Understanding Relevant Immune Pathways by the Oncologist

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Disclosures

iTeos Therapeutics  Scientific Advisory Board
eTheRNA  Scientific Advisory Board
PDC*line Pharma  Scientific Advisory Board
BMS  Educational tasks
Merck  Educational tasks
Sopartec-UCL  Revenues from licensing
Looking for Killers

- The classical view:
Principal immune effectors in cancer immunotherapy: cytolytic T lymphocytes

Exquisite specificity
Memory
Cytolytic T lymphocytes (CTL) recognize on the surface of target cells **peptides** presented by **HLA class I** molecules (HLA-A, B, C).
Looking for Killers

- The classical view: CD8$^+$ CTL > tumor antigen on HLA class I

- Expected results:
Metastatic melanoma, resistance to checkpoint blockade through inactivation of antigen presentation

anti-CTLA-4
anti-CTLA-4

![Survival analysis graph showing the effect of B2M LOH on patient survival. The graph compares survival rates between patients with and without B2M LOH, with a Log-rank P value of 0.01.]
Looking for Killers

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- Expected results: loss of surface HLA class I expression > resistance
Looking for Killers

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- Unexpected results:
<table>
<thead>
<tr>
<th>% of tumors (n = 72)</th>
<th>β2 microglobulin</th>
<th>HLA class I</th>
<th>HLA class II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Co-staining for PAX5 to identify the PAX5\textsuperscript{dim+} malignant Hodgkin Reed-Sternberg cells
## HLA expression (protein) in classic Hodgkin lymphoma

<table>
<thead>
<tr>
<th>% of tumors (n = 72)</th>
<th>β2 microglobulin</th>
<th>HLA class I</th>
<th>HLA class II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>7</td>
<td>7</td>
<td>39</td>
</tr>
<tr>
<td>Decreased</td>
<td>22</td>
<td>29</td>
<td>32</td>
</tr>
<tr>
<td>Negative</td>
<td>71</td>
<td>64</td>
<td>69</td>
</tr>
</tbody>
</table>

Roemer et al. - 2018 – JCO
HLA expression and outcome after PD-1 blockade in Hodgkin

β2 microglobulin

Numbers of patients (Best Overall Survival)

HLA class I

Roemer et al. - 2018 – JCO
HLA expression and outcome after PD-1 blockade in Hodgkin

HLA class II

<table>
<thead>
<tr>
<th></th>
<th>Negative (n = 20)</th>
<th>Decreased (n = 22)</th>
<th>Positive (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD</td>
<td>1 (2)</td>
<td>3 (4)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>SD</td>
<td>4 (6)</td>
<td>16 (23)</td>
<td>4 (6)</td>
</tr>
<tr>
<td>PR</td>
<td>14 (20)</td>
<td>3 (4)</td>
<td>13 (19)</td>
</tr>
<tr>
<td>CR</td>
<td>1 (2)</td>
<td>8 (12)</td>
<td></td>
</tr>
</tbody>
</table>

Roemer et al. - 2018 – JCO
Looking for Killers

- The classical view: CD8+ CTL > tumor antigen on HLA class I
- Expected results: loss of surface HLA class I expression > resistance
- Unexpected results:
  - Hodgkin: clinical responses despite absence of surface HLA class I
Surface HLA class I staining patterns on CRC

Loss  Regular  Weak

confirms previous observations:

1998  Cabrera et al. (F. Garrido) Tissue Antigens 52:114
2005  Kloor et al. (S. Ferrone) Cancer Res 65:6418
2007  Dierssen et al. BMC Cancer 7:33
Surface HLA class I staining patterns on CRC according to MMR-d/p
Surface HLA class I staining patterns on CRC according to MMR-d/p CMS subtypes
Surface HLA class I staining patterns on CRC according to MMR-d/p CMS subtypes metastatic sites

Ijsselsteijn et al. - 2019 – Br J Cancer
Genetic alterations in genes involved in HLA class I pathway

Ijsselsteijn et al. - 2019 – Br J Cancer
Genetic alterations in genes involved in HLA class I pathway

