Expression of Novel Immunotherapeutic Targets in Triple Negative Breast Cancer

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Diclosures

• Barbara A. Pockaj, MD
  – Nothing to disclose

• Collaborators are employees at Caris Life Sciences
Learning Objectives

• Discuss immune checkpoints and their ramifications in human cancers
• Evaluate the presence of PD-L1 expression in a large breast cancer population
• Identify immune and molecular pathway associations with PD-L1
• Review preliminary validation data
Introduction – Immune Checkpoints

• Immune checkpoints regulate the duration and level the T-cell response
  – Cytotoxic T-lymphocyte Antigen-4 (CTLA-4) functions as an “off” switch to T-cell activity in the priming phase
  – Programmed Death (PD-1) regulates T-cell activity during the effector phase and can shut down antigen-specific T cells in the tumor microenvironment
    • Tumor cells can block the immune response via the PD-1 checkpoint by expressing programmed death ligands (PD-L1) and inactivating T-cells
Introduction – Immune Mechanisms

• IDO1 indoleamine 2,3-dioxygenase 1 (IDO-1) – catalyzes the first and rate-limiting step in tryptophan catabolism
  – Important to immune tolerance and immunosuppression
  – IDO-1 inhibitors are available
    • Current trials are now underway
Clinical Application of Immunotherapy

• Several drugs have been developed which block the CTLA-4 and PD1 Immune Checkpoints
  – Anti-CTLA-4
    • Ipilimumab
    • Tremelimumab
  – Anti-PD1
    • Nivolumab (BMS936558/MDX-1106)
    • Lambrolizumab (MK-3475)
  – Anti-PD-L1
    • BMS-936559, MDX-1105
    • MPDL3280A/RG7446
    • MEDI4736
    • AMP-224
    • Pidilizumab (CT-011)
<table>
<thead>
<tr>
<th>Trial</th>
<th>Drug</th>
<th>Tumor Type</th>
<th>Response Rate</th>
<th>Immune Correlates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topalian, NEJM, 2012</td>
<td>Nivolumab (BMS-936558)</td>
<td>Melanoma, NSCLC, Renal Cell, Colorectal, Prostate</td>
<td>18% NSCLC 28% Melanoma 27% Renal</td>
<td>Response related to PD-L1 tumor expression 36% Response PD-L1+ 0% Response PD-L1-</td>
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<tr>
<td></td>
<td>Anti-PD-1</td>
<td></td>
<td></td>
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<tr>
<td>Wolchok, NEJM, 2013</td>
<td>Nivolumab + Ipilimumab</td>
<td>Melanoma</td>
<td>40% Concurrent Therapy 20% Sequential Therapy</td>
<td>PD-L1 Expression did not correlate to response for concurrent therapy but did for sequential therapy</td>
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<td></td>
<td>Anti-PD-1 and Anti-CTLA-4</td>
<td></td>
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</tr>
<tr>
<td>Daud, AACR, 2014</td>
<td>MK-3475 Anti-PD-L1</td>
<td>Melanoma</td>
<td>ORR 41%</td>
<td>ORR correlated with PD-L1 expression 52% ORR PD-L1+ 6% PD-L1-</td>
</tr>
</tbody>
</table>

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Methods

• 3993 formalin fixed, paraffin embedded breast cancer samples (Caris Life Sciences)

• Gene expression was performed using Illumina HumanHT-12 v4 BeadChip

• The Comprehensive R Archive Network was used for statistical computing and graphics

• The study was IRB approved
Breast Cancer
N=3993

ER-
- HER2-
  - PR- N=515
  - PR+ N=125
- HER2+
  - PR+ N=33
  - PR- N=271

ER+
- HER2+
  - PR+ N=133
  - PR- N=125
- HER2-
  - PR+ N=1867
  - PR- N=924
Methods

