Expansion and Activation of T-cells via the Targeting of the Immunosuppressive Ligand Phosphatidylserine: Combination Strategy with Other Checkpoint Inhibitors

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Immune Checkpoint Inhibitors: Validate Novel Pathways, Discover Predictive Biomarkers, Optimize Clinical Strategy
Combination Therapy is the Future
More Patients Responding to Therapy

1. Why do only a sub-set of patients respond to IO Therapy?
   - Role of the immunosuppressive tumor microenvironment
   - Lack of T-cell activation and myeloid cell differentiation

2. How can this patient pool be expanded?
   - Ways a PS blockade changes this environment
   - Consequences of PS targeting -- activation and expansion of T-cells

Combination Treatment *
* Hypothetical – for illustrative purposes only

**Checkpoint Blockade**
Yervoy® and Opdivo® are registered trademarks of Bristol Meyers Squibb. Keytruda® is a registered trademark of Merck.

**Targeted Therapy**
Conventional Therapy
Immune Activity in the Tumor Environment

**Tumor-Promoting Phenotype:**
Patients unable to benefit from checkpoint inhibitors

- **Myeloid-Derived Suppressor Cell**
  - Highly immunosuppressive
  - Secrete anti-inflammatory cytokines

- **M2 Macrophage**
  - Secrete anti-inflammatory cytokines and growth factors

- **Immature Dendritic Cell**
  - Incapable of antigen presentation to T-cells

- **T-Regulatory Cell**
  - Inhibits cytotoxic T-cells

- **Cytokines**
  - Inhibit anti-tumor immune responses
  - Keep Immune cells in quiet state

**Tumor-Rejecting Phenotype:**
Patients more likely to respond to checkpoint inhibitors

- **Myeloid-Derived Suppressor Cell**
  - Reduction is current goal of immunotherapy

- **M1 Macrophage**
  - Secrete inflammatory cytokines
  - Kill tumor cells through ADCC

- **Mature Dendritic Cell**
  - Present tumor antigens to T-cells
  - Co-stimulatory signaling for T-cells

- **Cytotoxic (CD-8+) T-cell**
  - Receptor specific tumor cell killing
  - Increased PD-1 expression

- **Cytokines**
  - Stimulate anti-tumor immunity
Immune Suppression in the Tumor Environment Creates a High Hurdle for Immunotherapies

- MDSCs M2 Macrophages
  - Maintain an immunosuppressive tumor environment
- TGF-β IL-10
  - Lack of antigen presentation
  - T-cells do not activate
  - Low levels of PD-1 expression
- Immature DC Naïve T-cells
  - Ineffective response to tumors

- Poor Response to Tumor
Bavituximab: Immune System Activation with Broad Applicability

Immune Signaling Target

- Monoclonal antibody targeting PS

Broad Applicability

- Activity in multiple cancer types and infectious disease

Significant Clinical Experience

- 19 studies, 500+ patients treated with bavituximab, currently in Phase III
- Good safety profile alone and in combination with other therapies (no IR safety events)

Immune Activation MOA

- Removes the “Brakes” and
- Hits the “Accelerator”
PS Ligand Engages Multiple Receptors

- Donna L. Bratton and Peter M. Henson, Apoptotic Cell Recognition: Will the Real Phosphatidylserine Receptor(s) Please Stand up? Current Biology Vol 18 No 2

Mechanisms of PS Immunosuppression Mediated by TAM and TIMs Receptor Signaling

TIM Receptors

- **CD4⁺**
  - Tim-1
  - Th2 bias
  - ↓Th1 response
  - T cell exhaustion
  - Tolerance

- **CD8⁺**
  - Tim-3
  - ↓Th1 response
  - T cell exhaustion

- MΦ
  - Tim-4
  - Tolerance

A apoptotic cells and debris with exposed PS

Innate immunosuppressive actions stimulated by ligand PS complexes

TAM Receptors

- Apoptotic cells polarize to M2 macrophages

- Activated T cell feedback inhibits innate immunity

TAM signaling inhibits NK anti-metastatic effects

Strong TAM Kinase Domain Activation Requires PS

[Diagram showing activation states of TAM kinases]

Courtesy of Lew et. al., and Lemke; eLife 2014;3:e03385
PS Signaling Pathway
Placing the “Brakes” on the Immune System