<table>
<thead>
<tr>
<th>HLA class I loss</th>
<th>HLA-A</th>
<th>HLA-B</th>
<th>HLA-C</th>
<th>B2M</th>
<th>TAP1</th>
<th>TAP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRC1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CRC2</td>
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<td>CRC4</td>
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<td>CRC5</td>
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<td>CRC6</td>
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<tr>
<td>CRC7</td>
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<tr>
<td>CRC8</td>
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<tr>
<td>CRC9</td>
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<tr>
<td>CRC10</td>
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<td></td>
</tr>
<tr>
<td>CRC11</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CRC12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **STOPgain**: Red
- **Frameshift InDel**: Orange
- **Non-frameshift InDel**: Purple
- **Splice-site**: Blue
- **Missense**: Green
Cytolytic T lymphocytes (CTL) recognize on the surface of target cells peptides presented by HLA class I molecules (HLA-A, B, C).

Antigens recognized by CD8 T cells on the surface of tumor cells
Genetic alterations in genes involved in HLA class I pathway
Genetic alterations in genes involved in HLA class I pathway
HLA class I antigen presentation pathway

Golgi

Endoplasmic reticulum

TAP

ERAP1

ERAP2

Tapasin

Calreticulin

ERp57

MHC class I

26S proteasome

Cytosolic proteases

Ubiquitinated Intracellular protein

adapted from Vigneron et al. - 2018 – Mol Immunol
Genetic alterations in genes involved in HLA class I pathway

Ijsselsteijn et al. - 2019 – Br J Cancer
Clinical response to PD-1 blockade in CRC patients with MSI tumors and loss of β2m

Middha et al. - 2019 – JCO Precis Oncol
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- Other lytic cells than CD8⁺ CTL have to be involved in these clinical responses
  - should be tumor-specific, or at least tumor-selective
  - should be able to express PD-1
Lytic effector cells

- CTL CD8⁺
- CTL CD4⁺ (antigen presented on HLA class II molecules)
- Natural Killer cells

Mechanisms of lysis:
- Lytic granules (Perforin, Granzymes)
- Tumor Necrosis Factor
- FasL
- Tumor necrosis factor Related Apoptosis Ligand (TRAIL)
NK cell effector functions

NK 'tolerance'

NK cell activation

Vivier et al. - 2011 – Science
NK cell receptors

**Activating receptors**

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Adaptors</th>
<th>Inhibitory receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>NKp46</td>
<td>CD3ζ, FcRγ</td>
<td>h KIR-L</td>
</tr>
<tr>
<td>CD16</td>
<td>CD3ζ, FcRγ</td>
<td>h LILRB1</td>
</tr>
<tr>
<td>h NKp30</td>
<td>CD3ζ, FcRγ</td>
<td>CD94/NKG2A</td>
</tr>
<tr>
<td>h NKp44</td>
<td>DAP12</td>
<td></td>
</tr>
<tr>
<td>h NKp80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NKG2D</td>
<td>FcRγ</td>
<td></td>
</tr>
<tr>
<td>h KIR-S</td>
<td>DAP10, DAP12, DAP12, DAP10</td>
<td></td>
</tr>
<tr>
<td>CD94/NKG2C</td>
<td>DAP12</td>
<td></td>
</tr>
<tr>
<td>CRACC</td>
<td>SAP, EAT2</td>
<td></td>
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<tr>
<td>Ly9</td>
<td>SAP</td>
<td></td>
</tr>
<tr>
<td>CD84</td>
<td>SAP, EAT2</td>
<td></td>
</tr>
<tr>
<td>NTBA</td>
<td>SAP</td>
<td></td>
</tr>
<tr>
<td>2B4</td>
<td>SAP, EAT2, ERT</td>
<td></td>
</tr>
</tbody>
</table>

**Chemotactic receptors**

- KLRG-1
- TIGIT
- CEACAM-1
- LAIR-1

**Cytokine receptors**

**Adhesion receptors**

Vivier et al. - 2011 – Science
Lytic effector cells

- CTL CD8+
- CTL CD4 +
- Natural Killer cells
- NKT cells
- \( \gamma \delta \) T cells
- Innate Lymphoid Cells

Mechanisms of lysis:
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- Other lytic cells than CD8\(^+\) CTL have to be involved in these clinical responses
  - should be tumor-specific, or at least tumor-selective
  - should be able to express PD-1

- Different mechanisms might lead to tumor regression and tumor stabilization

- Different killers, with different specificities, probably act on different tumors

- 'Finding the killers' might open new possibilities for currently resisting tumors