mechanism of action for patients with metastatic castrate-resistant prostate cancer undergoing treatment with sipuleucel-T.

**Background**

immunotherapy and prostate cancer

• 3 E's of immunoeediting: elimination, equilibrium, escape
• Emerging interest in promoting immune-mediated killing of cancer cells (cytotoxic, checkpoint inhibition, vaccines)
• Potential advantages in prostate cancer: slow growth, tissue-specific antigens and non-essential target, reliable serum marker of tumor burden
• B7-H3 is an immune checkpoint molecule widely expressed by prostate cancer cells associated with increased tumor aggression

Sipuleucel-T

• Autologous cell based immunotherapy (FDA approved 2010) used in the treatment of asymptomatic or minimally symptomatic metastatic castrate-resistant prostate cancer (mCRPC)
• Manufactured by ex vivo culture of patient’s peripheral blood mononuclear cells with PA2024 fusion protein: prostaglandin acid phosphatase (PAP) + GM-CSF
• Median OS benefit +4.1 months (IMPACT)
• Improved OS correlated with low PSA burden, ‘higher peripheral Ab-titer’

Current gaps

• How do we know it’s working? Despite OS benefit, there is no change in traditional markers of response to therapy (time to progression, PSA response)
• How does it work? Developed to activate Ag-specific T cell response by promoting differentiation of dendritic cells but no definitive evidence of this.

**Methods**

Clinical Trial NCT02036918 (see Trial Design)

Sample processing

• Serum samples were taken at baseline, prior to each sipuleucel-T lousepheresis session and after the last infusion
• Serum CBC with differential, PAP and PSA levels were obtained as standard lab evaluations

Immune assays

• Serum anti-PA2024, anti-PAP and anti-tetanus levels were measured using ELISA (performed by Dendreon Pharmaceuticals Inc.)
• Serum cytokine levels were measured using a 30-plex human cytokine panel developed for the Luminex Immunoassay system
• Serum soluble B7-H3 levels were measured using ELISA (performed by Dendreon Pharmaceuticals Inc.)

**Treatment summary**

<table>
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<th>Subject</th>
<th>Treatment</th>
<th>Time (wk)</th>
<th>Grade</th>
<th>Toxicity</th>
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<td>3</td>
<td>3.3</td>
</tr>
<tr>
<td>LNP003</td>
<td>Lap + Retroperitoneal</td>
<td>2</td>
<td>3</td>
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</tbody>
</table>

**Trial design**

Find evidence of treatment response, immune activation and mechanism of action for patients with metastatic castrate-resistant prostate cancer undergoing treatment with sipuleucel-T.

**Peripheral immune response - Th2 phenotype?**

- Ag-specific response
- Multiplex cytokine detection
- LNP002
  - Highest Ag-specific titers
  - Transient eosinophilia present
  - Known correlates with OS benefit
  - Decreased markers of disease aggression
  - Post-op inflammation increases sB7-H3

**Mechanistic framework for immunotherapy**

\[
\frac{dT}{dt} = k_{grow}[T] - k_{1}[T][E] + k_{2}[T][E]
\]

\[
\frac{d[T]}{dt} = -k_{1}[T][E] - k_{v}[T][E] + k_{d}[T][E]
\]

- *T* = tumor burden
- *E* = immune effector
- *k* = killing rate per immune effector
- *k* / *v* = apparent effector-tumor binding affinity
- *k* = decay rate of immune effector

**Connecting the dots**

Quantifying the immune effect

- How can we explain overall survival benefit with Sip-T?

**Conclusions**

Sipuleucel-T may act via Th2-dominant (humoral) pathway

- Estimated effector killer cell account for OS benefit noted in several clinical trials by targeting smaller populations of agressive, immunogenic tumor cells that may not correspond to the dominant clone(s) driving PSA changes
- Consistent with decreased markers of aggressive disease in LNP002

Hypothesis-generating mechanistic framework for immune action

- Based on equations developed previously
- Assumes immune killing is rate limiting
- Divergent clones have accumulated more mutations and are more immunogenic, Poly of ‘antigen spread’

**Future work**

- Completion of ongoing clinical trial, including measuring immune response of excised lymph nodes (EU-SIP07, TCP sequencing)
- Apply better quantitative methods and equations to assess and compare resistance to secondary therapies that decrease tumor burden in combination with Provenge +/- other immunotherapies

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