**PS Signaling**

- Exposed PS is a checkpoint that places brakes on the immune system

**Immune System Signaling**

- PS receptor signaling causes increased anti-inflammatory cytokine expression
  - MDSCs accumulate
  - DCs fail to mature
  - T-cells remain naïve
  - M1 Macrophages fail to develop

**Immune Suppression**

- PS is engaged by PS receptors
- Tumor Cell
- M2
- M1
- Immature DC
- Naive T-cell
- MDSC
- IL-10
- TGF-β

= Exposed PS
Bavituximab Facilitates Induction of Tumor-Specific Cytotoxic T-Cells

1. AACR 2012 Annual Meeting: Yin et al, Cure of castration-resistant prostate cancer in TRAMP mice by reactivating tumor immunity with a phosphatidylserine-targeting antibody. Department of Pharmacology, The University of Texas Southwestern Medical Center at Dallas, Dallas, TX

Tumor Response to Bavi Equivalent Involves Increased CD8 T cells and Decreased MDSCs

**Graph:**
- **X-axis:** Experimental Days
- **Y-axis:** Tumor Volume (mm$^3$)
- **Legend:**
  - C44
  - Ch1N11 (PS Targeting)
- **Statistical Information:**
  - P=0.025

**EMT-6 breast tumor model in Balb/c mice**
(N=10/group; mean ± sem)
Bavituximab Blocks Immunosuppression
Activates Immune Response

Blocks PS Signaling
- Blocks PS receptor immunosuppressive signal
- IL-10 and TGF-β levels decrease

Activates Immune Response
- Fc / Fcy receptor signaling
- MDSC differentiation
- M2 to M1 macrophage polarization
- DCs mature, present tumor antigens to T-cells

Sustained Immune Response
- Increased Inflammatory cytokines
- M1 macrophages kill tumor cells via ADCC
- Activated T-cells kill tumor cells

Promising and Consistent Pre-Clinical Data Support Phase III Development in Combination with Docetaxel

- **Rationale-based Combination**
  - Docetaxel has been shown to increase PS exposure (AACR 2013 Poster #2850)
  - Docetaxel has immunostimulatory effects (Kodumudi et al, Clin Cancer Res 2010;16:4583-4594)

- **Compelling Pre-clinical Data**
  - Tumor models show greater than 90% reduction in tumor growth with bavi equivalent plus docetaxel (Cancer Research 2005; 65: (10) 4408-4416)
Final Phase II 2nd Line NSCLC Overall Survival Data with Docetaxel

Overall Survival
Intent-to-Treat Patient Population

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Median (months)</th>
<th>HR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined Control</td>
<td>7.3 (5.6, 12.1)</td>
<td>0.662</td>
<td>0.113</td>
</tr>
<tr>
<td>Bavi 3 mg/kg</td>
<td>11.7 (5.2, 17.3)</td>
<td></td>
<td>0.128</td>
</tr>
</tbody>
</table>

Kaplan-Meier Estimate

Months

Combined Control: 80 49 34 14 5 0 0
Bavi 3 mg/kg: 41 25 16 11 3 1 0

23MAY13 - SAS Version 9.3
Global Phase III Registration Trial

- Positive EOP2 discussion with FDA and Ex-US Regulatory Agencies
  - Single Phase III global registration trial
  - > 150 sites (U.S., Europe and Asia Pacific)
  - Est. 24 month enrollment and 12 month follow-up
  - Powered to show approx. 2 month improvement in median overall survival
  - Enrollment initiated January 2014

- Patient Criteria:
  - Stage IIIb/IV Non–squamous NSCLC
  - Only one prior systemic therapy for advanced disease
  - Unselected for genetic mutations
  - Granted Fast Track Designation January 2014

- N = 582
  - Randomized 1:1
  - Placebo-Controlled

- Bavituximab (3 mg/kg)
  - Weekly

- Placebo
  - Weekly

- Docetaxel
  - Day 1, of 21-day cycles for up to 6 cycles

- Primary Endpoint:
  - OS

- Secondary Endpoints:
  - PFS, ORR, Safety
## Promising ORR, PFS and Survival Data in Breast Cancer

