Small-molecule inhibitors of the IDO pathway as immune modulators
In compliance with ACCME policy, the following are disclosed to the activity audience:

<table>
<thead>
<tr>
<th>Role</th>
<th>Organization</th>
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<tbody>
<tr>
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<td>NewLink Genetics, Inc.</td>
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<td>Consultant</td>
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<td>Scientific Advisory Board</td>
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</tr>
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</table>
Immunotherapy of Cancer: Statement of the problem

- Immunotherapy of cancer must do more than simply present antigens to the immune system...
  ... it must disrupt a pre-existing state of functional tolerance toward tumor antigens.
Tumor-induced tolerance

Tolerance is **acquired**, **active** and **dominant**

- **acquired** because even truly foreign antigens will become tolerated if introduced on tumors

- **active** because tolerance cannot be overcome simply by a good antigen and a strong adjuvant
  
  .... (i.e., responses are actively suppressed)

- **dominant** because even vaccination or adoptive transfer of pre-activated T cells is still subject to suppression
Indoleamine 2,3-dioxygenase (IDO)

- IDO is a natural endogenous molecular mechanism of immune suppression
  - involved in pregnancy, mucosal tolerance
- IDO can create acquired peripheral tolerance *de novo*
Haplo-mismatched allografts transfected with IDO are tolerated without additional immunosuppression

figure adapted from KA Swanson, David S. Wilkes et al

© 2004 [American Thoracic Society](https://www.ats.org)
IDO and malignancy

• IDO is expressed by cancer cells in a range of tumor types

• High IDO expression appears correlate with poor outcome in a number of cancers
  • ovarian cancer
  • AML
  • endometrial carcinoma
  • colon cancer
  • melanoma
IDO is also expressed by host cells in tumor-draining lymph nodes (human melanoma, breast cancer, etc)

Predictive value of abnormal IDO expression in human tumor-draining lymph nodes

- 40 patients with cutaneous malignant melanoma, no metastases
- sentinel lymph node obtained at time of initial diagnosis
- in collaboration with Scott Antonia at Moffitt Cancer Center

IDO-inhibitor (NSC-721782): 1-methyl-[D]-tryptophan (D-1MT)
1MT is synergistic with chemotherapy
(MMTV-neu tumor model – Prendergast lab)

Table 1 IDO inhibition enhances the efficacy of certain commonly used cancer chemotherapeutic agents

<table>
<thead>
<tr>
<th>Compound</th>
<th>Class</th>
<th>Mean ± s.e. (+1MT)</th>
<th>Mean ± s.e. (−1MT)</th>
<th>P</th>
<th>n</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>Alkylating agent</td>
<td>0.77 ± 0.18</td>
<td>1.7 ± 0.33</td>
<td>0.0419</td>
<td>7.8</td>
<td>1.0</td>
<td>i.v.</td>
<td>3×/week</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Alkylating agent</td>
<td>0.81 ± 0.12</td>
<td>1.4 ± 0.18</td>
<td>0.0269</td>
<td>5.5</td>
<td>100</td>
<td>i.v.</td>
<td>3×/week</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Antineoplastic antibiotic</td>
<td>0.79 ± 0.07</td>
<td>1.5 ± 0.25</td>
<td>0.0150</td>
<td>6.4</td>
<td>0.66</td>
<td>i.v.</td>
<td>3×/week</td>
</tr>
<tr>
<td>S-Fluorouracil</td>
<td>Antimetabolite</td>
<td>1.2 ± 0.20</td>
<td>1.1 ± 0.25</td>
<td>0.8926</td>
<td>8.7</td>
<td>50</td>
<td>i.v.</td>
<td>3×/week</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Antimetabolite</td>
<td>1.7 ± 0.28</td>
<td>1.7 ± 0.38</td>
<td>0.9047</td>
<td>3.3</td>
<td>1.0</td>
<td>i.v.</td>
<td>3×/week</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>Mitotic inhibitor (taxane)</td>
<td>0.68 ± 0.11</td>
<td>2.4 ± 0.43</td>
<td>0.0010</td>
<td>8.7</td>
<td>13.3</td>
<td>i.v.</td>
<td>3×/week</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>Mitotic inhibitor (vinca alkaloid)</td>
<td>1.3 ± 0.19</td>
<td>1.2 ± 0.18</td>
<td>0.7368</td>
<td>10.8</td>
<td>1.0</td>
<td>i.v.</td>
<td>3×/week</td>
</tr>
<tr>
<td>FTI</td>
<td>Signal transduction inhibitor</td>
<td>0.67 ± 0.11</td>
<td>1.0 ± 0.16</td>
<td>0.0979</td>
<td>8.8</td>
<td>40</td>
<td>i.p.</td>
<td>qdx11</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>Signal transduction inhibitor</td>
<td>0.97 ± 0.07</td>
<td>0.99 ± 0.25</td>
<td>0.9417</td>
<td>4.4</td>
<td>1.5</td>
<td>i.v.</td>
<td>qdx11</td>
</tr>
<tr>
<td>Tetrathiomolybdate</td>
<td>Antiangiogenic (iron chelator)</td>
<td>1.9 ± 0.52</td>
<td>2.0 ± 0.42</td>
<td>0.7996</td>
<td>3.4</td>
<td>40</td>
<td>p.o.</td>
<td>qdx11</td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
<td>1.7 ± 0.17</td>
<td>3.0 ± 0.44</td>
<td>0.0061</td>
<td>12.5</td>
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</tbody>
</table>