• Validation:
  – Immunohistochemistry (Caris Life Sciences)
    • Slides were stained using an automated system (Ventana Medical Systems, Tucson, AZ) as per manufacturer’s protocol with proprietary reagents.
    • IHC stained slides were scored by pathologists.
      – Tumor staining was scored for all markers except for PD1 which was scored in the tumor infiltrating lymphocytes.
  – BRCA 1 somatic mutation testing was performed by Next Gen Sequencing (Illumina Miseq platform)
    • Sequencing plots were read by board certified geneticists.
Methods

- Validation
  - 18 TNBC cases analyzed at Mayo Clinic in Arizona using array-based comparative genomic hybridization (aCGH) to evaluate genomic amplifications and deletion
RESULTS
PD-L1 Levels

Data was normalized by doing mean normalization (using mean value from control normal breast tissue)

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CTLA-4 and IDO1 Levels

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CTLA-4

IDO1
PD-L1 Associations

• Spearman correlation test
  – Positive correlation with immune regulators:
    • CTLA-4  correlation coefficient 0.528
    • IDO1   correlation coefficient 0.481
  – Mixed results with the Phosphatidylinositol 3-kinase (PI3-kinase) Pathway
    • PIK3CA  correlation coefficient 0.39
    • PTEN   correlation coefficient 0.11
AR Expression and PD-L1

- Anova p value = .05
- Suggests that there is a relationship between AR expression and PDL1 expression
  - AR- higher likelihood of expressing
- Similar finding with IDO1 and CTLA-4

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Differential expression of 144 genes based on T test between the high and low PD-L1 expressers:
• 4 distinct clusters noted in the PD-L1 low vs high population
Heatmap Analysis

- WebGestalt a "WEB-based GEne SeT AnaLysis Toolkit" was used to do enrichment analysis of the heatmap data
  - DNA repair genes were significant (adjusted p value=0.02)
    - BRCA1
    - Fanconi anemia, complementation group A
    - HUS1 checkpoint homolog (S. Pombe)
Validation

- 36 TNBC patients were profiled for PD1, PD-L1, AR, BRCA1 mutation.
  - PD-L1 expression 10 patients (28%)
  - PD-1 expression is present in 22 patients (61%)
  - Co-expression of PD-1 and PD-L1 was found in 7/10 patients (70%)
Validation

• AR expression found in 9 (25%) patients
  – Only 1 patient (11%) to be PD-L1+
• 33% of AR- TNBC were PD-L1+
• 90% PD-L1+ were AR-

• 3/3 BRCA1 mutated patients were PD-L1+

• 4/4 BRCA1+ (Mayo Samples) were PD-L1+
PI-3 Kinase Pathway

• Loss of PTEN expression was present in 19 patients (54%)
  – Only 4 of these patients were PD-L1+ (21%)
• PI3K mutation was present in 5 patients (14%)
  – Only 1 patient (20%) was PD-L1+
Validation

• cGH revealed over-expression of PD-L1 in 3/18 patients (17%)
  – All patients were AR- 3/11 (27%)
    • Mixed PI-3 Kinase pathway changes
Validation

CD274 (PD-L1)

Data provided by Michael Barrett, PhD

Chromosome 9

3.4N genome

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Conclusions

- A subset of TNBC patients express immune regulatory targets suggesting immunotherapy may be an effective option
  - PD-L1+ appears to be associated with
    - AR- TNBC
    - BRCA1 mutated TNBC
- High expression of PD-L1 in BRCA1 deficient as well as BRCA1 mutated patients indicate that anti PD-1/PD-L1 therapy in combination with platinum salts and/or PARP inhibitors may be a synergistic treatment strategy that warrants further study.

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Conclusions

• Consistent association with the PI-3 Kinase pathway are not found
• Further validation of findings are ongoing
  – Does PD-L1 overexpression by cGH lead to PD-L1 expression as seen by IHC?
  – Is PD-L1 expression correlated with BRCA1 mutation?
    • Would BRCA1 mutated patients benefit from immunotherapy?
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– 6,400 referring physicians
– All 50 states
– 30 countries

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– Pathologists and PAs
– Molecular Geneticists
– Consulting Medical Oncologists
– scientists
-- Entire lab staff

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