<table>
<thead>
<tr>
<th>Single-Arm Trials</th>
<th>n=</th>
<th>ORR</th>
<th>PFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Advanced/MBC – All Types (1 Prior Chemo)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bavituximab + Docetaxel</td>
<td>46</td>
<td>61%</td>
<td>7.4</td>
<td>20.7</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>29</td>
<td>41%</td>
<td>4.5</td>
<td>11.4</td>
</tr>
<tr>
<td><strong>Advanced/MBC – All Types (Front-Line)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bavituximab + Carbo/Paclitaxel</td>
<td>46</td>
<td>74%</td>
<td>6.9</td>
<td>23.2</td>
</tr>
<tr>
<td>Carboplatin/Paclitaxel</td>
<td>100</td>
<td>62%</td>
<td>4.8</td>
<td>16.0</td>
</tr>
<tr>
<td><strong>MBC (Her2 neg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bavituximab + Paclitaxel</td>
<td>14</td>
<td>85%</td>
<td>7.3</td>
<td>--</td>
</tr>
</tbody>
</table>

Bavi IST: Alison Stopeck
A HCC Responsive Patient Treated with Bavituximab in Combination with Sorafenib

Pt ID #P2-23 (7.98)

Pre

Post

H&E

IHC score: 2

IHC score: 3

CD68

IHC score: 3

IHC score: 3

CD4

IHC score: 2

IHC score: 3

FoXP3

IHC score: 1

IHC score: 0

CD8

IHC score: 2

IHC score: 3

GranzymeB

IHC score: 1

IHC score: 2

Increase in tumor immune activity: increase CD8+ & CD4+ T cells
Hypothesis: Bavituximab Can Potentiate Additional anti-PD-1 Responders

Phase II HCC Data: *Increased T-cell tumor infiltration*

- Best immune responses observed in patients with greatest % increase in PD-1 and CD8+ cells

![PD1 Expression Changes]

<table>
<thead>
<tr>
<th>Patients with pre- and post-tumor tissues available</th>
<th>TTP (months)</th>
<th>OS (months)</th>
<th>Immune Cell Type % Fold Increase Post Treatment (one cycle)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CD4</td>
</tr>
<tr>
<td>P2-27</td>
<td>4.0</td>
<td>6.0</td>
<td>3.0</td>
</tr>
<tr>
<td>P2-23</td>
<td>9.2</td>
<td>9.6</td>
<td>0.9</td>
</tr>
<tr>
<td>P2-13</td>
<td>5.3</td>
<td>5.3</td>
<td>0.5</td>
</tr>
<tr>
<td>P2-14</td>
<td>5.0</td>
<td>8.0</td>
<td>0.5</td>
</tr>
<tr>
<td>P2-21</td>
<td>2.4</td>
<td>3.0</td>
<td>1.5</td>
</tr>
<tr>
<td>P2-12</td>
<td>2.0</td>
<td>2.7</td>
<td>0.6</td>
</tr>
</tbody>
</table>

= poor immune responders
PD-L1/PD-1 Signaling = Suppression of T-Cell Function

Adapted from Nguyen et al, Nature Reviews: Immunology 15, 45–56 (2015) and Lee et al, Advances in Bioscience and Biotechnology; 2013, 4, 19-29
## Relationship of PD-L1 Expression and Activity in Tumor Biopsies Evaluable by IHC

### NSCLC

<table>
<thead>
<tr>
<th>PD-L1 Tumor Expression</th>
<th>N=</th>
<th>Responses</th>
<th>PFS at 6 mo.</th>
<th>OS at 6 mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>7</td>
<td>4 (57%)</td>
<td>67%</td>
<td>89%</td>
</tr>
<tr>
<td>Low</td>
<td>21</td>
<td>1 (5%)</td>
<td>11%</td>
<td>33%</td>
</tr>
</tbody>
</table>

### Melanoma

<table>
<thead>
<tr>
<th>PD-L1 Tumor Expression</th>
<th>N=</th>
<th>Responses</th>
<th>median PFS</th>
<th>OS at 6 mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>60</td>
<td>29 (48%)</td>
<td>10.6</td>
<td>93%</td>
</tr>
<tr>
<td>Negative</td>
<td>22</td>
<td>1 (5%)</td>
<td>2.9</td>
<td>75%</td>
</tr>
</tbody>
</table>

AACR 2014 Annual Meeting, abstract CT105
MK-3475 (anti-PD-1 monoclonal antibody) for non-small cell lung cancer (NSCLC): Antitumor activity and association with tumor PD-L1 expression

AACR 2014 Annual Meeting, abstract CT104
Antitumor activity of the anti-PD-1 monoclonal antibody MK-3475 in melanoma (MEL): Correlation of tumor PD-L1 expression with outcome
Upstream & Downstream Checkpoint Combinations