Take-home message:
even genetically-diverse, spontaneous tumors show widespread dependence on the IDO mechanism after chemoRx
D vs L isomer of 1MT (4T1 breast-tumor model)  
(courtesy of George Prendergast lab, from Hou et al, Cancer Res. 2007)
IDO and Tregs form a mutually-reinforcing system

Plasmacytoid DC

Activation of pre-existing Tregs

Differentiation of new Tregs

IDO and Tregs form a mutually-reinforcing system
IDO directly activates (Tregs) in TDLNs


Pre-activated Treg from TDLN

HY antigen

Antigen-specific readout assay
IDO-inhibitor (1MT) is synergistic with vaccine against established tumors.
IDO regulates re-programming of Tregs into TH17-like T-helper cells in tumor-draining lymph nodes

From Sharma et al Blood 113:6102, 2009
IDO regulates Treg conversion to TH17 cells in part via CGN2-mediated suppression of IL-6 expression.
Phase I Trial
of 1-methyl-D-tryptophan

PI: Scott Antonia MD PhD
Co PI: Hatem Soliman MD
Dan Sullivan MD

Moffitt Cancer Center/Southeast Phase II Consortium

Chuck Link MD
Nick Vanahanian MD
William Ramsey MD PhD

NewLink Genetics Inc
Combination of IDO-inhibitor drugs with chemotherapy and immunotherapy

Chemotherapy
- IDO appears to be a non-redundant mechanism needed by the tumor to re-establish the suppressive milieu following chemotherapy
- Blocking IDO may thus promote immune response against the tumor after chemotherapy

Immunotherapy
- Tumor-induced IDO acts as a fundamental antagonist to anti-tumor immune responses generated by immunotherapy
- Blocking IDO allows re-programming of Tregs into TH17-like helper cells following vaccination
Lessons and Take Home Messages

• **Key points**
  - IDO acts to create a suppressive milieu in tumor and tumor-draining LN
  - IDO *promotes* Treg activation, and *prevents* vaccine-induced reprogramming of Tregs into T-helper cells

• **Potential impact on the field**
  - IDO-inhibitor drugs can be synergistic with chemotherapy, and together may open a “window of opportunity” for vaccines and other immunotherapy
  - The combination of a vaccine plus an IDO-inhibitor drug may be able to re-program Tregs in situ into non-suppressive T-helper cells (which may offer an alternative to Treg depletion)
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Lankenau Institute