PS / PSR: Upstream “Immune Checkpoint”

Immunosuppressive Cytokines/ Enzymes
- TGF-β
- IL-10
- IL-21
- IDO

Downstream “Immune Checkpoints”
- PD-1
- PD-L1
- CTLA-4
- TIM-3

PS Receptors:
- Axl
- TIM-1
- TIM-4 Hokkaido University, Japan
- TIM-3

PS Receptors:
- Peregrine
- Genentech
- Celldex therapeutics
- Hokkaido University, Japan
- Novartis

Tumor Cell
- MDSC
- M2 Macrophage
- Immature Dendritic Cell
- T
- NK

Exposure:
- = Exposed PS

B7-H3
- B7-H3L
- PD-1
- PD-L1
- KIR
- TIM-3
PS Blockade Synergizes with Anti-PD-1

Mice bearing K1735 melanoma tumors were treated with anti-PD-1 or mch1N11 + anti-PD-1, 2.5 mg/kg 2x per week.
Combination Treatment Repolarizes Immune Profile in Spleen

Results of PS Blockade + anti-PD-1 Versus anti-PD-1 Alone (K1735 melanoma model)

CD8+ Cells

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8 cells</td>
<td>↑ 40%</td>
<td>0.056</td>
</tr>
<tr>
<td>PD-1+</td>
<td>No Change</td>
<td></td>
</tr>
<tr>
<td>IFN-γ+</td>
<td>↑ 40%</td>
<td>0.065</td>
</tr>
<tr>
<td>IL-2+</td>
<td>↑ 80%</td>
<td>0.034</td>
</tr>
</tbody>
</table>

CD4+ Cells

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 cells</td>
<td>No Change</td>
<td></td>
</tr>
<tr>
<td>T-reg</td>
<td>No Change</td>
<td></td>
</tr>
<tr>
<td>PD-1+</td>
<td>No Change</td>
<td></td>
</tr>
<tr>
<td>IL-2+</td>
<td>↑ 150%</td>
<td>0.018</td>
</tr>
</tbody>
</table>

MDSC / Macs

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD11b+</td>
<td>No Change</td>
<td></td>
</tr>
<tr>
<td>MDSC (via CD11b)</td>
<td>↓ 45%</td>
<td>0.001</td>
</tr>
<tr>
<td>PD-L1+ CD11b cells</td>
<td>No Change</td>
<td></td>
</tr>
<tr>
<td>PD-L1+ CD11b/Ly6G-</td>
<td>No Change</td>
<td></td>
</tr>
<tr>
<td>PD-L1+ CD11b/Ly6G-</td>
<td>No Change</td>
<td></td>
</tr>
<tr>
<td>DC (CD11c&lt;sup&gt;hi&lt;/sup&gt;/F480&lt;sup&gt;lo&lt;/sup&gt;)</td>
<td>↑ 30%</td>
<td>0.020</td>
</tr>
<tr>
<td>Mac (CD11b/F480&lt;sup&gt;hi&lt;/sup&gt;)</td>
<td>No Change</td>
<td></td>
</tr>
</tbody>
</table>

- PS Blockade + Anti-PD-1 shifts spleenocytes to immune active phenotype
- Data support potentiating additional PD-1 responders via PS blockade.
PS Targeting Treatment Dramatically Reduces Expression of PDL-1 on Tumor Isolated Leukocytes

85 to 90% of PD-L1+ cells are CD45+ leukocytes, less than 10-20% PDL-1+ cells are tumor cells in this analysis.
Combination Treatment Repolarizes Immune Profile in Tumor

Results of PS Blockade + Anti-PD-1 Versus anti-PD-1 Alone (K1735 melanoma model)

<table>
<thead>
<tr>
<th>CD8+ Cells</th>
<th>P =</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8 cells</td>
<td>30% 0.001</td>
</tr>
<tr>
<td>CD8 /T-reg Ratio</td>
<td>100% &lt; 0.01</td>
</tr>
<tr>
<td>PD-1+</td>
<td>50% 0.001</td>
</tr>
<tr>
<td>Lag-3+</td>
<td>90% &lt; 0.01</td>
</tr>
<tr>
<td>IFN-γ+</td>
<td>70% 0.017</td>
</tr>
<tr>
<td>TNF-α+</td>
<td>70% 0.007</td>
</tr>
<tr>
<td>Granzyme-B+</td>
<td>70% 0.017</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CD4+ Cells</th>
<th>P =</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 /T-reg Ratio</td>
<td>100% &lt; 0.01</td>
</tr>
<tr>
<td>PD-1+</td>
<td>No change</td>
</tr>
<tr>
<td>CD137+ (41BB)</td>
<td>60% 0.03</td>
</tr>
<tr>
<td>IFN-γ+</td>
<td>30% &lt; 0.05</td>
</tr>
<tr>
<td>TNF-α+</td>
<td>100% &lt; 0.01</td>
</tr>
<tr>
<td>IL-2+</td>
<td>60% 0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MDSC / Macs</th>
<th>P =</th>
</tr>
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<tbody>
<tr>
<td>MDSC (via CD11b)</td>
<td>40% 0.02</td>
</tr>
<tr>
<td>iNOS+ CD11b cells</td>
<td>90% 0.001</td>
</tr>
<tr>
<td>PD-L1+ TIL</td>
<td>80% &lt;0.01</td>
</tr>
</tbody>
</table>

- PS Blockade + Anti-PD-1 increases TIL; shifting TIL and cytokines towards tumor-rejecting phenotype.
- Data support potentiating additional PD-1 responders via PS blockade.
Key Takeaway: PS Blockade Optimizes Tumor Environment for Additional Anti-PD-1 Responders

Results of PS Blockade + anti-PD-1 versus anti-PD-1 alone (K1735 melanoma model)

- 40% reduction* of myeloid-derived suppressor cells
- 30% increase* in CD8+ T-cells
- 50% increase* in PD-1 expression
- 80% decrease* PD-L1 expression on TIL (CD45+)

* Statistically significant (p = < 0.02)
PS Targeted Combination Therapy in PD-1 Resistant Melanoma Increases the Anti-tumor Response Rate

B16F10 Melanoma Tumor Model in C57BL Mice (10/group)
**Immune Cell Changes in the Tumor Microenvironment in the Mouse B-16F10 Model**

<table>
<thead>
<tr>
<th>Treatment Type (Day 23, after 4 treatments)</th>
<th>CD45 (WBC)</th>
<th>CD3* (mature T-cells)</th>
<th>CD8** (Cyto T-cells)</th>
<th>CD4** (helper T-cells)</th>
<th>T reg</th>
<th>Tumor Size (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control mAb (2)</td>
<td>20.1</td>
<td>37.8</td>
<td>55.4</td>
<td>20.8</td>
<td>17.4</td>
<td>3325</td>
</tr>
<tr>
<td>ch1N11 (anti- PS) (7)</td>
<td>23.7</td>
<td>43.7</td>
<td>53.8</td>
<td>33.7</td>
<td>10.7</td>
<td>2165</td>
</tr>
<tr>
<td>Anti-CTLA-4 (5)</td>
<td>16.9</td>
<td>39.8</td>
<td>45.1</td>
<td>40.4</td>
<td>9.3</td>
<td>2204</td>
</tr>
<tr>
<td>Anti-PD-1 (6)</td>
<td>27.3</td>
<td>42.2</td>
<td>49.4</td>
<td>33.9</td>
<td>19.2</td>
<td>1797</td>
</tr>
<tr>
<td>ch1N11 + Anti-PD-1 (5)</td>
<td><strong>34.4</strong></td>
<td><strong>51.1</strong></td>
<td><strong>69.7</strong></td>
<td><strong>20.5</strong></td>
<td>17.3</td>
<td><strong>869</strong></td>
</tr>
</tbody>
</table>

* Percentage of CD45 (leucocyte) positive cells
** Percentage of CD3 (mature T-cell) positive cells

**Statistically significant change**
Key Observations - Summary

- Phosphatidylserine (PS) is externalized in the tumor microenvironment and is a major immunosuppressive signal.

- PS is a global immune checkpoint.

- Antibody-mediated blockade of PS signaling breaks immune tolerance reactivating innate and adaptive immunity and results in durable anti-tumor responses in multiple pre-clinical models.

- Combination pre-clinical studies in immuno-competent models with PD-1 and CTLA-4:
  - increase CD8 T cells and M1 macrophages
  - decrease MDSCs and M2 macrophages
  - demonstrate statistically significant anti-tumor responses.
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